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CONTENT

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INCIDENCE OF *ARMILLARIA* SPECIES IN AGRARIAN, FOREST AND ORNAMENTAL ECOSYSTEMS OF THE LAZIO REGION.

SUMMARY

A series of investigations to detect the incidence of the various species of *Armillaria* in the different ecosystems of the Lazio region of Italy have been carried out in the last fifteen years.

A. ostoyae has been found only in forests of *Abies alba* and *Pinus spp*, over 700 meters a.s.l.; *A. gallica* and *A. tabescens*, with a predominantly saprophytic character, have been found on very declining oak forests. The most numerous attacks have been ascribed to *A. mellea*, in various orchards, vineyards, olive groves, forests, wood plantations, shade trees and other ornamental ecosystems, on broadleaved trees and shrubs, but also on coniferous ones, often as a weakness parasite, but also with evident pathogenic capacity, especially in case of high inoculum pressure. The different predisposing factors have been discussed, with particular emphasis on the declining of the plants due to climatic change. The work concludes with some hints on the possible control strategies.

Keywords: *Armillaria*, orchards, forests, wood plantations, ornamental trees, predisposing factors, control.

INTRODUCTION

Armillaria is a fungal genus of the class Basidiomycetes, order Agaricales, necrotrophic, with rhizomorphs and, except for *A. tabescens*, with basidiomes equipped with rings (Figures 1-2). Its roles include primary pathogen, stress-induced secondary invader, and saprophyte. As pathogen, *Armillaria* invades the bark and cambium region of the root, collar, and basal part of the trunk, killing roots and trees of all sizes. Sign of its presence on the affected subject is the appearance, under the bark, of cream-white mycelium plaques with a characteristic fan shape, which can go up along the stem even for one or two

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meters (Figure 3), and the possible production, in the autumn months, of basidiomes in groups (Figures 1-2). Symptoms above ground are yellowish or undersized foliage, premature leaf drop, branch dieback in the upper crown, or rapid browning and death of the entire plant (see below), that can collapse to the ground for uprooting. As a root disease fungus, it is one of the most prominent killers and decayers of deciduous and coniferous trees and shrubs in orchards, natural forests, wood plantations, and ornamental ecosystems. The most important elements of diffusion and infection are rhizomorphs, as well as mycelium, passing through the soil (or by radical contact) from sick to healthy plants, often in an oil stain; infection rarely comes from basidiospores, usually through cut surface.

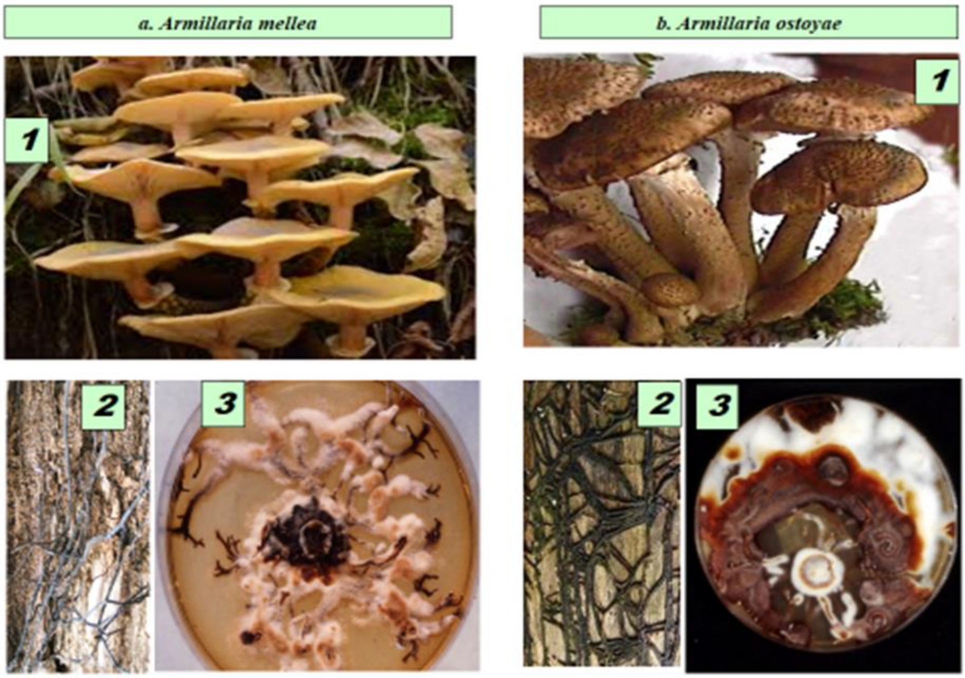

Fig. 1. Basidiomes (1), rhizomorphs (2) and in vitro colonies (3) of *Armillaria mellea* (a) and *Armillaria ostoyae* (b).

In the wake of studies on the cariological cycle and the tetrapolar etherotalism of the fungus conducted by Karl Korhonen (Korhonen, 1978) and subsequently by various other researchers, then validated by studies with isoenzymes (Anselmi *et al*., 1997) and, more recently, by molecular methods, in Italy 5 species of annulate *Armillaria* have been found: *A. cepistipes* Velenosky, saprophytes on conifers; *A. mellea* (Vahl: Fr) Kummer (Figure 1a), weakness parasite, but also primary pathogen, or saprophyte on broad-leaved trees and, less, coniferous species; *A. ostoyae* (Romagnesi) Herink (= *A. obscura* (Schaeffer Herink) (Figure 1b), primary or weakness parasite on conifers; *A. tabescens*

(Scop.) Emel. (Figure 2b) and *A. gallica* Marxmüller and Romagnesi (= *A. bulbosa* (Barla) Kile and Watling) (Figure 2a), weakness parasite or, above all, saprophyte on broad-leaved trees (Vannini and Magro, 1987; Intini, 1988, 1989, 1990, 1997; Anselmi and Lanata, 1989; Tirrò and Rapisarda, 1989; Ippolito *et al.*, 1989; Cellerino *et al.*, 1990; Shaw and Kile, 1991; Guillaumin *et al.*, 1993; Luisi *et al.*, 1996; Sicoli *et al.*, 2002; La Porta *et al.*, 2005).

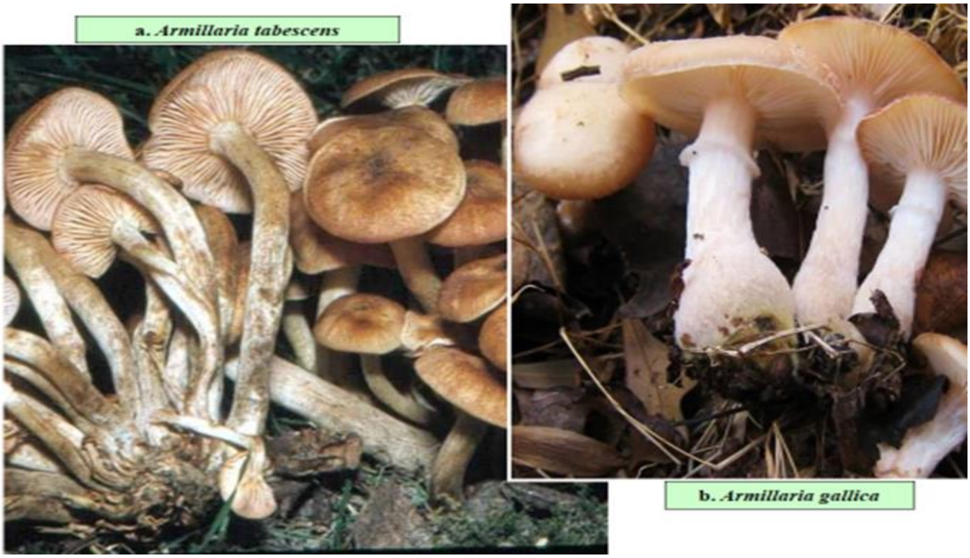

Fig. 2. Basidiomes of *Armillaria tabescens* (a) and *A. gallica* (b). (Carpofori di *Armillaria tabescens* (a) e *A. gallica* (b).

Fig. 3. Cream-white mycelium plaques of *Armillaria ostoyae* (a-b) and *A. mellea* (c-d) under the bark of basal part of the trunk of *Abies alba* (a), *Pinus nigra* (b) and *Prunus armeniaca* (c-d).

The most systematic territorial studies have involved northern and southern Italy and Tuscany, while various gaps remain regarding the lower part of central Italy, including Latium.

In the past, attacks of *Armillaria* in our country were complained mainly on orchards and shade trees, generally connected to soil with stagnant water, poorly drained, especially in the presence of high inoculum pressure. In recent decades, particularly in Mediterranean environments, the attacks of the fungus have become increasingly serious everywhere, even in forests, especially in dry areas, in parallel with the increasing phenomena of forest and tree declining, associated with climate change, often without clarity on the role of the fungus on the death of plants (Cellerino *et al.*, 1990; Luisi *et al.*, Eds, 1992; Luisi *et al.*, 1996).

Taking into account the Lazio region, heretofore neglected, in order to provide clarifications on the aforementioned aspects, this note aims to report the results of a series of investigations carried out in the last fifteen years to detect the incidence of the various species of *Armillaria* in various orchards, wood plantations, forests and ornamental ecosystems of the region and to identify the real predisposing factors for their attacks.

MATERIAL AND METHODS

The surveys have been conducted over the last fifteen years, on arboreal or shrubby agrarian, forest, or ornamental plants in various areas of the Lazio region, taking into account different stations from sea level to mountain areas, considering various sites per station. For some aspects the observations were carried out in stations already considered by other research projects, such as, for example, those relating to studies on “forest declining” (Figure 4) or on “parasitic attacks in deciduous plantations for valuable wood” (Figure 5).

Fig. 4. Stations of the region Latium in which investigations were carried out on the relationship between declining degree of oak forests and attacks of *Armillaria* species on the relative plants

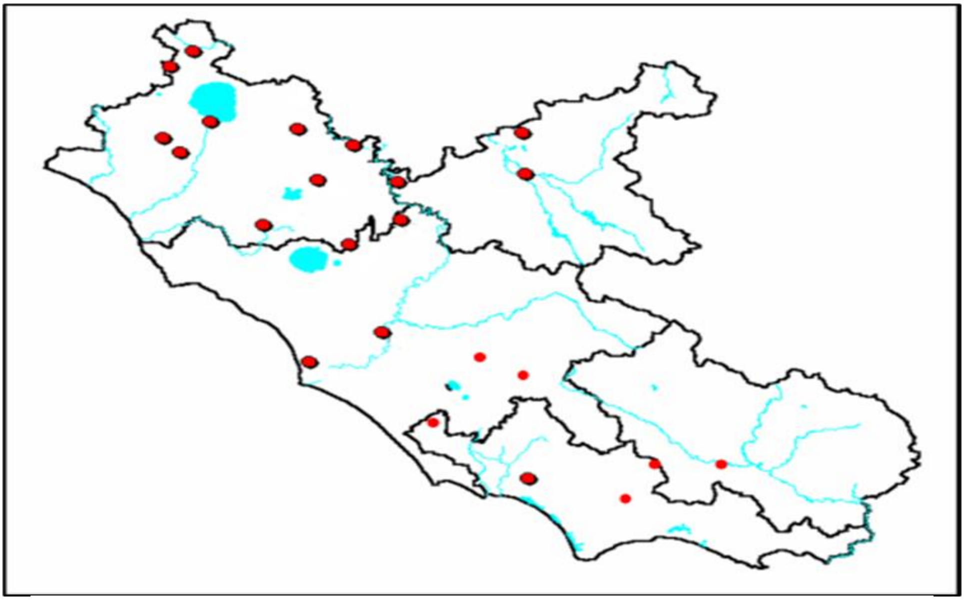

Fig. 5. Stations of the region Latium in which investigations on *Armillaria* attacks in valuable hardwood plantations were carried out.

Researche was directed at trees and shrubby plants with symptoms of suffering or decay, making observations on the proximal parts of the large roots, and on collar and the basal part of the trunk. In case of presence of *Armillaria*, for each station the following factors were noted: species, age and development of the affected plants, altitude, exposure, nature of the soil, presence of stagnant water, attacks by other parasites, damage from abiotic factors. In the case of agrarian plantations, the level of agronomic cures towards the plants, in particular fertilization, soil processing and irrigation, the crop preceding the plantation, the density of the plants and the eventual association with other trees or herbaceous crops were also noted. Similar observations were conducted for artificial wood plantations, where the dominance or not of the plants affected was also reported. In the ornamental field, the situation of the plant site, any excess or lack of irrigation, any critical situation that could cause suffering to plants, such as asphalting, cementing, pollution or other soil anomalies, atmospheric pollution, the state of the foliage, have also been recorded. In the case of surveys in the forest, the following additional factors were detected: turn of use, biodiversity, homogeneity or not of the plants; percentage of eventual desiccations to the canopy (declining index); incidence of grazing; presence of dead plants, including those collapsed to the ground. Observations on the development and integrity of mycorrhizae were also occasionally reported.

The identification of the *Armillaria* species was carried out on the basis of the rhizomorphs and above all the basidiomes appearance (Figures 1-2), and in the event of serious doubts, through observations on the appearance of the colonies (Figures 1.3) appropriately made to develop in Petri dishes (Intini, 1988; Anselmi and Lanata, 1989; Anselmi *et al*., 1990; Shaw and Kile, 1991).

During the processing of the surveys conducted, for some sites the monthly precipitation of the last eighteen years, collected by Meteorological Stations in Latium, has been analyzed.

RESULTS AND DISCUSSION

On the whole, in about 100 different stations (1500 sites), around 5000 plants apparently at risk of over 50 different agrarian, ornamental and forest species, arboreal or shrubby, were examined.

In ninety of these stations, the presence of *Armillaria* of four different species was detected: *A. mellea* (Figure 1a) and *A. ostoyae*, (Figure 1b) chiefly weakness pathogens, but often as primary pathogen, *A. tabescens* (Figure 2a) and *A. gallica* (Figure 2b), predominantly saprotrophs or, rarely, weakness pathogens.

Incidence of the various species.

Of the four species, *A. ostoyae* was the least widespread (6 stations), found only on conifers, and in particular in some woods of *Abies alba* (Figure 3a) and, above all, of *Pinus nigra* (Figure 6), at altitudes over 700 meters a.s.l.

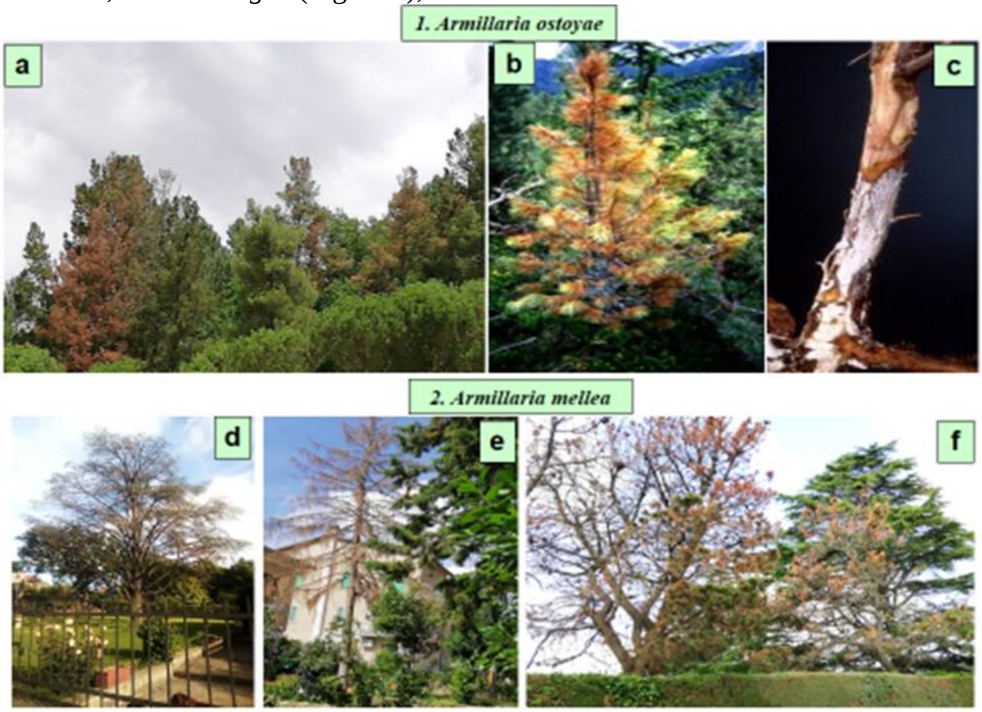

Fig. 6. Attacks of *Armillaria* on conifers: 1) *A. ostoyae* on *Pinus nigra* forest in Terminillo mountains. In b-c, infections on young plant. 2) *A. mellea* on ornamental trees of *Cedrus atlantica* (d) and *Abies alba* (e) incorrectly associated, and of *Pinus pinaster* attacked by *Tomicus* (f).

Its attacks have appeared to affect declining plants by strong drought, high competition with each other, or weakened by attacks of bark beetles. Some attacks have appeared to be associated with *Heterobasidion* root infections. In case of high inoculum pressure, the pathogen also affected young plants (Figures 6b-c), which soon ended up declining to death. A little more frequent were *A. tabescens,* (Figure 2a), found in 18 stations, with abundant basidiomes but rare rhizomorphs), and, less, *A. gallica* (Figure 2b), found in 12 stations, but with few basidiomes. These were usually found joined to *A. mellea*, with saprophytic character, in *Quercus* woods, either deciduous (*Q. cerris, Q. pubescens, Q. robur*, ecc.) or evergreen (*Q. ilex* and *Q. suber*), on very decaying or dead plants mainly due to "oak decline phenomena" (see below).

The most numerous attacks, however, were ascribed to *A. mellea*, on broadleaved trees and shrubs, but also on coniferous ones, both on orchards, forests, plantations and ornamental plants, in parks, gardens or shade trees, often as a weakness parasite, but also with evident pathogenic capacity, especially in case of high inoculum pressure. Infections in vineyards (Figure 7a), orchards (fig. 7b-c) or olive groves (Figure 7d) have appeared to be very diversified, depending on the cultivation status of the plant. The well-cultivated plantations were not much affected, except in areas with persistent water stagnation, on old plants or in the presence of old infected stumps of previous plants.

Fig. 7. Attacks of *Armillaria mellea* on agricultural arboreal plants: a. *Vitis vinifera*, b. *Prunus persica*, c. *Pirus communis*, d. *Olea europea*.

On grapevines, the attacks appeared as often confused with those from 'esca disease', much more widespread, and to which it often joins the same plant. *Olea europea* and *Amygdalus communis* have been attacked especially when associated with abundantly irrigated crops.

However, the most frequent attacks were found on old plants located in gardens or vegetable gardens, or in plantations little cared for, without sufficient irrigation, often set up and conducted as a hobby, or in abandoned plantations, with reduced reactivity of the plants. Here it would seem that the passing of years, with prolonged summer droughts probably connected with Global Change, played an important role (see below).

Prunus persica (Figure 7b) and *Prunus armeniaca* (Figures 3c-d), appeared to be the species most recurrently affected, followed in the order by *Citrus*, *Vitis* (Figure 7a), *Pyrus* (Figure 7c), *Malus, Actinidia*, *Corylus, Prunus domestica* and, finally, by *Castanea* and *Diospyros.*

More articulated were the attacks of *A. mellea* on forests, wood plantations (Anselmi, 2001) and ornamental plants, in their various types (parks, isolated shade trees, tree-lined roads or waterways) and to the most diversified predisposing factors. Some are common to agrarian contexts, while others are much more complex, in any case connected to the environmental conditions, often extremely critical, in which the aforementioned ecosystems are found. Here the attacks were conditioned, in addition to the factors previously indicated for the other species, also by incorrect plant associations (Figures 6d-e), by particular anomalies of the soil, such as excessive compaction for grazing (in forest) or asphalting, cementification (Figure 8b) or presence of pollutants, by damage from other parasites (Figure 6e) or abiotic factors, especially prolonged and repeated drought, linked to climate change.

Fig. 8. Attacks of *Armillaria mellea* on forestal broadleaved trees: a. *Populus nigra* ; b. *Quercus ilex*; c. *Prunus avium*; d. *Acer campestris*; e. *Robinia pseudoacacia*.

With respect to the past, however, our findings have shown a certain resurgence of the attacks of the fungus, particularly evident in forests (Figure 9), plantations or shade trees (Figures 8a-c-d-e) affected by the so-called "declining phenomenon" triggered by the strong aridity (Figure 10) related to aforementioned Climate change. These recrudescences have also been highlighted by the copious autumn productions of basidiomes at the base of the affected plants or on the relative stumps after the abatement or collapse on the ground.

Among the many species involved, those on which we have more frequently detected colonization of *Armillaria* to the collar are attributable to the genera (in order) *Populus* (Figure 8a), *Quercus* (Figure 9), *Salix, Fagus, Robinia* (Figure 8e), *Sambucus, Carpinus, Acer* (Figure 8d) , *Betula*, *Prunus* (Figure 8c), *Morus*, *Ligustrum* , *Eucalyptus, Fraxinus, Celtis, Juglans, Laurus*, etc., and, among the shrubs, *Spartium, Prunus laurocerasus, Viburnum timus, Syringa vulgaris*. Attacks on *Platanus, Alnus,* and especially on *Tilia* and *Ailanthus* (Sciré *et al.*, 2011) appeared as quite rare.

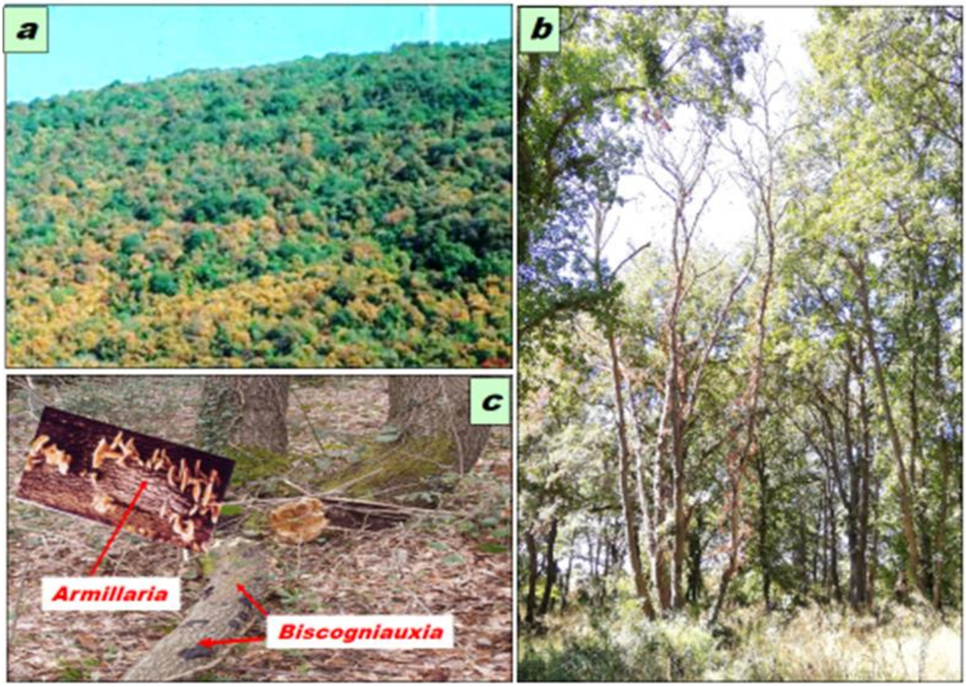

Fig. 9. Typical symptoms of the oak declining generally accompanied by attacks of *Armillaria* spp: *Quercus cerris* plants with strong yellowing of the foliage (a); dying plants (b) and trunks already fallen to the ground with stroma of *Biscogniauxia mediterranea* and basidiomes of *Armillaria* (c).

In many of these plants we have often noticed a degeneration of ectomycorrhizes (Pirazzi *et al.*, 1996), with the death of numerous mycorrhizal apices (Figure 11), which generally accompanies a real alteration of the useful microflora of the soil (Ambrosoli *et al.*, 1995). It is presumable that these phenomena of suffering of the symbionts and of the useful microflora in the soil reduce their antagonistic capacities towards *Armillaria*, favoring its aggressiveness and attacks.

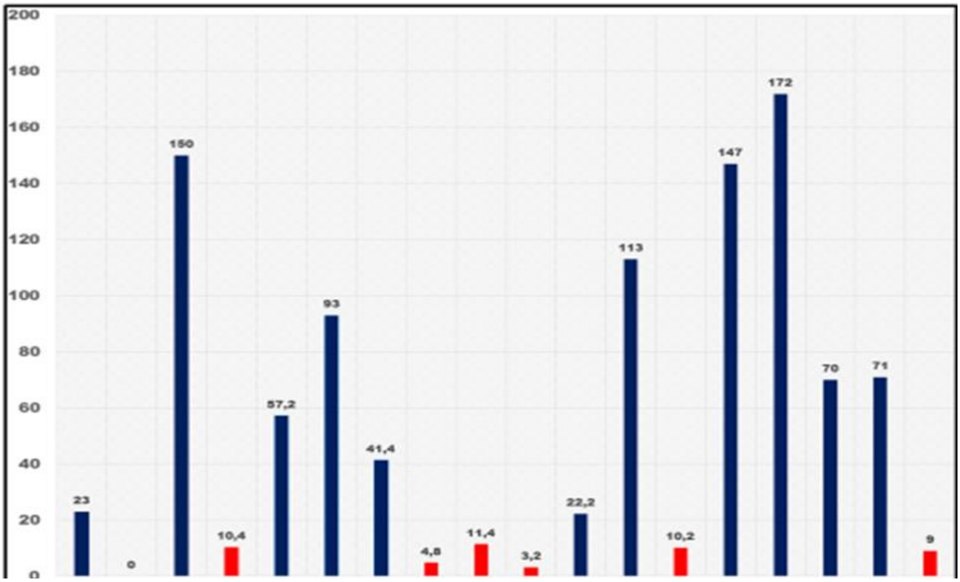

Fig.10. Cesano of Roma. Precipitations in millimeters in the period June-August of the last 18 years; in red the years considered to be very dry.

Fig.11. Mycorrhizae on *Fagus sylvatica* (1) and *Pinus pinea* (2) roots related to healthy (a) and declining (b) plants (from Torta *et al*., 2016). On some of these, attacks by *Armillaria mellea* have been detected.

Predisposing factors.

From the foregoing analysis it emerges that our investigations have on the whole highlighted aconsiderablenumber of factors that predispose the attacks of *Armillaria*, some of which are linked to a significant worsening of the state of health of the plants in the last decades. The 10 most recurrent factors that our findings have shown to be linked to the attacks of *A. mellea*, but which also include those related to the other species of the pathogen, are briefly analyzed below.

1. High inoculum pressure. Our investigations confirmed the important role of high inoculum pressure, especially if linked to accumulations of mycelium or rhizomorphs in the ground due to the presence of infected plants or of residues of old infected stumps (Figure 12a).

Fig.12. Some predisposing factors of *Armillaria* attacks: a. Thinning of a valuable hardwood plantation without removing the stumps: these will be easily colonized by the fungus and will act as an inoculum mass. If reduced in fragments (b), they will favor the diffusion of the inoculum in nearby areas. In c: old plant of *Fagus sylvatica* collapsed on the ground for attacks by *Armillaria mellea* in the very old beech forest of the Cimini Mountains.

If a tree is killed, the entire root system may become inoculum. In species in which the *Armillaria* is a primary pathogen, such as *A. mellea* and *A. ostoyae*, infection with a high level of inoculation generally resulted in rapid death even if the plants were vigorous before the attack, especially if young, close to relatively large food bases such as stumps. With stumps colonized by the fungus it would therefore be advisable to extract the whole stump and the infected material. On the other hand, their crushing with a shredding machine should be avoided because the numerous resulting fragments of infected wood (Figure 12 b) could be moved with the processing of the soil, potentially acting as new sources of inoculation.

2. Senescence of the plants. Undoubtedly plants that are too old, often compromised by the most varied factors, are not very reactive to pathogens and therefore more prone to encounter attacks of *Armillaria*, which are generally endemic and therefore always lurking. The phenomenon has emerged as particularly evident in various old forests (Figure 12c), parks or ornamental trees where, for historical, environmental and landscape reasons, we tend to conserve even very old plants. Truthfully, in some woods, especially if very old, the attacks of *Armillaria* are part of the natural balance of the ecosystem, participating slowly in the process of elimination of old, weaker plants, and creating spaces for a natural forest renewal.

3. Water stagnation in the soil. An excess of water in the soil on the one hand favors the fungus, enhancing its vitality and spread, while on the other creates stress to the plants, making them less reactive. Obviously, these stagnations appeared as particularly frequent in compact soils, in the valleys, in the floodplains, and in any case where soil drainage is poorly treated.

4. Incorrect associations of the plants. Even without water stagnation, which is always deleterious, a lot of water in the ground appeared anyway to favor the attacks of *Armillaria* towards those tree and shrub species that suffer water abundance, when they are affiliated to meadows or other species subject to intense irrigation, as it often happens in parks and gardens. The most recurrent cases concerned the species with xerophilic habitus, such as *Quercus ilex*, *P. pinea*, *P. pinaster*, *P. halepensis*, *Olea europea* (Figure d), *Laurus nobilis*, *Cupressus* spp. and various other species of the Mediterranean scrub, as well as species of mountainous origin that avoid excess water, such as *Abies alba* (Figure 6e) and *Picea abies*, usually residual of Christmas trees, and especially the different species of *Cedrus* (Figure 6a), one of the genes that are most affected by the pathogen. The abundant irrigations, favorable for the lawns or other herbaceous plants, but also for the diffusion of the pathogen, induce the tree plants not to deepen the root system. It follows that at the first interruption of irrigation (e.g., for rupture of the irrigation system) occurring in the summer season, the aforementioned plants quickly go into water shortages stress, favoring the attacks of *Armillaria*, ready to hit the suffering plants.

5. Attacks by other parasites. Many of the *Armillaria* attacks we found appeared as a result of the weakening of plants from attacks by other parasites, such as *Graphium* on *Ulmu*s, *Tomicus* (Figure 6f) and/or *Spaeropsis sapinea* on *Pinus*, *Cydalina* on *Buxus*, *Cytospora* on *Carpinus*, *Cryptosphaeria* (in short rotation) and *Agrilus* on *Populus*. The most recurring cases have concerned Mediterranean pines, which, in recent years, have been strongly attacked by *Tomicus* due to strong water shortages, such as that of 2017: about 15% of dead or dying plants showed attacks by *A. mellea*. As for the elm, decimated in recent years by tracheomycosis, while young recurs only rarely have shown the presence of *Armillaria*, the older plants, with a slower course of tracheomycosis, were colonized in at least 10% of the subjects.

6. Declining of the plants from climatic changes. A strong upsurge of the attacks of *Armillaria* appeared evident following the decline of the forest plants that has occurred in the last decades, due to prolonged and repeated summer droughts connected with well-known climatic changes (Cellerino *et al.*, 1990; Luisi *et al.* Eds, 1992; Anselmi *et al.*, 1998).

These have appeared exalted in dry soils or in very dense plantations, with plants in strong competition among themselves, in which the submissive subjects, less reactive, are particularly susceptible to the parasite. In Latium, the phenomenon was first highlighted in the forest, in particular on *Quercus* (Figure 9) and, to a lesser extent, on *Pinus nigra*, and in artificial wood plantations, especially *Populus* spp or *Prunus avium* (fig. 8c), but also in parks and other ornamental trees, as well as in some orchards which were not irrigated.

Predisposed by various factors, both intrinsic to the plant (genetic potential, senescence, etc.), or extrinsic, i.e. connected with environmental (e.g., not suitable soil) or anthropic (improper cultural interventions, excessive pasture, etc.) problems, the phenomenon is generally triggered by prolonged and repeated water shortages and results in the aggression of various weakness parasites, which progressively increase the pathological state of the plant until its death. Among these parasites an important role is often played by the *Armillaria* species, usually in combination with various secondary corticicolous agents, both fungal (e.g., among the most evident in Mediterranean areas, *Biscogniauxia* on *Quercus* (Figure 9), *Sphaeropsis* on *Pinus*), and insects (*Tomicus, Ips, Agrilus*, etc.). In recent years there has been a marked worsening of the phenomenon due to the severe summer droughts of 2001, 2003, 2007, 2008, 2009, 2012 and 2017 (Figure 11), which gave rise to an evident resurgence of the attacks of *Armillaria* species. This in fact, endemic everywhere, during the rainy periods reaches with the rhizomorphs the various plants, apparently harmless, to be unleashed on those rendered less reactive by the aforementioned stresses, probably also due to a strong suffering of mycorrhizae (Pirazzi *et al.*, 1996; Torta *et al.*, 2016) (Figure 10) and of the antagonist microflora (Ambrosoli *et al.*, 1995), which are therefore less in their braking action towards the pathogen (see above).

The phenomenon was particularly severe in many oak forests, especially *Quercus cerris* (Figure 9), not very tolerant of drought. In the oak woods with declining phenomenon, *A. mellea, A. gallica* and A. *tabescens* are often present together, usually on plants already killed or weakened by other agents. The incidence of the three species was correlated with the degree of forest declining (Table 1): while *A. gallica* and *A. tabescens* were found only in woods with high intensity of the phenomenon, *A. mellea* appeared almost everywhere, but with increasing incidence with the severity of declining

Stations	Dead plants %)	Incidence of *Armillaria*		
		mellea	*gallica*	*tabescens*
Antrodoco (RI) m 600 a.s.l.	0,5	-	no	no
Cori (LT) m 380 a.s.l.	0,5	-	no	no
S. Gregorio (RM) m 942 a.s.l.	1	-	no	no
Greccio (RI) m 900 a.s.l.	2	-	-	no
M. Flavio (RM) m 880 a.s.l.	2	-	no	no
Amatrice (RI) m 550 a.s.l.	3	-	-	-
Settefrati (FR) m 800 a.s.l.	5	+	-	no
Sabaudia (LT) m 17 a.s.l.	8	+	-	-
Cesano (RM) m 250 a.s.l.	17,5	++	++	++
Tolfa (RM) m 610 a.s.l.	23	+++	++	+
Monte Rufeno (VT) m 650 a.s.l.	30	+++	+	++
Tre Croci (VT) m 380 a.s.l.	32	+++	++	++

Tab.1. Incidence of the various species of *Armillaria* on plants of *Quercus cerris* affected by different degrees of declining: no: none; - rare; +: modest; ++: discrete; +++: high.

Strong water shortages, especially if combined with high summer heat, have in many parts triggered tree declining and related attacks by *A. mellea* even in isolated plants or groups of plants, especially along roads or in urban trees (Figures 8a-c-d-e).

7. Excessive pasture. In some old beech or oak woods (e.g., Lucretili and Tolfa mountains), the excessive trampling by grazing animals, particularly cattle, has created a certain suffering to the plants and has predisposed them to the attacks of pathogens of weakness, including *Armillaria*.

8. **Poor adaptation of the host species to the site.** Species that are not adapted to the site where they grow tend to be more prone to suffering and are therefore more susceptible to *Armillaria* attacks. *Quercus cerris*, for example, a plant which in the past was appropriate for the production of railway sleepers, was also widespread in areas not very suitable, instead of *Quercus pubescens*. Upon close observation, this species has proved to be the most susceptible to declining phenomena (see above) due to stress from water shortages, and also the most frequently prone to *Armillaria* attacks.

9. **Damages by other stresses.** Our investigations have shown various other examples of stress that, by causing declining and death of the interested plants, have favored the attacks of *Armillaria*. The most recurring cases are listed below: damage from salt antifreeze, as verified for *Robinia* and also *Ailanthus*

grown along mountain roads, where the snowplows more easily end up accumulating such pollutants under the plants; damage from saltiness as it occurs for some coastal pine forests; excessive asphalting or cementation of the soil around the trunk (Figure 8d), with water scarcity or root asphyxia, as it often happens in urban areas.

10. **Specific susceptibility.** At least with reference to *A. mellea*, despite its being an extremely polyphagous pathogen, our results confirmed (Raabe, 1979; Shaw and Kile, 1991) its greater aggressiveness on some species rather than on others. For example, in a plantation of different forest species of valuable wood, made up after an old vineyard affected by *A. mellea*, the incidence of the fungus seemed significantly to decrease on the various species in the following order: *Prunus avium*, *Alnus cordata*, *Fraxinus ornus* and *Juglans regia*. The 10 plants of *Juglans nigra* which were present, even in the middle of the affected plants, were not damaged at all.

Likewise, in a garden also built on land that had hosted an old abandoned vineyard affected by *A. mellea*, the tree species planted there at random ended up dying from attacks of the pathogen with the following decreasing "intensity": peach, plum, cherry laurel, privet trees, stone pine, holm oak.

In the orchards, while peach and apricot seem to be the most frequently affected species, chestnut and persimmon would be the least predisposed. However, in most cases, the apparent resistance of the host species to *Armillaria* is indirect, because it derives from the tolerance of that species to some predisposing factor. In the decline of the oak forest, for example, the greatest attacks of *Armillaria* on *Q. cerris* compared to those on *Q. pubescens* or *Q. ilex* are caused by its greater suffering due to shortage of water in the soil (see above).

It is worth mentioning that until now in Latium we have never encountered attacks by *Armillaria* on *Ginko biloba*, *Catalpa styraciflua*, *Juglans nigra*, *Paulonia tomentosa*, even when they were near sick plants.

Discussion

Our investigations, in addition to finding early evidence of the presence of *A. ostoyae* in the Latium region, have highlighted a certain resurgence of the attacks of *Armillaria* spp. in the last period, in particular on forest and ornamental plants, following the reduced reactivity due to the serious declining phenomena linked to recent climatic changes. Though in a way which is not totally clear, it seems that similar phenomena have interested, and could even more interest, even the orchards, especially poorly cultivated ones. It is not excluded that these outbreaks are destined to increase, also because the measures to combat the parasite are still complex and not always effective (Cellerino *et al.*, 1990; Anselmi, 1992; Guillaumin *et al.*, 1993; Anselmi *et al.*, 1998; Mazzaglia *et al.*, 2001). In fact, until now, no chemical fight intervention has offered good control of the attacks of *Armillaria*; moreover, their prevention is difficult because the fungus is very common in woody debris in the soil, boasts a wide range of guests and presents a non-specific model of parasitism.

Nonetheless, some precautions, preventive strategies or eradicating interventions can prevent or limit the attacks or reduce damage.

In artificial plantations, the most important strategies for the protection of orchards, wood plantations and ornamental trees consist in: planting suitable plants; removing the stumps and infected roots from the ground, thereby denying the fungus great food bases; promoting a good vigor of the plants through adequate irrigation (without stagnation), fertilization and pruning; and minimizing stress, also promoting the development of antagonistic organisms. In the establishment of new plantations, it is essential to choose species that adapt to the site and plants free from pathogenic attacks or from mycelium or rhizomorphs in earthen bread (certified plants). The physical removal of the inoculum involves the removal of diseased trees, the eradication of the stumps (no chopping), the destruction of the roots and the mixing of the earth up to a suitable depth, trying to bring to the surface as many fragments of roots as possible. In the case of circumscribed attacks in progress, suitable trenches sprinkled with lime or copper, or coated with plastic and refilled again, may represent excellent obstacles to isolate the infected area from healthy neighboring plants. On plants of great rhyzogenic capacity (for example, old olive trees) or of great value and development, when the attack is not too degenerate, it is often better to try the rehabilitation from the attacks by removing the soil around the collar and the buttresses of the big roots, scraping the tissues invaded by the mycelium and disinfecting the wounds with copper salts. The value of orchards, wood plantations or ornamental plants generally justifies the costs necessary for the aforementioned treatments, which however allow a valid form of defense against the pathogen.

Some suggest using biological control methods with products based on *Trichoderma harzianum* or fertilizers containing mycorrhizal products, on whose real effectiveness any further verification would be appropriate.

In extensive forests, it could be necessary to pursue an adequate distribution of available water and of the trophic resources among the components of the wood, thereby reducing as much as possible the radical competition among the plants. This would allow them to maintain greater vegetative vigor and therefore less susceptibility to attacks by weakness parasites. Partial cut or thinning can also increase host vigor and root resistance to the pathogen. In many areas it seems advisable to replace species with high climatic-stationary needs, which are therefore more vulnerable, with others being more tolerant: *Q. cerris*, for example, could be profitably replaced by *Q. pubescens*. In any case, maintaining good biodiversity through careful forest management ensures better tolerance of plants to biotic and abiotic stresses and consequently to *Armillary* attacks. Finally, the distribution of the pasture in proportion to the area and the production capacity of the forest could reduce the damage due to the constipation of the land and the bite of animals. This, in addition to improving the vegetative conditions of the forest, would favor the natural renewal of the plant species in the area.

CONCLUSIONS

Our research has shown a strong diffusion of the various species of *Armillaria* in Latium, with damages of particular importance by *A. ostoyae* on the coniferous forests, and above all *A. mellea* on the most varied host species and related ecosystems. It is likely that such recent discoveries concerning this region be also attributed to many other areas of our Country and of the Mediterranean in general. The evident resurgence of the pathogen predisposed by the increasing stress and by the declining of the plants – generally attributable to an increasingly intense anthropic pressure and above all to more and more frequent, prolonged and repeated droughts linked to climate changes – suggests an increasingly worrying future scenario.

In order to cope with these outbreaks, it could be useful to follow simple and effective control measures, which are currently unavailable. For these reasons we believe that, in addition to putting into practice every possible strategy aimed at stopping the Global changes phenomenon, it would be necessary to intensify the studies on the control measures against this pathogen, in particular the biological one, based on the antagonists and on the good mycorrhization of the plants.

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STUDY ON FUNGICIDAL ACTIVITY OF NEWLY SYNTHESIZED COMPLEX COMPOUNDS OF Cu (II), Zn (II) AND Ni (II) WITH PYRAZOLE-DERIVED LIGANDS AGAINST THE PHYTOPATHOGENIC FUNGUS *Phomopsis viticola Sacc*. IN LABORATORY CONDITIONS

SUMMARY

In the continuation of our detailed studies on pyrazole and its derivatives, Cu (II), Zn (II) and Ni (II) complexes were synthesized with ligand 4-Bromo-2-(1H-pyrazol-3-yl) phenol (HL) and their potential fungicidal activity against the phytopathogenic fungus *Phomopsis viticola* Sacc. (causal agent of Phomopsis cane and leaf spot disease of grapevine) was tested. Based on elemental (C, H, N) analysis and conductometric measurements, the formulas of complex compounds were determined. Biological research based on determining the inhibitory effects of commercial fungicide with active substances pyraclostrobin and metiram, ligand, and all newly synthetized complexes on *Ph. viticola* has been carried out.

Keywords: *4-Bromo-2-(1H-pyrazol-3-yl) phenol; complexes of Zn, Ni and Cu; Phomopsis viticola; active fungicidal substances, Phomopsis cane and leaf spot disease of grapevine*

INTRODUCTION

Grapevine production is very important for Montenegrin agriculture. Vineyards cover an area of 2783.2 ha (MONSTAT, 2017). Cultivation of grapevine is endangered by numerous diseases, and one of the most significant is Phomopsis cane and leaf spot caused by the phytopathogenic fungus *Phomopsis viticola* Sacc. (Latinovic and Latinovic, 2011). The disease occurs every year and makes more or less damages depending on the intensity of the disease, while in exceptional cases it can lead to vine declining. In conditions of Montenegro, the symptoms usually occur on canes and leaves (Latinovic, 2007), while in the

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world they can also appear on berries (Pscheidt and Pearson, 1989). According to Latinovic *et al*. (2003), particular problem is the fact that the most widespread varieties in Montenegrin vineyards Vranac and Kratosija are very susceptible to this disease. To control the disease, preventive measures are used that enable better aeration of the plant canopy and removal of infected plant parts, however treatments with fungicides are still essential. For this purpose, fungicides from the chemical groups' dithiocarbamates and strobilurins are the most commonly used (Latinovic, 2007; Gubler *et al*., 2015).

Pyrazole-based compounds and their transition metal complexes have attracted considerable research interest because of their potentially beneficial biological properties. The wide biological activity of this class of compounds (anticancer, antimicrobial, antiviral, anti-inflammatory, antifungal and others) is described in several reviews (Alex and Kumar, 2014; Kumar *et al*., 2013; Chimenti *et al*., 2006; Trofimenko, 1986). These results represent a part of our continued work with pyrazole-based complex (Jaćimović *et al*., 2013; Jaćimović *et al*., 2017a; Jaćimović *et al*., 2017b). In agriculture, they are in the use as pesticides (Lemaire *et al*., 2006; Vicentini *et al*., 2004; Singh *et al*., 2000).

In this paper, the syntheses of tree new Cu(II) complexes, Zn(II) and Ni(II) compounds of formulas $Cu(L\text{-}H)_2$, $[Cu(L\text{-}H)_2]Cl_2$, $[Cu(L\text{-}H)_2](NO_3)_2$, $Zn(L\text{-}H)_2$ and $Ni(L\text{-}H)_2 \cdot 4H_2O$ obtained in reaction of $Cu(OAc)_2$, $CuCl_2$, $Cu(NO_3)_2$, $Zn(OAc)_2$ and $Ni(OAc)_2$ with 4-Bromo-2-(1H-pyrazol-3-yl)phenol (HL) is described. Their activity was examined to the mycelial growth of *Ph. viticola in vitro*. Obtained results were compared with the commercial fungicide whose one active substance is pyraclostobin that belongs to pyrazole derivates.

MATERIAL AND METHODS

Preparation of complexes

Microcrystals of the complex of formula $Cu(L\text{-}H)_2$ were obtained by mild heating in the reaction on warm methanolic solutions of $Cu(OAc)_2 \cdot H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with methanol.

Microcrystals of the complex of formula $[Cu(L\text{-}H)_2](NO_3)_2$ were obtained by mild heating in the reaction on warm methanolic solutions of $Cu(NO_3)_2 \cdot 3H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. After two days, the formed microcrystals were filtered and washed with methanol.

Microcrystals of the complex of formula $[Cu(L\text{-}H)_2]Cl_2$ were obtained by mild heating in the reaction on warm ethanolic solutions of $CuCl_2 \cdot 2H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with ethanol.

Microcrystals of the complex of formula $Zn(L\text{-}H)_2$ were obtained by mild heating in the reaction on warm ethanolic solutions of $Zn(OAc)_2 \cdot 2H_2O$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with ethanol.

Microcrystals of the complex of formula $Ni(L\text{-}H)_2 \cdot 4H_2O$ were obtained by mild heating in the reaction on warm methanolic solutions of $Ni(OAc)_2$ and ligand 4-Bromo-2-(1H-pyrazol-3-yl)phenol in molar ratio 1:2. The formed microcrystals were filtered after 24h and washed with methanol.

Elemental analysis (C, H and N) of air-dried compounds was carried out by standard micromethods.

The molar conductivity of freshly prepared $1 \cdot 10^{-3}$mol dm^{-3} solutions of the complexes in DMF was determined at room temperature using a digital conductivity meter Jenway 4510.

These complexes were used to study the inhibition of *Ph. viticola* mycelial growth in laboratory conditions. A commercial fungicide which contains pyrazole derivate pyraclostrobin and methiram as active components is used as a standard. Potato dextrose agar (PDA) was prepared as a nutrient medium to test the growth of the fungus. After sterilization of the medium, it was cooled in a water bath at 60 °C when aqueous solutions of the complexes or a commercial fungicide in certain concentrations were added. For each complex and for a fungicide, five different concentrations were made: 0.12; 0.06; 0.03; 0.015; 0.0075 (%). 10 ml of a solution of each chemical complex and a fungicide in certain concentration was added in 100 ml of PDA. After homogenization of the solution of chemicals or fungicide with PDA, the medium was poured into 9cm Petri dishes. As a control, the medium with no amendments was used. After agar solidification, mycelial fragments 0.6 cm in diameter (taken from the edge of 10-day old fungal culture) were placed in the centre of Petri dishes. Inoculated Petri dishes were maintained in incubator at 25 °C. Ten days after the inoculation, mycelial growth of *Ph. viticola* was measured (in the control the fungus had covered 2/3 of the Petri dish).

Diameters of fungal mycelium as parameters of the growth inhibition effect were statistically analysed by analysis of variance, and mean values were compared using LSD test. If their difference was greater than the LSD test, they were considered statistically significant (Stankovic *et al.*, 1990). The percentage of fungal inhibition in treatments compared to the control was also calculated for each chemical and for each concentration (Kaiser *et al.*, 2005).

RESULTS AND DISCUSSION

Results of elemental analysis (C, H and N) and molar conductivity is given in Table 1.

Based on the obtained results, it can be concluded that the synthesised complexes and a commercial fungicide have shown statistically significant inhibition of *Ph. viticola* in comparison to control. The growth of fungal mycelium (cm) depending on the applied chemical and concentration is given in Table 2.

Average percentage of inhibition achieved by different chemical compounds and certain concentration in comparison to control is presented in Table 3.

Table 1. Elemental analysis and molar conductivity results for obtained complexes compounds

Formulas of the complex's compounds	C (%) Found ( ) Calculated	N (%) Found ( ) Calculated	H (%) Found ( ) Calculated	λ_M(DMF) $Scm^2 mol^{-1}$
$Cu(L\text{-}H)_2$	(39.82) 40.06	(10.36) 10.38	(2.33) 2.24	5.95
$[Cu(L\text{-}H)_2] Cl_2$	(32.70) 33.20	(9.11) 8.61	(1.95) 1.86	33.10
$[Cu(L\text{-}H)_2](NO_3)_2$	(32.10) 32.54	(12.15) 12.66	(1.59) 1.82	41.00
$Zn(L\text{-}H)_2$	(40.02) 39,89	(10.42) 10.34	(2.40) 2.23	3.60
$Ni(L\text{-}H)_2{\cdot}4H_2O$	(35.97) 35.63	(8.90) 9.23	(2,84) 3.32	4.20

Table 2. The growth of fungal mycelium (cm) depending on the applied chemical and concentration

Concentrations %	**Studied compound**					
	Commercial fungicide	$[Cu(L\text{-}H)_2] (NO_3)_2$	$Cu(L\text{-}H)_2$	$Zn(L\text{-}H)_2$	$Ni(L\text{-}H)_2{\cdot}4H_2O$	$[Cu(L\text{-}H)_2] Cl_2$
0.12	0.0	5.2	5.1	3.3	3.2	1.6
0.06	0.0	6.1	5.5	4.1	4.6	3.6
0.03	0.0	6.6	5.8	5.4	5.2	5.2
0.015	1.0	6.7	6.4	5.6	5.5	5.5
0.0075	1.1	6.8	6.7	6.5	5.6	6.0
Control	7.5	7.5	7.5	7.5	7.5	7.5
$LSD_{0,01}$	0.208	0.995	0.526	0.544	0.316	0.383

Compared to the complex compounds studied, a commercial fungicide expressed the best inhibitory effect. All applied concentrations of a commercial fungicide have shown statistically significant inhibition of *Ph. viticola* colony growth in relation to control.

The studied complex compounds had a lower inhibitory effect than a commercial fungicide but with obvious inhibition activity. $[Cu(L\text{-}H)_2] Cl_2$ complex expressed the best inhibitory effect at concentration of 0.12%.

Concerning $[Cu(L\text{-}H)_2] (NO_3)_2$ complex, concentrations of 0.12 and 0.06% exhibited inhibitory activity, while in the other three concentrations used, inhibition was not expressed at the level of a significant statistical difference in regard to control. $Cu(L\text{-}H)_2$ complex showed better inhibitory activity than the previous one since all five applied concentrations expressed inhibition effects that were statistically significant compared to control. The best inhibition achieved was at concentration of 0.12%. In relation to similar Cu (II) complexes examined in previous studies (Jaćimović *et al*., 2013; Jaćimović *et al*., 2017a; Jaćimović *et al*., 2017b), the tested Cu complexes in this research showed significantly higher inhibition of the growth of *Ph. viticola*. In previous studies, inhibition in relation to control at the highest concentration of the complexes (0.12%) was only 9.3%, 12.1% and 14.7%.

Table 3. Average % of inhibition achieved by different chemical compounds and certain concentration in comparison to control

Concentrations %	**Studied compound**					
	Commercial fungicide	$[Cu(L\text{-}H)_2](NO_3)_2$	$Cu(L\text{-}H)_2$	$Zn(L\text{-}H)_2$	$Ni(L\text{-}H)_2 \cdot 4H_2O$	$[Cu(L\text{-}H)_2]Cl_2$
0.12	100.0	30.7	32.0	56.0	57.3	78.7
0.06	100.0	18.7	26.7	45.3	38.7	52.0
0.03	100.0	12.0	22.7	28.0	30.7	30.7
0.015	86.7	10.7	14.7	25.3	26.7	26.7
0.0075	85.3	9.3	10.0	13.3	25.3	20.0

In comparison to $[Cu(L\text{-}H)_2]$ $(NO_3)_2$ and $Cu(L\text{-}H)_2$ complexes, $Zn(L\text{-}H)_2$ and $Ni(L\text{-}H)_2 \cdot 4H_2O$ complexes expressed better inhibitory activity. Complex of formula $Zn(L\text{-}H)_2$ caused fungal inhibition growth at all concentrations, which differed statistically from the control, and between each individual concentration, except between the third and the fourth. Maximum inhibition was achieved by the first concentration and it was 56.0%. A similar percentage of inhibition was obtained also in the previous study, where Zn complex expressed an inhibition of 53.5% at the highest concentration (Jaćimović *et al*., 2013). In the testing of Ni(L-H)2·4H2O complex, the compound applied in all investigated concentrations expressed significant inhibition compared to control. The best results were obtained at 0.12 and 0.06% concentrations, while at the other three concentrations applied inhibitory effects were lower and relatively uniform. Compared with the inhibition achieved by the similar Ni complex previously examined (14.7%) by Jaćimović *et al*. (2017b), the percentage of inhibition in this study is almost 4 times higher and amounts to 57.3%.

The complex of formula $[Cu(L\text{-}H)_2]Cl_2$ achieved the best results from other studied complexes (excluding a commercial fungicide), and especially at concentration of 0.12% when it inhibited the growth of fungal mycelium at a level of 78.7%, which is the best result in this experiment compared to other synthesised complexes and concentrations and also in comparison to previously reported experiments.

CONCLUSIONS

In the reaction on warm alcoholic solution (methanol or ethanol) of metal salts $Cu(OAc)_2$, $CuCl_2$, $Cu(NO_3)_2$, $Zn(OAc)_2$ and $Ni(OAc)_2$ and 4-Bromo-2-(1H-pyrazol-3-yl)phenol (HL) as ligand in molar ratio 1:2, tree new Cu(II) complexes, Zn(II) and Ni(II) complexes with formulas: $Cu(L\text{-}H)_2$, $[Cu(L\text{-}H)_2]Cl_2$, $[Cu(L\text{-}H)_2](NO_3)_2$, $Zn(L\text{-}H)_2$ and $Ni(L\text{-}H)_2 \cdot 4H_2O$ were obtained. Based on elemental analysis (C, H and N) and conductivity measurements, formulas of complex compounds were determined.

All complex compounds used in the experiment showed inhibitory activity against the growth of the phytopathogenic fungus *Phomopsis viticola*, causal agent of Phomopsis cane and leaf spot disease of grapevine. The best inhibition was achieved by the complex $[Cu(L\text{-}H)_2]\ Cl_2$ at concentration of 0.12%. At this concentration the inhibition reached 78.7%. These results are of importance for the preparation of the pyrazole compound which will have satisfactory fungicidal activity against *Ph. viticola*, since it has shown the best results so far both in this experiment and in previous studies carried out by the same researchers.

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STATUS AND PERSPECTIVES OF SUNFLOWER PRODUCTION IN CROATIA

SUMMARY

Sunflower, along with soybean and oilseed rape, is a very important oilseed crop in Croatia. In the period 1999-2018, it was grown on average of 32,741 ha with an average grain yield of 2.54 t ha^{-1}. The largest area under sunflower was in 1999 (49,769 ha) while the highest grain yield was achieved in 2013 (3.20 t ha^{-1}). In general, sunflower production is characterized by significant variations in areas and grain yields, due to number of reasons: agroecological conditions in previous growing season, prices of raw materials and repurchase, incentive and support system, applied technology and knowledge of the producers. However, in the last few years, there has been a slight increase in the areas and sunflower grain yield. Agroecological conditions, choice of hybrids, the possibility of crop rotation expansion, high grain yields in relation to almost all EU countries, processing capacities and repurchase security are in favour of the possibility of more intensive increase of areas and sunflower grain yield in Croatia.

Key words: sunflower hybrids, production, agroecological conditions, grain yield

INTRODUCTION

Agriculture is a strategic part of the economy in Croatia. In the total mainland area, agricultural area in 2017 was 26.4 percent or 1,496,663 ha. Arable areas and gardens dominated with 815,323 ha (54.5 percent), followed by permanent grasslands with 607,555 ha (40.6 percent), while orchards, vineyards, olive groves, vegetable gardens, nurseries, etc. were significantly lower (4.9 percent). Of the total agricultural area, cereals are sown on 461,483 ha or 30.8 percent, and oilseeds on 170,901 ha or 11.4 percent. Soybean is the most

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represented oilseed and is sown on 85,133 ha, followed by oilseed rape with 48,616 ha, while sunflower is represented with 37,152 ha (Croatian Bureau of Statistics, 2020). Sunflower production in Croatia has a long tradition. As in other European countries, the sunflower was originally grown as an ornamental plant. In the second half of the 19th century, it became interesting as a plant for oil production. In Croatia, it began to expanding with the construction of oil factories in Zagreb and Čepin in the mid-1930s. Until the appearance of Russian varieties, mainly seeds of local populations were used for sowing, but also varieties created in institutes in Zagreb and Novi Sad (Krizmanić, 2012). They were characterized by high susceptibility to biotic and abiotic stresses which usually resulted in low yields. At the end of the 1950s, varieties from the former USSR (Peredovik, VNIIMK 8931, Smena, etc.) were introduced, which showed slightly higher grain yield and oil content. The dominant problem of sunflower varieties in that period was its susceptibility to diseases, primarily downy mildew, black stem and white rot. Therefore, after increase in production areas under sunflower, due to the appearance of the diseases, they decreased again (Vratarić, 2004). With the introduction of hybrids in the second half of the 1970s, sunflower production began to stabilize. The reasons for this are the advantages of hybrids compared to varieties: more uniform growth, and thus more successful application of agrotechnical measures, lower and thinner but stronger stems, shorter vegetation, better resistance to lodging, drought, diseases and pests, and lastly, higher grain yield and oil content. Although these hybrids had showed a certain susceptibility to the parasite *Diaporthe (Phomopsis) helianthi*, sunflower breeders solved this by incorporating genetic tolerance so that hybrids are still sown today.

The aim of this investigation is to analyze the production and yields level of sunflower seed in Croatia over the past 20-year period (from 1999 to 2018) in terms of impact of weather conditions (mainly precipitation and air temperature) on sunflower cultivation and the perspective and possibilities of expanding sunflower production in the future.

MATERIAL AND METHODS

The paper uses data from the Croatian Meteorological and Hydrological Service, the Statistical Yearbook of the Republic of Croatia and the FAOSTAT database for the period from 1999 to 2018. The monitored time period was divided into five-year periods.

The analysis included: weather conditions (precipitation and air temperature), rain factor (RFm) according to Gračanin (1950) and areas with sunflower grain yields. Data of precipitation and air temperature refer to the Osijek locality (Osijek-Baranja County) which is the most represented in terms of areas under sunflower in Croatia. According to humidity, RFm classifies the months into: perarid (pa, less than 1.6), arid (a, 1.7 – 3.3), semiarid (sa, 3.4 – 5.0), semihumid (sh, 5.1 – 6.6), humid (h, 6.6 – 13.3) and perhumid (ph, more than 13.3).

RESULTS AND DISCUSSION

The results shown in Table 1 refer to precipitation amounts.

Table 1. Monthly precipitation (mm) for Osijek

Year	I – III*	IV	V	VI	VII	VIII	IX	I – IX*
1999	124	45	89	150	95	74	51	628
2000	73	28	26	10	63	5	23	228
2001	181	72	60	240	77	7	195	832
2002	69	64	135	37	59	84	82	530
2003	87	12	18	44	60	42	51	314
Mean (1999 – 2003)	107	44	66	96	71	42	80	506
2004	141	137	65	80	44	107	42	616
2005	156	55	51	110	171	238	75	856
2006	134	87	79	78	15	134	11	538
2007	148	3	56	33	27	45	65	377
2008	120	49	67	76	68	47	82	509
Mean (2004 – 2008)	140	66	63	76	65	114	55	579
2009	115	19	39	63	14	61	10	321
2010	165	71	121	234	32	111	108	842
2011	79	19	81	50	74	5	16	324
2012	87	46	94	68	48	4	32	379
2013	231	45	119	63	37	33	124	652
Mean (2009 – 2013)	135	40	91	96	41	43	58	504
2014	123	81	161	91	66	54	69	645
2015	181	13	113	17	26	106	41	497
2016	204	40	63	100	111	72	43	633
2017	167	50	51	45	64	30	80	487
2018	215	21	27	127	132	36	27	585
Mean (2014 – 2018)	178	41	83	76	80	60	52	570
Mean (1999 – 2018)	140	48	76	86	64	65	61	540
Minimum	69	3	18	10	14	4	10	228
Maximum	231	137	161	240	171	238	195	856
Variability	162	134	143	230	157	234	185	628

* sum of precipitation

Table 2. Mean monthly air temperature (°C) for Osijek

Year	I – III	IV	V	VI	VII	VIII	IX	IV – IX
1999	3.2	12.6	17.3	20.3	21.9	21.3	18.8	18.7
2000	3.2	14.8	18.4	22.4	21.7	23.7	16.7	19.6
2001	5.6	10.8	18.4	18.2	21.6	22.7	14.9	17.8
2002	4.7	11.2	18.6	21.1	22.3	20.9	15.4	18.3
2003	0.2	11.3	20.0	24.2	22.1	23.6	15.9	19.5
Mean (1999 – 2003)	3.4	12.1	18.5	21.2	21.9	22.4	16.3	18.8
2004	2.2	11.7	14.6	19.2	21.5	21.0	15.5	17.3
2005	0.3	11.5	17.0	19.5	21.6	19.3	17.1	17.7
2006	1.7	12.7	16.2	20.1	23.5	19.2	17.8	18.3
2007	6.8	13.3	18.3	22.3	23.8	22.2	14.5	19.1
2008	4.6	12.5	18.1	21.5	21.8	21.8	15.6	18.6
Mean (2004 – 2008)	3.1	12.3	16.8	20.5	22.4	20.7	16.1	18.2
2009	2.7	14.6	18.3	19.2	23.2	22.9	19.1	19.6
2010	2.5	12.4	16.5	20.4	23.2	21.7	15.6	18.3
2011	2.7	13.2	16.7	20.8	22.2	23.0	20.3	19.4
2012	2.3	12.5	16.9	22.5	24.8	24.1	18.9	20.0
2013	3.4	13.1	16.7	20.0	22.9	22.9	15.9	18.6
Mean (2009 – 2013)	2.7	13.2	17.0	20.6	23.3	22.9	18.0	19.2
2014	6.3	13.2	16.1	20.5	21.9	20.8	17.0	18.3
2015	4.3	12.1	17.8	20.8	24.6	23.7	17.9	19.5
2016	5.1	13.1	16.5	21.0	22.8	20.6	18.1	18.7
2017	2.9	11.3	17.5	22.4	23.4	23.7	16.1	19.1
2018	3.2	16.5	20.1	21.0	22.1	23.6	17.4	20.1
Mean (2014 – 2018)	4.3	13.2	17.6	21.1	23.0	22.5	17.3	19.1
Mean (1999 – 2018)	3.4	12.7	17.5	20.9	22.7	22.2	16.8	18.8
Minimum		10.8	14.6	18.1	21.5	19.2	14.5	17.3
Maximum		16.5	20.1	24.2	24.8	24.1	20.3	20.1
Variability		5.7	5.5	6.1	3.3	4.9	5.8	2.8

In summary, in the period from January to September the most precipitation fell in 2005 (856 mm), then in 2010 (842 mm) and 2001 (832 mm), and least in 2000 (228 mm), then in 2003 (314 mm), 2009 (321 mm) and 2011

(324 mm), which indicates enormous differences in the amount of precipitation between years. The difference in the amount of precipitation for the same month in different years is also evident. Good examples of precipitation variation are August 2012 and 2005 (4 mm and 238 mm) as well as June 2000 and 2001 (10 mm and 240 mm).

Differences are also evident for air temperatures (Table 2). The average temperature refers to temperatures in the growing season (April to September). The lowest temperatures were determined in 2004 (17.3°C), followed by 2005 (17.7°C) and 2001 (17.8°C), and the highest in 2018 (20.1°C), and then 2012 (20.0°C). Even more pronounced are the differences between individual months in different years that range from 5 to 6°C (the difference between the maximum and minimum mean monthly temperatures for the same month). Based on five-year averages, a slight upward trend in average temperatures by months can be observed (Table 2). Increasingly, the occurrence of high daily maximum temperatures, but also fluctuations in weather parameters on a daily and weekly basis (Mijić *et al.*, 2017), which can be expected in the future as well (Jug *et al.*, 2018). This is supported by the research of Debaeke *et al.* (2017) who point to increasingly frequent weather changes that are characterized by: higher temperatures, elevated CO_2 concentrations in the atmosphere, the occurrence of weather extremes, and water scarcity in agriculture.

Aware of the fact that precipitation and air temperature, as environmental factors, have an important role in the growth and development of every plant species, including sunflower, we can understand the complexity and demanding work of producers and breeders, creators of sunflower hybrids. Achieving the optimum of agronomic traits in a wide range of different agroecological conditions is objectively an extremely complex process that requires a lot of knowledge. Numerous authors have written about the influence of precipitation and temperature on the most important agronomic traits of sunflower (Šimić *et al.*, 2008; Černý *et al.*, 2011, 2013; Mijić *et al.*, 2012, 2017.; Černý and Veverkova, 2012; González *et al.*, 2013; Milošević *et al.*, 2015; Liović *et al.*, 2017; Jocković *et al.,* 2019).

Rain factor according to Gračanin (RFm)

The relationship between precipitation and air temperature and their quantification can be seen through calculation of the rain factor according to Gračanin (RFm). Monthly averages of this factor, which refer to the twenty-year period, indicate that months in the vegetation period are perarid, semiarid and arid. The exceptions are 2005 and 2010, which had a higher degree of humidity compared to other years (Table 3). However, analyzing the five-year results, and in particular, the individual values of the rain factor, a wide range from peraridity to humidity was obtained. In the last five-year period (2014 – 2018), the largest number of months indicates aridity, which confirms the occurrence of increasingly dry years with less precipitation and higher air temperatures.

Table 3. Rain factor according to Gračanin (RFm)

Year	IV	V	VI	VII	VIII	IX
1999	3.6 *sa*	5.1 *sh*	7.4 *h*	4.3 *sa*	3.5 *sa*	2.7 *a*
2000	1.9 *a*	1.4 *pa*	0.4 *pa*	2.9 *a*	0.2 *pa*	1.4 *pa*
2001	6.7 *h*	3.3 *a*	13.2 *h*	3.6 *sa*	0.3 *pa*	13.1 *h*
2002	5.7 *sh*	7.3 *h*	1.8 *a*	2.6 *a*	4.0 *sa*	5.3 *sh*
2003	1.1*pa*	0.9 *pa*	1.8 *a*	2.7*a*	1.8 *a*	3.2 *a*
Mean (1999 – 2003)	3.8 *sa*	3.6 *sa*	4.9 *sa*	3.2 *a*	2.0 *a*	5.1 *sh*
2004	11.7 *h*	4.5 *sa*	4.2 *sa*	2.0*a*	5.1 *sh*	2.7 *a*
2005	4.8 *sa*	3.0 *a*	5.6 *sh*	7.9*h*	12.3 *h*	4.4 *sa*
2006	6.8 *h*	4.9 *sa*	3.9 *sa*	0.6*pa*	7.0 *pa*	0.6 *a*
2007	0.2 *pa*	3.1 *a*	1.5 *pa*	1.1*pa*	2.0 *a*	4.5 *sa*
2008	3.9 *sa*	3.7 *sa*	3.5 *sa*	3.1*a*	2.2 *a*	5.3 *sh*
Mean (2004 – 2008)	5.5 *sh*	3.8 *sa*	3.7 *sa*	3.0 *a*	5.7 *sh*	3.5 *sa*
2009	1.3 *pa*	2.1 *a*	3.3 *a*	0.6 *pa*	2.6 *a*	0.5 *pa*
2010	5.7 *sh*	7.3 *h*	11.5 *h*	1.4 *pa*	5.1 *sh*	6.9 *h*
2011	1.4 *pa*	4.9 *sa*	2.4 *a*	3.3 *a*	0.2 *pa*	0.8 *pa*
2012	3.7 *sa*	5.6 *sh*	3.0 *a*	1.9 *a*	0.2 *pa*	1.7 *a*
2013	3.4 *sa*	7.1 *h*	3.2 *a*	1.6 *pa*	1.4 *pa*	7.8 *h*
Mean (2009 – 2013)	3.1 *a*	5.4 *sh*	4.7 *sa*	1.8 *a*	1.9 *a*	3.5 *sa*
2014	6.1 *sh*	10.0 *h*	4.4 *sa*	3.0 *a*	2.6 *a*	4.1 *sa*
2015	1.1 *pa*	6.3 *sh*	0.8 *pa*	1.0 *pa*	4.5 *sa*	2.3 *a*
2016	3.1 *a*	3.8 *sa*	4.8 *sa*	4.9 *sa*	3.5 *sa*	2.4 *a*
2017	4.4 *sa*	2.9 *a*	2.0 *a*	2.7 *a*	1.3 *pa*	5.0 *sa*
2018	1.3 *pa*	1.3 *pa*	6.0 *sh*	6.0 *sh*	1.5 *pa*	1.6 *pa*
Mean (2014 – 2018)	3.2 *a*	4.9 *sa*	3.6 *sa*	3.5 *sa*	2.7 *a*	3.1 *a*
Mean (1999 – 2018)	3.7 *sa*	4.3 *sa*	4.1 *sa*	2.7 *a*	3.0 *a*	3.6 *sa*
Minimum	0.2	0.9	0.4	0.6	0.2	0.5
Maximum	11.7	10.0	13.2	7.9	12.3	13.1

**a*= arid, *h*= humid, *sa*= semiarid, *sh*= semihumid, *pa*= perarid, *ph*= perhumid

Areas and amount of grain yield

In the last two decades (1999 – 2018) in Croatia, the sunflower was grown on an average of 32,741 ha with grain yield of 2.54 t ha^{-1} (Figure 1). Unfortunately, production is characterized by variations, both in terms of sown areas (20,615 – 49,769 ha) and grain yields (1.57 – 3.20 t ha^{-1}). The reasons for this are multiple and primarily relate to: growing conditions for sunflower in previous growing season (unfavourable agroecological conditions – this usually means less sown areas next year), prices of raw materials (fertilizer, seed, pesticide, fuel etc.) and repurchase, incentive and support system, applied technology and knowledge of the producers. However, it can be said that there is a slight trend of increasing area and average grain yield, and that some producers, equipped with quality machinery, often achieve yields above 4 and even 5 t ha^{-1}.

As can be seen in Figure 1, the differences between the minimum and maximum grain yield are over 100 percent, i.e. the lowest grain yield was achieved in 2005 (1.57 t ha^{-1}), and the highest yield in 2013 (3.20 t ha^{-1}). According to the research of Iljkić *et al.* (2019) who analyzed the six field crops sown on the largest areas in Croatia (maize, wheat, soybeans, barley, sunflower and oilseed rape), the highest variability in grain yield was found in sunflower.

The low grain yield in 2005 can be partly explained by the excessive amount of precipitation during vegetation. Namely, in addition to the large reserve of winter moisture, which proved to be very important in the production of sunflowers in our agroecological conditions, there were months with above-average precipitation and lower temperatures. As can be seen from Table 3, RFm indicates semihumidity (May and June) or even humidity (July and August), which is extremely unfavourable in sunflower production. In such microclimatic conditions, sunflower plants become hypersensitive to the development of diseases. This fact is supported by the research of Duvnjak *et al.* (2008) who point out that the occurrence and intensity of a disease in certain years significantly reduce the grain and oil yield in Croatia. Likewise, precipitation often coincided with flowering time, and since sunflower is typically a cross-pollinated, entomophilous plant, pollination success was lower. In addition, the flowering time of each hybrid is short (about ten days), which limits the visit of pollinators. In conditions of continuous, more intense precipitation, the flight of insects is smaller or even absent. Also, in conditions of increased humidity, the development of thermophilic weeds often occurs, above all ragweed (*Ambrosia artemisiifolia* L.), rough cocklebur (*Xsantium strumarium* L.) and velvetleaf (*Abutilon teophrasti* Med.). Weeds and cultivated plants compete for soil and air biofactors, which certainly leads to a decrease in the value of the most important agronomic traits, primarily grain and oil yields. In addition, it should be emphasized that these weeds can host various pathogens and serve as a source of inoculum and potential infection (Vrandečić *et al*., 2007, 2008). Weed control is one of the most critical elements in optimizing sunflower yield and quality (Smatana *et al*., 2014).

On the other hand, the high grain yield in 2013 is based on large winter moisture reserves and a favourable distribution of precipitation during the growing season. The values of the RFm were in the range of perarid (July and August), arid (June) and semiarid (April) with the exception of May when there was slightly more precipitation. Increased amounts of precipitation during May generally did not adversely affect the growth and development of sunflower, as it did not develop a lush vegetation mass and closed the rows. Air temperatures did not deviate significantly from the multi-year average, nor did the number of days with extremely high temperatures.

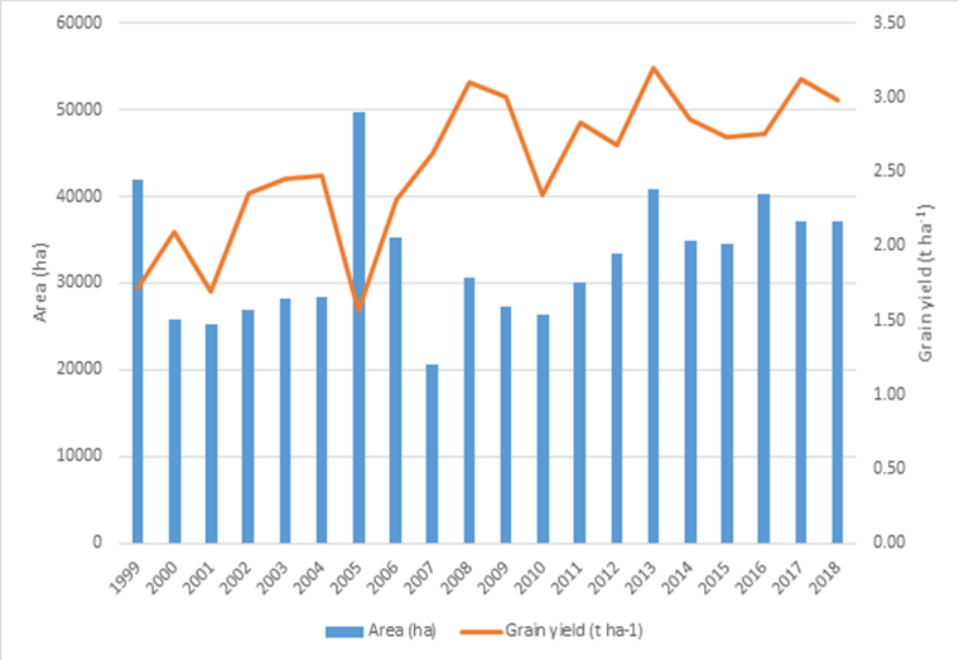

Figure 1. Sunflower growing areas and grain yields from 1999 to 2018 year in Croatia

Perspectives of sunflower production

The part of oilseeds (11.4 percent) with share of sunflower (2.5 percent) in the total agricultural area in Croatia is low, which indicates the need to increase this crop on our fields in the future. It should be noted that Croatia, according to the multi-year average (1999 – 2018), is the second country in terms of grain yield per ha in the European Union (FAOSTAT, 2020). The appearance of increasingly dry vegetation seasons, especially in summer with lower precipitation, higher temperatures and daily temperature maximums support the need to increase areas under sunflower. The reason lies in the fact that sunflower, of all field crops, best absorbs unwanted stress conditions (García-López *et al.*, 2014). Sunflower has a strong and branched root of large absorbency potential and a specific anatomical structure of the stem and leaves that enables it to achieve satisfactory grain and oil yields under stressful conditions (Gadžo *et al.*, 2011).

Sunflower is mostly grown in the eastern part of the Republic of Croatia, in Osijek-Baranja and Vukovar-Syrmia Counties, which is confirmed by the research of Zmaić *et al.* (2014), Iljkić *et al.* (2019) and Markulj Kulundžić (2019). However, on the basis of the given agroecological conditions of the rest of Croatia and the large choice of hybrids, it can be concluded that there are no obstacles for greater spreading of sunflower in other areas, especially in the remaining three Slavonian Counties: Brod-Posavina, Virovitica-Podravina and Požega-Slavonia County. Furthermore, crop rotation is a problem for a large number of crop producers. Most crops are grown in cycles of each three years, often two years, on the same area. The choice of sunflower, as another additional crop in the crop rotation, would certainly contribute to better efficiency of individual producers, including family farms. Sunflower leaves the field early and enables timely sowing of winter crops, leaves the soil without weeds, in good pedophysical condition, and sunflower production fits well into the optimal use of machinery (Gadžo *et al.*, 2011).

The fact that the Oil factory Čepin, as the most important company for the repurchase and processing of oilseeds in Croatia, continuously invests in modernization and capacity widening, which is a guarantee for repurchase of this important oilseed plant in a longer period, certainly supports the spreading of sunflower areas in Croatia. At the moment, the Oil factory Čepin can repurchase and process the entire sunflower production in Croatia which can be a strongly supportive moment for additional expansion of sunflower growing.

CONCLUSIONS

Sunflower is sown in Croatia on 32,741 ha (multi-year average), mainly in its eastern lowlands part. The average grain yield is about 2.5 t ha^{-1} and the contribution of sunflower growing area in the total agricultural land is very low (2.5 percent). Production is characterized by large variations both in terms of areas as well as grain yield. In the last few years, there has been a slight increase in the growing areas and sunflower grain yield. Agroecological conditions, choice of hybrids, the possibility of crop rotation expansion, satisfactory grain yields in relation to other EU countries, processing capacities and repurchase security are in favour of the possibility of more intensive increase of areas and sunflower grain yield in Croatia.

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SENSORY-BIOLOGICAL-CHEMICAL PROTECTION OF STATES PERSONS IN MONTENEGRO

SUMMARY

In this paper we presented the results of a study on sensory-bio-chemical checks in facilities on the territory of Montenegro visited, regardless of the reason, by national states persons and foreign states persons during their visits to Montenegro. The checks concern the total number of checks, results of compliance, structure of the premises and relations within the checks. The checks were carried out by specialized police officers, officers of inspectorates and national laboratories. We analysed 965 checks over 11 years, with 551 checks showing compliance with the requirements or 57.1% and 414 checks showing non-compliance with the requirements or 42.9%. Detection of non-compliant swabs and samples using scientific and technological analyses prevented poisoning of states persons. Most of the checks were carried out in hotel-tourism facilities, then in residential-type facilities and the lowest number of checks was carried out in other facilities. The total number of checks rose by 6.11% on average per annum, on the basis of which a forecast of checks by 2022 was given. Based on results of the study, proposals are given in the conclusion, aimed at further improvement of work of the police and other authorities. There is also a need for modernization of capacities in terms of human resources and technical equipment and for continuous monitoring of hazards in the field of sensory-biological-chemical protection with a view to preventing possible threats for state persons.

Key words: Protection; states person; safety; checks; food; Montenegro.

INTRODUCTION

Historically, there are numerous examples were famous people were poisoned. For centuries, in order to protect them, dogs that sniffed or were the

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first to taste the food were kept on courts of rulers. Of course, there were also specific people, so-called food tasters, who were to ingest food first and thus “detect” poisons (Thompson et al, 2014).

Even in modern times, the hazard of various substances and means in food and harmful consequences for human safety exists. Acknowledging the importance of food safety, US President George W. Bush issued on 30 January 2004 a presidential directive (HSPD-9) establishing a national policy to defend the agriculture and food system against terrorist attacks. It notes that the food system is vulnerable to disease, pest, or poisonous agents and due to its complex structure provides potential targets for attack (Rasco & Bledsoe, 2019).

Contrary to deliberate attempts to put protected persons in danger - the food hazards occur also in absence of bad intentions, so certain protective measures have to be taken (Manning and Soon, 2016; Lopez-Hernandez et al., 2014). This is particularly noticeable if we take into account that hazards concerning water, air, soil and food occur on daily basis (Koumolou et al, 2013; Todd et al., 2010; Spalevic, 2011; Chalise et al., 2019).

In Europe, one of the most significant achievements when it comes to food safety policy is issue concerning food labelling (Boqvist et al., 2018). Consumer have the right to be informed, including the data what the food they buy on the market *contains* (Puster, 2018) where it originates in, how to *store it safely* and prepare it and *use by/best by date* (Proso & Sokanovic, 2009; Keener et al, 2014). Therefore, food is a product intended for human consumption, drinks and water used in food (Bel et al., 2019; Drew and Clydesdale, 2014).

Apart from humans, animals and plants are also exposed to various diseases and harmful effects. We are surrounded by modern chemical and biological hazards and diseases that in everyday life we recognize as lumpy skin disease, avian influenza, swine flu, and bluetongue disease, various types of salmonellae, trichinella, tapeworms, and anthrax. If we add also radioactive substances, plant and animal toxins and inorganic toxins (arsenic-rat poison, potassium cyanide) and physical hazards in food (glass, metal particles) then legal, institutional, organizational, scientific-educational, pedagogical, human resources, operational-tactical and other measures need to be undertaken in order to protect people, facilities and objects (Ntemiri et al., 2019, Carpenter, 2018; Corradini et al., 2018; Froude et al., 2011). All the measures stated above are implemented through cooperation and exchange of information among police, laboratories, inspectorates and other authorities. However, despite all the measures undertaken, when it comes to protection of security of states persons, sensory-bio-chemical protection is provided by specialized inspectors of special organizational unit of the police[2], either independently or in cooperation with competent inspectorates

[2]The Sector for the Protection of Persons and Facilities has one post systematized - independent police inspector bio-chemical protection engineer. http://www.mup.gov.me/biblioteka/pravilnici?alphabet=lat%3fquery%3dsistemat&sortDirection=desc&pagerIndex=2 accessed on 27 March 2019.

(e.g. sanitary, veterinary) and with representatives of the national reference laboratory.

Before each activity, in order to protect protected persons covering the highest offices in the state - in accordance with the legislation in force and standards of profession, the police officers, in cooperation with competent officers of inspectorates and laboratories, have to provide SBHP. Activities of protected persons concern visits to hotel-tourism and hospitality facilities, business, commercial and religious buildings, state administration buildings, activities in residences, etc. Therefore, these activities vary from operational, through local political activities to receptions for foreign officials.

With regard to various hazards and manner of checks, the activity concerning the protection of persons we shall name - sensory-bio-chemical protection (SBCP). SBCP is a preventative activity encompassing the control of facilities visited by states persons regardless of the reason, in terms of control of health safety of food and water, food hygiene, medicinal products, safety of air and general use items, health and sanitary status of staff and fuels.

In practical terms, the SBCP is carried out by sensory-bio-chemical checks (SBCC). Therefore, we can say that SBCC is a preventative direct practical activity for implementation of sensory-bio-chemical protection. It is carried out by sensory and various biochemical scientific and technological analyses with a view to identification and elimination of hazards - risks (by taking samples and swabs) which may pose health risk for protected persons.

SBCC includes health examination of people, food checks, and checks of general use items, anti-radiation examination and analysis of fuels in order to establish their compliance with requirements. SBCC is carried out as follows: *sensory (organoleptic), laboratory examination of samples and swabs taken, detection on-the-spot, by examination of the premises and means of transport and documentary checks* (Spalevic, 2016; Spalevic, 2010). Where checks or techniques used reveal inadequate safety levels, the use of facilities or food serving is prohibited.

MATERIAL AND METHODS

The subject of the study is SBC checks in Montenegro in the period 2007-2017. The scope of the set is final and covers 965 SBC checks carried out on the territory of Montenegro (Figure 1) in the period 2007-2017.

The objective of the study is to analyze the level of health hazard or risk of states persons when ingesting food in certain facilities. All the analyses were performed at the laboratories of the Institute of Public Health of Montenegro in Podgorica and Ministry of the Interior of the Government of Montenegro.

Pursuant to the Article 12 of the Law on Food Safety (Official Gazette 14/07), the Ministry of Health of the Government of Montenegro adopted the Rulebook on Microbiological Criteria for Food, which prescribes microbiological criteria on permitted types and quantities of microorganisms, bacterial toxins and histamines dangerous to health, as well as microbiological criteria for process hygiene, methods of determination and assessment, i.e. rules that must be followed in accordance with the implementation of general and special hygiene requirements.

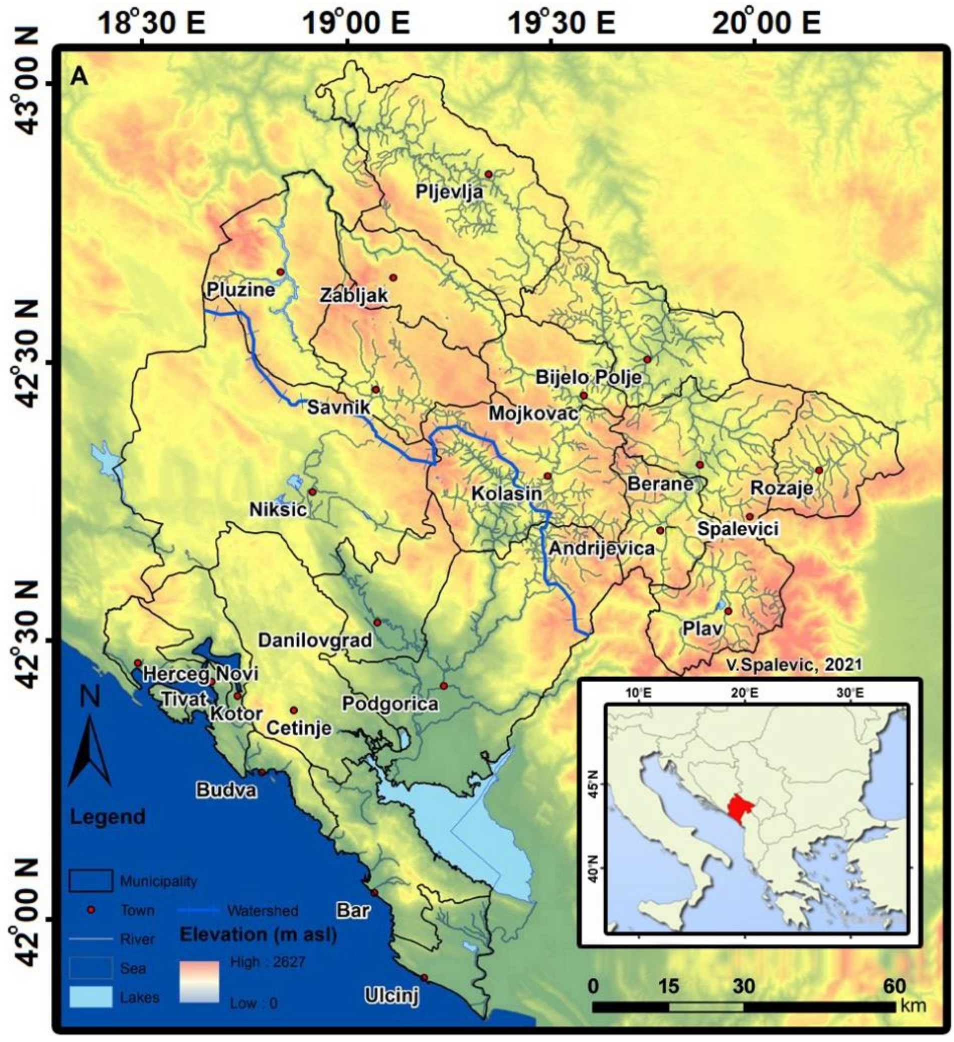


Figure 1. Study area where the Sensory-Biological-Chemical protection of States Persons was implemented

The competent authority responsible for carrying out official controls checks compliance with applicable regulations, but also requires further sampling and testing to prove the presence or determination of other micro-organisms, their toxins or metabolites in the context of risk analysis, in terms of work verification or when there is reasonable doubt in the safety or suitability for use of some type of food. According to the Law on Food Safety (Official Gazette 14/07), Article

39 defines that the issue of food safety is monitored at all stages of production, processing and distribution, and that they are under the constant control, so it must be ensured that food meets food regulations that are important for their business and health safety. During the subject controls, the control of general hygiene requirements was performed, fulfilling special hygiene requirements in all phases of food trade and in terms of physical, chemical and microbiological criteria, compliance of temperature regimes with requirements in individual phases, maintenance of refrigeration chain, sampling and testing, compliance with products. The system has also established an ongoing control procedure based on the Hazard Analysis and Critical Control Points (HACCP) system, which allows the identification of microbiological, chemical and physical factors that may be harmful to human health. Control analyzes were performed in compliance with the Microbiological hygienic Standards for objects, surfaces and hands that come into contact with food.

Standards have been determined in accordance with ISO 18593: Microbiology of food and animal feeding stuffs — Horizontal methods for sampling techniques from surfaces using contact plates and swabs. The frequency and number of samples were determined taking into account the purpose and scope of control work.

Requirements for food safety assessment are based on the Table: Certain microbiological requirements that are consistent with the values that make food unsafe in accordance with Article 24 of the Food Safety Act, with indications of quantities that may endanger human health (in this case protection of the State Persons). If, during the control, a microorganism or its toxins or metabolites were found in the food that are not listed in the table, and could be dangerous to human health the samples would be further assessed for microbiological control. In case that the defect is found in the sample, the food must be withdrawn or recalled.

A ***statistical method*** separating statistical description and statistical analysis was used in this paper. The methods are grouped into: statistical analysis of occurrence (in the period 2007-2017) and occurrence dynamics analysis (analysis of occurrence trends 2007-2017 with tendency forecast by 2022).

The statistical data collected were classified into the time series, presented in tabular and graphic form and subject to statistical analysis for the purpose of highlighting the structure, significant characteristics and composition of the statistical population. In dynamic analysis of time series of the following are used: Graphical method, index number method, development rate and trend analysis method. The study methods selected provided information and results that are presented in the discussion of this paper.

RESULTS AND DISCUSSION

Statistical analysis of occurrence

The information on the structure of the occurrence contains data on the composition and the extent of the occurrence (i.e. on the frequency of the occurrence in the statistical set) and data on internal relations between elements of the occurrence (configuration of the occurrence).

The composition and the extent of SBCC occurrence concern the territory of Montenegro in the period 2007-2017, where in 551 facilities compliance with the requirements were identified, accounting for 57.1% of the total number of checks, while non-compliance with the requirements was identified in 414 establishments, accounting for 42.9% of the total number of checks.

Table 1. Results of checks in establishments, by compliance status [1]

Samples	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	∑	%
SBCP, correct	35	38	59	45	100	70	44	26	28	46	60	551	57.1
SBCP, not correct	23	39	42	54	39	36	64	21	25	26	45	414	42.9
Total of SBCP	58	77	101	99	139	106	108	47	53	72	105	965	100

[1] *Annual report of the Group for Antiterrorism Checks of the Sector for the Protection of Persons and Facilities of the Police Administration of Montenegro, 2007-2017.*

The maximum number of total SBCC in facilities as well as the maximum number of SBCC establishing compliance with the requirements was achieved in 2011, the year where 139 checks were made in total, establishing compliance in 100 facilities. The minimum number of facilities checked was carried out in 2014, with 47 checks establishing compliance in 26 facilities and non-compliance in 21 checks.

All SBCC were carried out in facilities structurally divided into: closed-type facilities, hotel-tourism and hospitality facilities and other facilities, such as commercial and religious buildings, company premises, etc.

Table 2. Sensory-bio-chemical checks in facilities in the period 2007-2017.

No	Structure	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
1	Closed type	17	17	23	19	30	15	16	5	9	10	31
2	Hotel facilities	41	60	70	73	103	85	81	26	41	49	68
3	Other facilities	0	0	8	7	6	6	11	16	3	13	6
	Total	58	77	101	99	139	106	108	47	53	72	105

By structure of the facilities checked, the maximum number of closed-type facilities checked (residential buildings, etc.) was carried out in 2017, 31 checks, and the minimum number of facilities checked was in 2014, with 5 checks.

The maximum number of hotel-tourism and hospitality facilities checked was in 2011, with 103 checks, while the minimum number of facilities checked was in 2014, with 26 checks.

The maximum number of checks of other facilities was carried out in 2014, with 16 checks and the minimum number of checks was carried out in 2007 and 2008, when there were none.

Having regard of the fact that mean values represent a significant group of statistical indicators in research and analysis, i.e. that they are a quantitative represent of all individual modality values, we calculated them. Apart from being fundamental in statistical analysis, middle values are used also in dynamical analysis.

The selection of the middle value we will apply in a research depends also on the level of data relatedness and homogeneity in a series on occurrence that is observed. The table below shows the results of monitoring of data homogeneity in the series on all check categories. We calculated first the simple arithmetic mean using the following formula:

$$\overline{X} = \frac{\sum_{i=1}^{N} x_i}{n} \qquad (1)$$

then standard deviation

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y - y_i)^2} \qquad (2)$$

And variation coefficient in the end

$$V_{[\%]} = \frac{\sigma}{\overline{X}} \cdot 100 \qquad (3)$$

Table 3. SBC checks homogeneity analysis by annual values.

Type of inspection	Mean value	Standard deviation	Coefficient of variation
Total number of SBC checks	87.73	27.11	30.91%
Number of SBC checks, correct	50.09	20.42	40.77%
Number of SBC checks, incorrect	37.64	12.89	34.26%

Since variation coefficient is above 30% in all averages stated, we can conclude that average values do not represent the occurrence properly. Variability of 30-50% is considered moderate. The lower the coefficient of variation, the lower the level of dispersion around the arithmetic mean, and vice versa.

Further homogeneity analysis of checks in certain structures of facilities, presented in Table 4, shows that average annual number of checks do not represent the occurrence properly, as their variation coefficient is above 30%.

Table 4. Homogeneity of checks in certain structures of facilities (annual values)

Structure of facilities	Mean value	Standard deviation	Coefficient of variation
Structure of Closed type	17.45	7.77	44.54%
Hotel tourist facilities	63.36	21.58	34.06%
Other facilities	6.91	4.78	69.13%

The analysis of homogeneity of total number of checks by years, presented in Table 5, show relatively strong non-homogeneity in 2014 (variation coefficient above 50%), as well as pronounced non-homogeneity in other years (variation coefficient above 70% in all), which shows a pronounced difference in number of checks depending on the type of the facility.

Table 5. Homogeneity of total checks per years.

	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Mean value	19.3	25.7	33.7	33.0	46.3	35.3	36	15.7	17.7	24.0	35.0
Standard deviation	16.8	25.2	26.4	28.7	41.2	35.3	31.9	8.58	16.7	17.7	25.5
Coeff. of variation	57%	98%	78%	87%	89%	100%	89%	55%	94%	74%	73%

When we compare the average number of checks of facilities by years with checks by years we can see that the deviation from the average is high, so other middle values need to be calculated, which is median in this case. In simple series for an uneven number of data, median is found using the formula

Therefore, in a sequence from the highest to the lowest number of SBC checks establishing **compliance**, 50% of checks made is above 45 per annum, and 50% of checks made is below 45 checks per annum.

In a sequence from the highest to the lowest number of SBC checks establishing non-compliance, 50% of checks made is above 39 per annum, and 50% of checks made is below 39 checks per annum.

When it comes to the **total number** of SBC checks, in a sequence from the highest to the lowest, 50% of the checks made was above **99** checks per annum, and 50% of the total checks are below 99 checks per annum.

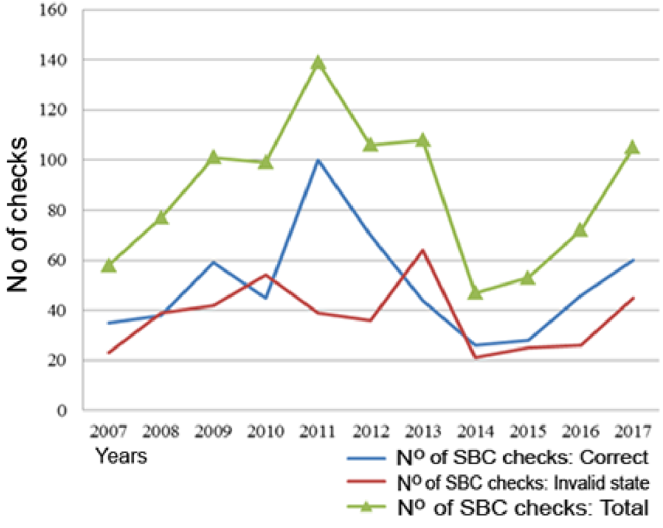
Following the analysis of the structure of the occurrence, we will analyze is configuration as well. We can conclude from Table 6 that the share of SBC checks is the highest in hotel-tourism facilities, followed by closed-type facilities, while the lowest number of SBC checks was carried out in other facilities.

Table 6. An overview of frequency of SBC checks of establishments in Montenegro in the period 2007-2017.

Structure of facilities	2007 [%]	2008 [%]	2009 [%]	2010 [%]	2011 [%]	2012 [%]	2013 [%]	2014 [%]	2015 [%]	2016 [%]	2017 [%]
Structure of Closed type	29.31	22.08	22.77	19.19	21.58	14.15	14.81	10.64	16.98	13.89	29.52
Hotel tourist facilities	70.69	77.92	69.31	73.74	74.10	80.19	75.00	55.32	77.36	68.06	64.76
Other facilities	0.00	0.00	7.92	7.07	4.32	5.66	10.19	34.04	5.66	18.06	5.71
Total [%]	100	100	100	100	100	100	100	100	100	100	100

Occurrence dynamics

Dynamical analysis follows the occurrence over a period of time and shows the developmental tendency of an occurrence. SBC checks chronology is presented in Graph 1 where we can see that the total number of SBC checks is growing in the period 2007-2011, decreasing by 2014 and then increasing again by 2017.



Graph 1. The trend of the total number of SBC checks in Montenegro in the period 2007-2017.

The occurrence dynamics will be analyzed using absolute growth indicators and occurrence development, such as: growth of the occurrence, increase of the occurrence and mean increase of the occurrence.

The growth of the occurrence is calculated using the formula $P_i = N_i - N_b$, where based on Table 7 we can conclude that in 2014 and 2015 the number of SBC checks decreased compared to the reference year 2007, while in other years their number rose. The highest rise in total number of SBC checks was recorded in 2011.

Table 7. Absolute growth indicators (reference year 2007).

SBC checks	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Correct findings	-	3	24	10	65	35	9	-9	-7	11	25
Irregular state	-	16	19	31	16	13	41	-2	2	3	22
Total	-	19	43	41	81	48	50	-11	-5	14	47

Increase of the occurrence is calculated by

$$A_i = N_i - N_{i-1} \quad (5)$$

where based on data from the Table 8 we can see that the highest increase in the total number of SBC checks was in 2011, while the major decline in total number of SBC checks was recorded in 2014.

Table 8. Absolute development indicator.

SBC checks	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Correct findings	-	3	21	-14	55	-30	-26	-18	2	18	14
Irregular state	-	16	3	12	-15	-3	28	-43	4	1	19
Total	-	19	24	-2	40	-33	2	-61	6	19	33

Table 9. shows that the mean increase in the total number of SBCC in facilities was 4.7 checks for the period 2007-2017, showing the development trend of the occurrence.

Table 9. Mean increase of SBC checks.

SBC checks	Mean increase
Correct findings	2.5
Irregular state	2.2
Total	4.7

The occurrence dynamics will be analyzed also using the relative growth indicators and occurrence development including: growth index, growth tempo, growth rate, mean growth tempo and average growth rate of the occurrence.

Growth index (R_i) of the occurrence, as an indicator of changes in the occurrence observed expressed as percentage is calculated using the formula:

$$R_i = \frac{N_i}{N_b} \cdot 100 \tag{6}$$

In order to compare the changes in number of SBC checks by years compared to 2007. Table 10 below shows that the total number of SBC checks in 2017 was by 81.03% higher compared to the baseline year 2007, while, for example, in 2014 it was by 18.97% lower than in baseline year 2007.

Table 10. The growth index (reference year 2007) of SBC checks in Montenegro in the period 2007-2017.

SBC checks	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Correct	-	108.57	168.57	128.57	285.71	200.00	125.71	74.29	80.00	131.43	171.43
Irregular	-	169.57	182.61	234.78	169.57	156.52	278.26	91.30	108.70	113.04	195.65
Total	-	132.76	174.14	170.69	239.66	182.76	186.21	81.03	91.38	124.14	181.03

Growth tempo ***(E_i)*** as an indicator of change in development of the occurrence in the period 2007-2017 is presented in Table 11 and is calculated using the following formula

$$E_i = \frac{N_i}{N_{i-1}} \cdot 100 \tag{7}$$

Table 11. Growth tempo of SBC checks in Montenegro in the period 2007-2017.

SBC checks	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Correct	-	108.57	155.26	76.27	222.22	70.00	62.86	59.09	107.69	164.29	130.43
Irregular	-	169.57	107.69	128.57	72.22	92.31	177.78	32.81	119.05	104.00	173.08
Total	-	132.76	131.17	98.02	140.40	76.26	101.89	43.52	112.77	135.85	145.83

Growth rate (S_i) of the occurrence as an indicator of a relative (percentage) extent of the occurrence measured against the extent of the occurrence in the previous year is calculated using the following formula:

$$S_i = \frac{A_i}{N_{i-1}} \cdot 100 = \frac{N_i - N_{i-1}}{N_{i-1}} \cdot 100 = E_i - 100 \tag{8}$$

Table 12 shows that the highest growth rate was recorded in 2017, i.e. that the total number of SBC checks in 2017 was by 45.83% higher than in 2016, while the total number of SBC checks in 2014 was by 56.48% lower than in 2013.

Table 12. Growth rate of SBC checks in Montenegro, period 2007-2017 [%]

SBC checks	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Correct	-	8.57	55.26	-27.73	122.22	-30.00	-37.14	-40.9	7.69	64.29	30.43
Irregular	-	69.57	7.69	28.57	-27.78	-7.69	77.78	-67.2	19.05	4.00	73.08
Total	-	32.76	31.17	-1.98	40.40	-23.74	1.89	-56.5	12.77	35.85	45.83

Mean growth tempo (E_s) of the occurrence is an indicator of growth and development of the occurrence expressing the general direction of the occurrence trends in the period 2007-2017 and is calculated by the following formula:

$$E_s = \sqrt[k-1]{E_1 \cdot E_2 \cdots E_i \cdots E_{k-1}} = \sqrt[k-1]{\prod_1^{k-1} E_i} \qquad (9)$$

or

$$E_i = \sqrt[k-1]{\frac{N_k}{N_1}} \qquad (10)$$

Average growth rate (S_p) of the occurrence in Table 13 provides information on average changes in the occurrence, expressed as percentage, in each year of the period 2007-2017. It is calculated by subtracting 100 from the mean value of development index of the occurrence, expressed in percentage.

Table 13. Mean growth tempo and average growth rate of SBC checks in the period 2007-2017.

SBC checks	**Mean growth tempo**	**Average growth in %**
Correct findings	105.54	5.54
Irregular state	106.94	6.94
Total	106.11	6.11

The data on the occurrence dynamics presented, we can conclude that in the given period, 2007-2017, the total number of SBC checks in Montenegro rose by 6.11% on average. It is important to underline that it is an average annual change, not the overall change at the annual level.

Based on the average mean growth tempo for the total number of SBC checks, amounting to 6.11%, we can forecast the value of number of SBC checks over the next five years. From the formula used to calculate the mean growth tempo

$$E_i = \sqrt[k-1]{\frac{N_k}{N_1}}, \; N_k = N_1 \cdot E_s^{(k-1)} \tag{11}$$

so the next forecast value is

$$N_{k+1} = N_1 \cdot E_s^{k} \tag{12}$$

Forecast values are shown in the table below.

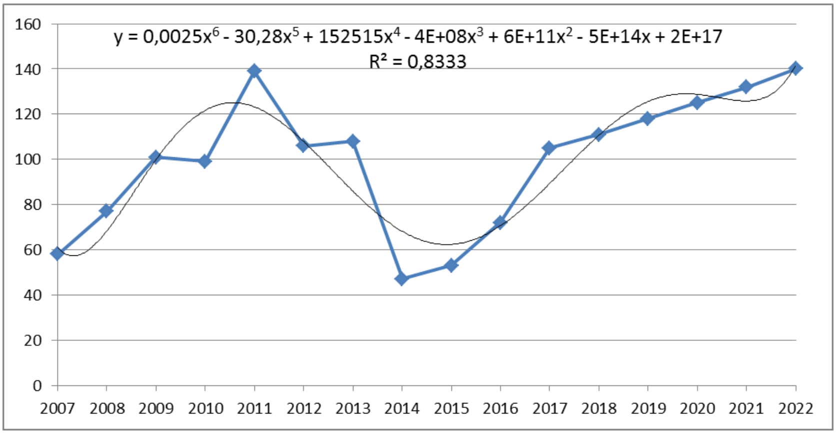
Table 14. Trends of SBC checks in Montenegro by 2022.

Year	2018	2019	2020	2021	2022
Forecast value of SBC checks	111	118	125	132	140

This forecast makes sense only if it is assumed that the factors of relevance for this occurrence would not change substantially in future. Only in that case the occurrence would maintain the same trend.

Extrapolation by trend line extension was made in Graph 2 on reliability of the trend model gives determination coefficient of 0.83. Based on the value of the determination coefficient (R^2), we will know whether the trend line was chosen properly, i.e. whether it adequately represents the occurrence tendency in the period under observation. Determination coefficient is calculated using the following formula:

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(y_i - y_i^t)^2}{\sum_{i=1}^{n}(y_i^2 - \bar{y}_i)} \tag{13}$$



Graph 2. The flow of the occurrence with the trend line and forecast of SBC checks in Montenegro in the period 2007-2022.

The value of the determination coefficient of 0.83 or, expressed in percentage 83.33%, obtained by polynomial function means that 83.33% of periodical or regular changes in number of the total number of SBC checks was explained by the given polynomial function.

CONCLUSIONS

Analysis of the structure of the occurrence and the frequency of SBC checks in Montenegro in the period 2007-2017 should be of use to an efficient management, particularly with regard to prevention.

The homogeneity of total number of checks by years shows relatively strong non-homogeneity in 2014 (variation coefficient above 50%), as well as pronounced non-homogeneity in other years (variation coefficient above 70% in all). Non-homogeneity of checks is a result of various activities of protected persons at the annual level. We will corroborate that with noting that the total number of SBCC in facilities in 2011 was 139, while in 2014 it was 47.

Of the total number of SBC checks, the highest share was carried out in hotel-tourism facilities, followed by closed-type facilities, while the lowest number of SBC checks was carried out in other facilities.

The highest growth in total number of SBC checks compared to the reference year 2007 was recorded in 2011, while the greatest decline in the total number of SBC checks was recorded in 2014. As regard the increase of occurrence of the total number of SBCC, the highest number was recorded in 2011, while the major decline in the total number of SBCC was recorded in 2014. Mean increase in the total number of SBCC in facilities was 4.7 checks for the period 2007-2017, showing the development trend of the occurrence.

Compared to the baseline year 2007, the number of SBCC in 2017 was by 81.03% higher, while for example, in 2014 it was lower by 18.97%. (Growth index - Table 10).

The highest growth rate was recorded in 2017, when the total number of checks was by 45.83% higher compared to 2016. The total number of SBC checks in 2014 was by 56.48% lower than in 2013.

The data on the occurrence dynamics presented, we can conclude that in the given period, 2007-2017, the total number of SBC checks in Montenegro rose by 6.11% on average. It is important to underline that it is an average annual change, not the overall change at the annual level.

Based on the average mean growth tempo for the total number of SBC checks, amounting to 6.11%, we can forecast the value of number of SBC checks over the next five years. Taking into account the data presented, increase in number of police officers in charge of SBCP should be considered in order to improve the quality of SBCP, and hence the protection of persons.

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ENHANCEMENT OF WHEAT VIRUS-RESISTANCE AT APPLICATION OF THE SE NANOPARTICLES CITRATES AND CONSORTIUM OF SOIL MICROORGANISMS

SUMMARY

The influence of pre-sowing seed treatments of selenium nanocitrates (SeNPs) and application of soil formed microorganism consortium (biological preparation (BP) Extrakon) on wheat plants infected with wheat streak mosaic virus (WSMV) were investigated in greenhouse and fields conditions in 2018-2019 (on territories of Zabolotny Institute of Microbiology and Virology of NASU).

The pre-sowing seed treatments of 1% selenium nanocitrates (SeNPs) with application of BP Extrakon initiating the growth of juvenile wheat in laboratory experiments were found.

In the field experiment on variants of WSMV-infected plants, two weeks after inoculation (tillering stage), we observed the appearance of characteristic symptoms of yellow mosaic with intermittent strokes on the leaves. In the boot phase, a lag in the growth of WSMV-infected plants compared with intact plants was already clearly visible, which was confirmed by the ELISA test.

It was shown, increase of the quantum efficiency of PS II (F_v/F_p) of WSMV-infected plants at pre-sowing treatment of 1% SeNPs and application of microbial consortium (BP Extrakon) compared with virus-infected plants without treatment.

In plant tissue of all experimental variants increase activity of superoxide dismutase was shown. It was shown more significant increase of the SOD-activity on the variant of WSMV- infected plants with application of BP Extrakon

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and SeNPs. Estimation of 1000-grain weight and grain productivity confirmed the effectiveness of the combined use of BP Extrakon with pre-sowing treatment of SeNPs on both intact and WSMV-infected plants. Thus, mixed application both SeNPs pre-sowing treatment and adding in soil of soil forming microbial consortium has a bio protective effect on wheat plants enhancing the resistance of plants cells to viral damage.

Keywords: Wheat, WSMV, Se nanoparticles, consortium of soil microorganisms, chlorophyll a fluorescence induction

INTRODUCTION

The wheat streak mosaic virus (WSMV) causes wheat mosaic, a disease of cereals and grasses, which threatens incomplete harvest wheat worldwide. Infected plants lag behind in growth and have a yellow mosaic with intermittent strokes on the leaves. It is known that WSMV affects winter and spring wheat (*Triticum aestivum* L.) and depending on environmental conditions and cultivation technology, the loss of wheat yield from viral damage can reach 60% or more (Hadi *et al*., 2011; Singh *et al.* 2018). In general, studies of the effects of plant damage by pathogens have focused on the effects of nutrient availability, on plant susceptibility to infections and disease transmission rates (Dordas, 2008; Butsenko *et al*., 2018; Dankevych *et al*. 2018). This effect depended on the interaction between the phytopathogen and the host, as well as on the type of fertilization of plants. Investigations of the effect of adding macronutrients on virus replication have shown that the effects vary depending on the nutrients and the pathological system, with the addition of nitrogen and phosphorus usually increases the titer of the virus (Pennazio and Roggero, 1997; Miller *et al.*, 2015).

In addition, a modern direction in crop production is the use of nanotechnology (Prasad *et al.*, 2017; Liu and Lal, 2015; Ljubičić, N. et al., 2020), in particular to reduce the percentage of infection by phytopathogenic microorganisms, including viruses – in resource-saving technologies. Promising agents for the control of phytopathogenic microorganisms can be selenium nanoparticles (SeNPs), which are used in biomedicine and agriculture due to their high biological activity, including antioxidant and antimicrobial characteristics, and highness of biocompatibility and biosafety too (Wadhwani *et al.*, 2016).

It should be noted that with the current deterioration of soils at the global level it is extremely important to use microbiological technologies for its reproduction and in eco-technologies for growing plants (Kumar *et al*., 2015). It is known that agronomically useful soil microorganisms are used in crop production as bio stimulants, bio fertilizers and bio protectors, due to complex mechanisms of plant-microbial interaction that improve plant growth, development and stability (Nihorimbere *et al*., 2011).

Therefore, the aim of our work was to study the role of pre-sowing seed treatment with selenium nanoparticle citrates together with using soil-forming microbial consortium in enhancing virus resistance and productivity formation in conditions of WSMV infection.

MATERIAL AND METHODS

In laboratory, field experiments and experiments in the greenhouse used: 1% solution of citrates of nanoparticles Se – SeNPs (Se content: 100 g/l) and soil-forming microbial consortium (biological product (BP) "Extrakon" (Ukraine)). BP Extrakon consists of a consortium of soil cellulolytic and heterotrophic microorganisms inoculated into a peat-like substrate, which are in a functionally active state and are closely connected by trophic bonds: (*Sporocytophaga mixococcoides*, *Sorangium cellulosum*, *Cellvibrio mixtus*, *Trichoderma viride*, *Pseudomonas fluorescens*, *P. putida*, *Bacillus subtilis*, *B. sphaericus*, *B. megaterium*, *B. pumilus*).

In fields of conditions, plants of wheat *Triticum aestivum* L. variety Pecheryanka were grown on an experimental plot of the D.K. Zabolotny Institute of Microbiology and Virology of NASU in 2018-2019. The area of the experimental plot is 50 m^2, the soil is sod-podzolic. Repetition in the experiment four times. $N_{90}P_{90}K_{90}$ (ammonium nitrate, granular superphosphate, and potassium chloride) was applied to the soil before sowing.

The scheme of experiments: 1 – control (intact plants); 2 – WSMV-infected plants; 3 – pre-sowing treatment (p.-s. treatment) of 1% SeNPs solution; 4 – addition in soil of BP Extrakon + p.-s. treatment of 1% SeNPs solution; 5 – p.-s. treatment of 1% SeNPs solution + WSMV; 6 – BP Extrakon + p.-s. treatment of 1% SeNPs solution + WSMV.

The scheme of experiments in the greenhouse: 1 – control (intact plants); 2 – WSMV-infected plants; 3 – BP Extrakon; 4 – BP Extrakon + WSMV-infected; 5 – p.-s. treatment of 1% SeNPs solution + BP Extrakon; 6 – p.-s. treatment of 1% of SeNPs solution + BP Extrakon + WSMV.

In laboratory experiments 1: in Petri dishes decomposed of seed grains of 50 pcs, in three replications. The scheme of experiments: 1 – control (intact plants); 2 – p.-s. treatment of 1% of SeNPs solution.

After 7 days, the effect of this measure on the growth of the roots of juvenile plants in comparison with intact plants was determined.

In a laboratory experiment 2, using the roll method evaluated the effect of p.-s. treatment of 1% SeNPs solution together with BP Extrakon application. To do this, 50 seeds were laid out on rectangular filter paper, size 75x15 cm, it was tightly twisted and immersed in filtered water. The scheme of laboratory experiments 2: 1 – control (intact plants); 2 – p.-s. treatment of 1% of SeNPs solution; 3 – p.-s. treatment of 1% SeNPs solution + BP Extrakon.

In field experiments and in the greenhouse, inoculation of wheat plants with freshly prepared virus-containing material was carried out in stage of the second pair of true leaves, by the method of mechanical inoculation of leaves with preliminary dusting with carborundum. Isolation of viral material was performed by homogenization of freshly cut leaves of diseased plants with clear symptoms of WSMV with the addition of 0.1 M phosphate buffer pH 7.0. The plant homogenate was filtered through a nylon sieve and used to mechanically infect the plants. Infection of plants was carried out using a glass spatula or fingers in disposable gloves soaked in inoculum. Excess inoculum was washed off with water (Wetzel, 1984). Antigen detection was performed by enzyme-linked immunosorbent assay (ELISA) using diagnostic sera for WSMV (Bradwell *et al*., 1990; Gnutova, 1993). Solid-phase ELISA (sandwich variant) was performed using commercial test systems to WSMV "Loewe" (Germany). The reaction results were recorded on a reader of Termo Labsystems Opsis MR (USA) with Dynex Revelation Quicklink software at wavelengths of 405/630 nm. Values that exceeded the negative control three times were considered reliable (Crowther, 1995).

Studies of the activity of the antioxidant enzyme superoxide dismutase (SOD) were performed in leaf tissue on the stage of heading – flowering. The activity of SOD was determined by the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium (Beyer and Fridovich, 1987). The portion of plant material weighing 150–200 mg was ground in a cooled mortar in 300–400 μl of extraction buffer. The resulting homogenate was centrifuged at 12,000 g for 5 minutes. For each sample made two identical tubes with the reaction mixture and extract. One test tube was placed in the dark, the other – in the light. In addition, control tubes were prepared without enzymatic extraction – to calculate the maximum formation of formazan. The reaction was started by adding 20 μl of 0.025% riboflavin to all tubes, which were stirred rapidly.

Tubes to determine the maximum formation of formazan, placed in a dark place. The remaining tubes were installed under two fluorescent lamps. The reaction lasted 15 minutes. At the end of the time, the reaction was stopped by turning off the light. The optical density is measured at a wavelength of 560 nm. The enzyme activity was calculated by determining the percentage inhibition per min. The increase in absorbance without the enzyme extract was taken as 100%. Fifty percent of inhibition was taken as equivalent to 1unit of SOD activity. To calculate the activity of SOD per gram of dry weight (units/g dry wt) (Giannopolitis and Ries, 1977; Chakrabarty *et al*., 2009).

The photochemical activity of the leaves was determined by the biophysical method of chlorophyll a fluorescence induction (ICF) using a portable device "Florotest" (Ukraine).

The device is equipped with a liquid crystal display (128 × 64 pixels) and a remote optoelectronic sensor with a wavelength of 470 ± 15 nm, the area of the irradiation spot not less than 15 mm^2 and illumination within it not less than 2.4 W/m^2. The spectral range of fluorescence intensity measurements is in the range from 670 to 800 nm. The data measured by the device was transferred to a PC via the USB port of the computer using the software "Floratest", which comes with the device and allows you to display this data in tabular or graphical form. Dark adaptation of leaves before measurements was in range: 6-20 minutes. Replications measurements on each variant – threefold. The evaluation of the influence of experimental factors was performed by changes in the value of the quantum efficiency parameter PS II: F_v / F_p ($F_v = F_p - F_0$), where F_0 – the minimum fluorescence; F_p – maximum (peak) fluorescence, F_v – variable fluorescence (Sharma *et al*., 2015; Lichtenthaler *et al*., 2007; Huliaieva, *et al*. 2018).

Accounting for grain productivity in the experimental plots was done at the end of the growing season at full grain maturity. Statistical processing of the obtained results was performed using computer programs MS Excel.

RESULTS AND DISCUSSION

In the laboratory experiment, it was found that pre-sowing treatment of wheat seeds with 1% solution of SeNPs increased root length from 4.16 cm in the control to 7.99 cm, and therefore 1.92 times. It should be noted, that pre-sowing treatment of 1% SeNPs and application of BP Extrakon several-fold enhanced the growth-stimulating effect of pre-sowing treatment of 1% SeNPs by showing a synergistic effect. Increase of 16.5% of juvenile plants leaf weight at pre-sowing treatment of 1% SeNPs and adding of Extrakon was shown.

In the field experiment on variants of virus-infected plants, two weeks after inoculation, we observed the appearance of characteristic symptoms: yellow mosaic with intermittent strokes on the leaves (tillering phase) (Fig. 1 A). In the boot phase, the lag in the growth of infected compared to intact plants was already clearly visible (Fig. 1 B)

Fig. 1. The WSMV-infected wheat plants in different growth stage: the tillering phase (A) and the boot phase (B), the milk-wax ripeness phase (C).

The ELISA results showed that with the defeat of WSMV, the content of antigens on the 14th day after infection increased significantly – 5 times compared to the control (Fig. 2).

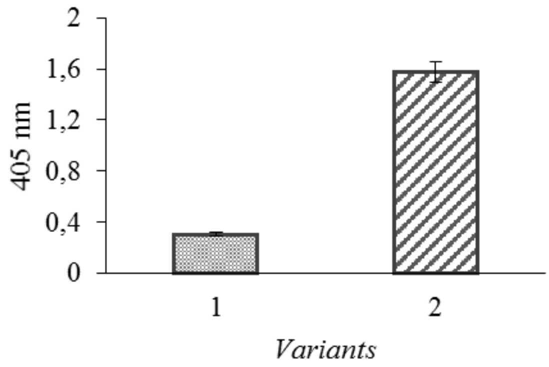

Fig. 2. The effect of artificial contamination of WSMV of spring wheat on the content of antigens, ELISA data 14 days: 1 – the intact plants (treatment with water, control 1); 2 – the WSMV-infected plants (treatment with water).

With ICF of method, we found decrease of quantum efficiency of PSII (Fv/Fp), which reflects decrease of quantity of photochemically active centers in PSII, in the leaves of virus-infected wheat plants (14 days after infection) (Fig. 3).

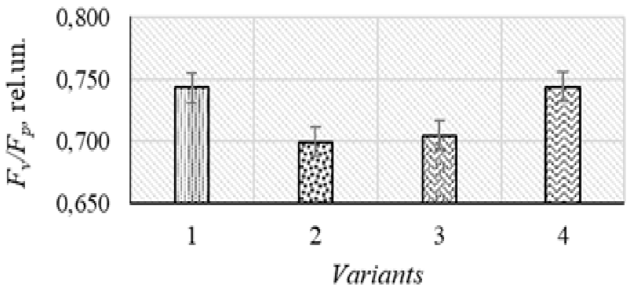

Fig. 3. The quantum efficiency of PSII of leaves of WSMV-infected wheat plant at pre-sowing treatment of 1% SeNPs and adding BP Extrakon, the tillering phase: 1 – Control (intact plants); 2 – WSMV-infected plants; 3 – WSMV + BP Extrakon; 4 – WSMV + p.-s. treatment of 1% SeNPs + BP Extrakon (experiment in the greenhouse).

It is known that under the action of stresses of different nature, including the action of phytopathogenic microorganisms, in cells there is an accumulation of reactive oxygen species (ROS), in particular: superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), etc. One of the main antioxidant enzymes involved in the regulation of ROS levels while maintaining resistance to adverse factors is superoxide dismutase (SOD), which catalyze the dismutation of superoxide radicals to molecular oxygen and hydrogen peroxide (Tyagi *et al.*, 2019; Tanase and Popa, 2014; Choudhury *et al.*, 2013).

Therefore, as a marker of resistance to the action of experimental measures, we determined the change in the activity of SOD in the tissues of the leaves. Evaluation of SOD activity (in the field experiment) in wheat leaves in the heading – flowering growth period showed its growth in all experimental variants in the following order: control (intact plants) > SeNPs + WSMV > BP Extrakon + SeNPs > SeNPs > WSMV > BP Extrakon + SeNP. Thus, the most significant increase in the activity of SOD, indicating an increase in plant resistance, was found in the variant Extrakon + SeNPs + WSMV (Fig. 4).

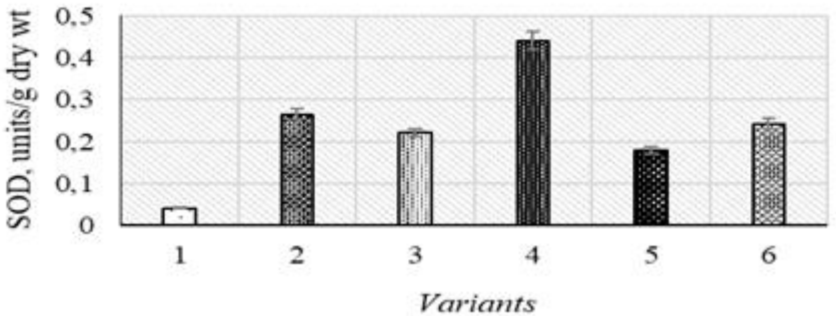

Fig. 4 The activity of SOD in the tissues of the leaves of WSMV-infected spring wheat under the influence of pre-sowing treatment of 1% SeNPs and adding of BP Extrakon (field experiment): 1– intact plants; 2 – WSMV- infected plants ; 3 – BP Extrakon + 1% SeNPs; 4 – BP Extrakon + 1% SeNPs + WSMV; 5 – 1% SeNPs + WSMV; 6 – 1% SeNPs.

Accordingly, in variants with WSMV-infected plants, a significant decrease in grain productivity and the thousand-kernel weight was observed compared to the control – by 29.7% (Table 1).

Table 1. The grain productivity of spring wheat at application of 1% SeNPs pre-sowing treatment, use of BP Extrakon and WSMV-infection

Variants	The thousand-kernel weight, g	The grain productivity, $kg/10m^2$
Control (intact plants)	34,8±1,39	3,17±0,12
WSMV-infected plants without treatment	21,4±0,86	2,23±0,11
1% SeNPs	34,2±1,37	3,12±0,15
1% SeNPs+ BP Extrakon	36,2±1,45	3,41±0,14
1% SeNPs+ WSMV	22,1±0,89	2,37±0,09
1% SeNPs+ BP Extrakon + WSMV	27,5±1,11	2,52±0,10

On variants with pre-sowing treatment with 1% solution of SeNPs and WSMV-infected plants was showed a tendency to increase grain productivity compared to WSMV-infected plants without treatment. The better effect on grain productivity was detected on variants of intact plants wheat with pre-sowing treatment of 1% SeNPs solution with BP Extrakon application, where productivity increased by 7.6% relative to control. The application of pre-sowing treatment of 1% SeNPs and BP Extrakon on the variants with WSMV-infected plants allowed an increase of grain production by 13% compared with virus-infected plants without treatment, partially reducing losses from viral infection.

The effect of experimental treatment on the thousand-kernel weight had a similar tendency. Its effect it is possible be arranged in the following order: SeNPs + BP Extrakon > Control (intact plants) > SeNPs > SeNPs + BP Extrakon + WSMV > SeNPs + WSMV > WSMV-infected plants (see Table 1).

Thus, pre-sowing treatment of wheat seeds with selenium-nanocitrates with soil-forming microbial consortium application has not only bio stimulating, but also antioxidant and therefore – bio protective effect on plants – increasing the resistance of cells to viral damage.

CONCLUSIONS

Thus, the better effect in growth stimulating wheat plants at mixed application both 1% SeNPs and consortium of soilformed microorganisms (BP Extrakon) was found in laboratory conditions on the young plants.

In the field experiment on WSMV-infected wheat plants, two weeks after inoculation (in the tillering phase), we observed the appearance of characteristic symptoms: yellow mosaic with intermittent strokes on the leaves. In the boot phase, a lag in the growth of infected compared to intact plants was already clearly visible, which was confirmed by the ELISA test. It was shown, increase of the quantum efficiency of PS II (F_v/F_p) of WSMV-infected plants at mixed application both pre-sowing treatment of 1% SeNPs and microbial consortium (BP Extrakon) compared to virus-infected plants without treatment.

In plant tissue of all experimental variants increase activity of superoxide dismutase was shown. It was shown more significant increase of the SOD-activity on the variant of WSMV- infected plants at mixed application both application of BP Extrakon and SeNPs. Estimation of 1000-grain weight and grain productivity confirmed the effectiveness of the combined use of BP Extrakon with pre-sowing treatment of SeNPs on both intact and WSMV-infected plants.

Thus, mixed application both SeNPs pre-sowing treatment and adding in soil of soil-forming microbial consortium has not only bio stimulating, but also antioxidant and therefore – bio protective effect on wheat plants – increasing the resistance of cells to viral damage.

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EPIDEMIOLOGICAL PATTERNS, PREVALENCE AND SEASONAL DYNAMICS OF DIFFERENT VIRUSES IN SUSCEPTIBLE CUCURBIT CROPS

SUMMARY

Vegetables represent valuable food source and are cultivated worldwide. Cucurbits are of great importance for farmers, and viruses endanger their profitable production. Monitoring of vegetable viruses in Ukraine showed that cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus (WMV) were the most harmful and common viruses in reference regions. This work was focused on establishing sources of virus introduction and/or maintenance in a population of susceptible crops, as well as on assessing relative changes in virus-infected symptomatic plants during the vegetative season in open field conditions. Samples were collected based on visual symptoms on leaves and fruits. For virus indication, commercial DAS-ELISA kits for CMV, ZYMV, and WMV were used (Loewe, Germany). Following this work, epidemiological patterns for viruses infecting vegetable crops based on their biological properties were discovered. Seed transmission was confirmed as an important way of spreading of ZYMV and CMV, and often neglected source of viruses in the ecosystems requiring special control for pathogens with wide host range (CMV). CMV was the commonest virus found in symptomatic plants (21%) followed by ZYMV (14%) and WMV (8%). A large portion (18%) of mixed virus infection was typically induced in Ukraine by the following virus groups: CMV/ZYMV, CMV/WMV, ZYMV/WMV, and rarely CMV/ZYMV/WMV. Field experiments showed steep decline of healthy plants from the end of May until the end of August, in parallel to an increase of virus-infected cucurbits. CMV was detected starting from the beginning of the experiment. Similarly, WMV was also found during the duration of screening with sharp increase of diseased plants by the end of the season. ZYMV was detected only in July with subsequent decrease in the number of ZYMV-positive

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samples as opposed to WMV. Artificial ecosystems were demonstrated as important factors maintaining populations of viruses infecting vegetable crops.

Keywords: cucumber mosaic virus, zucchini yellow mosaic virus, watermelon mosaic virus, cucurbit, Ukraine.

INTRODUCTION

In Ukraine, vegetable crops are considered strategically important as they account for a very significant amount of gross plant production and value reaching ~20%. The areas used for production of these crops and production volumes (including cultivation in glasshouse conditions) increase annually. In addition, many vegetables (tomato, melon, squashes, cucumber, etc.) traditionally form an important article for international trade (http://minagro.gov.ua/).

Viruses are the major cause of losses both in terms of yield quantity (15-100% decrease, ~20% on average) and quality of plant production (loss of marketable appearance/shortening of the storage period with resulting sharp decrease of commercial value). Since viral diseases of plants are incurable, the means of their control are mostly limited to routine diagnostics of production plants and prevention of virus spread on the fields through cultural practices, vector control and use of resistant cultivars when they are available.

Cucurbits and other vegetable crops are known hosts for several dozens of viral pathogens, and the larger part of these is transmitted by flying vectors – mostly aphids. Knowing profile of viruses and their respective vectors, as well as other means of their transmission, for a given ecosystem and cultivation technology is the absolute must-do for introducing efficient control measures for such diseases.

During the recent years, vegetable viruses attain growing attention. Several major factors are deemed to contribute to wider profile of viruses appearing in the ecosystems. The most important ones are global warming leading to migration of flying vectors, and especially aphids, to earlier unfavorable (colder) regions coupled with global trade involving plant and seed material together with their pests.

In Ukraine, several previously absent new highly damaging vegetable viruses have been described including zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) on zucchini, squash and pumpkin plants; pepper mild mottle virus (PMMoV) and pepino mosaic virus (PeMV) on tomatoes and sweet pepper; turnip mosaic virus (TuMV) on many wild-growing and domesticated brassicas; onion yellow dwarf virus and leek yellow stripe virus on various onions (Tsvigun *et al*., 2016; Shevchenko *et al*., 2018a; Snihur *et al*., 2019; Sherevera *et al*., 2019). The phylogeny of several viral pathogens infecting fruits and vegetables in Ukraine was also studied in detail showing that both known and novel isolates circulate in the region (Budzanivska *et al*., 2016; Shevchenko *et al*., 2018b; Kutsenko and Budzanivska, 2020), confirming the significant role of interregional plant transportation in virus spread and evolution.

CMV is a representative of *Bromoviridae* family and has icosahedral particles with tripartite genome (King *et al*., 2012). CMV was first described nearly 90 years ago and remains a devastating pathogen with extremely wide host range (>16 plant families) endangering production of a vast majority of vegetables including tomato, spinach, and cucurbits all over the world (CABI Datasheet – Cucumber mosaic virus). CMV is efficiently transmitted by aphids in a non-persistent manner, by mechanical inoculation, and by seed.

On the other hand, ZYMV and WMV belong to the large *Potyviridae* family, were first isolated in 1960-70-ies and then identified in all continents within a decade (CABI Datasheet – Zucchini yellow mosaic virus; CABI Datasheet – Watermelon mosaic virus). They represent a major constraint in the world-wide production of cucurbits, from cucumbers to watermelons. Contrary to ZYMV, which is naturally restricted mainly to cucurbits, WMV has wider host range. Both viruses induce persistent symptoms (yellow and green leaf mosaic, fruit mosaic, spotting and deformation). The viruses can cause massive damage (to total loss) to the crops (especially in co-infection) and prevent their growth in certain areas (Gal-On, 2007). ZYMV and WMV are mainly transmitted by aphids non-persistently, mechanically from plant to plant, as well as with planting material. ZYMV was also confirmed seed-born in zucchini, squash, and holl-less seeded oil pumpkin, which could have contributed to its rapid spread worldwide (Tobias and Palkovics, 2003).

Ukraine is one of the largest European countries enjoying strategic position between the eastern EU states and Black Sea/Middle East region. Our previous work showed that cucumber mosaic virus (CMV), ZYMV and WMV were the commonest viruses infecting cucurbits in Ukraine, where ZYMV and WMV were first detected in 2006 both in mono- and mixed infection, whereas CMV was known for decades but was officially ‘reconfirmed’ back in 2015 (Rudneva *et al*., 2006; Shevchenko *et al*., 2015).

This paper describes comparative analysis of viruses infecting cucurbits which are truly devastating for profitable production of these crops in Ukraine, in order to establish their epidemiological patterns, prevalence and seasonal dynamics in susceptible crops to recommend simple and reliable means for controlling virus spread.

MATERIAL AND METHODS

Plant sampling was conducted during vegetative seasons (summer) of 2016-2019 in open field conditions on plots annually used for cultivation of various cucurbit crops predominantly including squash, zucchini, and pumpkin in central part of Ukraine (Poltava region) considered an important area for commercial cultivation of seasonal cucurbits. Cucurbit plants were visually examined; samples (leaves and fruits) were collected from plants with virus-like symptoms.

Cucurbit plants (squash, zucchini, etc.) were visually examined; approx. 260 samples (leaves and fruits) were collected from plants with virus-like symptoms.

Collected samples were tested for CMV, ZYMV, and WMV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), as described previously by Clark and Adams (1977), using specific polyclonal antibodies purchased from Loewe (Germany) and following the manufacturer's recommendations. Briefly, 0,5 g leaf tissue was ground to a powder with a mortar and pestle in 10 mL phosphate-buffered saline, pH 7,4, containing 0,05% Tween 20, 2,0% polyvinylpyrrolidone (MW 40 000) and 0,2% bovine serum albumin. In the meantime, microtitre plates (Greiner Bio-One, Germany) were coated with virus-specific broad-spectrum polyclonal antibodies (1:200) in carbonate buffer according to the manufacturer's instructions. Leaf extracts were then added to the plates in duplicate wells and incubated overnight at 4°C. The presence of virus(es) in the samples was detected in 200 μL homogenate by virus-specific antibodies conjugated to alkaline phosphatase using *p*-nitrophenyl phosphate substrate (Sigma, USA). Absorbance values at 405 nm were measured using a Thermo Labsystems Opsis MR microtitre plate reader (USA). Absorbance values, measured 60 min after adding the substrate, greater than three times those of the negative controls were considered positive.

For transmission electron microscopy (TEM), copper grids (Sigma, USA) were coated with chloroform-dissolved 0.2% polyvinyl formaldehyde (Serva, Germany) and dried overnight on filter paper at room temperature. The samples deposited onto grids were stained with 2.5% uranyl acetate and 0.02 N lead citrate (Serva, Germany), and examined using JEM 1400 (JEOL, Japan) transmission electron microscope. The samples were photographed at a magnification of 5,000-60,000x (Mendgen, 1991).

RESULTS AND DISCUSSION

On sampled cucurbits (zucchini, squash, and pumpkin) virus-like symptoms typically included leaf mosaic and yellowing, leaf/fruit mottling and deformation, plant stunting, etc. Typical symptoms for mono-infected plants are shown on Fig.1.

Using DAS-ELISA, symptomatic plant samples were subsequently screened for CMV, ZYMV, and WMV. Overall, 61% of symptomatic plants were shown positive for one or more viruses whereas 39% were negative. CMV has been detected in large part of the collected plant samples (21%) in every cucurbit species tested. This was followed by ZYMV monoinfection (14%) and WMV monoinfection (8%) found in pumpkin, squash, and zucchini. A large portion (18%) of mixed virus infection was typically induced by the following virus groups: ZYMV/WMV (6%), CMV/ZYMV (5%), CMV/WMV (5%), and rarely CMV/ZYMV/WMV (2%) (Fig.2).

Figure 1. Virus-like symptoms on sampled plants in field conditions later confirmed as virus-infected: A – leaf yellowing/mosaic/deformation and plant stunting of CMV-positive pumpkin, B – yellowing and ringspots on fruit of ZYMV-positive squash, C – dark green veinal mosaic of leaves of WMV-positive squash

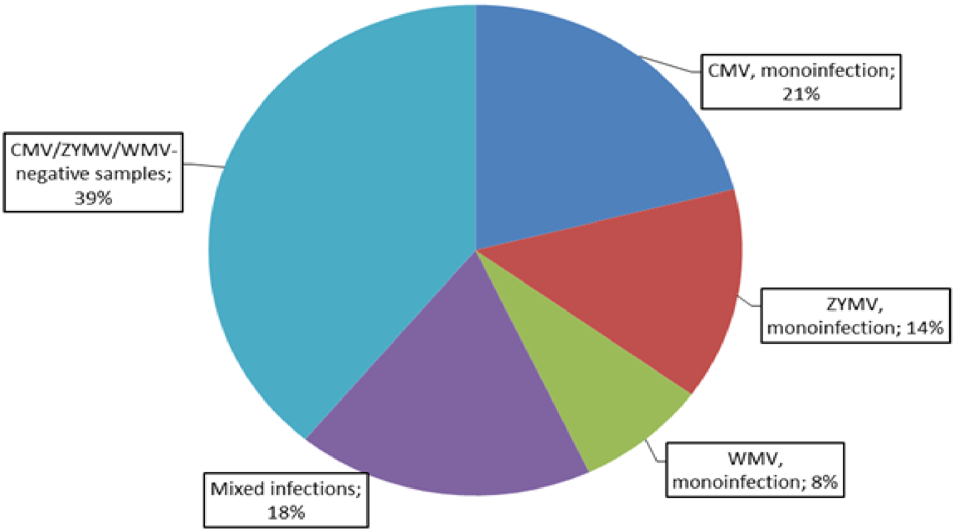

Figure 2. Double-antibody enzyme-linked immunosorbent assay for the detection of cucumber mosaic virus, zucchini mosaic virus and watermelon mosaic virus in mono- and mixed infection in cucurbits in Ukraine

In line with ELISA results, transmission electron microscopy of plant sap confirmed the presence of spherical (~25-35 nm in diameter) and filamentous virions (720-850 x 16 nm) typical for cucumoviruses and potyviruses, respectively (Fig.3).

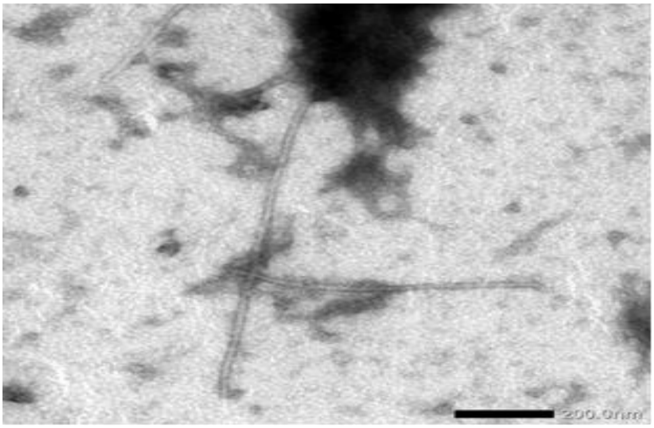

Figure 3. Electron micrograph of partly purified sap of CMV/ZYMV-positive squash showing small icosahedral CMV particles and filamentous ZYMV particles. Scale bar corresponds to 200 nm

The pattern of virus occurrence and prevalence (Fig.2) follows general knowledge on these viruses (King *et al*., 2012; Tsvigun *et al*., 2016; Shevchenko *et al*., 2018b) and probably reflects their biological and epidemiological properties (namely host range, means of transmission and stability in the environment) rather than plant insusceptibility/resistance to virus infection. In this context, CMV benefits from a very wide host range, diverse transmission routes including seed transmission (absent for WMV and most of ZYMV isolates), and highly stable virions. ZYMV and WMV, on the contrary, have significantly narrower host range and do not demonstrate notable stability in the environment.

Surprisingly high level of mixed infections can be explained differently and needs more analysis. However, the symbiotic interactions between CMV, ZYMV and WMV are probably unavoidable in artificial ecosystems, especially if these are routinely used for cultivation of the same crops annually allowing virus accumulation in the environment (plan debris, concomitant weeds and substrate). Expectedly, ZYMV and WMV co-infection was most frequent (6%) followed by CMV/ZYMV and CMV/WMV (5% each), and occasionally by CMV/ZYMV/WMV (2%) – the latter rare combination inducing more severe symptoms on infected plants (Fig.4E).

According to available literature data, CMV, ZYMV and WMV are often found in co-infection. For example, CMV mostly interfere with another virus(es) including WMV and ZYMV (Sano and Kojima, 1989; Barbosa *et al.*, 2016). In contrast, WMV and ZYMV frequently co-occur in cucurbits (Desbiez *et al.*, 2020) and share the same aphid vectors. Interestingly, ZYMV replication rates did not depend on the type of infection (mono- or mixed with WMV) but ZYMV enhanced aphid recruitment to infected plants. However, WMV did not much affect plant-vector relationships, while its strains were shown to accumulate to significantly lower levels in the presence of ZYMV. Despite poor in-plant competition with ZYMV, WMV readily transmitted from mixed infections (Salvaudon *et al.*, 2013). The line of evidence suggests that lower occurrence of WMV shown in this study may be conditioned by its wider host range (ability to survive in neighbour weeds thus avoiding the competition) and antagonism with ZYMV.

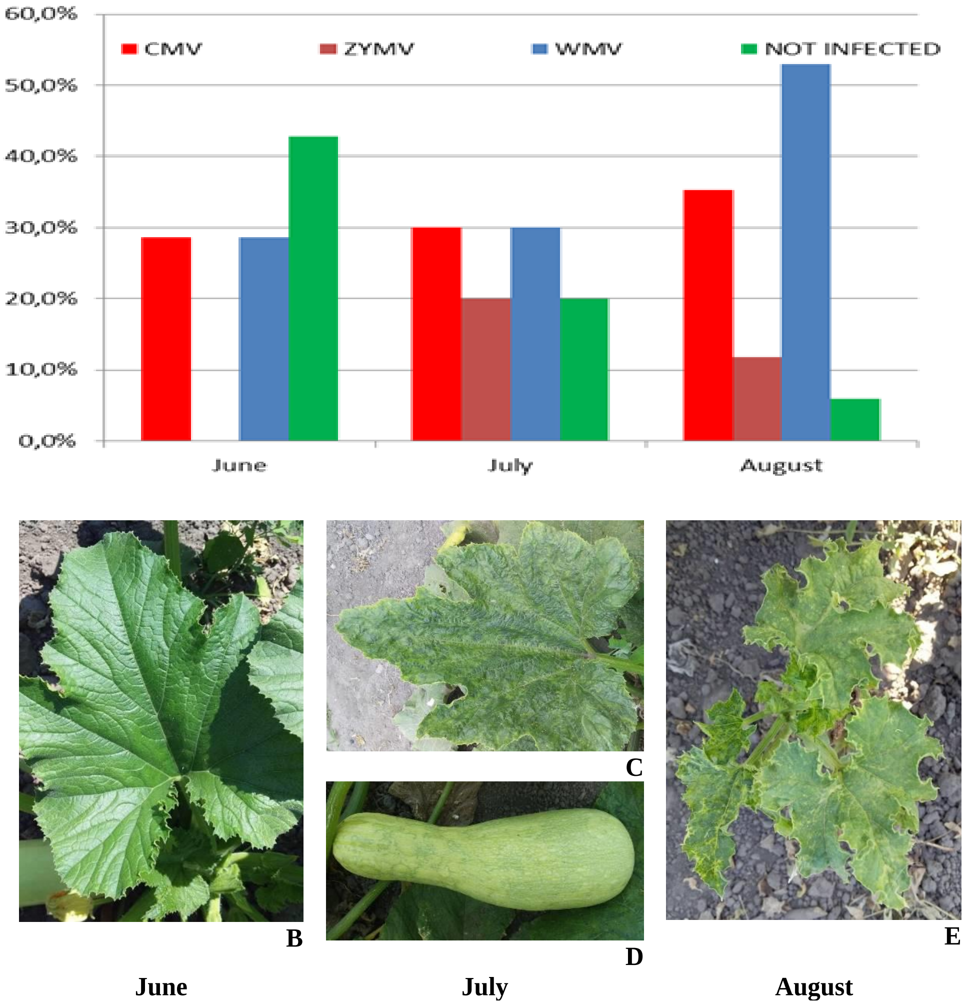

Figure 4. Seasonal dynamics of CMV, ZYMV and WMV in susceptible cucurbit crops as percentage of infected plants on experimental plot (A) and intensification of visual disease symptoms on such plants (B-E) during summer months of 3 consequential years: B – healthy-looking leaf of WMV-infected squash, C – dark green veinal leaf mosaic of ZYMV-infected squash, D – irregularly shaped ringspots on fruit of ZYMV-infected squash, E – severe leaf mosaic, yellowing and stunting of CMV/ZYMV/WMV-infected squash

Following this lead, an experimental plot annually used for cultivation of various cucurbit crops was regularly screened for CMV, ZYMV and WMV to elucidate their seasonal dynamics during vegetative period (typically June-August in open field conditions of central Ukraine (Fig.4).

From Figure 4 it follows that the percentage of healthy plants (green color) steeply declined from the beginning of June until the end of August, in parallel to an increase of virus-infected cucurbits. CMV was detected starting from the beginning of the experiment, with gradual increase of CMV-positive plants during the experiment. Similarly, WMV was also found during the whole duration of screening with sharp elevation of diseased plants by the end of the season. However, ZYMV was first detected only in July with subsequent decrease in the number of ZYMV-positive samples as opposed to WMV.

Expectedly, visual virus-induced symptoms became more pronounced with time, from healthy-looking leaves (Fig.4B) to severely diseased plants showing complex and systemic symptoms, especially in case of mixed infection (Fig.4E).

The results suggest long-term persistence of CMV and possibly WMV in the ecosystem where these pathogens may circulate off-season by infecting other available non-cucurbit hosts. Nevertheless, ZYMV has the narrowest host range and obviously requires susceptible cucurbits for massive multiplication occurring mostly in the midsummer after crop sawing and mass aphid infestation in May-June. Such narrow and disadvantageous ‘window of opportunity’ for ZYMV may be compensated by its active spread by vectors in the midseason which may be sufficient for maintaining virus population.

Altogether, obtained results clearly demonstrate the correlation between epidemiological properties and seasonal dynamics of cucurbit viruses in susceptible crops, and underline the importance of routine control of planting material and cultivated crops on a regular basis which remain highly efficient measures in preventing the spread of the mechanically and aphid-transmitted virus and reducing consequential damages.

CONCLUSIONS

Visual and serological screening of cucurbit crops showed that 61% of symptomatic plants were positive for one or more viruses, where CMV has been detected in 21% of samples of every species tested followed by ZYMV (14%) and WMV (8%) found in pumpkin, squash, and zucchini. A large portion (18%) of mixed virus infection was typically induced by ZYMV/WMV (6%), CMV/ZYMV (5%), CMV/WMV (5%), and rarely CMV/ZYMV/WMV (2%). The pattern of virus occurrence and prevalence reflects their biological and epidemiological properties. The outcomes of studying seasonal dynamics of CMV, ZYMV and WMV in susceptible crops suggest long-term persistence of CMV and possibly WMV in the ecosystem where these pathogens may circulate off-season as opposed to ZYMV which obviously requires susceptible cucurbits for massive multiplication. Obtained data confirm that CMV, ZYMV and WMV remain damaging vegetable viruses in Ukraine, and raise questions of their proper control.

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Tetiana IVANOVA[1]

ADVANCED RECEPTIONS IN THE METHODOLOGY OF EXTRACTION TOTAL AND VIRAL NUCLEIC ACIDS FROM BASIDIOMYCETES

SUMMARY

Existing governmental programs aimed to increase mushrooms production and significant market demand for mushrooms contributed to the expansion of such production. Meanwhile, until now practically don`t exist highly precised molecular diagnostics for the detection and identification of mycoviruses that strike the Basidiomycetes in Ukraine. The purpose and objectives are to study modern methods of extraction of total RNA from Basidiomycetes and their modifications and improvements as well as the molecular biological systems test for the identification of Basidiomycetes mycoviruses. This research was carried out by way of with follow techniques: molecular biological (the method of extraction of total RNA, dsDNA, precipitation, amplification); biotechnological (obtaining and subcultivation of samples of mycelium in vitro using electrophoresis in agarose and polyacrylamide gels (PAGE); microbiological (obtaining pure mushroom culture, determining the hydrogen index (pH) of the nutrient medium), mycological (a measurement of growth), virology, microscopy. Interpretation statistics data was carried out with the help of computer software. Screening of mushroom`s samples has been performed in the Kyiv region. The virus infection and prevalence of viral diseases of mushrooms have been researched. The modification of method of extraction total and viral nucleic acids from Basidiomycetes has been proposed. It is based on the acceleration of the stage of cellulose-chromatographic purification of nucleic acids and fractionation of dsRNA. It is proposed to propagate the first stage of low-speed centrifugation at high speeds from 6000g to 8000g. Such centrifugation is performed according to the method after the first phenol isolation of total nucleic acids. In the future it is proposed to perform only one instead of two stages of the cellulose gradient for double-stranded ribonucleic acids of the test sample. As a result of the modification a larger number of nucleic acids has been received. An alternative step was introduced for reducing the loss of RNA from natural abrasives by increase the weight of samples to 10 g, 30 ml STE buffer for initial washing, 50 % of the phenol`s volume, 17 ml of chloroform, and 2 ml of isoamyl alcohol.

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Under conditions of allocation double-stranded viral RNA appears to confirm the effectiveness of the proposed method of diagnosis and identification of viral RNA in Basidiomycetes.

Keywords: Extraction, Basidiomycetes, viral disease, methodology, total and double-stranded RNA, diagnostics

INTRODUCTION

Mushrooms are well known for their nutritional good values worldwide. Interest in them is at the peak because immunity and cellular protection are important issues for health- conscious consumers. Generally, mushrooms belong to Basidiomycetes. It included mushroom species with a diversity of metabolites of nutraceutical and therapeutic significance. They have been reported that it is the most valuable ones for humans (Pereyma, 2017; Patyka V.P. *et al*., 2019; Vanina and Ivanova, 2019)

International Meeting of the Conservation and Utilization of Genetic Resources of Mushrooms proposed the collection and conservation of the wild useful mushroom germplasm.

The main prerequisites for high yields of mushrooms are introduction and using of high-quality spawn without pathogens and disease resistant (Grogan and Tomprefa, 2002; Grogan *et al.*, 2003; Maffettone, 2007; Ivanova, 2017).

The mushroom (*Agaricus bisporus* (J. Lge) Imbach) is the most widely cultivated mushroom culture in the world. It accounts for 35–45 % of the total production of mushrooms. In recent years, viruses are made deleterious diseases for mushroom cultures. (Grogan *et al*., 2003; Maffettone, 2007; Elibuyuk,and Bostan, 2010;Ivanova, 2015, 2018, 2019, 2020, Klyachenko and Prysiazhnuk 2019).

Diagnosis has made considerable sense in assessing the source and breeding material for resistance to viral diseases. Several methods are used for diagnostic and identification of mycoviruses. The most relevant and accurate are molecular-biological and biochemical methods for diagnosing pathogens which affect Basidiomycetes.

Agaricus bisporus has over ten described pathogenic viruses. Heretofore practically do not exist highly precised molecular diagnostics for the detection and identification of mycoviruses that strike the Basidiomycetes in Ukraine.

The most dangerous among them is La France (LFIV or LIV). This isometric virion by the size of 35 nm. The virus has been the subject of extensive epidemiological studies and fight, so now La France disease is rare (Grogan, and Tomprefa, 2002; Grogan *et al*., 2003). The British scientists reported a reduction in yield mushrooms, which causes failure to install. The symptoms were absent, but the mycelium was almost with no signs of growth fruiting bodies. Some farms were affected by a similar ethicology increased. Some symptoms of the La France disease, but the diagnostic tests showed negative results for the spherical virus. Recently, the disease was associated with the presence of double-stranded RNA new elements (Maffettone, 2007). However, dsRNA different from previously described dsRNAs elements characteristic of the La France disease. It was suggested that the disease-induced previously undescribed virus with characteristic dsRNA genome. It was called "virus X", and then Mushroom Virus X (MVX). It is proved that the viral disease fruiting bodies of mushrooms changed they're morphologically losing its taste, have reduced shelf life, and are often dangerous to use.

As a result, increasing environmental problems are leading to a decrease in the resistance of mushroom grown in industrial conditions to abiotic and biotic factors. Therefore, recently the development of the mushroom industry has focused on studying the ways of transmission of fungal, bacterial, and viral diseases and creating a methodology which capable of responding quickly to stress and further regulation (Grogan *et al*., 2003; Maffettone, 2007).

The purpose of the research is to adapt and improve the method of separation, purification, and identification of viral total and double-stranded RNA from Basidiomycetes for their diagnosis and identification by highly pathogenic mycoviruses.

MATERIAL AND METHODS

The experimental part of the work was performed from 2011 to 2017 on the basis in the Problem Laboratory of Phytovirology and Biotechnology of the National University of Life and EnvironmentalSciences of Ukraine (Kyiv, Ukraine). Some researches were carried out in the research Department of molecular diagnostic researches of the Ukrainian Laboratory of Quality and Safety of Agroindustrial Complex Products (Kyiv, Ukraine), in the Laboratory of mycelium production of Agro-Industrial Complex (Kyiv, Ukraine), at the private mushroom enterprises of Kyiv region.

Screening of viral diseases of mushrooms

The samples with and without specific symptoms were used to study viral pathogens of mushrooms Table 1 (Ivanova, 2019.

Table 1. Detection of viral diseases of mushrooms in the Kyiv region

Research area (spread) of viral diseases	External symptoms of fruiting bodies	Detection of viruses by dsRNA isolation	Lesions, %, M ±
Kyiv region, Vasylkiv district, Private enterprise	Dark brown spots, watery legs	+	8.5 ± 0.5
Kyiv region, Kyiv-Sviatoshynskyi district Private enterprise	Missing	+	7.0 ± 0.3
Kyiv region, Vasylkiv, Private enterprise	Brownish-dark fruiting bodies	+	6.3 ± 0.3
Production agro-industrial complex, Kyiv	Brownish-brown spots	+	1.6 ± 0.6

Examination of fruiting bodies of dicotyledonous mushrooms in mushroom farms of Vasylkiv, Kyiv-Sviatoshynsky and Makariv districts of Kyiv region and industrial mushroom enterprises of Kyiv in the period from 2011 to 2017 took more 740 samples, of which 87 revealed symptoms characteristic of viral diseases.

We have selected to analyze the fruiting bodies with disease symptoms. The fruiting bodies that didn`t have symptoms according to a visual assessment and electron microscopic analysis (Grogan and Bostan, 2002; Ivanova, 2019) were as control.

Total and viral RNA isolation

These methodologies were carried out the detection of total and double-stranded RNAs in Basidiomycetes (Valverde and Guttierez, 2005).

Molecular biological method of extraction total DNA, cDNA, reverse transcription of PCR, amplification, sequencing of viral cDNA, and dsRNA were performed according to the method described previously (Morris and Dodds, 1979; Melnychuk and Valverde, 2001). Biotechnological (obtaining and subcultivation of samples of mycelium in vitro using electrophoresis in agarose and polyacrylamide gels (PAGE) (Melnychuk and Valverde, 2001), determining the hydrogen index (pH) of the nutrient medium), mycological (a measurement of growth).

Statistical analysis of data

Statistical analysis of data has been carried out with the help of statistical and other computer software.

RESULTS AND DISCUSSION

The essence, uniqueness and practicality of the method lies in the isolation from the total number of nucleic acids, which is inherent only in the virus is dsRNA. The method was first proposed and published by Morris and Dodds. Its specificity is to detect a double-stranded form of viral nucleic acid, which is also a nucleic acid of viral origin. Later, Valverde and Melnychuk improved the technique. The improved method of diagnosis and identification of RNA-containing phytoviruses involves the following stages: 3.5 g of plant sample (affected areas of leaves, stems, roots, seeds, etc.) to homogenize in a buffer solution of STE (0.1M NaCl, 0.05M tris-HCl, 1.0 mM EDTA, pH 7.0) under liquid nitrogen. (Figure 1)

After homogenization, there is a stage of phenolic isolation and purification of total nucleic acids and two cycles of cellulose fractionation, which include several stages using 16% ethanol solution. The dsRNA fraction after cellulose filtrate was transferred to STE buffer without ethanol and concentrated in two volumes of cooled 95% ethyl alcohol and 0.1 volume of 0.2 M sodium acetate, pH 5.5.

Insofar as the research the method of diagnosis and identification of RNA-containing phytoviruses that methodology of isolation in the case of Basidiomycetes didn't fully ensure the purity. Noticeable, there was not sufficient concentration of nucleic acids and their double-stranded form.

Impressive data has been accumulated on the novel techniques, but the potential methodology will still remain renewed.

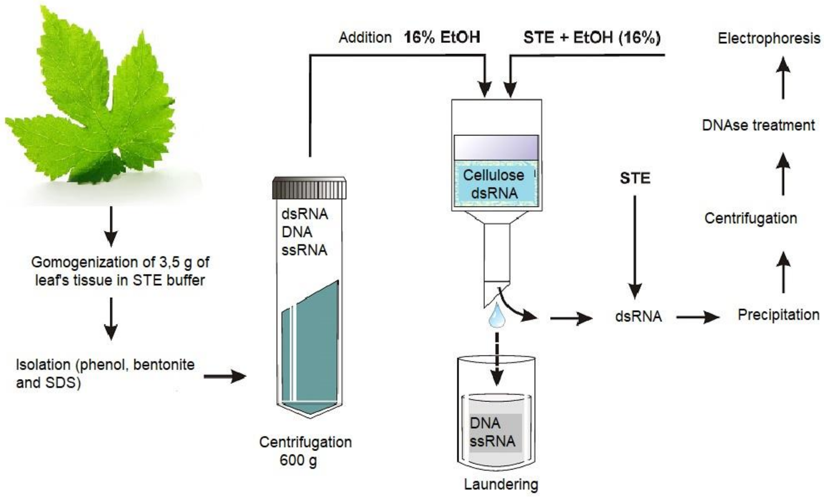

Figure 1: General scheme of isolation and chromatographic purification of double-stranded RNA

Isolation of nucleic acids.

The molecular characterizations of MVX dsRNAs were locally initiated and developed by the research. Fresh diseased fruiting bodies samples with MVX symptoms were finely grounded under liquid nitrogen in a mortar. The powder then was transferred to tubes and the protocol was followed for isolation of dsRNAs. Isolation of total nucleic acids from fruit bodies and spawn run compost was achieved via the procedures described according to the new protocol: then this powder transferred to centrifuge with 12 ml 2x STE buffer (H_2O – 500 ml, NaCl – 29h, tris – 30,5h, EDTA – 1,85h), 1 ml of 1 % SDS (H_2O – 100 ml of SDS – 10 g) and 1 mL of bentonite. Next step is mixing this composition on a shaker for 15 min until smooth. Then the addition 17 ml STE-phenol (H_2O – 500 ml, 2 x STE – 500 ml, pH 4.5) plus 17 ml of chloroform-isoamyl (24:1). It should be centrifuge for 2500 g/min for a 20 min. After centrifugation the aqueous phase was retrieved and again centrifugation at 8000 g/min for 10 minutes. After that, the 1.5 g cellulose and 3 ml of absolute ethanol are added to retrieving precipitate. These samples stirred for 15–20 minutes. Further, it transferred to the ice at temperature - 15 °C for 30 minutes. The contents of the tubes were poured into a column and added buffer STE-OH (STE – 100 ml ethanol – 174 ml H_2O – 726 ml) and 20 ml of STE buffer – 20 ml. 30 ml of ethanol was added to these filtrates. The centrifugation at 8000 g for 30 minutes. Samples were dried on filter paper for 2 hours at a temperature of +18–20 °C. To the same tube was added 200 ml 10XRNA-buffer (0.35 % (w/v) Orange G, 30 % (w/v) Ficoll 400, 1 mM EDTA). To the resulting solution applied 1 ml $MgCl_2$ and was transferred to a thermostat +37 °C for 1 hour.

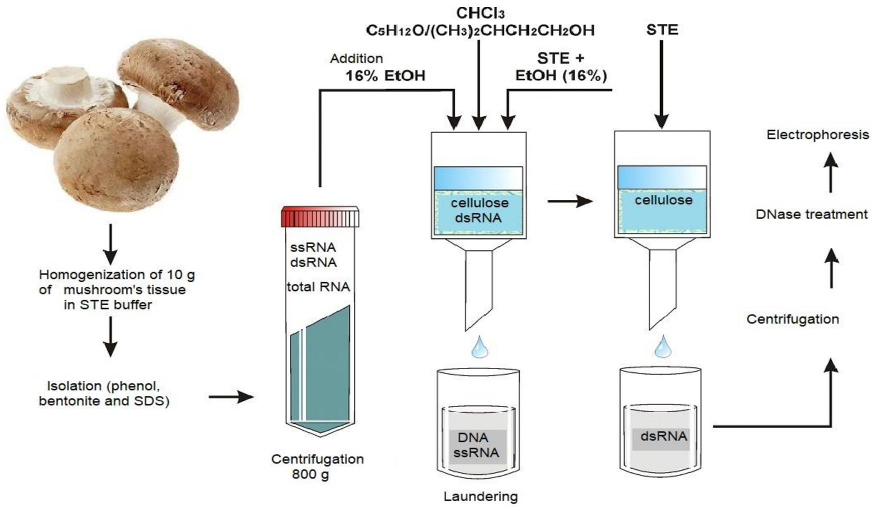

Figure 2: Scheme Advanced in the methodology of extraction total and viral nucleic acids from mushroom

In consequence of an alternative step for reducing the loss of RNA from natural abrasives by increasing the weight of samples to 10 g, STE buffer volume (30 ml) for initial washing, adding 50% by volume of phenol, 17 ml of chloroform, and 2 ml of isoamyl alcohol. Under conditions of allocation double-stranded viral RNA appears to confirm the effectiveness of the proposed method of diagnosis and identification of total and viral RNA in Basidiomycetes.

It's generally known that scientists studied the FISH method as a controversial and interesting point for our research works. A limited understanding exists of the localization and mobilization of viruses within the mycelium of A. bisporus. To this end, a non-destructive fluorescence in situ hybridization (FISH) method was developed (Coates, Eastwood, Fitzpatrick, Grogan, 2020) for in situ targeting of AbV6 and AbV16 in A. bisporus mycelium. Localization of the two viruses in MVX-infected cultures appears independent, as both viruses were found in completely discrete areas of the mycelium in differential patterns. FISH detected the low-level presence of the two viruses, AbV6 and AbV16 in a number of cultures that had tested negative for the viruses by RT-PCR. This suggests that FISH may be more sensitive at detecting viruses at low levels than molecular methods.

Simultaneously, proposing our methodology with updating techniques extraction demonstrates a powerful tool in the field of mycovirology.

Regarding molecular biological identification of fragments, dsRNA is constant for specific representatives of mycoviruses pathogen. It is a method of diagnosis and identification of RNA-containing in other stages of transport carriers (fungi, insects, nematodes, etc.) as opposed to the FISH method. The results of isolation and identification of total and mycovirus`s dsRNA in combination with classical methods of virus diagnostics allow studying the issue of localization and transfer of the virus in mushrooms.

Weighty use of modificated method can be used for mushroom phytosanitary control systems, quarantine, Ukrainian customs.

Our research has shown the analysis of dsRNA which isolated from mushrooms imposed the presence of viral infection. This method ensures the high quality of diagnosis and identification of RNA-containing viruses in mushrooms.

To intensify the industrial production of mushrooms on a virus-free basis and prevent the spread of mycoviruses we recommended in the early stages of growing to conduct a comprehensive diagnosis of virus mycelium. Development of test systems based on PCR will be the next step in our research.

CONCLUSIONS

Proposed own modification of this method is based on acceleration stage of cellulose chromatographic purification of nucleic acids and dsRNA fractionation. It has been proposed to conduct the first stage of low speed centrifugation at high speeds from 6000 g to 8000 g. Such centrifugation is performed according to the method after the first phenolic isolation of common nucleic acids. In the future, it has been proposed to conduct only one instead of two stages of cellulose gradient for double-stranded ribonucleic acids of the studied sample. As a result of modification, a large number of studied nucleic acids are stored.

As a result of the dsRNA analyses that are isolated from the fruiting bodies of mushrooms a viral infection has been found. Based on the experiments it has been recommended to use aliquot amount 10 g, the amount STE buffer for primary washing 30 ml, adding 50 % by volume of phenol, 17 ml of chloroform and 2 ml isoamyl alcohol. This method provides high quality diagnostics and identification of RNA-containing viruses in mushrooms.

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STRUCTURE OF AGRICULTURAL LAND LOSSES IN BOSNIA AND HERZEGOVINA

SUMMARY

As the world's population grows, so does the pressure on agricultural land. Consequently, there is less and less agricultural land. Bosnia and Herzegovina is one of the countries whose population is decreasing from year to year, partly owing to negative natural increase and partly owing to the migrations of young people to developed western countries. However, despite the depopulation trend, there has been a significant decline in agricultural land in the first two decades of the twenty-first century. The reasons should be sought in various socio-economic processes that crucially affect spatial development. In order to understand the changes that are happening in the field, the paper analyzes the structure of agricultural land losses in B&H based on the database of the European project CORINE Land Cover. In relation to the five main land cover categories (artificial surfaces, agricultural areas, forests and semi-natural areas, wetlands and water bodies), the data show that in the period from 2000 to 2018 artificial areas increased spatial coverage, forests and semi-natural areas as well, while agricultural land decreased.

The aim of the research is to identify the spatially largest changes of agricultural land subclasses into other types of land cover, in order to determine the causes and areas with the most intense pressure. This is essential given the fact that agricultural land is becoming an increasingly important natural resource over time. Based on the recent demographic and socio-economic trends, it is to be expected that the trend of decreasing agricultural land will continue in the future, but with a reduced intensity.

Key words: agricultural land, losses, B&H, CLC, CHA, database.

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INTRODUCTION

Bosnia and Herzegovina (B&H) is a country in the Western Balkans, with an area of 51 209 km^2. The Dayton Peace Agreement from 1995 created a new state structure consisting of the Federation of B&H (FB&H) and Republika Srpska (RS), and since 2000 the Brčko District (BD). According to the last census from 2013, B&H has about 3.53 million inhabitants (ASB&H, 2016), which is a significant loss in comparison with the previous census from 1991, when it had 4.37 million inhabitants. Almost 100 000 people lost their lives in the 1992-1995 war and a large number fled to other countries from which some never returned. There are two dominant demographic trends which are becoming intense during the first two decades of the new millennium: negative natural increase and migration of mostly young people to developed western countries.

The process of depopulation in B&H is especially intense in the second decade of the 21st century. RS has been recording a negative natural increase since 2002, and in the period 2013-2018 averaged to - 4 732 annualy (RSIS, 2020). The FB&H had a negative natural increase for the first time in 2013, while losses in the period 2013-2018 averaged to - 4 580 annualy (FB&H BS, 2020). Official estimates of entity statistics bodies say that in the period 2013-2018 RS lost 23 277 and FB&H 22 898, in total of over 46 000 inhabitants. According to the FB&H Bureau of Statistics, 24 154 citizens or 4 026 per year left B&H in the same period. The total demographic losses amount to almost 13 500 inhabitants per year, with a tendency of constant growth.

Agriculture plays an important socio-economic role in rural areas of B&H; the rural population accounts for 61% and almost half of the rural households is still engaged in agriculture (Luketina *et al*, 2018). Statistical data indicate that the lack of a clear vision of development of agrarian and land policies in the country, lack of investments and incentives, fragmentation of the holdings as well as numerous other problems along with large deagrarization resulted in having over 50% of arable land uncultivated. The share of agricultural in the total population decreased to just 20.4% which means that every fifth or sixth person is engaged in agriculture. Trends in changes in total agricultural land by all categories of use were not linear and were considerably affected by the socio-economic development and sectoral policies (Ljuša and Čustović, 2019). Budget allocations for agricultural support in B&H as well as their structure are inadequate and insufficient and do not encourage farmers to invest in the production in which they would be more efficient (Makaš *et al*, 2018).

The biggest cause of changes in the land cover types are socio-economic processes. People change space and adapt it to their needs, spatially and temporally with stronger or weaker intensity. Where the intensity is weak or absent, natural processes dominate (overgrowing of abandoned agricultural land into shrubs/bushes, transformation of coniferous forest into mixed, etc.). Natural processes are much slower and can be observed by visualization over long periods.

The changes that appear on the Earth's surface are visible on satellite images, and over the course of time they can be compared and thus determine the differences and directions of spatial development. Copernicus is the European Union's Earth Observation Programme. The Copernicus Land Monitoring Service (CLMS) based on data from a constellation of six families of satellites, provides geographical information on land cover and its changes, land use, vegetation state, water cycle and Earth's surface energy variables to a broad range of users in Europe. Pan-European database provides land cover and land use (LC/LU), land cover and land use changes and land cover characteristics. The CORINE Land Cover (CLC) is provided for 1990, 2000, 2006, 2012, and 2018. This vector-based dataset includes 44 land cover and land use classes. The time-series also includes a land change layer, highlighting changes in land cover and land-use (CLMS, 2019).

The first CLC project for B&H started in 1998 and was successfully completed in 2000. The result was the creation of the B&H CLC 2000 database, which included the identification of the types of surface cover at the level of the main classes, and also the second and third level subclass with a detailed description of the structural characteristics. Subsequently, the CLC 2006, CLC 2012, and CLC 2018 as well as CLC Changes CHA 2000-2006, CHA 2006-2012 and CHA 2012-2018 databases were created with the aim to monitor the dynamic changes in the land cover (Drašković *et al*, 2020). In 2019, the European Environment Agency (EEA) published an updated illustrated guide to the nomenclature of land cover types with a structural classification at three hierarchical levels and a differentiated level of detail (details at: EEA, 2019).

The objectives of this research are to define the processes that most endanger agricultural land, to identify the subclasses of agricultural land that are most endangered as well as those subclasses that are most widespread at the expense of agricultural land.

MATERIAL AND METHODS

The basic principles of the CLC project are the visual interpretation of satellite images and as supplementary data are used: aerial images, topographic maps, statistical data, field work, etc. The basic trends of spatial development have been identified by analyzing changes in the field in the period from 2000 to 2018 in B&H. The three most significant trends are: 1) growth of artificial surfaces, 2) growth of forests and semi-natural areas and 3) reduction of agricultural areas (Drašković, 2020). The largest changes are related to the decrease in agricultural land, which recorded a decrease of 3.65% in absolute terms in comparison with the initial year. The reasons for reduction of agricultural land are complex and mainly related to various socio-economic processes. Vector-based database on land cover types (CLC 2000, CLC 2006, CLC 2012 and CLC 2018) as well as on changes for three periods (CHA 2000-2006, CHA 2006-2012 and CHA 2012-2018) were used. CLC uses a Minimum Mapping Unit (MMU) of 25 hectares (ha) for areal phenomena and a minimum width of 100 m for linear phenomena. The time series are complemented by change layers, which highlight changes in land cover with an MMU of 5 ha (CLMS, 2019).

Data for the B&H territory were extracted using GIS software and changes in the form of polygons for all three periods were visualized. The data are exported to Microsoft Excel and classified by type of change. Using the Sort & Filter and SUBTOTAL SUM tools, individual sum of areas of all land cover types and changes that occurred by periods were calculated. By processing this data, we get a spatial and temporal insight into all the processes that take place in the field. With regard to the aim of the paper, the changes related to agricultural land have been singled out and particularly analyzed.

In general, common problems was overestimated class 2.4.3 (Land principally occupied by agriculture, with significant areas of natural vegetation), position of boundaries and a need for better separation of certain classes within agricultural areas. High resolution images from 2017/18 were extremely useful in identification of certain classes within agricultural areas that were not visible previously due to small parcel size and low resolution of images (e.g. separation of 2.2.2 in agricultural areas). In addition to this, one of the reasons for having overestimated class 2.4.3 is that post war period was characterized by abandoned land (EEA, 2018).

RESULTS AND DISCUSSION

Based on CLC 2000, CLC 2006, CLC 2012 and CLC 2018 databases, it was established that artificial areas increased spatial coverage from 1.35% to 1.7%, agricultural areas decreased from 36.81% to 33.15%, forests and semi-natural areas have increased from 61.08% to 64.36%, while wetlands and water bodies are at about the same level (Drašković, 2020).

When it comes to subclasses of agricultural land according to the CLC 2018 database, Complex cultivation patterns (2.4.2) dominates with 43.46% (growth in comparison with 2000 of 2.58%), Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) with 26.11% (decrease by 4.8%, explanation in the last pasus of Materials and methods), Pastures (2.3.1) with 19.09% (decrease by 2.58%) and Non-irrigated arable land (2.1.1) with 10.39% (growth of 4.49%) (Table 1).

A more detailed analysis of the changes including agricultural land participation shows that they cover 12% of all changes. Three types of changes in which agricultural land participates are: conversion of agricultural areas into other land cover types, conversion of other land cover types into agricultural areas and internal conversion (transition from one subclass of agricultural land to another). The largest part in the structure of changes refers to losses. The total loss of agricultural land (without internal conversion) for 18 years is 14 073 ha. In the same period, the total benefit (transition of other types to agricultural land) amounted to 2 768 ha. Thus, the absolute loss is 11 305 ha (Table 2).

When analyzing the changes by periods, it is noticeable that the largest net losses were recorded in the first period (2000-2006) with 9 165 ha. In the next two periods, there was a significant reduction in losses to 2 000 ha (2006-2012) and 2 908 ha (2012-2018). The conversion of other types into agricultural land had a significantly lower intensity in all periods. In the first period, it was only 552 ha, in the second 1 106 ha and in the third 1 110 ha. Internal conversion was initially intensive and later gradually decreased: in the first period 4 563 ha, in the second 1 247 ha and in the third 425 ha. New agricultural land obtained by cultivating other types of land cover amounts to only 13.63% of the losses.

Table 3 shows that more than half of all changes or 51.51% (10 465 ha) over 18 years relate to the conversion of agricultural land into artificial surfaces, while the conversion to forests and semi-natural areas amounts to 19.52% (3 965 ha). The internal conversion within agricultural land accounts for 30.69% (6 235 ha) and the conversion of agricultural land into water bodies 1.88% (382 ha).

Table 1: Agricultural land subclasses in B&H for 2000 and 2018 (Level 3)

		CLC 2000		CLC 2018	
Code	Land cover type	P (ha)	P (%)	P (ha)	P (%)
2.1.1	Non-irrigated arable land	111 161	5.90	176 459	10.39
2.1.2	1.Permanently irrigated arable land	2 904	0.15	2 252	0.13
2.2.1	Vineyards	1 460	0.08	3 021	0.18
2.2.2	Fruit trees and berry plantations	4 696	0.25	7 997	0.47
2.3.1	Pastures	408 597	21.67	324 206	19.09
2.4.1	2.Annual crops associated with permanent crops	0	0.00	52	0.00
2.4.2	Complex cultivation patterns	773 587	41.04	740 732	43.62
2.4.3	Land principally occupied by agriculture, with significant areas of natural vegetation	582 720	30.91	443 276	26.11
	Total	1 885 125	100.00	1 697 995	100.00

Source: CLC database for B&H, CLMS, 2019.

Table 2: Structure of relative changes of agricultural land by periods in (ha)

Changes*	2000-2006	2006-2012	2012-2018	Total
2xx-xxx	13 728	3 247	3 333	20 308
xxx-2xx	5 115	2 353	1 535	9 003
2xx-2xx	4 563	1 247	425	6 235
xxx-2xx**	552	1 106	1 110	2 768
2xx-xxx**	9 165	2 000	2 908	14 073
			Loss	11 305

* x can be any number from 1 to 4 and also 5 for the first column.
**Without internal conversion (without 2xx-2xx).

During the first period, 53.01% of changes accounted for the conversion of agricultural land into artificial land, 33.24% belonged to internal conversion and only 11.65% was the conversion into forests and semi-natural areas. Other conversion i.e. change of agricultural land into wetlands or water bodies accounts for 2.1%. Thus, the influence of socio-economic processes during this period was dominant (internal migrations, the refugee return process etc.).

Table 3: Changes with the participation of agricultural land by periods

	CHA 2000-2006		CHA 2006-2012		CHA 2012-2018		Total	
	P (ha)	P (%)	P (ha)	P (%)	P (ha)	P (%)	P (ha)	P (%)
2xx-1xx	7 278	53.01	1 205	37.10	1 982	59.34	10 465	51.51
2xx-3xx	1 600	11.65	730	22.48	905	27.10	3 965	19.52
2xx-2xx	4 563	33.24	1 247	38.39	425	12.72	6 235	30.69
2xx-5xx	288	2.10	66	2.03	28	0.84	382	1.88
Total	13 729	100.00	3 248	100.00	3340	100.00	20 317	100.00

Source: CHA database for B&H, CLMS, 2019.

Table 4: Top 10 largest changes with the participation of agricultural land by periods

	CODE 2 2000-2006			CODE 2 2006-2012			CODE 2 2012-2018		
	CHA	P (ha)	P (%)	CHA	P (ha)	P (%)	CHA	P (ha)	P (%)
1	242-112	3 655	26.62	243-222	647	19.92	243-324	559	16.74
2	231-242	2 724	19.84	243-324	418	12.87	231-211	370	11.08
3	211-112	1 226	8.93	242-131	298	9.17	242-122	337	10.09
4	231-211	811	5.91	231-324	295	9.08	231-131	305	9.13
5	243-112	721	5.25	243-131	248	7.64	243-131	258	7.72
6	231-324	559	4.07	242-222	167	5.14	242-131	246	7.37
7	221-324	259	1.89	211-221	129	3.97	231-334	166	4.97
8	222-242	255	1.86	242-243	91	2.80	211-122	134	4.01
9	231-131	239	1.74	211-121	84	2.59	231-324	133	3.98
10	231-112	237	1.73	242-122	81	2.49	243-133	113	3.38

Source: CHA database for B&H, CLMS, 2019.

Urbanization in developing countries in comparison with developed countries has been accelerating and this has caused by unplanned physical expansion results in more harmful undesirable effects on natural environments and the agricultural lands around and the trouble which is happening a serious treatment of urban-rural prospective (Parsipour *et al*, 2019). The conversion of agricultural land into artificial areas (2xx-1xx) is caused by the intensive human influence and urbanization, i.e. settlement construction, commercial buildings, infrastructure, etc. In the first period, the greatest threat to agricultural land was the expansion of suburban settlements, which accounted for about 43% of lost agricultural land. There is often a lack of quality agricultural land in the vicinity of densely populated zones, so the damage due to losses is proportionally greater.

The conversion of agricultural land to forest may be the result of natural forest expansion or tree planting (FAO, 2016). The process of leaving rural areas and overgrowing agricultural land into bushes and shrubs is taking place along with the urbanization. The conversion of agricultural land under the influence of natural processes is constantly growing. These include changes of different types of agricultural land to the Transitional woodland/shrub (3.2.4) or Natural grassland (3.2.1). This is supported by the fact that in the third period the largest change was recorded in the conversion of Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) in the Transitional woodland/shrub (3.2.4) in the amount of 559 ha or 16.74% of all changes. Agro-socio-economic processes that take place with more or less human influence cause internal conversion. The conversion of Pastures (2.3.1) into Non-irrigated arable land (2.1.1) or Fruit trees and berry plantations (2.2.2) into the area of Complex cultivation patterns (2.4.2) is an example of such changes.

Speaking of individual changes, Table 4 shows that in the period from 2000 to 2006, the change of agricultural land into other types is most intensive in the conversion of Complex cultivation patterns (2.4.2) into a Discontinuous urban fabric (1.1.2), a total of 3 655 ha or 26.62% of all changes. The zones with the largest spread of discontinuous urban area due to complex cultivation were recorded in the vicinity of Sarajevo (195 ha and 106 ha), Tuzla (168 ha, 165 ha and 100 ha) (Figures 1 and 2) and Zvornik (111 ha).

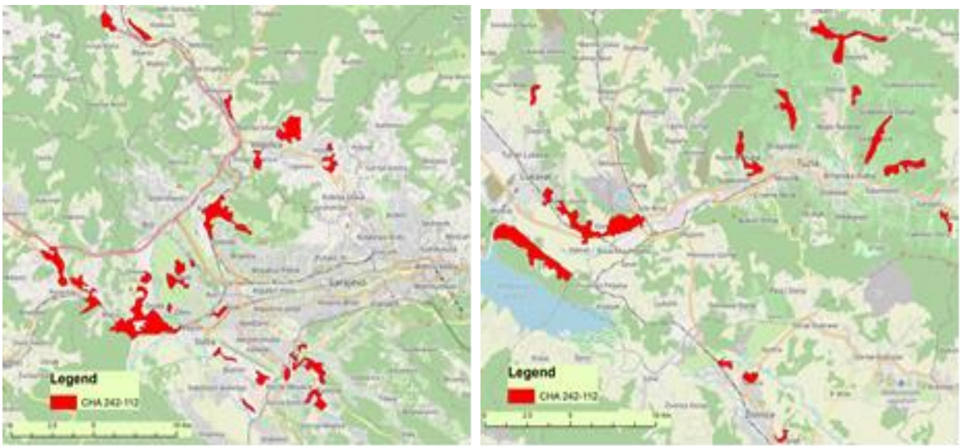

Figure 1 and 2: Spreading of discontinuous urban areas (1.1.2) to areas covered by complex cultivation (2.4.2) near the cities of Sarajevo and Tuzla

The next biggest change in that period refers to the internal conversion within agricultural land, i.e. Pastures (2.3.1) were transferred to Complex cultivation patterns (2.4.2) in the amount of 2 724 ha or 19.84%. This does not represent a loss since one type of agricultural land has been qualitatively replaced by another. Furthermore, there is a significant loss of Non-irrigated arable land (2.1.1) in the Discontinuous urban fabric (1.1.2) in the amount of 1 226 ha or 8.93% of changes. The next largest change relates to the internal conversion of Pastures (2.3.1) into Non-irrigated arable land (2.1.1) in the amount of 811 ha or 5.91%. Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) are also transferred to a Discontinuous urban fabric (1.1.2) in the amount of 721 ha or 5.25%. Among other external changes, mention should be made of the conversion of Pastures (2.3.1) and Vineyards (2.2.1) into the Transition woodland/shrubs (3.2.4) and Pastures (2.3.1) into a Discontinuous urban fabric (1.1.2).

Changes during the second and third period were less intense (Figure 3). The spread of discontinuous urban area over agricultural land has almost disappeared. The reasons are to be found in the mainly completed refugee return process, the 2008 global economic crisis and negative demographic trends.

During the second period (2006-2012), internal conversion was the most dominant process with 38.39% of the total changes involving agricultural land. The largest change was recorded in the conversion of Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) into Fruit trees and berry plantations (2.2.2) in the amount of 647 ha or 19.92% of changes. Internal conversion is among the top 10 biggest changes represented by the conversion of Complex cultivation patterns (2.4.2) into Fruit trees and berry plantations (2.2.2), Non-irrigated arable land (2.1.1) into Vineyards (2.2.1) and Complex cultivation patterns (2.4.2) in Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3). The largest losses of agricultural land were related to the conversion of Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) and Pastures (2.3.1) into the Transition woodland/shrubs (3.2.4). The conversion of agricultural land into forests and semi-natural areas has doubled in comparison with the previous period to 22.48%. Natural processes without human activities become more intense. Conversion to water bodies accounts for 2.03%. Concerning artificial areas, agricultural land was most endangered by Mineral extraction sites (1.3.1) built in areas with Complex cultivation patterns (2.4.2) and Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) in the amount of 298 ha and 248 ha.

Anyway, the pattern of agricultural development in B&H is quite different comparing with previous period. In particular, consumption of pastures, the most significant trend in previous period, ended up. The main reason for this decrease of pasture consumption intensity is the significant slowdown of urban residential sprawl and of internal agricultural conversion from pasture to arable land and permanent crops, as these flows were the main consumers of pasture land in the previous period (EEA, 2017).

During the third period (2012-2018), the dominant process is again the expansion of artificial areas at the expense of agricultural land in the amount of 59.34 percent of all changes. Natural processes of conversion of agricultural land into forests and semi-natural areas continue to grow continuously with a share of 27.1% of all changes. Internal conversion within agricultural land decreased to only 12.72%. The remaining conversion is 0.84% (Table 3).

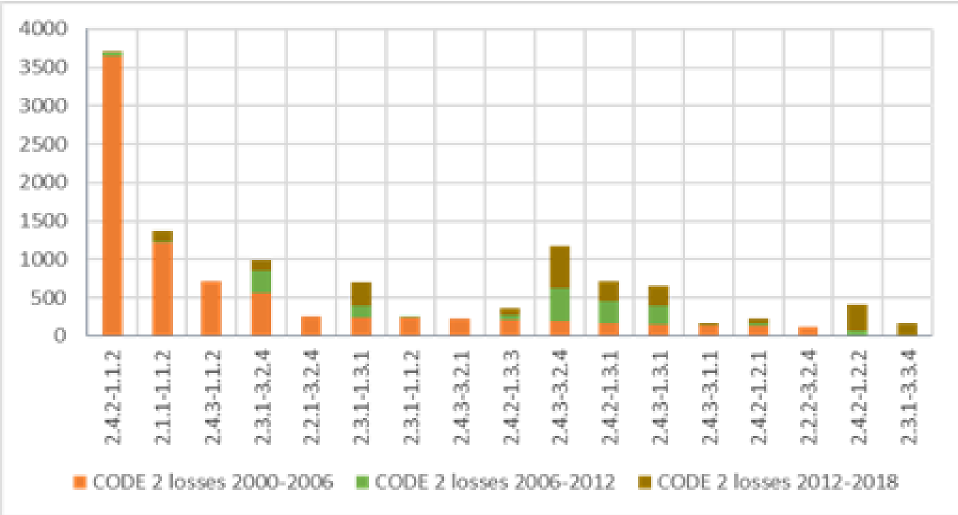

Figure 3: Structure of the agricultural land losses by periods

The largest change was recorded in the conversion of Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) in the Transition woodland/shrubs (3.2.4) with 599 ha. Mineral extraction sites (1.3.1) occupied the former areas under Pastures (2.3.1) with 305 ha, Land principally occupied by agriculture, with significant areas of natural vegetation (2.4.3) with 258 ha and Complex cultivation patterns (2.4.2) with 246 ha. In addition, Complex cultivation patterns (2.4.2) and Non-irrigated arable land (2.1.1) were replaced with Road and railway network and associated land (1.2.2) in the amount of 337 ha and 134 ha (mainly related to the construction of the highway Banja Luka - Doboj). It is important to mention the losses of Pastures (2.3.1) in the Burnt areas (3.3.4) with 166 ha and the Transition woodland/shrubs (3.2.4) with 133 ha. Higher internal conversion was recorded by changing Pastures (2.3.1) into Non-irrigated arable land (2.1.1) to 370 ha.

CONCLUSIONS

The analysis of the structure of changes with agricultural land participation in B&H shows that the largest changes were recorded during the first period (2000-2006). In that period, there was the largest decrease of agricultural and, mainly owing to the expansion of discontinuous urban areas in the amount of 43% of total changes. In the second period (2006-2012), the external conversion of agricultural land into other types of cover in absolute terms was significantly reduced. The expansion of urban zones over agricultural land was minimal, but the construction of mines and construction sites on agricultural land was intensified. The conversion caused by natural processes has doubled. The largest losses were recorded by the expansion of the transitonal woodland/shrub area. In the third period (2012-2018), the conversion of agricultural land into artificial land increased to a record 59.34%. The road and railway network, as well as mines and constuction sites, were mainly expanded at the expense of agricultural land. At the same time, there was a further increase in the conversion of agricultural land into forest and semi-natural areas, of which most land principally occupied by agriculture, with significant areas of natural vegetation and pastures into the transitional woodland/shrub. Part of the area under pastures was affected by fires and converted into a burned zone.

Two parallel processes are dominant: urbanization and deagrarization. The population is concentrated around larger cities while rural areas are sparsely populated. The biggest losses of agricultural land occur owing to the expansion of artificial areas. However, as demographic losses increase over time, natural processes become more intense in an increasing area. Former agricultural lands become uncultivated and over time grow into shrubs and bushes.

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Kostić, M., Ljubičić, N., Ivošević, B., Popović, S., Radulović, M., Blagojević, D., Popović, V. (2020): Spot-based proximal sensing for field-scale assessment of winter wheat yield and economical production. Agriculture and Forestry, 67 (1): 103-113.

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SPOT-BASED PROXIMAL SENSING FOR FIELD-SCALE ASSESSMENT OF WINTER WHEAT YIELD AND ECONOMICAL PRODUCTION

SUMMARY

The study was conducted on a test field with an area of 255 ha including four winter wheat varieties (Basmati, Farinelli, Balaton, and NS40S). The objective of this research was to evaluate the suitability of NDVI and soil ECa data in the modeling of selected winter wheat properties. The results in this paper are based on observations of a plant canopy at 40 locations using an NDVI sensor measured at four stages (BBCH65, BBCH75, BBCH83, and BBCH89), as well as soil electromagnetic conductivity using an EC probe before analysis had commenced. The strongest relation between yield components and NDVIs was observed in the milk growth stage (R ranged from 0.30 to 0.67). Poor correlation was determined between soil ECa and wheat traits. Ordinary least squares regression gave models where average NDVI and soil ECa described 75% variation in plant height of the Balaton variety; 74% of Farinelli plant height changes were characterized by NDVIBBCH65 and EC; 73% of Basmati yield was explained by NDVIBBCH75 and EC. Sufficient rainfall during the growing season, high fertility of the soil and appropriate temperature regime lowered the influence of spatial heterogeneity on final crop outcomes due to optimal water and nutrition uptake.

Keywords: geostatistics, NDVI, soil apparent electrical conductivity, wheat, yield prediction.

INTRODUCTION

The efficiency of soil and plant management is strongly dependent on the method of data collection hence data validity and data usability in the decision-making process. Huge scientific resources have been allocated in the

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development of farmer-friendly sensing devices (low price, easy to handle, high efficiency), algorithms and PC software that could be equally efficient in various agro-ecological environments. The most advanced techniques used in temporal studies have been provided from informatics (big data, machine learning, geostatistics, etc.). Some authors have worked on the development of algorithms for distinguishing “good” from “bad” data, on the form and pattern recognition (Moshou et al., 2002; Pantazi et al., 2016), designs of self-driving robots, etc. Optical sensors have become very popular in crop scouting and scientific research. Simple handling and non-destructive measurement make them suitable for wide range of applications on different crops, and instant data can be collected without biomaterials destruction (Raun et al., 2002; Magney et al., 2016; Ljubičić et al, 2017). The results of studies based on remote or proximal optical sensors offer a vast number of vegetative indices which could be used in the prediction of crop maturity, yield potential, plant health estimation, detection of weeds and pests, etc. Spectral analysis of reflected waves from either plant canopy or soil is valuable in recognition of spectral “fingerprints”, which help identify some biotic or abiotic processes that are otherwise undetectable by human or machine. Multispectral sensors use natural source of light which highly depends on the sun exposure, cloudiness, architecture and reflective characteristics of scanned objects that could jeopardize recording stability in time, especially if large field area is observed which requires a lot of time (Oberti et al., 2014; Whetton et al., 2017). From empirical point of view, commercial NDVI devices that use artificial source of light are more convenient solution for spectral reflectance sensing. They are more confident in all weather conditions, easy to use, provide instant data, can be hand held, attached to sensor based variable rate applicators or carried by UAVs. NDVI maps ensure insight into crop spatial variability, but single parameter is not sufficient to reveal the real causes of yield variability. Due to the complexity of soil and its influence on plant development, additional examinations in form of standard scientific measurement or proximal sensing increase the quality of spatial modeling and robustness of gained solutions (Huete et al., 2012; Kostić et al., 2016; Magney et al., 2016). Winter wheat is suitable for spectral scanning due to high plant population and early soil coverage. Successive NDVI measurement during the wheat growing period might offer reliable yield prediction modeling (Raun et al., 2002). Also measurements of apparent electrical conductivity (ECa) using electromagnetic induction (EMI) give information related of soil physical properties, and it is broadly used to delineate management zones of yield potential (Bramley, 2001; Rodrigues et al., 2015; Quinta-Nova and Ferreira, 2020).

The objectives of this research were to evaluate the suitability of NDVI data and soil ECa data in the modeling of selected winter wheat properties.

MATERIAL AND METHODS

The study was carried out at the field located in the northern part of the Republic of Serbia (45.563785N, 19.876757E) on calcic chernozem type of soil during the 2015/2016 growing season. It is used in traditional wheat-maize-soybean crop rotation with conventional deep plowing or chiseling as primary tillage. According to Kovačević et al. (2012), Serbia climate conditions could be characterized with higher precipitation sums in autumn and winter, and significantly lower in spring, which is the critical period for wheat development. Table 1 shows significantly higher amount of rainfall in the 2015/2016 growing season compared to an average value obtained from long-term climate data. Field area grown with winter wheat covers 255 ha in total. Four varieties of winter wheat were used in the experiment (Basmati, Farinelli, Balaton and NS40S). The sowing was started on 15th October and finished 12 days later in 2015, with a seeding rate 255 kg/ha and 15 cm row to row distance.

Table 1. Climate parameters for observed field in 2015/2016 growing season

Month	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Total
Temp. (ºC)	11.3	7.8	3.2	1.2	7.3	7.9	14.3	16.7	21.6	91.3
Precip. (mm)	74.6	56.1	3.6	51.6	49	65.3	74.2	84.6	143	669.32

Soil sampling was done by a specialized vehicle equipped with a GPS receiver and automatic sampling device (340 samples were taken). Additional observations of soil and plants in the field were conducted at 40 common locations (10 per variety) which were scattered in order to ensure approximately equal coverage considering the irregular subfield shape (Figure 3b). Apparent soil electrical conductivity (ECa) was measured prior to sowing using an EM38-MK2 device (Geonics Ltd., Ontario, Canada). Measurement was done in vertical mode covering the surrounding area of the observed locations as well. Average ECa values for each location were obtained and subsequently compared with other parameters. NDVI measuring was performed with a commercial hand-held sensor (GreenSeeker, Trimble USA) in four growing stages of wheat: full flowering (BBCH65), medium milk (BBCH75), early dough (BBCH83) and fully ripe stage (BBCH89). NDVI measurements were taken by holding GreenSeeker about 60 cm horizontally above the crop canopy and moving in zig-zag pattern including approximately 10x10 m of sensed area. The obtained values were averaged to get a single representative value of the location. Yield components (plant height, spikes/m^2, ear length and grain yield) were assessed from the plant samples collected at full maturity stage from one square meter in five repetitions at the observed area (40 locations, Figure 3a). Also, yield maps were generated using the data collected by the harvester monitors. Since the absolute accuracy of yield monitor varies with different wheat varieties due to uneven grain properties, which cause different response of the embedded sensors, the collected yield values were post-calibrated to reduce possible data discontinuity. Unprocessed yield data could skew the results; the values can be underestimated or overestimated depending on the source of the error. Method for data cleaning was based on statistical data interpretation and minimum and maximum yield thresholds. Yield monitoring was done at 1.2-1.3 m distance according to combine average speed and measurement frequency. In order to compare the data recorded by yield monitors and yield data obtained from samples, certain yield monitor data were manually extracted using GIS. The selection was done on the map by choosing the points-data within 10 m diameter around each location of observation, and the mean value was calculated afterwards (Figure 2a). The obtained linear models given in Table 2 show how well are fitted grain yield data from yield monitor and from samples. The quality of adjusted yield values was evaluated by using the RRMSE parameter which indicates that differences are regular for all varieties, and confirms the confidence of the models.

Table 2. Comparison of wheat yield data collected with yield monitor and standard sampling for postharvest yield monitor data correction.

Variety	$Q_{Ym}(y)$	$Q_{Ob}(x)$	Q_{adjust}	Model	$R^2(x, y)$	RMMSE
Basmati	7246.4[a]	7672.8[a]	7673.3	Q_{adjust}=1.05Q_{Ym}+76.8	0.97	2.03
Farinelli	8884.7[b]	9587.6[c]	9587.74	Q_{adjust}=0.97Q_{Ym}+945.8	0.86	4.77
Balaton	8149.6[b]	8464.6[ab]	8464.76	Q_{Ym}=0.91Q_{Ob}+512.7	0.89	2.01
NS40S	8326.2[b]	8536.8[b]	8536.85	Q_{Ym}=0.92Q_{Ob}+502.2	0.74	2.97

Note: [a,b,c] different classes obtained by ANOVA (p=0.05); Q_{Ym}(y)-average yield recorded by monitor; Q_{Ob}(x)-average yield calculated from data collected from samples; Q_{adjust}- adjusted yield data.

The regression analysis was used to observe individual contribution of wheat yield components to sensor readings. All statistical analyses were carried out using STATISTICA software (StatSoft Inc., Tulsa, OK, USA). The geostatistical tools were engaged for spatial data analysis. Spatial dependence of the data ware observed and modeled by variogram functions. The kriging interpolation was used for data interpolation, as well as for the mapping. Ordinary least squares (OLS) regression was conducted to determine the relation of NDVIs and soil ECs with yield components. The significance of each explanatory variable was checked with OLS, as well as the normality of distribution of residuals and confidence of the model (Terrón et al., 2011). All geostatistical operations were done in ArcGis software.

RESULTS AND DISCUSSION

Interpretation of 95% confidence intervals was used to assess the difference between mean values given in Figure 1. The results of analysis of the observed parameters revealed that phenotypic divergence of the selected wheat varieties were appropriate for this kind of research in which the reliability of crop and soil scouting technics should be proven. From Figure 1a, it is clear that NDVI reached the highest value (~0.85) around flowering stage when the plant photosynthesis and leaf area were on the top level causing saturation effect and already reported by several authors (Aparicio et al., 2000; Erdle and Schmidhalter, 2013). Afterwards, it gradually decreased until maturity (0.13-0.4) when the reflectance of visible wavelengths increased and reflectance of NIR decreased as a consequence of less absorption of visible light in the leaves (Babar et al., 2006; Naser, 2012; Reynolds et al., 2012b; Sultana et al., 2014).

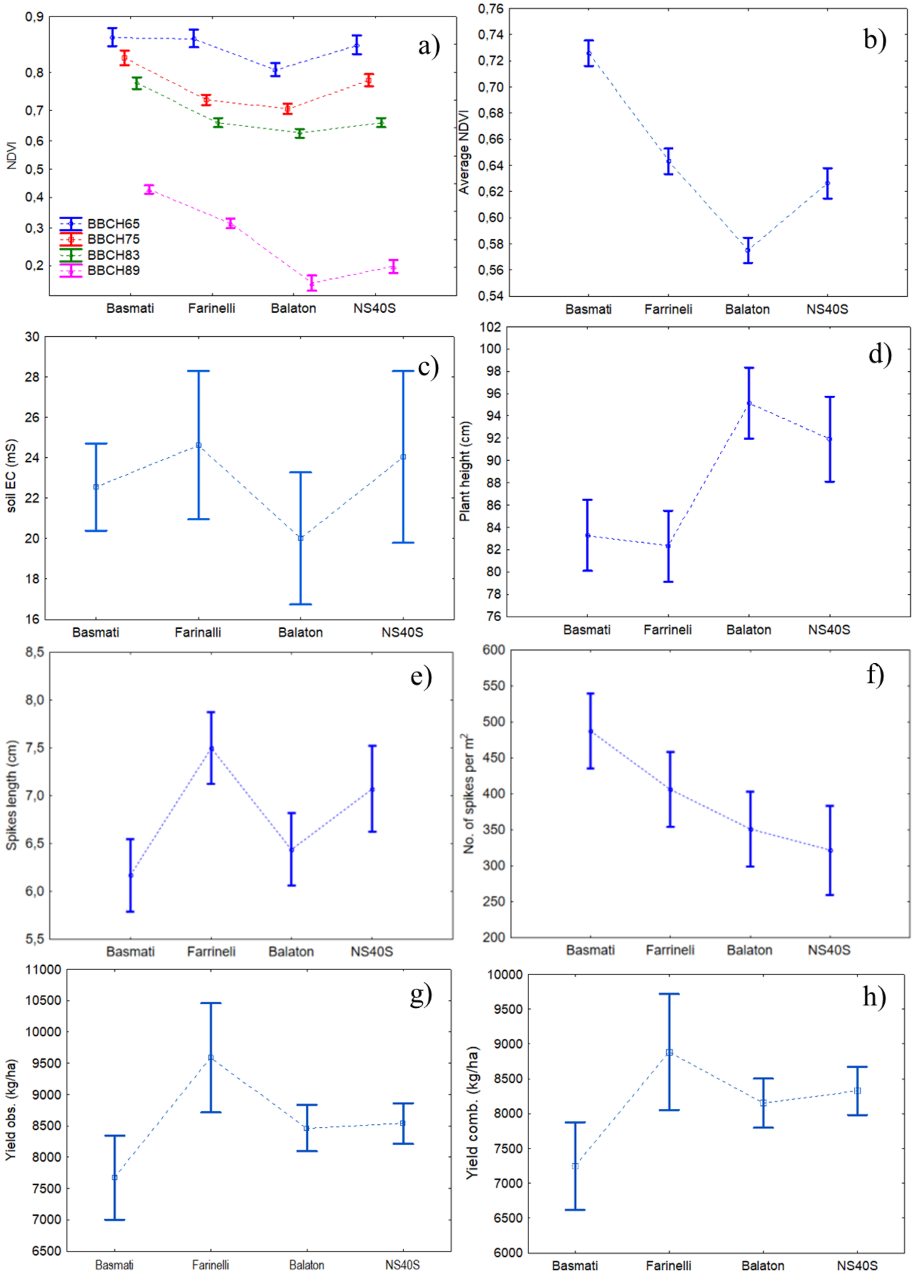

Figure 1. The overall mean values of the observed crop parameters, soil apparent electrical conductivity (ECa) and normalized difference vegetation index (NDVI)

In general, NDVI for all genotypes was slightly decreasing from BBCH65 stage to BBCH83 stage; thereafter, NDVI decrease was more rapid. Slower reduction of NDVI index of Basmati plot (Figure 1a) can be explained by the fact that during maturation period, volume reduction of vegetative parts did not uncover the soil surface as much as in case of other varieties which had less plant density. Hence, the spectral characteristics of reflected light from Basmati plot include more NIR lights and higher average NDVI at all. The Basmati variety had the highest average value of NDVI and spike/m^2 (Figure 1b,f), likewise the lowest grain yield of all included varieties (Figure 1g,h). It could be stated that high average NDVI readings of Basmati plants are induced by higher plant density (spike/m^2) which caused the lower reflection from soil surface (in comparison to other three varieties). According to range of NDVI downtrend of each variety as shown in Figure 1a and their yield performance (Figure 1f,g), the NDVI declining characteristics could be good indicator of tillering and yield potential. Farinelly plants had the longest spikes hence the highest yield. Also, Farinelli had smaller reduction of NDVI index during maturation than Basmati and NS40S.

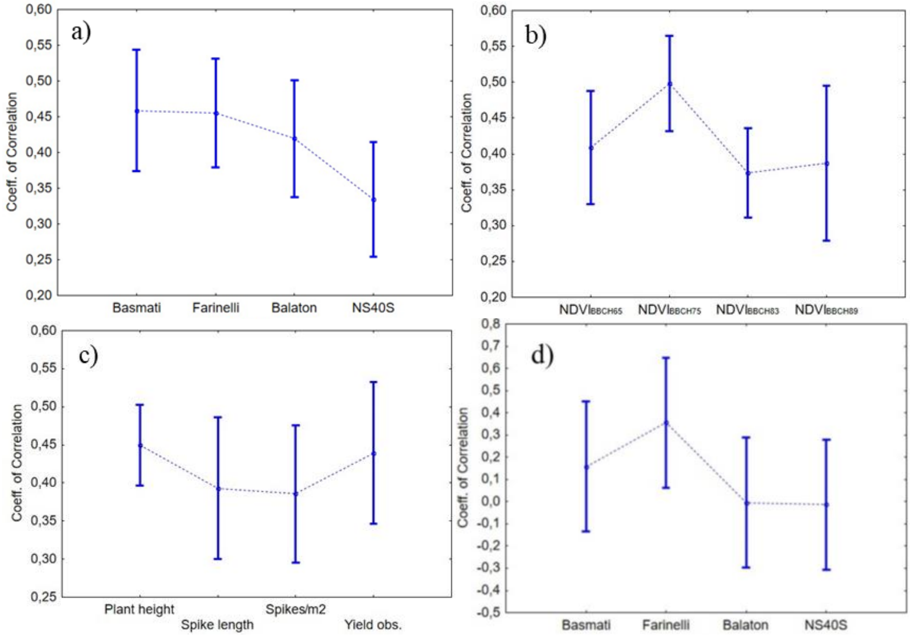

Figure 2. Comparison of R coefficient calculated between selected wheat traits and sensors recordings

According to the range of confidence intervals of soil ECa (Figure 1c), certain data dissipation is evident which implies absence of data normality. Various ECa values of subfield could be the result of elevation impact, hence water and clay distribution over soil layer. The Farinelli subfield had the highest value of soil ECa with very wide 95% confidence intervals. Due to a range of intervals, no meaningful difference was recorded between soil ECa data groups. Concerning plant height feature, the highest average value was obtained for Balaton variety (95 cm), which were similar to NS40S variety (92 cm, Figure 1d). There were no differences between Basmati (83 cm) and Farinelli (82 cm), although these two varieties statistically differed from Balaton and NS40S. The mean values of spike lengths ranged from 6.2 cm for Basmati to 7.5 cm for Farineli, as shown in Figure 1e. It could be noted that similar average values were obtained for Basmati (6.2 cm) and Balaton (6.4 cm) varieties, also between Farinelli (7.5 cm) and NS40S (7.1 cm) varieties. The greatest spikes/m^2 were observed on Basmati plot (480), followed by Farinelli (420), Balaton (350) and NS40S (310) varieties. The last two graphs in Figure 1 are presented to show how well the data on wheat yield from observed locations is matched comparing manual collection with data from harvester yield monitor. For better understanding of relationship between selected parameters and sensors data, the R values are presented as 95% confidence intervals of mean values (Figure 2). The Figure 2a shows that the highest average correlation between NDVI and wheat traits was achieved on Basmati plot (R=0.46) while the weakest correlation was observed between NDVI and wheat traits of NS40S (R=0.33).

If consider the average correlation among NDVI index and phenotypic parameters (Figure 2b), it is clear that stage from full flowering to medium milk appears as the best for the early assessment of wheat outcomes (Hansen et al., 2002, Magney et al., 2016). Based on the width of the confidence interval of the coefficient R (Figure 2c), it can be noted that the parameter plant height was best recognized (R=0.45) considering all varieties and time of measurement. Almost the same value was obtained in case of yield parameter but with lower confidence due to wider confidence intervals. Soil ECa didn’t reach any meaningful correlation with tested varieties or their components (Figure 2d), although the yield of Farinelli could be distinguished from others (R=0.35).

Spatial analysis

The thematic maps are generated to enable additional inspection of spatial arrangement of measured parameters (Figure 3). The map of humus shows that the zones with similar values are randomly grouped in the field without any relation to a particular plot or direction of the field. Certain zones with slightly higher humus content were formed on a subplot with Farinelli variety and they matched with higher grain yield zones. The maps of potassium and phosphorus show certain regularity in spatial distribution. The zone with highest content of P_2O_5 and K_2O in soil is located in the south-west part and changes gradually to opposite direction. This fact is contradictory to the yield map since the greatest yields were achieved in the south-east part. The yield maps visualize spatial variability of gathered yield, which was ranged at most from 6.5 to 10.5 t ha^{-1} (Table 3). The low value zone is concentrated along the right-bottom side of the field and its position coincides with the position of Basmati subplot. Higher yield associated with dark green color overlaps the area where Farinelli variety was grown. On NS40S and Balaton subplots, there is erratic distribution of raster grid with sporadic changes of high and low yield. Field elevation map comprises higher altitude in the bottom zone (Basmati field) and lower in the middle of Farinelli subplot. Spatial structure of altitudes describes a relief, which is one of the greatest contributing factors for crop yield. It is indicative that water availability in soil, and consequently the nutrients, has a significant impact on wheat yield. Considering the results given in Figure 1, it could be concluded that Farinelli and Basmati varieties have the widest confident intervals, which matches the presented spatial pattern of yield map (Figure 3). The results of OLS analysis of the sensor data as the indicator of yield components are presented in Table 3.

Table 3. Characteristics of OLS analysis of selected wheat features and sensed parameters

Yield (Basmati)						
Variables	Coeff.	StdError	t-Stat.	p	VIF	R^2
Model intercept	-1548.69	480.95	-3.22	0.015*	--------	0.73
$NDVI_{BBCH75}$	2268.47	555.52	4.08	0.005*	1.10	
ECa	6.77	2.46	2.76	0.03*	1.10	
Plant height (Farinelli)						
Variables	Coeff.	StdError	t-Stat.	p	VIF	R^2
Model intercept	70.76	9.24	7.66	0.00005*	--------	0.74
$NDVI_{BBCH65}$	10.90	10.78	1.01	0.35	1.05	
ECa	0.09	0.03	2.75	0.03*	1.05	
Plant height (Balaton)						
Variables	Coeff.	StdError	t-Stat.	p	VIF	R^2
Model intercept	-67.88	37.02	-1.83	0.11	--------	0.75
$NDVI_{AV}$	275.24	62.33	4.42	0.003*	1.04	
ECa	0.349	0.21	1.62	0.15	1.04	

VIF greater than 7.5 indicates redundancy in the explanatory variable
*Statistically significant at 0.05 level.

Models with other combinations of explanatory variables (sensor data) in which variables did not reach significant level of contribution were omitted from further analysis. The best correlation, R^2=0.73, was determined between yield and sensor data for Basmati variety which implies that the explanatory variables in their joint interaction have a relatively good potential for wheat yield prediction. The values given in the Coefficient column indicate the degree and type of correlation that exists between the explanatory variable and dependable variable. Statistically significant variables are marked with an asterisk ($p<0.05$). It can be concluded that $NDVI_{BBCH75}$ variable was most influenced by wheat yield, and not so significant relation can be observed between yield and soil EC. This statement supports the R analysis presented in Table 2.

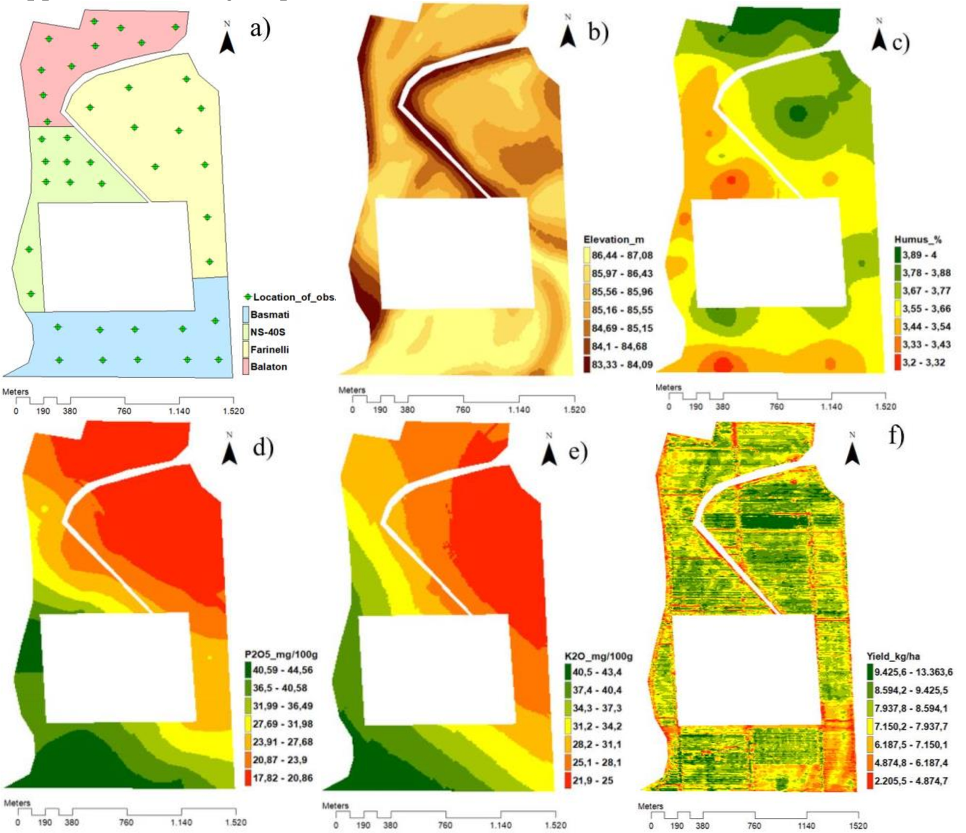

Figure 3. The maps of wheat variety plots, soil properties and wheat yield presented over real geographical proportions

The OLS model found that ECa could be a good predictor of wheat height when considered jointly with NDVIBBCH65. Yield model for Balaton was best explained (75%) with soil ECa and NDVIAV variables, although NDVIAV proved to be equally meaningful in modeling. With OLS analysis, model fitting showed better results compared to the comparison of single parameters. In order to make more confident conclusions, regardless of geographic location and period of observation, more elements need to be taken into consideration followed by various data analysis methods. Without that, the given conclusions could be relevant but only for the specific moment of measurement and the constellation of natural factors that are highly changeable over time and space.

CONCLUSIONS

The presented results of multivariable analysis confirmed that the combined measurements of NDVI and soil apparent electrical conductivity have the potential to identify the differences in soil conditions, crop stand and wheat traits. The best associations between yield components and NDVIs were achieved in milk growth stage with R2 ranged from 30% to 67%. Soil EC didn't match certain correlation with wheat traits and in this case couldn't be characterized as meaningful parameter. Geostatistics partly confirmed the correlations obtained by standard statistics. The quantity of dominant factors and complexity of its interrelations impose constraints in terms of robustness of given models. Uncommon climate condition manifested with sufficient rainfall during growing season diminish the influence of observed spatial heterogeneity on final crop outcomes hence uncovering of certain relationships.

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IDENTIFY OF MACRO AND TRACE ELEMENTS IN GRAIN OF SOME BARLEY VARIETIES AND INTERPRETATION WITH BIPLOT TECHNIQUE

SUMMARY

The purpose of this study was to determine the content of some macro and trace elements in grain of spring barley cultivars, and to state the relationships between those elements. In this study macro and trace elements (Ca, K, Mg, Fe, Zn, Mn, Si, Sn, Cu, Cr, Cd, Ni, V, Pb, As and Se) of barley cultivars were determined by inductively coupled plasma optic emission spektometry (ICP-OES) using grain. The grain samples were digested by microwave system, as well as. As and Se were determined by hydride system. The result of study showed that the content Si of barley cultivars are quite high, however, the concentrations toxic heavy metals of Cd, Pb and as were determined to be below the limit values.

The biplot indicated that three group occurred among macro and trace element and the correlation of Zn with Sn, Cr with Ca and Fe, Ca with Fe and Pb was significant and positively, while V with Si was significant and negatively. On the other hand, the study showed that Samyeli is the best cultivar based on macro and trace element concentrations and this variety can be used in animal husbandry.

Keywords: Cultivar, food, macro, ICP-OES, Heavy metal

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INTRODUCTION

Barley is the most important food source for animal con-sumption, and historically has been an important food source in many parts of the World, as well as Turkey (Jākobsone *et al*., 2018; Kendal *et al*., 2019). Transfer of macro and trace elements to the feeding chain of animals are significantly affected by the geological origin of the soils and the ground water basin as well as the environmental conditions and genetic difference of Barley varieties (Markova Ruzdik *et al*., 2015). Since heavy metals are mobile and easily absorbed by plants in the environment, they are transmitted to the animal body through nourishment.

The studies on the transfer of chemical contaminants through the food chain provide useful information for the development of surveillance programs aimed at ensuring the safety of the food supply and minimising human exposure to toxic agents (Cubadda and Raggi, 2005). It is well known that foods take up trace metals from soils, fertilizers, air, and industrial process, transportation, and package materials. Heavy metals are mobile and easily taken up by plants in the environment (Khairiah *et al*., 2004; Chojnacka *et al*., 2005; Demirel *et al*., 2008) When considering different kinds of soil pollutants, heavy metals represent a special hazard because of their persistence and toxicity (Adriano, 2001). For the human body, certain heavy metals are essential for the biological systems as structural and catalytic components of proteins and enzymes like zinc (Zn) and copper (Cu), and others are contaminants such as cadmium (Cd), arsenic (As), lead (Pb), chromium (Cr), nickel (Ni) and so on (Rana,2008; McLaughlin, 1999). Soil behaves as a sink for heavy metals arriving by the aerial deposition of particles emitted by urban and industrial activities (Bermudez *et al*., 2010; Schuhmacher *et al*., 2009) as well as from agricultural practices (Chen *et al*., 2008; Mico *et al*., 2006). High pollution levels in soils can lead to phytotoxicity and result in the transfer of heavy metals to the human diet from crop uptake or soil ingestion by grazing livestock (Abrahams, 2002; Kabata-Pendias and Mukherjee 2007). Food and Agriculture Organization (FAO) and World Health Organization (WHO), European Commission (EC) and other regulatory bodies of other countries strictly regulate the allowable concentrations or maximum permitted concentrations of toxic heavy metals in foodstuffs (FAO/WHO 1984, EC, 1989). Reference daily intakes for 9 significant elements have been established: calcium (1000 mg), chromium (120 μg), copper (2 mg), iron (18 mg), potassium (3500 mg), magnesium (400 mg), manganese (2 mg), selenium (70 μg) and zinc (15 mg) (Mindel, 2000, Cernohorsky *et al*., 2008). However, heavy metal levels in the soil for lead (300 mg/kg), cadmium (3 mg/kg), chromium (100 mg/kg), Copper (140 mg/kg), nicel (75 mg/kg), zinc (300 mg/kg) were established (Anonymus 1). The impact of heavy metals on the environment is greatly dependent on their speciation in soil solution and solid phase which determine their environmental availability, toxicity, migration – accumulation phenomena, geochemical transfer and mobility pathways (Druteikienė *et al*., 2002; Pinto at al., 2004). Uptake and bioaccumulation of heavy metals by plants is of importance because of an impact on soils by anthropogenic emissions and its consequence for human uptake (Bradl, 2005).

MATERIAL AND METHODS

In the study, four barley cultivars (Altıkat, Samyeli, Şahin-91, Sur-93) used and the samples of grain of these cultivars was taken from the Department of field crops department of GAP International Agricultural Research and Training Center.

Table 1. Sample digestion program for barley and soil

Step(barley)	Time(min)	T (^{o}C)	Power(W)
1	20:00	180	1200
2	20:00	180	1200
Step(soil)			
1	20:00	180	500
2	15:00	180	500

Table 2. Instrumental Operating Conditions Using Thermo ICAP 6300 ICP-OES

Parameter	**Normal**	**Hydride System**
Power	1150 W	1350 W
Pomp speed	50 rpm	30 rpm
Purge gas	Argon	Argon
Coolant Gas		
Flow	12 L/min.	16 L /min.
Auxiliary gas		
Flow	0.5 L/min.	0.5 L/min.
Torch	Axial, Radial	Axial
Auto sampler	Cetac ASX-260	

The nearly 0.25 g dried and ground sample of grain barley was put into burning cup and 8 ml % 65 HNO_3 and 2 ml % 30 H_2O_2 added on this grain samples. The samples were dissolved in a Milestone Smart D microwave oven according to program showing at Table 2. Soil sample was weighed 0.25 g in TFM containers and 6 ml % 65 HNO_3, 1 ml % 30 H_2O_2 and 3 ml % 40 HF added (Table 1). After dissolving of soil sample 1,2 g H_3BO_3 added in burning cup and dissolved.

Samples dissolved and diluted a certain volume with ultra-pure water (Elga ultra-Pure water system). The Concentrations were determined by ICP-OES (Thermo ICAP, 6300).

Total element concentration of soil was determined and showed in Table 3.

According to determined data in the soil Se content found below dedection limit. On the other hand, macro elements in the soil were showed normal concentration but micro element content such as Fe was found higher than other elements.

Table 3. Micro and trace element content in soil sample (n=3, mean±standart deviation, (mg/kg) dry weight)

Elements	Soil	Elements	Soil
Ca	9550±33	Cr	119±6
K	4083±44	Cu	23.8±2.3
Si	1971±32	Sn	10.8±0.7
Fe	16594±102	Pb	10.2±0.1
Zn	44.5±1	V	70.6±6.8
Mn	520±10	As	8.92±0.32
Ni	64.6±0.8	Se	<0.0045
Cd	0.56±0.01		

Statistical analysis (GT)

The GT biblot analeyses were carried out using GT biplot software to assess micro and macro element content (Yan and Rajcan, 2002; Kendal *et al*., 2019). In multi-traits (MT) for cultivars, biplots were constructed by plotting the first two principal components (PC1 and PC2) derived from centered micro and macro element content data to singular value separation. Also, with the GT biplot analysis graphs in the study: It was aimed at revealing relation among examined micro and macro elements content and cultivars means by scatter plot (Fig. 1), and grouped micro and macro elements content and performance of each cultivars at each trait (Fig. 2), the stable and high performance of gcultivars micro and macro elements content by ranking model (Fig. 3), compare the desirable cultivars to ideal center based on micro and macro elements content by comparison model (Fig. 4).

RESULTS AND DISCUSSION

The result of element concentrations of barley cultivars showed in Table 4. According to these data, the concentrations of macro and trace elements were very variable in barley cultivars.

The consentration of Ca, Mg, K, Fe, Cr, V and Pb were changed from 471-521 mg·kg-1, 637-846 mg·kg-1, 2611-3116 mg·kg-1, 36.6-68.7 mg·kg-1,0.55-0.97 mg·kg-1,0.010-0110 mg·kg-1, 0.034-0140 mg·kg-1 respectivelly. The concentrations of these elements in Samyeli cultivar were higher than other three cultivars, while the concentration of Si (134 mg/kg 1) in Samyeli cultivar were quite lower. On the other hand, the concentration of Si (239 mg·kg-1) and Cu (5.83 mg·kg-1) in Sur 93 were quite higher than other three cultivars, while the concentration of Vanadium content (0.010 mg·kg-1) in Sur-93 quite lower than other cultvars. Moreover, the concentration of Se was changed from 0.210-0.380 mg·kg 1, and the consentration of this element was higher in Altıkat than other cultivars. Meanwhile Arsenic concentration of all cultivars found below, compare with dedection limits. The elements of Pb benefical for nutrition animal and human food. When the results of this study compare to other literature results, some elements concentration of barley taked from this study were higher and some of them were lower than other results and some of them were like some results of other studies. This differencies is normal, because these differences are estimated to be caused by climatic factors, varieties, genotypes, and soil factors (Ereifej *et al.*, 2001 Salama and Radwan, 2005). On the other hand, Jâkobsone *et al.* (2018) reported that barley products can provide necessary macro and trace elements, especially of Mn, Mg, Fe, and Zn (7.8–16.1; 1024–1249; 29.2–52.9, and 20.5–33.7 mg·kg-1, respectively). Jakobsone *et al.* (2015) reported that the obtained data from trace and macro elements will expand the opportunity for food and nutrition scientists to evaluate content of the examined elements in grain products, and dietary consumption (bioavailability) of the examined macro-elements and trace elements.

Table 4. Concentrations of macro and trace elements in barley cultivars (n=3, mean±standart deviation, (mg/kg), dry weight)

Elements(mg.kg^{-})	Cultivars			
	Altıkat	Samyeli	Şahin-91	Sur-93
Ca	471±12	521±15	480±13	488±8
Na	449±39	415±9.4	415±8.5	489±16
Mg	637±14	846±11	720±90	844±23
K	2837±172	3116±78	2611±153	2547±143
Si	160±35	134±11	171±12	239±3
Fe	36.6±5.6	68.7±1.8	38.3±2.4	47.7±0.6
Zn	19.4±0.2	23.4±1	28.4±0.25	21.3±0.08
Mn	13.7±0.07	15.1±0.37	14.2±1.3	21.4±0.3
Ni	0.53±0.07	0.54±0.04	0.44±0.01	0.52±0.02
Cd	0.041±0.004	0.023±0.006	0.029±0.006	0.032±0.002
Cr	0.55±0.02	0.97±0.05	0.55±0.004	0.61±0.008
Cu	3.90±0.3	4.81±0.2	4.47±0.45	5.83±0.02
Sn	16.6±0.7	17.3±1.1	18.3±1.8	16.8±0.2
Pb	0.090±0.02	0.140±0.01	0.121±0.08	0.034±0.01
V	0.090±0.005	0.110±0.02	0.063±0.006	0.010±0.001
As	<0.002	<0.002	<0.002	<0.002
Se	0.380±0.24	0.210±0.2	0.311±0.1	0.312±0.12

Graphically the performance of cultivars based on macro and trace elements and correlation among macro and trace elements

Principal component analysis was used to show the distribution of cultivars based on macro and trace elements. The two-dimensional PCA score plot, derived from macro and trace elements and accounted for 78.37% (45.32% and 33.05% for PC1 and PC2, respectively) of the total variation (Figs. 1-4).

The relationship each cultivar by each macro and trace element showed by cultivar vectors and macro and trace element vectors are drawn in Fig. 1, so that the specific interactions between a cultivar and a trait can be seen.

Therefore, this figure can be used (1) to rank the cultivar based on performance in any trait, and (2) to rank macro and trace elements on the relative performance of any cultivar. The interpretation of performance a cultivar in a trait is better than average if the angle between its vector and the element's vector is <90°; it is poorer than average if the angle is >90°; and it is near average if the angle is about 90° (Yan and Thinker, 2009; Dogan *et al*., 2016). The results of traits showed that there is high variation among cultivars based on elements. According to results, there was high correlation by Zn with Sn (r=1.00), Cr with Ca and Fe (r=098), Ca with Fe (r=0.99) and Pb (0.45) was significant and positively, while V with Si (r=-0.99) was significant and negatively (Fig.1; Table 5).

Moreover, Samyeli cultivar related with especially Cr, Ca and Fe, Sur 93 related with Mn and Cu, Altıkat with Se, Cd and Şahin 91 with Zn and Sn elements (Fig.1). On the other hand, the scatter plot indicated that three groups were occurred among macro and trace elements and cultivars showed a wide distribution on macro and trace element, and also The biplot indicated that three group occurred among macro and trace elements(Fig. 2). First group was occurred among V, Pb, Sn, Zn, K, Cr, Ca, Fe, Mg and related with Samyeli cultivar. The second group occurred among Cu, Mn, Na, Cd and related with Sur 93 cultivar. The Altıkat and Şahin 91 did not related with any group of macro and trace elements (Fig1 and Fig 2).

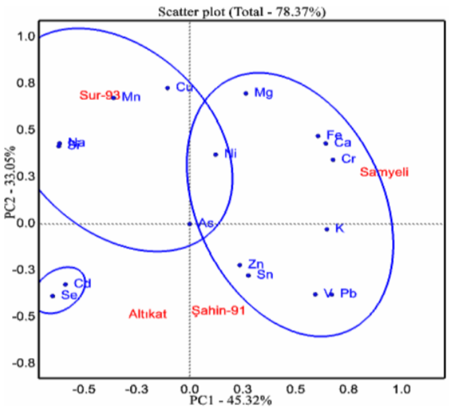

Figure 1. Relation among cultivars and macro and trace elements content.

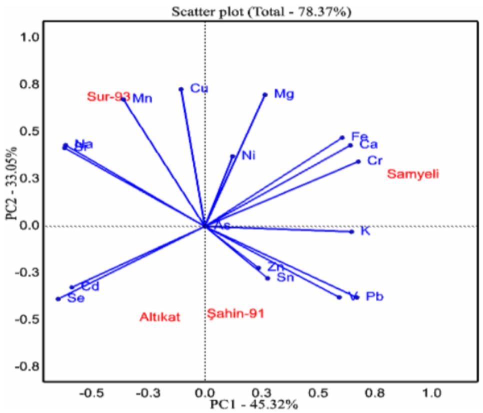

Figure 2. Groups of macro and trace elements content based on cultivars.

The third group occurred only Cd, Sa and did not related with any cultivar. The GT biplot mainly allows the visualization of any crossover GT interaction, which is very important for the breeding program (Kilic *et al*., 2018).

The ranking biplot indicate that the cultivars located close to the origin of the coordinate system of the Biplot graph (Fig. 3) are considered more stable and the opposite is equivalent, the greater the distance from the origin, the less the stability based on mean of trace and macro elements (Kendal and Dogan, 2015). The Samyeli cultivar can be considered stable based on mean of trace and macro elements, when compared other cultivars, while, Altıkat and Şahin 91 were undesirable, because these two cultivar located under mean line of trace and macro elements, and Sur 93 was favorable but unstable because it was located far from origin of the coordinate line axis. Therefore, we can be used the Samyeli cultivar based on macro and trace elements for animal food.

Table 5: The correlation among trace and macro elements content

	Ca	Na	Mg	K	Si	Fe	Zn	Mn	Ni	Cd	Cr	Cu	Sn	Pb	V
Na	-0.34														
Mg	0.79	0.17													
K	0.68	-0.55	0.12												
Si	-0.37	0.87	0.28	-0.83											
Fe	**0.99****	-0.25	0.79	0.70	-0.33										
Zn	0.08	-0.62	0.08	-0.23	-0.16	-0.05									
Mn	0.10	0.82	0.67	-0.50	0.88	0.15	-0.26								
Ni	0.41	0.35	0.27	0.59	-0.10	0.53	-0.87	0.22							
Cd	-0.86	0.49	-0.77	-0.35	0.25	-0.78	-0.57	-0.09	0.11						
Cr	**0.98***	-0.40	0.65	0.82	-0.51	0.98*	-0.04	-0.05	0.52	-0.75					
Cu	0.41	0.56	0.88	-0.32	0.70	0.43	-0.03	0.93	0.17	-0.45	0.24				
Sn	0.09	-0.69	0.02	-0.16	-0.25	-0.05	**1.00****	-0.35	-0.86	-0.55	-0.01	-0.12			
Pb	**0.45***	-0.99	-0.12	0.69	-0.93	0.37	0.49	-0.82	-0.18	-0.50	0.52	-0.55	0.57		
V	0.37	-0.78	-0.28	0.89	**-0.99***	0.36	-0.01	-0.84	0.25	-0.17	0.53	-0.69	0.09	0.86	
Se	-0.97	0.44	-0.80	-0.56	0.34	-0.93	-0.32	-0.08	-0.18	0.96	-0.91	-0.43	-0.32	-0.50	-0.30

******: Probability value is significiant at P<0.01 level, *****: Probability value is significiant at P<0.05 level.

The discriminating and representativeness of genotypes-based traits are visualizing the “ideal center” over the mean values of the environments and offers the opportunity to evaluate genotypes according to their proximity or distance to this center (Kendal, 2020). If the genotypes are located in the center, they are the most ideal, if they are located above the average vertical axis, but far from the center, it means that they are ideal, if they are located below vertical axis, it means that they are undesirable. Based on this overview the *Fig. 4* explained that the Samyeli located near center of AEA, and so, it is more desirable than other cultivars, while Altıkat and Sahin 91 are the poorest cultivars, because these two cultivars are located under mean axis. The term “ideal genotype” is meaningful only when associated with mean performance. According to *Fig. 4*, the Samyeli is highly “ideal”, Sur 93 is desirable genotypes, and because of Samyeli took places in center of AEA and Sur 93 took places on above averages of macro and trace elements axis, and so it means that it is just good for macro and trace elements.

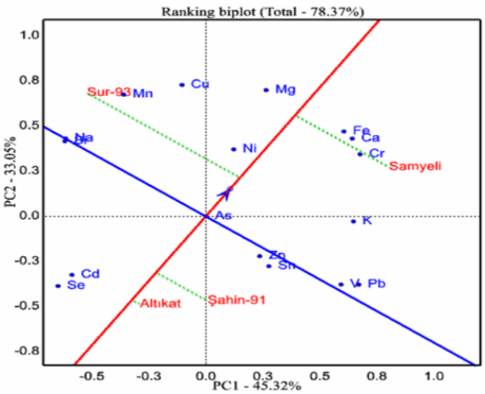

Figure 3. Ranking of cultivars based on macro and trace elements content.

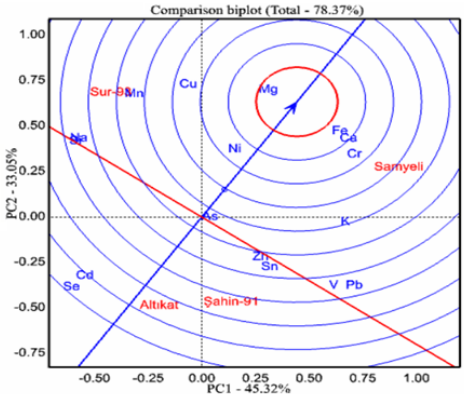

Figure 4. Comparison of cultivars based on macro and trace elements content

CONCLUSIONS

The According to result of study, there were differences among cultivars in terms of macro and trace elements. These differences are caused by climatic factors, varieties, cultivars, and soil factors. The study showed that Samyeli cultivar is the best in terms of majority macro and trace elements which examined in the study, while it was poor in terms of concentration of Si. On the other hand, Sur 93 was good based on some macro and trace elements, while Şahin 91 and Altıkat cultivars were poor based on majority macro and trace elements. The study showed that barley is importance to research and raise the quality of fattening. According to the biplot techniques indicated that there is high correlation between Zn with Sn. Also, the study showed that the biplot technique is a very suitable method for visually understanding and evaluating the relationship between varieties and macro and trace elements.

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SOIL DISTRIBUTION IN CRNA RIVER BASIN AND ITS IMPORTANCE FOR AGRICULTURAL PRODUCTION

SUMMARY

This paper is a result of many years of field and laboratory research of the soils in Crna River Basin, spread out on 497 514,81 ha, with the altitudes ranging from 150 to 2601 m above sea level with the main goal of gaining better understanding of the productive capacities of soils and soil conservation measures for their improvement. The catchment area of the Crna River is a spatial area that extends in two states in the southwest of the Republic of North Macedonia and the northern part of the Republic of Greece. The filed research of the soils and preparation of soil samples has been done according to ISO 10381-1 and ISO 10381-2 protocols. Soil samples were analysed in the laboratory: hygroscopic moisture; mechanical composition; pH of the soil solution; humus content and total nitrogen; content of carbonates; available nutrients P_2O_5 and K_2O. The mechanical composition and chemical properties of the soils were determined by standard methods. Physical-geographical conditions of the studied area are heterogeneous, with numerous relief forms; different expositions and inclinations, and with great differences of altitude. There are several geological formations of a very heterogeneous petrographic-mineralogical composition and climate-vegetation zones. Long-term effects of human participation should also be noted. The vast diversity of the factors required for soil formation in the catchment area of the Crna River are the reason for the formation of many different soil types as well as the lower taxonomic units. There are 14 (fourteen) different soil types distributed in the Crna River Basin together with a considerable amount of subtypes, varieties and forms. The most significant soil types are: Fluvisol and Cambisol. These types of soils are characterized by different properties (chemical, physical, physical-mechanical and productive). Therefore, they have varied effects on agricultural production (field crop, viticulture and fruit production). The aim of this research was to present combined measures of soil conservation for soils from mountain terrains, lake terraces and undulating-hilly terrains, including from sloping terrains and from plain terrains, based on the research that was implemented.

Key words: Soil types, Crna River Basin, Agricultural production.

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

INTRODUCTION

Today, the greatest success in agriculture will be to achieve the desired increase in production by reducing the negative environmental conditions. This can only be achieved by implementing sustainable methods and sustainable solutions in agriculture. The fact that the agricultural activities and practices are compatible with the environment and being permanent is great importance in terms of contributing to the sustainability of the ecology (Tuğrul, 2019).

Soils, which form at the interface between the atmosphere, lithosphere, hydrosphere, and bio-sphere (Targulian *et al*., 2018), are at the constant pressure of Land degradation. Land degradation, either natural or induced by humans, is a continuing process. It has become an important issue through its adverse effects on national natural resources, food security, and the livelihood of the world population. Much has been said and documented about land degradation but there are still gaps of knowledge, due to the fact that only a few countries have really developed cost-effective technologies for mitigation. Inappropriate land use is a major cause of declining soil quality (Sestras *et al*., 2019). In many countries there is continuous stress on the limited land resources due to population pressure. Food security is directly related to the ability of land to support the population (Nabhan *et al*., 1997; Kavian *et al*., 2018; Tavares *et al*, 2019; Chalise *et al*, 2019; Dudic *et al*, 2020; Sakuno *et al*., 2020).

In this paper are presented the main aspects of the soil geography. Soil geography seeks to determine the distribution of soils on the earth's surface, to understand the soil-forming processes and environmental factors, and to suggest the most satisfactory methods of using soils. In the first place is based directly upon field mapping and description. Secondly, a broad theoretical background is necessary to understand physical, chemical and biological aspects. Thirdly, field characteristics need to be interpreted in the light of laboratory assessment of soil properties. Fourthly, soil map interpretation for agriculture, forestry, land use planning is part of the soil geographer's map work (Bridges, 1977).

The agro technical and ameliorative measures are determined based on the properties and processes of the various soil types found in this area with the goal of improving their productive capacity to further increase agricultural production.

Finally combined measures of soil conservation for soils from mountain terrains, lake terraces and undulating-hilly terrains, including from sloping terrains and from plain terrains, based on the research that was implemented were presented.

Inevitably there will be many different opinions about the content and measures suggested, but the idea is to provide relevant basis for discussion upon which further studies can be built.

MATERIAL AND METHODS

The area of the Crna River Basin is an area that extends into two states in the south-western part of the Republic of North Macedonia and the northern part of the Republic of Greece. Map of the Study area of the Crna River basin presented at the Figure 1.

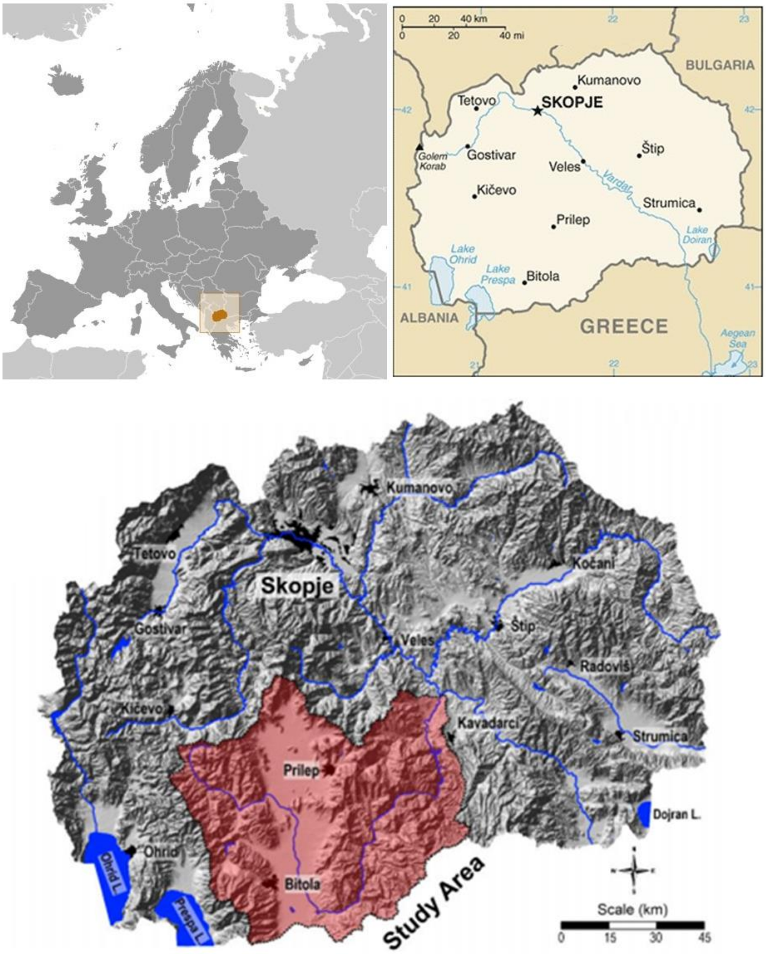


Figure 1. Map of the Republic of North Macedonia indicating the Study area of the Crna River basin (Source of the Maps: The World Factbook 2021. Washington, DC: Central Intelligence Agency, 2021.

On the territory of the Republic of North Macedonia, the Crna River basin extends between 40°51'56" and 41°36'20" north latitude and 20°56'45" and 22°4'58" eastern longitude. Its total length is 207 km with the total area of the catchment area in both countries of 5775 km^2, of which to the Republic of North Macedonia belongs the largest part of 4870 km^2, while the catchment area in the Republic of Greece is 905 km^2.

In the river basin of Crna River the influences of the Mediterranean, Mediterranean-altered, temperate-continental and mountainous climate are present. The mean annual air temperature ranges from 8.4 ºC to 13.5 ºC over a period of 23 years. The average absolute maximum air temperatures in the Crna River are within the limits of 32 ºC. The absolute minimum air temperature for the same period is within the limits of –15ºC. The amplitudes of the extreme temperatures are quite large and they range from 51.5 ºC to 66.6 ºC (Filipovski, 1996 and Koteski, 2009). The warmest months for all meteorological stations in the Crna River basin are July and August and the coldest month is January. According to the regions of this basin, in Tikveš Valley the influence of the sub-Mediterranean climate is generally present, in the central part of the basin (Pelagonia Valley) the continental climate is represented, while the influence of the moderate continental climate appears in the upper part of the basin (Tomovsk *et al*., 2017).

The Crna River basin belongs to three geotectonic structural units: the upper western part lies in the area of the West-Macedonian zone, the middle part is on the Pelagonian zone and the lower part to the Vardar zone (Stafilov *et al*., 2016). The upper western part which lies in the area of the West-Macedonian zone is built mainly from Paleozoic and Triassic formations, primarily from crystalline schists and limestones (marbles and dolomites), as well as from granites. The middle part which belongs to the Pelagonian geotectonic zone is dominated by Precambrian rocks, such as: micas and marbles, as well as Neogene deluvial and alluvial formations. In the lower part of the flow of Crna River, which belongs to the Vardar zone, covering the areas of the eastern part of Mariovo and part of the Tikveš Valley, the most present are the crystalline schists, granites and granodiorites, flysch sediments, volcanic breccias, limestones, marble dolomites etc. (Koteski, 2009; Stafilov *et al*., 2016).

This area (Crna river basin) is very heterogeneous, with numerous relief forms, with different expositions and inclinations, and with great differences of altitude. Additionally, there are several geological formations of a very heterogeneous petrographic-mineralogical composition and climate-vegetation zones. Long-term effects from human involvement should also be noted. The vast diversity of the factors required for soil formation in this area is the reason for the formation of many different soil types as well as the lower taxonomic units.

The soils in the area also appear in the complexes that are presented on the soil (pedological) map. These types of soils are characterized by different properties (chemical, physical, and physical-mechanical, productive). Therefore, they have varied effects on agricultural production. In this paper are presented the main aspects of the soil geography. The agrotechnical and meliorative measures are determined based on the properties and processes of the various soil types found in this area with the goal of improving their productive capacity to further increase agricultural production.

The filed research of the soils has been done according to methods described by Filipovski *et al*. (1967). The filed research of the soils and preparation of soil samples has been done according to ISO 10381-1 and ISO

10381-2 protocols. In laboratory, the following analyses have been carried out on the soil samples: hygroscopic moisture; mechanical composition; pH of the soil solution; humus content and total nitrogen; content of carbonates; available nutrients P_2O_5 and K_2O. The mechanical composition and chemical properties of the soils have been determined by standard methods.

The mechanical composition and chemical properties of the soils have been determined by standard methods described by Bogdanović *et al*. (1966), Mitrikeski and Mitkova, T (2006); Resulović *et al*. (1971), Džamić *et al*. (1996).

RESULTS AND DISCUSSION

Geography of soils

Distribution of soil types and complexes.

The formation, the distribution and the soil properties in this area are in close correlation with the environmental conditions, i.e. the soil genesis conditions, such as the geographical position and the relief, the hydrography, the parent material, the climate, the vegetation, the time period and the human factor. The soil (pedologic) map, figure 2; figure 3 and 4 together with Table 1 on the distribution of the soil types, differentiates the following properties in the geography of soils.

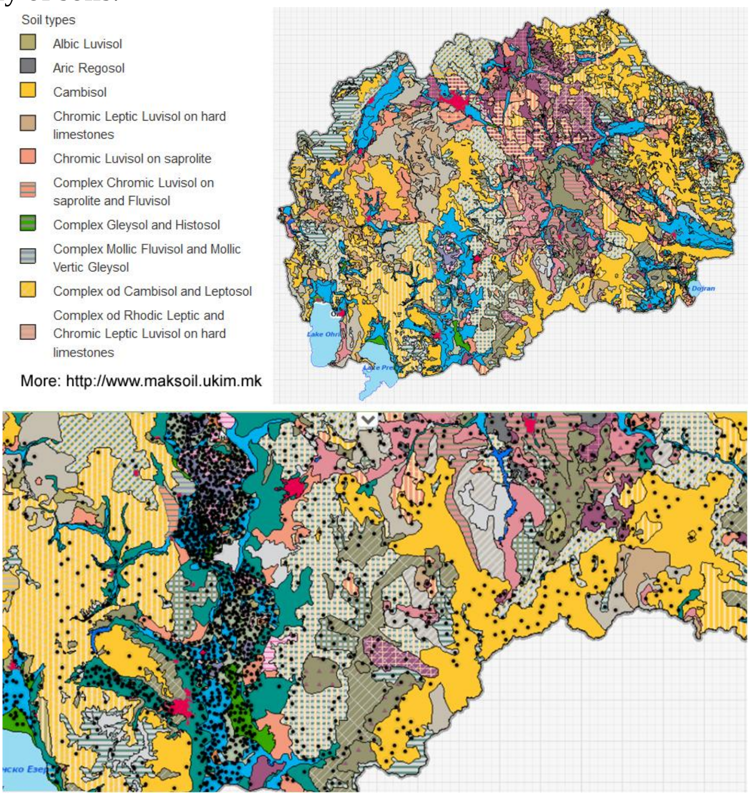


Figure 2. Soil map - Soil types and complexes distribution in the catchment area of the Crna river (http://www.maksoil.ukim.mk/masis/)

In the catchment area of the Crna river, there are 40 (forty) soil types and a number of subtypes, varieties and forms.

Depending on the dominant influence of individual soil forming factors, most part of the areas are covered with soils that are also following the local climate – vegetation zoning (Chromic Luvisols on Saprolite, Cambisols, Humic Eutric and Umbric Regosols, Albic Luvisols), which is also combined with the influence of other soil forming factors (parent material, relief).

Some of the soils demonstrate strong lithogenous character (Leptosols, Regosols, Humic Calcaric Regosols, Vertisols), whereby the influence of other factors (relief) is also significant. Some of the soils have topogenous – hydrologic origin, related to the consequences of the erosion processes (Fluvisols, Gleysols, Fluvisols-Colluvial Soils). Lately, due to the newly planted seedlings (orchards, vineyards), some of the soils also have antropogenous origin (Aric Regosols).

The papers of Filipovski (2015), address these conditions in details.

Table 1. Soil types and complexes distribution in the catchment area of the Crna River (ha and %)

SOIL TYPES AND COMPLEXES (WRB Soil Classification)	[ha]	[%]
I. SOILS OF THE PLAINS		
Fluvisol	28212.55	5.67
Mollic Vertic Gleysol	6627.15	1.33
Complex of Solonchak and Solonetz	6515.91	1.33
Gleysol	4764.08	0.96
Complex Mollic Fluvisol and Mollic Vertic Gleysol	1025.87	0.21
Mollic Fluvisol	16234.24	3.26
Urbisol	2786.81	0.56
Total	**66166.61**	**13.30**
II. SOILS OF COLLUVIAL FANS		
Fluvisol (Colluvial Soils)	**54664.44**	**10.99**
III. SOILS OF LAKE TERRACES AND OF UNDULATED HILLY RELIEF		
Regosol	4128.85	0.82
Albic Luvisol	3017.5	0.61
Aric Regosol	3574.23	0.72
Chromic Luvisol on saprolite	12590.99	2.53
Humic Calcaric Regosol	16915.98	3.40
Vertisol	1930.17	0.38
Planosol	1168	0.23
Complex of Regosol and Vertisol	10369.59	2.08
Complex of Humic Calcaric Regosol and Regosol	12248.4	2.46
Complex of Chromic Luvisol on saprolite, Humic Calcaric Regosol and Regosol	2470.79	0.49
Complex of Vertisol and Humic Calcaric Regosol	240.8	0.05
Complex of Humic Calcaric Regosol, Regosol and Vertisol	2006.21	0.40
Complex of Chromic Luvisol on saprolite and Regosol	5169.29	1.04
Complex of Humic Calcaric Regosol, Regosol and Leptosol	6248.43	1.26
Complex Chromic Luvisol on saprolite and Fluvisol	1365.25	0.27
Complex of Vertisol, Regosol and Leptosol	4083.91	0.82
Complex of Chernozem and Humic Calcaric Regosol	322.77	0.06
Complex of Humic Calcaric Regosol and Leptosol	105.03	0.02
Total	**87956.19**	**17.68**

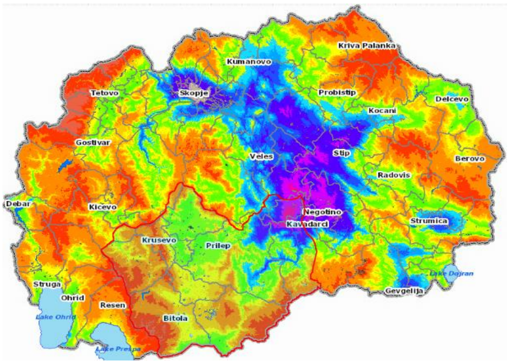
IV. MOUNTAIN SOILS		
Complex of Humic Eutric and Umbric Regosol	13837.12	2.71
Leptosol	12186.31	2.45
Complex of Regosol and Leptosol	26253.86	5.07
Complex of Humic Eutric and Umbric Regosol and Regosol	15576.94	3.13
Complex of Cambisol and Regosol	6492.52	1.30
Rendzic Leptosols	21205.35	4.26
Cambisol	62214.37	12.50
Complex of Humic Eutric and Umbric Regosol, Regosol and Leptosol	45873.64	9.22
Complex of Cambisol, Humic Eutric and Umbric Regosol and Regosol	3708.64	0.75
Complex of Cambisol, Humic Eutric and Umbric Regosol	9741.03	1.96
Complex of Rendzic Leptosol and Leptosol	13101.45	2.63
Chromic Leptic Luvisol on hard limestones	901.29	0.18
Leptosol, Calcaric	61.96	0.01
Complex of Cambisol, Leptosol and Regosol	57573.09	11.57
Total	**288727.6**	**58.03**
Grand Total	**497514.81**	**100**

Table 1 contains data on the soil types and complexes distribution according to the relief forms in the catchment area of the Crna river in ha and %. It can be seen from the Table that the soils spread on lake terraces and of undulated hilly relief dominate in the catchment area and cover an area of 87956.19 ha, or 17.68% of the area, followed by the soils spread on mountainous terrains with 288727.6 ha, or 58.03%. The soils on plains and sloping terrains (colluvial fans) cover small areas (66166.61 ha, or 13.30% and 54664.44 ha or 10.99% of the area).

The plain terrains are mostly covered by Fluvisols (28212.55 ha or 5.67 %), while the percentage of other soils is under 1%. The sloping terrains are mostly covered by Fluvisols (Colluvial Soils) (54664.44 ha or 10.99%). The undulating-hilly terrains and the lake terraces are mostly covered by Humic Calcaric Regosol (16915.98 ha or 3.40 %), followed by Chromic Luvisols on saprolite (2.53%), as well as Regosol (0.82%), and Vertisol (0.38). Most of the mountainous terrains are covered with Cambisols (62214.37 ha or 12.50%) and Rendzic Leptosols (21205.35 ha or 4.26 %), as well as Leptosols with 12186.31ha or 2.45%. On the basis of the many processed data on the map of the graphs, the soil reaction models, organic matter and soil texture of the soils in the Crna River basin are given. (Figure 3, 4 and 5).

The erosion processes, i.e. the human factor are strongly reflected in the geography of the soils in the area. The area of soils that occurred from erosion processes (Leptosols, Regosols, Fluvisols-Colluvial Soils and their complexes) is more than 25% of all areas and unfortunately, the spreading process for these areas is still active, (Markoski *et al*., 2018).

The individual terrain forms differ from each other by their terrain, geological structure, their climate – vegetation and hydrographic conditions and by the degree of anthropogenization. This is reflected on the geography of soils and their properties, as well as on the degree of their utilization and the measures that need to be undertaken in the agricultural production of the area, (Mitkova *et al*., 2017).



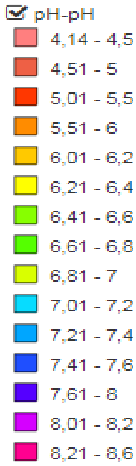
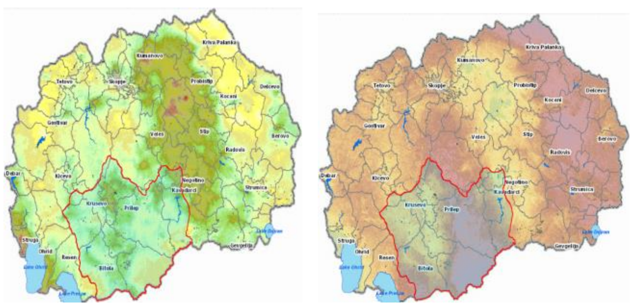


Figure 3. Soil map – pH-reaction distribution in the catchment area of the Crna River (http://www.maksoil.ukim.mk/masis/)

Legend

Figure 4. Soil map – organic matter distribution in the catchment area of the Crna River (http://www.maksoil.ukim.mk/masis/)

Legend



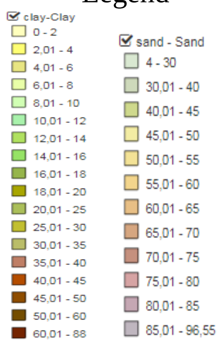


Figure 5. Soil map – soil texture distribution in the catchment area of the Crna River (http://www.maksoil.ukim.mk/masis/)

Legend

Common properties of the soils according to terrain (relief) forms

Common properties of the soils on the mountainous terrains.

The soils spread on mountainous terrains cover 288727.6 ha, or 58.03 % of the area. These are: Leptosols, Humic Eutric and Umbric Regosols, Cambisols, as well as their complexes, in combination with Regosols. Their common properties are: a) very pronounced erosion processes; b) weak chemical weathering resulting in shallow solum over some substrates, and lack of deep regolith and poor clay content; c) absence of carbonate, pronounced acidification (weaker in the soils over acidic rocks than in the basic rocks); d) absence or very poor textural differentiation of the solum; e) clearly expressed changes in the soil properties and the intensity of some processes as the altitude increases; f) clearly expressed dependence of the soil properties from the substrate: soils over acidic rocks contain less clay, they are more acidic and are less texturally differentiated, unlike the soils formed over basic rocks, g) absolute domination of the silicate over the carbonate substrate.

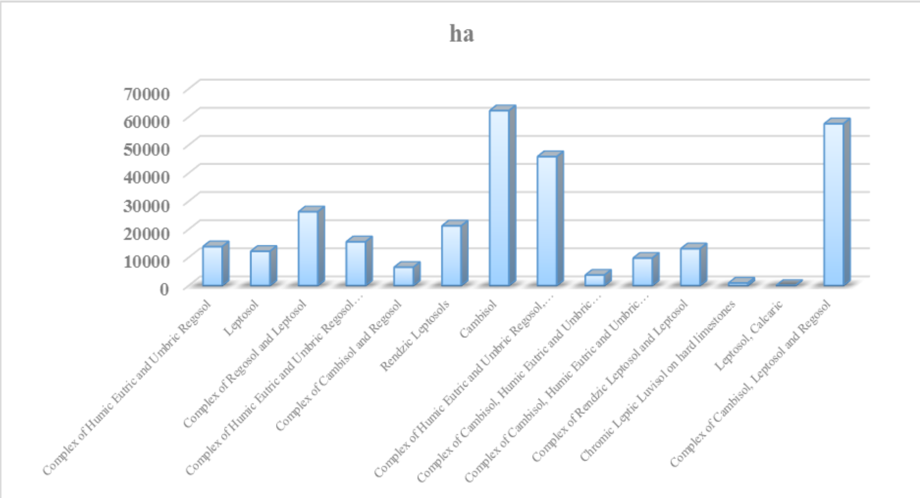


Figure 6. Mountain soils in [ha]

Common soils properties from the lake terraces and of undulated hilly relief.

The following soil types are present on this terrain form: Regosols, Humic Calcaric Regosols, Vertisols, Chernozems, Chromic Luvisols on saprolite and Albic Luvisols. The occurrence of these types of soils is in close co-relation with the substrate, the relief, the climate-vegetation conditions and the degree of erosion. The soils on this terrain (relief) form have the following common properties:

a) very pronounced erosion (occurrence of Regosols and erosion of horizon A or part thereof, in the soils with A-C, A-(B)-C and A-E-B-C profile type);

b) absence of compact rocks as substrate and soil genesis over clastic sediments, resulting in deep solum and physiologically active profiles;

c) greater presence of clay resulting from the substrate or the argilogenesis within

the profile;
d) presence of smectites in some soils (Vertisols, Vertic Chromic Luvisols on saprolite) arising mainly from the substrate and partially from the soil genesis, and in relation to that, deterioration of the physical properties of the soils;
e) occurrence of textural differentiation at some soils (Vertisols and Albic Luvisols), and in relation to that, deterioration of the physical properties;
f) greater presence of the silicate – carbonate substrate in the soil genesis, in comparison to the substrate of the mountainous terrains;
g) relative dryness of the soils (which is lower at the lake terraces), caused from insufficient quantity of rainfalls, surface water flow and very deep underground water;
h) insufficient quantity of humus and nutrients (especially N and P);
i) relatively good chemical properties (the high content of carbonates of Humic Calcaric Regosols and the acidity of the Albic Luvisols are an exception).

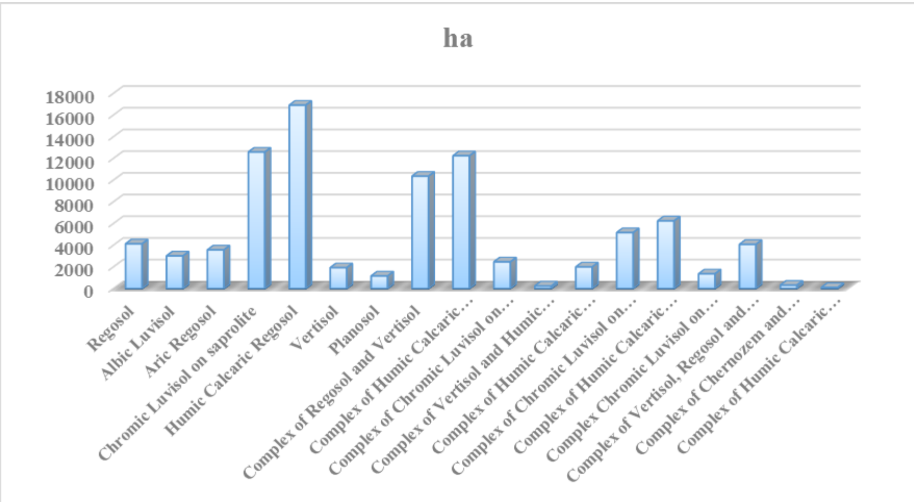


Figure 7. Soils of lake terraces and of undulated hilly relief in [ha**]**

Common properties of soils from sloping terrains

These terrains are covered with Fluvisols (Colluvial Soils), which are characterized by:
a) horizontal and vertical (according to depth of profile) heterogeneity in the mechanical and mineral-petrographic composition;
b) low content of clay, drainage, dryness, good aeration;
c) poor humus content, insufficient stability of the aggregates;
d) good chemical properties with insufficient nutrient elements;
e) increasing the finer particles by descending to the lower parts of the cones ("fans");
f) short duration of soil genesis (the youth of the soils);
g) unregulated water regimen (floods and sedimentation of coarse material). Common properties of the soils of the plains.

Fluvisols with varying degrees of gleyzation are present in the flat bottom deep in the profile and the Gley soils (Gleysols and Mollic Vertic Gleysols) have the following characteristics:
a) the appearance of non-saline underground waters at different depths;
b) the appearance of a physiologically deep profile;
c) gleyzation at the bottom of the profile;
d) a significant amount of organic matter (especially in Gleysols), where it is of hydromorphic origin;
e) occurrence of unregulated water regimen (floods, riverbad erosion, deposition of coarse sediments, regeneration of fertility by application of fine sediment);
g) absence of texture differentiation of the profile (no occurrence of (B) or Bt horizons);
h) favorable physical and chemical properties.

The occurrence of Halomorphic soils (Solonchak and Solonetz) on small areas (total 6515.91 ha or 1.33%) is characteristic of these terrains in the area. Their formation is related to the presence of salts in the sediments, drier climate conditions and relief-topographical conditions in which there are shallow and saline underground waters.

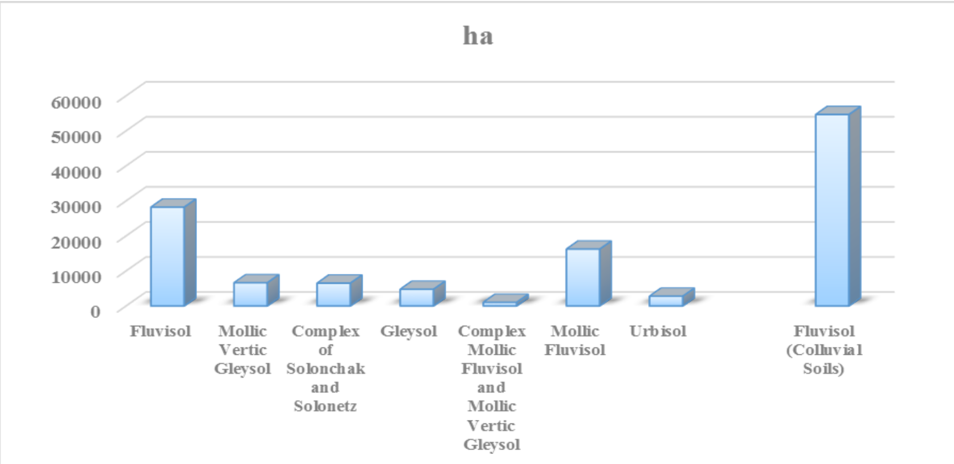


Figure 8. Soils of the plains and soils of colluvial fans in [ha]

Soil significance according to relief forms for agricultural production

The properties (mechanical composition and chemical properties) of individual soils formed in the area are described in detail in the papers of, (Filipovski, 2015) and (http://www.maksoil.ukim.mk/masis). Here, together and according to relief forms, we will explain their significance for agricultural production in the area, the measures for improving their productive ability will be explained in the conclusions.

In mountain reliefs, part of the Humic Eutric and Umbric Regosols is under summer pastures, a smaller part under forests, and a small part is cultivated. Fields are mostly abandoned, and some produce potato and seed material for it, then rye and oats, and at lower altitudes some fruit trees are cultivated. As for the use of Cambisols, it can be said that they have the greatest significance for forestry, because they produce and then exploit most of the wood mass in our country. By deforestation some of these soils are converted into pastures or into now abandoned fields. A very small part is cultivated, used as fields, and a smaller part as pastures. Potatoes are most commonly cultivated field crops, some forage field crops can be successfully grown, as well as crops for green fertilization. Some of these soils can be successfully turned into artificial grasslands. Several fruit crops can be successfully grown (chestnut, walnut, plums, apples, pears, raspberries, blackberries, ribes).

Among the soils formed on lake terraces and of undulated hilly relief, Vertisols and Humic Calcaric Regosols are characterized by greater productivity in comparison with Regosols, Chromic Luvisols on saprolite and Albic Luvisols. Depending on the conditions for irrigation these soils have heterogeneous use. Field crops, vegetable crops, forage crops, industrial crops, vineyards, orchards are cultivated on them.

Fluvisols (Colluvial Soils) are significantly less productive than Fluvisols (with which they border. They are less sorted, do not have a flat relief, have higher impact from drought, contain less nutrients, and do not supply water from groundwater.

In the plain terrains of the area, Fluvisols are of the greatest significance for agricultural production. This is due to the favorable physical and chemical properties, the deep solum, the provided conditions for irrigation and the presence of available forms of P_2O_5 and K_2O. They provide relatively high yields of all agricultural crops. Mollic Vertic Gleysol and Gleysols are potentially fertile. The former have good chemical properties, but poor physical properties, and the latter have relatively good properties, but have shallow underground waters, occasional floods at some sites, anaerobic conditions and due to this, poor nitrification.

CONCLUSIONS

There are 40 (forty) different soil types distributed in the Crna river basin together with a considerable amount of subtypes, varieties and forms. They are formed on four relief forms (plain terrains, sloping terrains, mountain terrains and undulating-hilly terrains and lake terraces) that have different significance for agricultural production. In order to increase their productive ability, the following joint measures should be undertaken according to relief forms:

- Joint measures for soils from mountain terrains: protection from erosion, fertilization with organic and mineral fertilizers, proper tillage, liming if necessary;

- Joint measures for soils from lake terraces and undulating-hilly terrains: deep tillage, humization: organic fertilizers and phytomeliorations, intensive use of mineral fertilizers N and P_2O_5, and for plants that need potassium during the entire year and for obtaining much higher yields and K-fertilizers, anti-erosion measures, proper irrigation method;

- Joint measures for soils from sloping terrains: anti-erosion protection measures, irrigation, humization, intensive use of mineral fertilizers;

- Joint measures for soils from plain terrains: regulation of the water regimen, lowering of the level of underground water-drainage, tillage and creating a deep fallow, fertilization with mineral and organic fertilizers with previous soil fertility control, proper irrigation.

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FLOWERING BEHAVIOR OF FRENCH OLIVE CULTIVAR 'PICHOLINE' UNDER SYRIAN COAST CONDITIONS

SUMMARY

The study was conducted in Bouka center for research and plant production, Syria, in order to study flowering behavior of French olive cultivar 'Picholine'. Average number of flowers per inflorescence, pistil abortion (%) and fertile inflorescence (%) were studied in three positions of flowering shoots (apical, middle and basal position) in 2018 and 2019 seasons. Chilling units, flowering dates and growing degree days were also estimated in 2017, 2018 and 2019 seasons. Results showed that the number of flowers per inflorescence was not changing between seasons, but it was influenced by inflorescence position on the shoot. Pistil abortion was significantly lower in the basal position compared to inflorescences located in the apical and middle position of the shoots. Ratio between the number of flowers per inflorescence and the number of flowers with aborted pistils differed depending on inflorescence position on the shoot and season. In total, the number of flowers per inflorescence was responsible for 11% of the variation in pistil abortion in 2018, and 37% in 2019 season. Chilling units differed considerably among seasons, 2018 had the lowest chilling units, while 2017 had the highest. 'Picholine' flowered regularly even under low chilling conditions showing to have lower chilling requirements and consequently could adapt well to areas with relatively warm winters. This could be an advantage under global climatic change.

Keywords: pistil abortion, chilling, growing degree days, Picholine, inflorescence position.

INTRODUCTION

Olive (*Olea europaea* L.) is an iconic tree of the Mediterranean. Olive flowers on shoots from previous year growth in inflorescence or sometimes called "panicle" or "cluster", where the quantity and quality of the flowers could determine the commercial yield (Rapoport *et al*., 2012).

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Olive is andromonoecious crop, i.e. having both hermaphrodite and staminate flowers on the same plant. Staminate flowers occur as a result of different level of pistil abortion, a phenomenon differs considerably between olive cultivars and could influence fruit set and consequently tree yield (Rosati *et al*., 2012; Selak *et al*., 2019).

Flowering characteristics could affect fruit characteristics and yield, because yield depends on fruit number and weigh, two parameters influenced by flower quality and quantity (Rosati *et al*., 2012). Moreno-Alias *et al*. (2013) mentioned that flower quality has significant influence on yield, so it's an important parameter in olive breeding programs. The same researchers reported that the new olive cultivar 'Sikitita' which resulted from the hybridization /'Picual' (♀) × 'Arbequina' (♂)/ had the same number of flowers per inflorescence of the father and intimidate between the two fathers in hermaphrodite flowers ratios.

Studies reported many factors which could affect pistil abortion such as genetic factor, Alagna *et al*. (2016) reported many genes involved in pistil abortion of olive such as *BAM 1*, *Inv-cw*, *Inv-V* and *GBSSI* which involve in starch and sucrose metabolism and *NLP1*, *SPDS* and *ADC* which involve in polyamines biosynthesis. In addition, environmental conditions like drought, mineral deficiency and previous year yield could affect flower gender in olive (Rapoport *et al*., 2012; Rosati *et al*., 2012; Beya-Marshall and Fichet, 2017).

Garcia-Mozo *et al*. (2009) mentioned that phenological data is an important and sensitive tool in evaluating the adaptability of plants in local environments and how those plants respond to climate change. Olive cultivars reported to require sufficient level of chilling (below 7.2° C) for bud differentiation and olives growing under stable temperature above 15.5° C do not flower at all (Hartmann and Porlinglis, 1957).

Olive cultivars differ in chilling requirements between low chilling requirements like 'Koroneiki' and high like 'Sevillano' (Aybar *et al*., 2015; Malik and Bradford, 2006). Koubouris *et al*. (2019) reported a decrease in the number of flowers/inflorescences in Greek olive cultivars 'Koroneiki' and 'Mastoides' resulted from low chilling, while no effect was found on inflorescence length or width. Any way the threshold used in calculating chilling requirements differed among authors, where some used 7.2°C (Hartmann and Porlinglis, 1957; Zouari *et al*. 2017), while others used 9, 10, 12.5, 15 and/ or 16°C (Alcalá and Barranco, 1992; Haberman *et al*., 2017; Selak *et al*., 2018; Koubouris *et al*., 2019). Other studies reported different thresholds depending on latitude, so thresholds are lower in olive orchards located at higher latitudes (Aguilera *et al*., 2014).

In this study, flowering behavior of French olive 'Picholine' was studied; also chilling and heat requirements provided by study area were calculated in order to evaluate the response of this cultivar to different chilling units throughout three concessive years characterized by different climatic conditions.

MATERIAL AND METHODS

Study location and Plant material:

The study was conducted in Bouka center for research and plant production, Latakia province, Syria, 40 m above sea level. A 30 years old trees of French olive cultivar 'Picholine' was studied.

Methods:

In 2018 and 2019, four uniform sized trees of olive cultivar 'Picholine' with high level of flowering were chosen for the study (category 5) according to (Ramirez-Santa Pau *et al*., 2002). a sample of 75 inflorescences was taken from random one-year old shoots distributed around the canopy at white bud stage in each tree as follows: 25 inflorescence from the apical part of the shoot, 25 inflorescence from the middle part of the shoot and 25 from basal part of the shoot. For each part of the shoot the following parameters were evaluated:

Average number of flowers per inflorescence = total number of flowers in the sample/ number of inflorescences.

Pistil abortion (%) = (number of flowers with non-functional pistils/ total number of flowers) ×100.

Fertile inflorescences (%) = (number of inflorescences containing at least one hermaphrodite flower/ total number of inflorescences) × 100 (Sanchez-Estrada and Cuevas, 2020).

The start of flowering (stage 61), and the end of flowering (stage 69) was estimated according to (Sanz-Cortés *et al.* 2002). Maximum and minimum daily air temperatures for three years (2017, 2018 and 2019) were obtained from meteorological station located 3-4 km from the study orchard. Flowering dates (FD) were calculated as the number of days from January 1 until the start of flowering in each year.

Chilling accumulation (CU) for each study season was calculated as follows:

$$CU = ((T-m)/(M-m)) \times 24,$$

where T is the threshold air temperature (7.2 and 12.5°C were used), M is the daily maximum air temperature, m is the daily minimum air temperature. The chilling period started in the first day in autumn when the mean daily air temperature dropped below the threshold temperature followed by subsequent days with air temperature below the threshold (Aron and Gat, 1991; Zouari *et al*., 2017; Selak *et al*., 2018).

Heat accumulation expressed as growing degree days (GDD) starts the day after chilling period stopped and continued until the start of flowering. GDD was estimated as follows:

$$GDD^\circ = \{(Tm + TM)/2\} - T0$$

Tm is the daily minimum air temperature; TM is the daily maximum air temperature; T0 the threshold temperature.

Experimental design and statistical analysis:

Two-way randomized blocks design was used with four replications. Data were arcsine transferred when necessary and subjected to ANOVA. Means were separated using Duncan multiple range test ($P \leq 0.05$). CoStat version 6.400 Copyright(c) 1998- 2008 CoHort software, CA, USA was used for statistical analysis.

RESULTS AND DISCUSSION

Number of flowers per inflorescence

Average data of the number of flowers per inflorescence are presented in Table 1. The data shows little influence of the position on the branch on inflorescence. The number of flowers per inflorescence was not changing significantly between apical and middle position on the flowering shoots, whereas different behavior was observed for the basal position. In 2018 season, basal position had significantly the highest number of flowers per inflorescence, while in 2019 season the opposite situation was observed. Anyway, the average of the two years data showed that the number of flowers per inflorescence was decreasing from the apical to the basal position of the shoot by almost 11%.

Table 1. Number of flowers per inflorescence of olive cultivar ‘Picholine’ depends on season and inflorescence position on the shoots

Inflorescence position on the branch	Season		Mean seasons
	2018	2019	
Apical	15.81 ab	17.23 a	16.52 a
Middle	14.69 b	16.97 a	15.83 a
Basal	17.17 a	12.30 b	14.74 a
Mean positions	15.89	15.5	15.70
P-value (seasons)	0.555		
P-value (positions)	0.117		

Means followed by different letters within columns indicate significant differences using Duncan test ($P \leq 0.05$).

Regardless of inflorescence position, season had no-effect on the number of flowers per inflorescence because both seasons gave almost the same number of flowers per inflorescence (15.89 and 15.5 flower/inflorescence in 2018 and 2019, respectively). The fluctuating results between apical and basal positions could refer to different nutritional and climatic conditions by the time of bud burst and inflorescence axil growing in the study seasons. Ghrisi *et al*. (1999) studied ‘Picholine’ at the Menara station in Morocco and they found that this cultivar had high average number of flowers per inflorescence compared with ‘Picholine Marocaine’, ‘Souri’, ‘Manzanilla’ and ‘Arbequina’; they also reported no significant differences in number of flowers per inflorescence between the two years of their study. Mhanna *et al*. (2020) also reported no significance differences in the number of flowers per inflorescence among seasons for the autochthonous Syrian olive cultivar ‘Dermlali’ in the same location.

Pistil abortion and fertile inflorescence

Quality of the flowers in two years period is presented in Table 2. Pistil abortion was significantly influenced by inflorescence position on the shoot in both seasons. Although no significant differences in pistil abortion were found between apical and middle position of the shoot, pistil abortion was higher in these two positions compared to the basal position. The pistil abortion increased from the base to the top of the shoot where pistil abortion reached the maximum level (63.98% as mean of the 2018 and 2019 seasons). This could refer to better nutritional position of the basal buds and inflorescence compared to apical positions where more competition for metabolites between flowers and vegetative growth could be found; also hormone distribution in the shoots could differ between apical and basal position and this could affect sex expression (Gupta and Chakrabarty, 2013). However, mentioned explanations need further study. Mhanna *et al*. (2019a) found no significant differences in pistil abortion between the apical, middle and the basal positions of inflorescence on the flowering shoots of Italian olive cultivar 'Coratina', this could refer to low pistil abortion ratio of this cultivar compared to 'Picholine', a phenomenon seemed to be cultivar dependent.

Table 2. Influence of season and inflorescence position on the shoots on pistil abortion (%) of olive cultivar 'Picholine'.

Position/season	Season		Mean seasons
	2018	2019	
Apical	50.24 a	77.72 a	63.98 a
Middle	50.12 a	66.30 a	58.21 a
Basal	28.47 b	45.15 b	36.81 b
Mean positions	42.94	63.06	53.00
P-value (seasons)	0.000		
P-value (positions)	0.000		

Means followed by different letters within columns indicate significant differences using Duncan test ($P \leq 0.05$).

Season also affected pistil abortion significantly, from 42.94% in 2018 to 63.06% in 2019.

Multiple comparisons showed significant interaction between season and inflorescence position on the shoot. Apical position of 2019 season had significantly the highest pistil abortion ratio (77.72%), while basal position in 2018 season had the lowest (28.47%).

Regarding the presence of the fertile inflorescence (Table 3), it was not changing significantly when analyzing each season alone, but factorial analysis showed that fertile inflorescence (%) on the basal position was superior to middle and apical position. The season also affected this parameter, in 2018 season there was significantly the highest fertile inflorescence (96.44%).

Significant interaction between season and position was found. The highest fertile inflorescence (%) was on the middle position of 2018 season (98.67), while apical position of 2019 season had significantly the lowest fertile inflorescence (68%).

Table 3. Influence of season and inflorescence position on the shoots on fertile inflorescence (%) of olive cultivar ‘Picholine’.

position/season	Season		Mean seasons
	2018	2019	
Apical	93.33 a	68.00 a	80.67 b
Middle	98.67 a	88.00 a	93.34 a
basal	97.33 a	86.77 a	92.05 a
Mean positions	96.44	80.92	88.69
P-value (seasons)	0.001		
P-value (positions)	0.019		

Figure (1) shows the relationship between the number of flowers per inflorescence and the number of flowers with aborted pistils in each shoot position. The apical part of the shoot (figure 1, A) showed significant correlation between the number of flowers per inflorescence and the number of flowers with aborted pistil (r= 0.29*, R^2= 0.087) in 2018.This correlation increased in 2019 (r=0.63***, R^2= 0.39) (figure 1, A′). In middle part (figure 1, B), low and insignificant correlation between number of flowers per inflorescence and the number of flowers with aborted pistil was found in 2018 (r= 0.08^{ns}, R^2= 0.006), while in 2019, this relation was significant (r= 0.4, R^2= 0.16) (figure 1, B′). In basal part of the shoot (figure 1, C, C′), the correlation was significant in both seasons (r= 0.32**, R^2= 0.10; r=0.29*, R^2= 0.08, respectively).

Correlation results indicated that the number of flowers per inflorescence influenced the pistil abortion, but its effect differed between seasons and inflorescence position on the shoot, maximal in the apical part, and minimal in the middle part of the flowering shoot. This indicates that in the apical position stronger competition between flowers could be found.

When merging all data together, correlation between the number of flowers per inflorescence and the number of flowers with aborted pistils was significant (r=0.34***, R^2= 0.11) in 2018 season (figure 1, D), and (r= 0.61***, R^2= 0.37) in 2019 season (figure 1, D′). Depending on coefficient of determination, the number of flowers per inflorescence was responsible for 11% of the variation in pistil abortion in 2018, and 37% in 2019 season, while the rest of variation could refer to other factors such as moisture, nutrients, etc.

Chilling accumulation, Flowering dates and heat requirements:

The start of flowering, flowering period extension, flower quality and quantity, fruit set, fruit development and ripening, all influenced by environmental conditions especially temperature and moisture (Bonofiglio *et al*., 2008; Rapoport *et al*., 2012). Figure 2 shows that 2018 season was warmer than 2017 and 2019 seasons. Table 4 shows that flowering date of ‘Picholine’ occurred on 109th day in 2017 season, and on 101st day in 2018 season what was earlier compared to 2017 but delayed for 15 days in 2019 season.

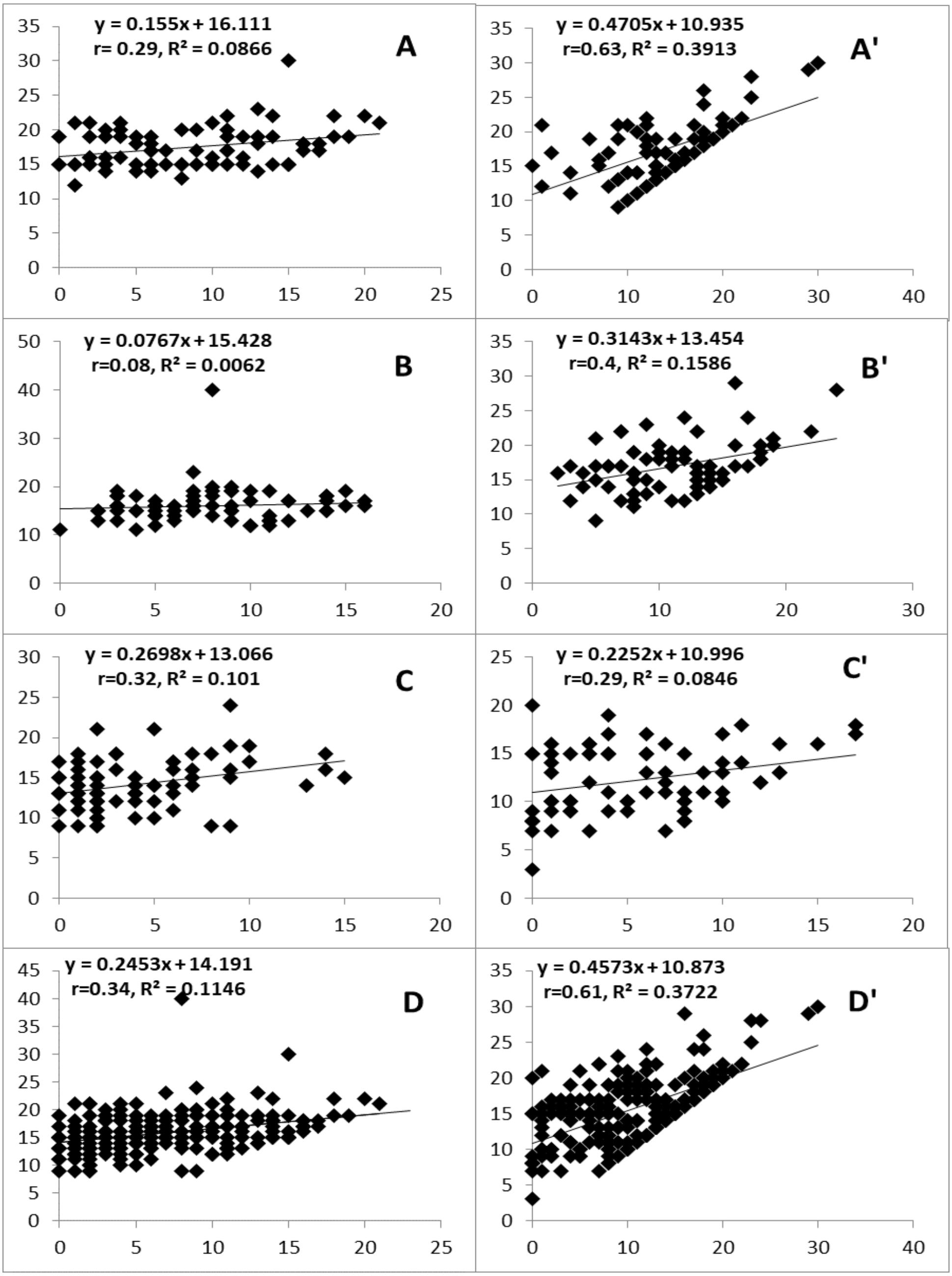

Figure 1. Linear relationship between the number of flowers per inflorescence (Y axis) and the number of flowers with aborted pistils (X axis) in different positions of inflorescence on the shoot: A: apical part, B: middle part, C: Basal part, in 2018 season (A′, B′, C′) in 2019 season.

Chilling units varied widely depending on the threshold used and seasons. When using the traditional threshold temperature (7.2°C), chilling units were low in general reaching 51.2 units in 2017, and only 16.6 u in 2019, but was null (0 unit) in 2018 season. When 12.5°C was used as threshold, chilling units were 1284.6 u in 2017, 326.4 u in 2018 and 612 u in 2019. In general, 2017 season had the highest chilling units, while 2018 had the lowest.

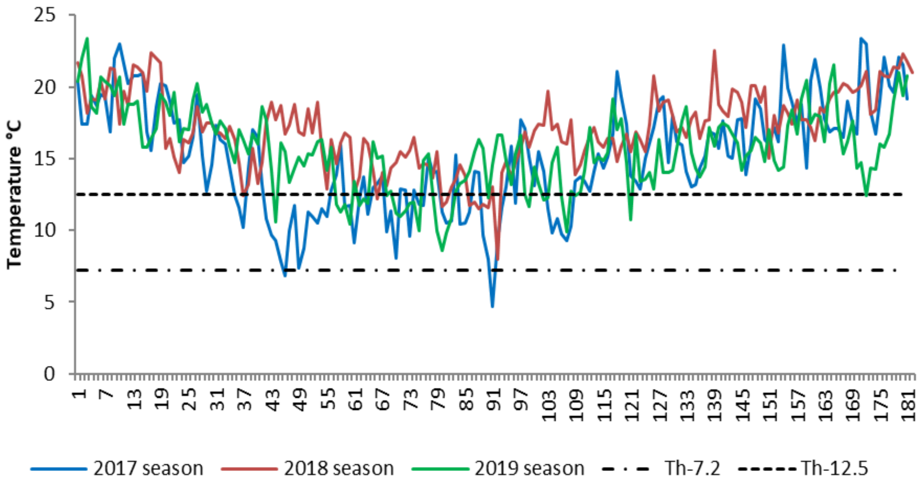

Figure 2. Mean air temperature (°C) in Bouka, Latakia for the period from 1st November to 30th April for 2017, 2018 and 2019 seasons.

Growing degree days (GDD) also differed considerably; it reached the maximum level in 2018 season (1197.4 and 316.2) depending on the threshold used, while 2019 season had the lowest GDD. No clear relationship between FD, CU and GDD was found. Zouari *et al*., (2017) studied chilling and heat requirements in Sfax (Tunisia) and they found inter-annual variability in chilling hours and the mean was 170.89 (threshold 7.2°C), also flowering date of 'Picholine' was fluctuating between April 23 to May 7 depending on the season; while the mean GDD for the period from 2008- 2012 was 231.87.

Several studies reported the necessity of chilling for olive flowering, Hartmann and Porlingis (1957) reported no flowering in olive when temperature was higher than 15.5°C, any way this seems to be cultivar dependent, Malik and Bradford (2006) reported that 'Arbequina' needs lower chilling requirements, while 'Ascolana' needs higher chilling requirements (Kailis and Harris, 2007).

In the present study, 'Picholine' seems to have low chilling requirements, so it could have normal flowering even in relatively warm winters (like 2018 season). This could be in advantage since global warming and increased earth temperature could affect chilling requirements and consequently olive growing negatively. Anyway, calculating the accurate chilling requirements for this cultivar needs further investigation under controlled environment.

Table 4. Flowering period, Flowering date (FD), Chilling units (CU) and Growing degree days (GDD) for 2017- 2019 seasons in Bouka, Latakia.

season	Flowering period*	FD	CU		GDD	
			7.2°C	12.5°C	7.2°C	12.5°C
2017	(20/5)- (3/ 6)	109	51.2	1284.6	842.4	226.4
2018	(12-25)/ 5	101	0.0	326.4	1197.4	316.2
2019	(12- 27)/ 6	116	16.6	612.0	773.1	127.3

*flowering period of 2017 season was adapted from (Mhanna *et al*., 2019).

CONCLUSIONS

Pistil abortion of olive differed depending on inflorescence position on the flowering shoots, where apical part had the highest pistil abortion ratio; also, season could significantly affect this phenomenon. Significant relationship was found between the number of flowers per inflorescence and the number of flowers with aborted pistils, the significance of this relation could differ depending on inflorescence position on the shoot and season. ‘Picholine’ seems to have low chilling requirements in Syrian costal area, and flowering parameters showed good adaptability to local conditions. Under global warming conditions, cultivars with lower chilling requirements could have an advantage in areas under threat of climatic change like the Mediterranean area.

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EVALUATION OF BREEDING IMPROVEMENT FOR SPRING BARLEY VARIETIES IN TERMS OF YIELD AND YIELD-RELATED TRAITS

SUMMARY

As a result of multi-year trial (2013–2017) there have been proved statistically significant breeding and genetic improvement of yield, its stability and level of manifestation of yield-related traits in the newly developed spring barley varieties Virazh, Talisman Myronivskyi, MIP Myrnyi, MIP Saliut, MIP Sotnyk, MIP Azart, and MIP Bohun. The spring barley varieties MIP Bohun and MIP Myrnyi were characterized with the optimal combination of yield and its stability according to the GGE biplot model. It was revealed that the spring barley varieties Virazh, MIP Saliut, and MIP Sotnyk had the highest genetic gain for thousand kernel weight, the variety Talisman Myronivskyi for number of productive tillers, and MIP Myrnyi for kernel number per spike and thousand kernel weight. The variety MIP Azart was differed from the other varieties in the ratio of main yield structural elements. Thus, the newly developed spring barley varieties had differences from each other in pattern of yield and yield-related traits manifestation. According to the GYT biplot model the spring barley variety MIP Bohun was the nearest to the "ideal genotype" in terms of yield*traits combination. The practical worth of the identified patterns is that the new varieties, due to the relatively different pathways of yield formation, will complement each other under unfavorable environmental factors in the production conditions. On the whole, it was shown effectiveness of combining statistical and graphical approaches to comprehensive evaluation the breeding improvement for yield and yield-related traits in new varieties compared to ones created in the previous period.

Key words: *Hordeum vulgare* L., genetic gain, GGE biplot, GYT biplot

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the major crops in world agriculture. Therefore, increasing barley grain yield production is an important

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aspect of human food security. The recent studies in different countries based on both statistical data and the results of comparative trials of barley varieties revealed a significant yield increasing due to breeding improvement (Laidig *et al*., 2017).

Grain yield is formed depend on influence of numerous different environmental factors. It rarely happens that the ranges of vital factors of growing conditions coincide with optimal for plants. At least one factor is limiting. Its effect on genes products (proteins–enzimes) leads to the epigenetic regulation and modifies the phenotypic manifestation of quantitative traits. The presence of the epigenetic regulation of genes expression leads to elementary adaptive reactions, which are stages in the path of the genotype hereditary realization and are ultimately expressed in quantitative and qualitative traits which specific to it (Vasylkivskyi and Gudzenko, 2017). Moreover, yield is a complex trait. It is a result of combination a number of quantitative yield-related traits – its structural components. Thereby yield increasing depends on genetic improvement and the optimal combination of these individual traits (Sharma *et al*., 2018; Hu *et al*., 2018). That is why the level of manifestation and interrelation between yield and its structural components has received considerable attention in breeding and genetic studies (Abdullah *et al*., 2018; Matin *et al*., 2019). The information about already achieved level of manifestation of yield-related traits in previously developed varieties has the practical worth for the purpose of consistent improvement of new varieties (Mirosavljević *et al*., 2016). The problem is that phenotypic manifestation of yield structural elements is largely determined by environmental conditions (Tamm *et al*., 2015). It is important for the breeder to have information about the genetically determined proportion in the overall phenotypic variability of yield structural components. In order to determine the breeding improvement for economically important traits there are widely used different statistical parameters of phenotypic and genotypic variation, heritability coefficient, and genetic gain (Tahar *et al*., 2015; Addisu and Shumet, 2015; Ahmadi *et al*., 2016; Hailu *et al*., 2016).

The aim of our research was to identify main patterns of breeding improvement in newly developed Myronivka spring barley varieties in terms of yield and yield-related traits when using statistical and graphical methods.

MATERIAL AND METHODS

The study was conducted in 2013–2017 growing seasons at the V.M. Remeslo Myronivka Institute of Wheat of NAAS. The objects of study were seven spring barley varieties Virazh, Talisman Myronivskyi, MIP Myrnyi, MIP Saliut, MIP Sotnyk, MIP Azart, and MIP Bohun, which have been registered in Ukraine since 2016. These varieties were compared with the first Myronivka variety Myronivskyi 86 (registered in Ukraine in 1994). The trial was laid out with complete randomized blocks in three replications. The individual plot size was 10 m^2. Genetic gain was calculated for number of productive tillers, kernel number per spike, and thousand kernel weight. Statistical analysis was performed using the breeding equation:

$$\Delta G = SD\ x\ H^2,$$

where ΔG is genetic gain, SD is selection differential, H^2 is heritability coefficient.

The selection differential was calculated with the formula:

$$SD = X_c – X_в,$$

where X_c is the individual trait level of manifestation/ in new variety, $X_в$ is the individual trait level of manifestation in the variety Myronivskyi 86.

The heritability coefficient was calculated with the formula:

$$H^2 = \sigma_q / \sigma_{ph},$$

where σ_q is the genotypic variance, σ_{ph} is the phenotypic variance.

Graphical analysis (GGE biplot, GYT biplot) was performed with accordance to Yan and Tinker (2006) and Yan and Frégeau-Reid (2018) using non-commercial software *GEA-R* version 4.1.

Meteorological conditions through the years of the trial were characterized with significant variability of hydrothermal parameters (Table 1). These meteorological fluctuations made it possible to comprehensive explore spring barley varieties for yield performance, its stability, and the level of manifestation of main yield-related traits.

Table 1. Meteorological conditions during the spring barley growing season

Year	Code	Monthly air temperature, °C				Monthly precipitation, mm			
		April	May	June	July	April	May	June	July
2013	E13	10.5	19.0	21.4	20.6	35.0	61.0	57.0	52.0
2014	E14	10.1	17.3	18.0	21.7	60.7	158.3	47.5	107.0
2015	E15	9.3	16.3	19.4	21.5	34.0	55.0	101.0	99.0
2016	E16	12.4	15.2	20.1	22.2	55.4	91.7	68.6	19.1
2017	E17	10.4	15.4	20.6	21.0	42.7	23.6	20.1	101.8
Long-term		8.8	15.0	18.0	19.7	42.1	51.2	85.2	86.5

RESULTS AND DISCUSSION

Grain yield and genotype plus genotype by environment interaction

According to multi-year trial data there was revealed that the spring barley varieties Virazh (G2), Talisman Myronivskyi (G3), MIP Myrnyi (G4), MIP Saliut (G5), MIP Sotnyk (G6), MIP Azart (G7), and MIP Bohun (G8) had reliable higher yield (1.09–1.60 t/ha) compared to the first Myronivka variety Myronivskyi 86 (Table 2). In general, there is a noticeable significant variability in the yield of genotypes through the years. Several other researchers also have reported about high year-to-year, or site-to-site grain yield variation in Eastern European countries (Pržulj and Momčilović, 2012; Mirosavljević *et al*., 2014; Pržulj *et al*., 2015; Solonechnyi *et al*., 2018). For accurate interpretation of the genotype by environment data it is necessary to use the most appropriate statistical models (van Eeuwijk *et al*., 2016). The genotype main effects plus genotype by environment interaction (GGE biplot) model has been the most widely used for this purpose in different countries (Kendal and Doğan, 2015;

Meng *et al*., 2016; Bilgin *et al*., 2018; Kendal *et al*., 2019; Hudzenko *et al*., 2019).

Table 2. Grain yield of newly developed spring barley varieties compared to the first registered Myronivka variety Myronivskyi 86, t ha^{-1}

Code	Variety	Years of trial (code)					Mean
		2013	2014	2015	2016	2017	
		E13	E14	E15	E16	E17	
G1	Myronivskyi 86	3.21	4.17	6.27	4.57	3.98	4.44
G2	Virazh	3.83	5.01	7.50	7.13	5.08	5.71
G3	Talisman Myronivskyi	3.52	4.95	7.17	7.05	4.97	5.53
G4	MIP Myrnyi	4.12	5.35	7.81	7.41	5.23	5.98
G5	MIP Saliut	3.67	5.32	7.27	7.16	5.01	5.69
G6	MIP Sotnyk	3.62	5.12	7.24	7.22	4.83	5.61
G7	MIP Azart	3.82	5.45	7.39	7.38	5.19	5.85
G8	MIP Bohun	4.70	5.48	7.34	7.21	5.45	6.04
LSD_{05}		0.18	0.35	0.26	0.28	0.17	0.25

The first two principal components of the GGE biplot explained 97.57 % of genotype by environment interaction (Fig. 1). The environment E16 was characterized by the greatest discriminating ability. The environment E14 was the most representative, whereas E13 was the least representative. The environments E13 and E16 were the most distant from each other. The environments E14 and E17 were similar among themselves. GGE biplot "which-won-where" polygon view is effective to visualize the interaction patterns between genotypes and environments (Fig. 2). In our case, the first mega-environment is formed by the environments E13, E17, and E14. The variety MIP Bohun (G8) had advantage in it. The second mega-environment is formed by the environments E15 and E16. The varieties MIP Azart (G7), Virazh (G2), and MIP Saliut (G5) were more adapted to it. The variety MIP Myrnyi (G4) was located on the line which separated these two mega-environments. It is indicating that the variety MIP Saliut (G5) showed high performance in both of them. The varieties Myronivskyi 86 (G1), Talisman Myronivskyi (G3), and MIP Sotnyk (G7) had no advantages in the formed mega-environments. The variety MIP Bohun (G8) was characterized the maximal mean yield according to "mean yield against stability" view (Fig. 3). The poorest performance of both yield and stability was noted in the variety Mironovsky 86 (G1). Ranking the spring barley varieties relative to a hypothetical "ideal genotype", which conventionally is represented as the center of centric circles, shows that the varieties MIP Bohun (G8) and MIP Myrnyi (G4) were the nearest to it (Fig. 4)

In general, it should be noted that the new spring barley varieties Virazh (G2), Talisman Myronivskyi (G3), MIP Myrnyi (G4), MIP Saliut (G5), MIP Sotnyk (G6), MIP Azart (G7), and MIP Bohun (G8) significantly exceeded over the variety Myronivskyi 86 in both yield performance and adaptive reaction to changeable conditions through different years of trial.

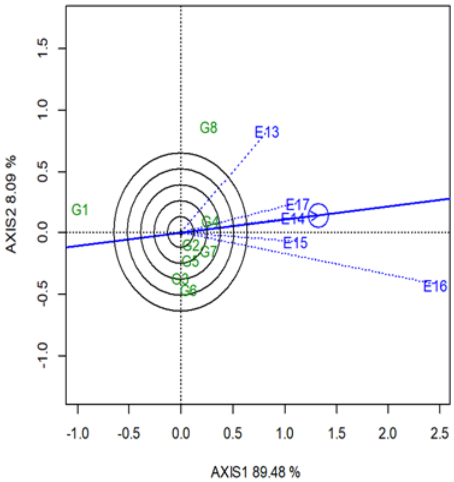

Figure 1. GGE biplot of discriminating ability and representativeness of test environments, 2013–2017

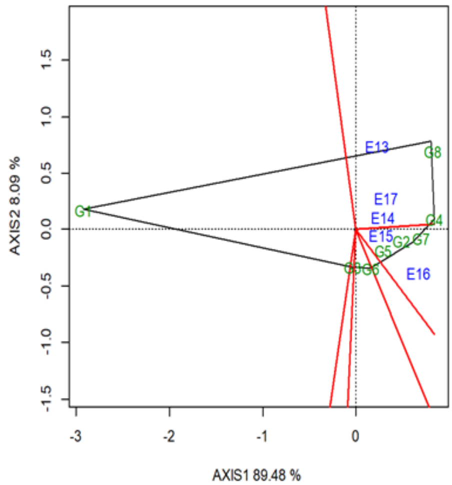

Figure 2. GGE biplot "which-won-where" polygon view for spring barley varieties and test environments, 2013–2017

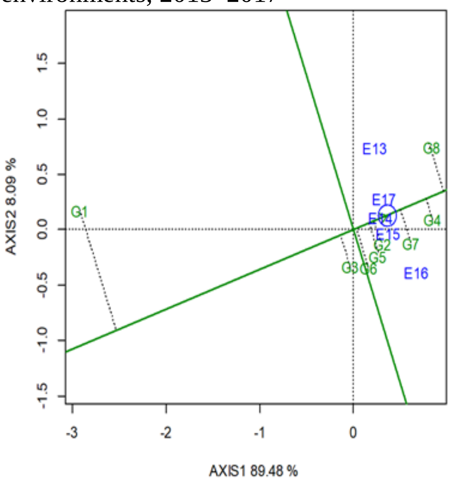

Figure 3. GGE biplot average environment coordination view of spring barley varieties for mean yield against stability, 2013–2017

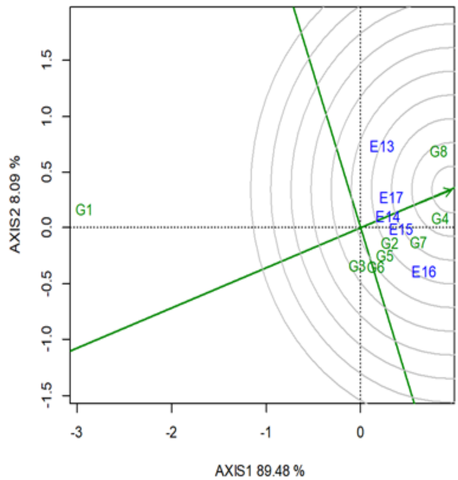

Figure 4. GGE biplot ranking spring barley varieties relative to an "ideal genotype", 2013–2017

Yield-related traits manifestation and genetic gain

All newly developed varieties predominated over the variety Myronivskyi 86 (G1) in number of productive tillers, despite the significant variability of this trait during years of trial (Table 3).

Table 3. Phenotypic manifestation and genetic gain for yield-related traits in newly developed spring barley varieties

Code	Years of trial, code					Mean	Statistical indices		
	E13	E14	E15	E16	E17		SD	H^2	ΔG
Number of productive tillers per plant (NPT)									
G1	1.40	1.59	2.11	2.02	1.60	1.74	-	0.80	-
G2	1.70	2.23	2.68	2.80	2.08	2.30	0.56		0.44
G3	1.97	2.89	3.38	3.42	2.67	2.87	1.12		0.90
G4	1.68	2.12	2.66	2.52	2.10	2.22	0.47		0.38
G5	1.64	2.11	2.63	2.51	2.05	2.19	0.45		0.36
G6	1.57	2.32	2.83	2.74	2.15	2.32	0.58		0.46
G7	1.91	2.41	3.17	2.95	2.35	2.56	0.81		0.65
G8	1.73	2.17	2.69	2.60	2.13	2.27	0.52		0.42
LSD_{05}	0.23	0.34	0.31	0.26	0.18	0.26	-	-	-
Kernel number per spike (KPS)									
G1	19.57	19.47	20.33	20.50	19.33	19.84	-	0.86	-
G2	22.03	23.80	24.43	23.57	22.50	23.27	3.43		2.95
G3	19.43	19.27	20.23	20.63	19.77	19.87	0.03		0.02
G4	26.37	29.60	28.47	27.37	26.60	27.68	7.84		6.74
G5	22.43	24.33	23.13	24.43	23.17	23.50	3.66		3.15
G6	22.65	24.13	22.63	22.63	22.20	22.85	3.01		2.59
G7	22.50	24.17	23.50	22.03	21.60	22.76	2.92		2.51
G8	23.47	26.37	23.57	23.57	24.47	24.29	4.45		3.82
LSD_{05}	1.95	1.99	1.73	1.48	1.30	1.69	-	-	-
Thousand kernel weight (TKW)									
G1	44.67	44.50	47.27	47.47	40.27	44.83	-	0.98	-
G2	51.00	51.00	52.90	51.73	44.67	50.26	5.43		5.32
G3	45.20	47.73	48.43	48.07	41.23	46.13	1.30		1.27
G4	50.37	49.57	51.37	50.13	46.13	49.51	5.29		5.19
G5	51.33	50.23	53.37	52.63	46.20	50.75	5.92		5.80
G6	47.97	47.40	49.33	48.73	42.10	47.11	2.27		2.23
G7	47.60	47.90	49.30	50.23	45.57	48.12	3.29		3.22
G8	48.18	47.67	49.13	51.83	46.33	48.63	4.25		4.17
LSD_{05}	0.45	0.36	0.56	0.46	0.43	0.45	-	-	-

It was observed the highest value of number of productive tillers in the variety Talisman Myronivskyi (G3) (2.87 tillers/plant). The selection differential for number of productive tillers varied from SD = 0.45 tillers/plant in the variety MIP Saliut to SD = 1.12 tillers/plant in the variety Talisman Myronivskyi (G3). The heritability coefficient was H^2 = 0.80. Thus, the genetic gain for this trait varied from ΔG = 0.36 tillers/plant in the variety MIP Saliut (G5) to ΔG = 0.90 in the variety Talisman Myronivskyi (G3). The varieties Virazh (G2), MIP Myrnyi (G4), MIP Saliut (G5), MIP Sotnyk (G6), MIP Azart (G7), and MIP Bohun (G8) had the advantage over the variety Myronivskyi 86 (G1) in kernel number per spike through all years of the trial. It was observed the highest value of kernel number per spike in the variety MIP Myrnyi (G4) (27.68 kernels). The same variety was characterized with the highest selection differential. The heritability coefficient for kernel number per spike was $H^2 = 0.86$. Accordingly, the genetic gain for this trait varied from $\Delta G = 6.74$ kernels in the variety MIP Myrnyi (G4) to $\Delta G = 0.03$ kernels in the variety Talisman Myronivskyi (G3). All new varieties had the advantage over the variety Myronivskyi 86 (G1) in thousand kernel weight. The maximal its value was noted in the varieties MIP Saliut (G5) (50.57 g) and Virazh (G2) (50.26 g). Thousand kernel weight had the highest heritability coefficient among the studied traits ($H^2 = 0.98$). The lowest genetic gain for this trait was in the variety Talisman Myronivskyi (G3) ($\Delta G = 1.27$ g). Its high value was noted in the varieties MIP Saliut (G5) ($\Delta G = 5.80$ g), Virazh (G2) ($\Delta G = 5.32$ g), and MIP Myrnyi (G4) ($\Delta G = 5.19$ g).

In general, in new spring barley varieties compared to the variety Myronivskyi 86 (G1) there was detected statistically confirmed genetic gain for studied traits. As an exception, it should be noted the variety Talisman Myronivskyi (G3), which had no genetic gain for kernel number per spike, and minimal its value in thousand kernel weight. Meanwhile, the Talisman Myronivskyi (G3) was characterized by the highest genetic gain in number of productive tillers. The most improved thousand kernel weight was noticed in the varieties MIP Salyut (G5) and Virazh (G2). The variety MIP Myrnyi (G4) combined the highest genetic gain in kernel number per spike with high its value in thousand kernel weight.

Genotype by yield*trait combination

To visualize the level of manifestation for a number of traits in genotypes some researchers used GT (genotype by trait) biplot (Al-Sayaydeh *et al.*, 2019). However, as it was mentioned, yield is the main integral trait which characterizes economic value of any commercial variety. Therefore, information about the combination of yield and other parameters is of significant practical importance. For genotype selection based on yield and trait complex combination a novel approach was proposed by Yan,and Frégeau-Reid (2018). It consists in modifying the experimental data of trials with multiplying yield performance and other economically important traits. For the first time in Eastern European conditions we have used this method for study of genetic gain in terms of yield*structural elements combination. In the first stage genotype and traits data were converted to GYT (genotype by yield*trait) table (Table 4). In this table the raw column is for yield by trait multiplication, the index column is for standardized GYT data. This is done by subtracting the mean and dividing the centered value by the standard deviation within the yield*trait combination. Mean GYT index is calculated from these standardized yield*traits data for each genotype.

The highest Mean GYT index was noted in the variety MIP Myrnyi (G4), the variety Myronivskyi 86 (G1) had the lowest one. The GYT biplot (Fig. 5, 6) graphically displays the standardized GYT data. The procedure for constructing GYT biplot are the same as for constructing GGE biplot except the term "environment" is replaced with "yield*trait" combination. In our case they are yield*number of productive tillers (YLD_NPT), yield*kernels per spike

(YLD_KPS), yield*thousand kernel weight (YLD_TKW). The first two principal components (AXIS1, AXIS2) of the GYT biplot explained 98.14 % variation of the genotype for yield*trait combination. The GYT biplot "which-won-where" shows that only two sectors contain both genotypes and yield*traits combinations (Fig. 5). The first sector contains the combinations YLD_KPS and YLD_TKW, as well as the varieties MIP Myrnyi (G4), MIP Bohun (G8), Virazh (G2), and MIP Saliut (G5).

Thus, these varieties combined yield performance with higher level of manifestation in kernel number per spike and thousand kernel weight. The second sector includes YLD_NPT and the variety Talisman Myronivskyi (G3). That is, this variety was characterized by combination of yield and number of productive tillers. The variety MIP Azart (G7) was in a narrow sector, which lies between two sectors mentioned above.

Table 4 Characteristics of spring barley varieties with genotype by yield*trait combination, 2013–2017

Code	Raw and index (standardized) value of genotype by yield*trait						
	YLD_NPT		YLD_KPS		YLD_TKW		Mean GYT index
	raw	index	raw	index	raw	index	
G1	0.08	-2.19	0.88	-1.80	1.99	-2.22	-2.07
G2	0.13	0.05	1.33	0.13	2.87	0.50	0.23
G3	0.16	1.18	1.10	-0.86	2.55	-0.48	-0.05
G4	0.13	0.11	1.66	1.55	2.96	0.79	0.81
G5	0.12	-0.23	1.34	0.17	2.89	0.56	0.17
G6	0.13	0.00	1.28	-0.07	2.64	-0.20	-0.09
G7	0.15	0.81	1.33	0.15	2.82	0.33	0.43
G8	0.14	0.29	1.47	0.73	2.94	0.71	0.58
Mean	0.13	0.00	1.30	0.00	2.71	0.00	-
σ	0.02	-	0.23	-	0.32	-	-

Notes: σ – standard deviation, YLD_NPT – yield*number of productive tillers, YLD_KPS – yield*kernel per spike, YLD_TKW – yield*thousand kernel weight, Mean GYT index – superiority index.

Thus, the variety MIP Azart (G7) differed from the varieties from the first and second sectors in the pattern of manifestation of yield*structural elements combination. The varieties Myronivskyi 86 (G1) and MIP Sotnyk (G6) are located in the sector with no yield*trait combination. It is indicating that these varieties had poorer performance than characterized above genotypes in yield and its combination with individual structural elements. Accordingly, to the GYT biplot ranking relative to the "ideal genotype" it is noticeable that the new varieties (G2…G8) had significant advantage over the variety Myronivskyi 86 (G1) (Fig. 6).

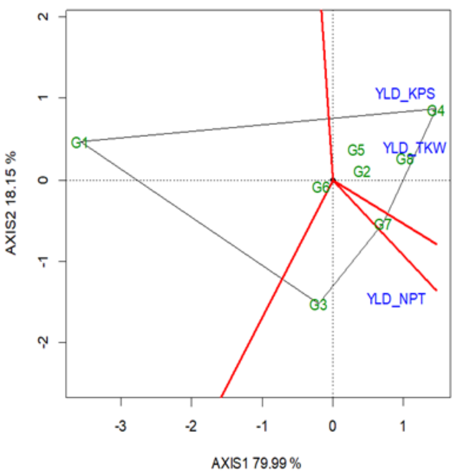

Figure 5. The "which-won-where" polygon view of the genotype by yield*trait (GYT) biplot

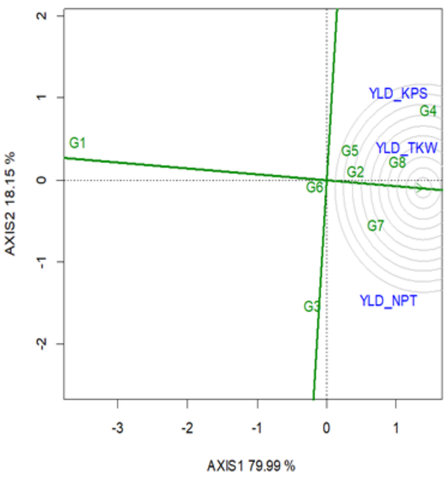

Figure 6. GYT biplot ranking spring barley varieties relative to an "ideal" genotype

The variety MIP Bohun (G8) was characterized the optimal combination of yield and its three main structural elements. The variety MIP Myrnyi (G4) was more displaced towards to YLD_KPS marker. The variety Talisman Myronivskyi (G3) was significantly displaced in the direction of YLD_NPT combination.

Thereby, the GYT biplot confirms the above-stated patterns for breeding and genetic improvement in the new spring barley varieties revealed when using statistical parameters for yield-related traits. In addition, it complements statistical indices with visual ability to analyze combination of yield and its structural elements.

CONCLUSIONS

As a result, our study proved statistically significant breeding and genetic improvement for yield and level of manifestation of yield-related traits in the newly developed spring barley varieties Virazh, Talisman Myronivskyi, MIP Myrnyi, MIP Saliut, MIP Sotnyk, MIP Azart, and MIP Bohun compared to the first Myronivka variety Myronivskyi 86. The spring barley varieties MIP Bohun and MIP Myrnyi were characterized with optimal combination of yield and its stability according to the GGE biplot model. However, using statistical and graphical analysis we revealed that the spring barley varieties Virazh, MIP Saliut and MIP Sotnyk had the highest genetic gain for thousand kernel weight, the variety Talisman Myronivskyi for number of productive tillers, and the variety MIP Myrnyi for kernel number per spike and thousand kernel weight. The variety MIP Azart was differed from the other varieties in the ratio of main yield structural elements. Thus, the newly developed spring barley varieties significantly differed from each other with their combination of yield and its structural elements. According to GYT biplot model the spring barley variety MIP Bohun was the nearest to the “ideal genotype” in terms of yield*traits combination. The practical worth of the identified patterns is that the new varieties, due to the relatively different pathways of yield formation, will complement each other under unfavorable environmental factors under production conditions.

On the whole, it is shown effectiveness of combining statistical (breeding equation) and graphical (GGE biplot, GYT biplot) approaches to comprehensive evaluation the breeding improvement for yield and yield-related traits in new genotypes compared to varieties created in the previous period.

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GERMINATION, SEEDLING GROWTH AND PHYSIO-BIOCHEMICAL INDICES OF BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES UNDER PEG INDUCED DROUGHT STRESS

SUMMARY

The establishment of seedlings at early growth stages of plants is one of the important determinants for higher yield potential under stress conditions. Therefore, high germination rate and vigorous seedling growth under water stress conditions is preferred to increase productivity. Considering this issue, an experiment was conducted to know the response of drought stress on the germination and seedling growth traits of available wheat cultivars. The study was carried out at the Laboratory of Horticulture in Agriculture Faculty, Cukurova University, Turkey. Seeds of five bread wheat genotypes viz. Wafia, Lucilla, Envoy, DZ19-2 and DZ19-1 were placed and grown in Petri-dishes under polyethylene glycol (PEG 6000) induced drought stresses of 0 (control=distilled water), -0.2, -0.4 and -0.6 MPa conditions for evaluation the properties of germination and seedling growth. Regarding the root length and dry weight, the genotypes Wafia, Lucilla and DZ19-1were found superior and seemed to be relatively tolerant to drought stress over other genotypes. Regarding biochemical analysis, the proline content increased with increasing drought levels, where the maximum proline was observed in the DZ19-1 genotype. Due to drought stress, the shoot proline content increased in drought-tolerant wheat genotypes whereas the proline level decreased in the sensitive ones compared to the control. Considering the study, the genotype DZ19-1 can be treated as drought tolerant genotype, and further investigations are needed, however, to support our understanding of the drought stress effects during the whole life cycle of wheat.

Keywords: Water stress, growth indices, biochemicals, wheat genotypes, proline

INTRODUCTION

Crop production is usually restricted by abiotic stress factors such as drought, temperature, and salinity. During crop production, seed germination and seedling establishment are the most sensitive stages to abiotic stresses particularly

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to water deficit stress (Patade *et al*., 2011; Kizilgeci *et al*., 2020). The incidence of drought is increasing day by day owing to global warming which ultimately affecting the agriculture. Drought stress at germination stage reduced the seedling emergence and establishment (El Sabagh *et al*., 2019; Salih and Tuncturk, 2020). Drought stress delays seed germination declines germination rate, and also significantly changes the physiology and biochemistry in seedling (Bateman *et al.*, 2016). Extreme water shortage causes a considerable reduction of morpho-physiological and metabolic activities in plants, and ultimately reduces yield and quality of crop (Aslam *et al*., 2013). In some cases, plants exhibit adaptation to withstand water stress (Mickky and Aldesuquy, 2017). Under drought stress, the morphology, physiology, biochemistry in a plant would be adversely changed (Duan *et al*., 2017). Germination, a very sensitive stage in plants life cycle (as it determines the degree of crop establishment), is severely affected by salinity (Vardar *et al*., 2014).

Wheat is a major cereal crop in many parts of the world, and it is commonly known as king of cereals. Wheat grain yield is depressed by environmental stresses such as drought, heat, low temperatures, low fertility (especially nitrogen) and soil salinity (Barutcular *et al*., 2017; Dončić *et al*., 2019; Panfilova, and Mohylnytska, 2019; Guo *et al*., 2020; Zaman *et al*., 2020). Therefore, a great attention has been made to produce wheat under various environmental stresses (Mickky and Aldesuquy, 2017; Mubeen *et al*., 2020 Popović *et al*., 2020). Drought is a deleterious factor among environmental stresses that can reduce wheat yield (Abbasi *et al*., 2015; Barutçular *et al*., 2017).
There are many factors which are greatly influenced the growth and productivity of crops by alleviating the drought stress effects, and selection of and cultivation drought tolerant cultivars is the most important one. Therefore, existing wheat varieties should be evaluated under drought stress conditions at seed germination and early seedling growth stages, and promising drought tolerant varieties should be recommended for crop cultivation (Alom *et al*., 2016). Hence, screening of wheat genotypes should be made under naturally or artificially induced drought stress, and polyethylene glycol (PEG 6000) is used to induce artificial drought stress. PEG is a flexible, water-soluble polymer, and creates very high osmotic pressures. These properties make PEG one of the most useful molecules for applying osmotic pressure in biochemistry and bio-membranes experiments, in particular when using the osmotic stress technique (Partheeban *et al*., 2017; Wikipedia, 2020). The present study was, therefore, undertaken to evaluate and select tolerant wheat genotypes for verifying their responses under different levels of PEG induced drought stress during the seed germination and seedling growth stages.

MATERIAL AND METHODS

The study was carried out at the Horticulture Laboratory, Faculty of Agriculture, Cukurova University, Turkey. The form of experiment was factorial which was laid out in a completely randomized design (CRD) with thrice in replication. Five bread wheat genotypes (Wafia, Lucilla, Envoy, DZ19-1 and

DZ19-2) were placed to petri dishes under four levels of PEG induced drought stresses viz. control (distilled water), -0.2, -0.4 and -0.6 MPa under laboratory conditions to study the properties of germination and early seedling growth.

Seeds of different wheat genotypes were treated with a 5% solution of sodium hypochlorite for 10 min, and residual chlorine effect was removed by washing the seeds thoroughly with distilled water. The 20 seeds were placed on two layers of Whatman no. 2 filter paper with 12 cm diameter Petri-dishes. Day and night lengths were 18/6 h, with 24±1°C. Drought conditions were created by using PEG-6000 according to the treatment specification.

The seedlings were grown up to 7 days and then harvested. The germination and seedling growth traits like seed vigor, shoot and root lengths, coleoptile length, fresh weight of shoot and root fresh weight were measured. Turgor weight, shoot and root dry weight were measured for each plant after drying the samples in an oven at 72°C temperature for 48 h, and the relative water content (RWC) was calculated by using the following formulae:

RWC = (FW - DW) x 100 / (TW - DW)

Where, FW: Fresh weight, DW: Dry weight and TW: Turgid weight.

The proline content was measured following the protocol of Bates *et al.* (1973). The optical density of the extracted sample was recorded at 528nm wavelength in spectrophotometer, and the proline was calculated using a calibration curve of L-proline concentration (0- 5µg/ml).

The chlorophyll (a, b, and total), and carotenoid contents were measured following the method developed by Agarwal (1986) with a slight modification by Higazy *et al.* (1995). The absorbance reading was taken from spectrophotometer at 480, 649 and 665 nm wavelengths.

The data were analyzed by partitioning the total variance with the help of a computer using MSTAT-C program (Gomez and Gomez, 1984). Duncan's Multiple Range Test was used to compare the treatment means (Duncan, 1955).

RESULTS AND DISCUSSION

The results of the germination and various growth characteristics of wheat seedlings under different drought stresses are shown in Table 1. Data revealed that drought stress adversely affected the seed vigor, root, coleoptile and seedling lengths, fresh and dry weights of root and shoot, RWC, proline, chlorophyll, and carotenoid content. The result of the present findings corroborates with the results of Partheeban *et al*. (2017), Kizilgeci *et al.* (2017), Guo *et al.* (2020), where they also noted that water deficit stress suppressed the membrane integrity by stimulating the lipid peroxidation as significant increase of membrane leakage.

Germination under drought condition is very important because it reflects the seedling establishment and crop yield (Mwale *et al*., 2003). Drought is a serious threat in agriculture among the abiotic stresses that limits the seed germination, seeding growth, plants growth and yield (Aslam *et al.*, 2013; El Sabagh *et al*., 2019). The response of drought stress on the wheat cultivars varied regarding the germination rate, and germination index, used as drought tolerance indices at germination stage (Öztürk *et al.*, 2016).

Table 1. Analysis of variance for seedling growth properties for genotypes and PEG concentrations

SOV	*df*	RL	CL	SL	RFW	SFW	RDW	SDW	RWC	SV	Proline	*Chl-a*	*Chl-b*	Cd	Total Chl
Genotypes	4	**	**	**	*	**	**	**	ns	**	**	**	**	**	**
Stresses	3	**	**	**	**	**	**	**	**	**	**	**	**	**	**
G×S	12	ns	ns	ns	**	*	ns	**	**	ns	**	**	**	**	**
CV (%)		13.71	8.02	14.25	25	13.2	18.18	9.09	18.75	10.57	24.4	9.61	17.39	12.18	9.26

*= 5% level of significance; ** = 1% level of significance; ns: not significant SOV: Source of variance, RL: Root length, CL: Coleoptile length, SL: Seedling length RFW: Root fresh weight SFW: Seedling fresh weight RDW: Root dry weight SDW: Seedling dry weight RWC: Relative water content SV: seedling vigour *Chl-a : chlorophyll-a Chl-b: chlorophyll-b* Cd: Carotenoid

Root length

Under drought stress condition, behaviour of roots is an important trait that triggers to other growth traits, and plants under drought stress enhance deeper roots to absorb available water in the soil which enables higher drought tolerance (Španić *et al*., 2017). The ANOVA of root length of bread wheat genotypes during the germination period under different levels of PEG-6000 induced stresses is presented in Table 1, whereas its mean values are given in Table 2. The differences between genotypes and concentrations were found significant at 1% level. Statistically insignificant interaction between genotypes × levels of PEG-6000 induced stress was observed. In the present study, the root length decreased significantly with increasing drought stress among the genotypes (Table 2). However, the shortest root length (8.45 cm) was recorded at DZ19-2 genotype as compared to other genotypes as they showed statistically similar root length. The genotype DZ19-1 showed the longest root lengths numerically (10.65 cm). The longer root length provides an advantage of plant roots to go deep in soil for uptake of water and nutrients. In general, it can be argued that DZ19-1, Wafia and Lucilla genotypes tolerated drought at all levels of drought stress. Nature of root length under drought stress is considered as an important index to select drought tolerant genotypes (Turner, 1997), considering the root morphology and growth rate (Malik *et al*., 2002). Remarkable decrease in root length with increasing PEG concentrations has been observed by Jajarmi *et al*. (2009). Under drought stress, plant could show the relative adaptability in terms of morphological, physiological, and biochemical changes in order to ensure the relatively stable growth of plant, for example, reduced leaf area and increased root activity (Mwadzingeni *et al.*, 2016; Duan *et al.*, 2017). Root is the main organ of plant to absorb water and nutrient, which reacts against drought stress at first (Janiak e*t al.*, 2015; Duan *et al*., 2017).

Table 2. Response of PEG 6000 induced drought stress on the root length (cm) of wheat genotypes

		Levels of stress (MPa)				
	Genotypes	**0 (control)**	**-0.2**	**-0.4**	**-0.6**	**Mean**
Root length (cm)	**DZ19-2**	12.62	7.92	7.55	5.72	8.45 b
	Lucilla	12.86	12.02	9.03	6.24	10.04 a
	DZ19-1	14.29	11.49	9.81	7.03	10.65 a
	Envoy	12.80	11.53	9.31	6.23	9.97 a
	Wafia	14.83	11.61	8.30	5.70	10.11 a
	Mean	13.48 a	10.91 b	8.8 c	6.18 d	

Means within columns followed by different lowercase letters are significantly different

Table 3. Response of PEG 6000 induced drought stress on the coleoptile length (cm) of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Coleoptile length (cm)	**DZ19-2**	2.65	2.74	3.17	2.81	2.84 a
	Lucilla	2.41	2.55	2.75	2.43	2.54 c
	DZ19-1	2.75	3.05	2.96	2.96	2.93 a
	Envoy	2.79	2.89	2.86	2.48	2.76 ab
	Wafia	2.49	2.73	2.82	2.52	2.64 bc
	Mean	2.62 c	2.79 ab	2.91 a	2.64 bc	

Means within columns followed by different lowercase letters are significantly different

Table 4. Response of PEG 6000 induced drought stress on the seedling length (cm) of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Seedling length (cm)	**DZ19-2**	12.15	10.13	10.85	7.11	10.06 b
	Lucilla	14.73	12.19	10.69	7.43	11.26 ab
	DZ19-1	15.81	13.65	12.46	8.16	12.52 a
	Envoy	12.54	12.19	10.01	6.90	10.41 b
	Wafia	16.10	10.67	9.68	6.83	10.82 b
	Mean	14.27 a	11.77 b	10.74 b	7.28 c	

Means within columns followed by different lowercase letters are significantly different

Coleoptile length

The differences between genotypes and stress levels were determined at 1% level of significance but the interaction between genotypes × PEG-6000 stress was found statistically insignificant (Table 1). The coleoptile length of wheat cultivars was significantly influenced by the imposition of drought stress, and it decreased considerably with the increasing concentration of PEG6000 induced stresses (Table 3). However, the coleoptile lengths were seen to increase up to-0.4 MPa stress, and thereafter decreased at -0.6 MPa stress, but the values were higher over control condition. This shows that wheat genotypes tend to increase coleoptile lengths in response to drought stress. Wheat genotypes showed differential responses due to magnitude of drought stresses. The genotype DZ19-1 produced the highest coleoptile length which was statistically similar to Envoy and DZ19-2 under drought stress, while the lowest was recorded for Lucilla. Drought stress suppressed the seed germination which retarded the growth of young seedling, reduced the length of plumules and radicles, fresh weight of seedlings and relative water content of wheat genotypes (Mickky and Aldesuquy, 2017).

Seedling length

The variations among the genotypes as well as stress levels were found to be significant at 1% level, but the interaction between genotypes × PEG-6000 concentrations was found to be statistically insignificant (Table 1). Wafia genotype had the longest shoot lengths (16.10 cm) under control conditions followed by DZ19-1 (15.81 cm). As drought stress increased, there was a noticeable decline in seedling length. The shortest shoot length (6.83 cm) was observed in Wafia genotype under severe stress condition, which had the highest shoot length under control conditions demonstrating its sensitivity to drought conditions (Table 4). The genotype DZ19-1 had the longest shoot lengths of 8.16 and 12.52 cm under control condition and drought stress of -0.6 MPa, respectively indicating that DZ19-1is resistant to drought stress. Similarly, in their research, Kizilgeci *et al.* (2017) reported that PEG induced stress of -1.2 MPa caused a drastic reduction of seedling length in comparison to -0.3 MPa stress. Drought stress severely reduced the seedling growth of wheat genotypes (Španić *et al*., 2017).

Root fresh weight

The root fresh weight of all wheat genotypes was markedly decreased with the increasing PEG induced stress over control. The mean root fresh weights of genotypes decreased with increasing the drought stress concentration (Table 5). The genotype Wafia had the highest root fresh weight under the control condition which was followed by DZ19-2 and Lucilla, while the highest root fresh weight (50.0 mg) was observed in DZ19-1, and the lowest (16.8 mg) was in Lucilla genotype at -0.6 MPa stress condition. DZ19-1 was observed to maintain the maximum root fresh weight compared to other genotypes under increased drought stress, although it maintained the minimum root fresh weight (46 mg) at the control condition and can be treated as drought stress resistant genotype.

Seedling fresh weight

The Seedling fresh weight was significantly affected by the PEG induced drought stress, genotypes, and their interaction (Table 1). The differences among the genotypes and stress levels were highly significant (1% level), and their interaction effect was also significant (5% level). There was no statistical difference between control and -0.2 MPa induced stress on the seedling fresh weight, while the average seedling fresh weight decreased as the stress level increased. Nonetheless, DZ19-1 genotype obtained the highest seedling fresh weight (42.9 mg) under severe drought stress, indicating its tolerance to drought stress (Table 6). These results were adhering to the results of Sayar *et al*. (2010) and Španić *et al*. (2017), who reported that the seedling fresh weight of wheat genotypes decreased with increasing the osmotic stress.

Root dry weight

The root dry weight was influenced significantly (1% level) by different genotypes, and stress levels whereas no remarkable variation regarding the root dry weight was observed by the interaction effect of genotypes and stress levels (Table 1)

Table 5. Response of PEG 6000 induced drought stress on the root fresh weight (mg)of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Root fresh weight (mg)	**DZ19-2**	68.2a	44.0ef	50.7cf	21.6gh	46.1bc
	Lucilla	68.4ab	53.1c-f	45.5def	16.8h	45.9bc
	DZ19-1	46.0def	36.1fg	37.8fg	50.0c-f	42.5c
	Envoy	61.8a-d	47.7c-f	58.1a-e	43.0ef	52.7ab
	Wafia	73.8a	64.2abc	48.9c-f	38.0fg	56.2a
	Mean	63.7a	49.0b	48.2b	33.9c	

Means within columns followed by different lowercase letters are significantly different

Table 6. Response of PEG 6000 induced drought stress on the seedling fresh weight (mg) of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Seedling fresh weight(mg)	**DZ19-2**	67.2a	67.8ab	59.4bcd	33.7h	57.0a
	Lucilla	41.4fgh	54.2cde	49.6d-g	35.6h	45.2b
	DZ19-1	72.1a	63.5abc	56.1bcd	42.9e-h	58.7a
	Envoy	74.4a	56.8bcd	52.9c-f	37.4gh	55.4a
	Wafia	64.4abc	62.7abc	49.5d-g	37.7gh	53.6a
	Mean	63.9a	61.0a	53.5b	37.5c	

Means within columns followed by different lowercase letters are significantly different

The root dry weight decreased with the increasing drought stress levels. The genotype Envoy maintained the highest and lowest root dry weight (15.7 and 11.1 mg) both at control and -0.6 MPa PEG conditions, respectively (Table 7). The minimum root dry weight was recorded in Wafia genotype at severe water stress (-0.6 MPa) which reduced root dry weight by 42.85% over control, whereas the reduction was only by 31.25% in Envoy genotype at same condition. In root development, Envoy, DZ19-1 and Wafia genotypes showed better results in stress conditions compared to other genotypes when examined in length and age. The decreasing trends of the shoot and root dry weight with increasing drought stress was reported previously by Chachar *et al*. (2014) and Molla *et al*., (2019). Our results are line with the results of Jatoi *et al.* (2014), who concluded that the root dry weight of wheat genotypes was reduced gradually with the increasing PEG concentrations.

Seedling dry weight

Seedling dry weight is the consequence of plant physiological and biological activities. Seedling dry weight values were decreased with the increase of PEG6000 concentrations in all wheat genotypes. Differences among the genotypes, stress levels, and their interaction were found to be significant at 1 % level. According to the results, the shoot dry weight ranged from 8.2 mg to 16.8 mg owing to the interaction between stress levels and genotypes (Table 8). When the differences among water stress levels were analysed, the seedling dry weight decreased statistically with the imposing of moderate and severe (-0.6 MPa) drought stresses. The Wafia genotype, which had the highest seedling dry weight under control conditions, was found to reduce drastically (52.94%) at -0.6 MPa compare to other genotypes. Islam *et al.* (2018) depicted that the seedlings dry weight decreased with the increasingPEG6000 induced stress levels in rice genotypes. Rana *et al.* (2017) found a wide difference among wheat genotypes to stress tolerance based on their tolerance index for seedling dry weight.

Relative water content

Relative water content (RWC) was used as a measure of drought tolerance indicator. PEG-induced drought stress considerably reduced the RWC in the shoot over control (Table 9). The differences among the stress levels were significant regarding the RWC at the level of 5%, and the interaction between PEG-6000 induced stress and genotypes was found significant at the level of 1%. However, the differences among the genotypes were found statistically insignificant. From the study, it is shown that the RWC decreased with the increment of drought stresses. Among all, the genotype Lucilla showed the highest RWC at severe stress conditions. Many researchers (Yassin *et al*., 2019; Monsur *et al*., 2020) confirmed that proline is accumulated in crops under stress environment. Drought stress induced by PEG decreased the water status in shoot which is consistent with the study of Guoxiong *et al.* (2002), who reported that PEG induced drought stress significantly decreased the RWC. It is reported earlier that larger seeds produced longer root lengths that help to uptake more water resulting in higher RWC in shoot (Kaydan and Yagmur, 2008)

Seed vigour

Vigour test does not only measure the percentage of viable seeds in a sample, it also reflects the ability of those seeds to produce normal seedlings under adverse growing conditions alike to field conditions. The differences among the genotypes and water deficit stresses regarding the seed vigour were found significant at 1% level, but their interactions were found statistically insignificant (Table 1).

Table 7. Effect of PEG 6000 induced drought stress on the root dry weight (mg) of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Root dry weight (mg)	**DZ19-2**	11.3cde	8.3e	8.2e	7.6e	8.8d
	Lucilla	15.2ab	16.7a	15.6ab	9.1de	14.1a
	DZ19-1	13.2abc	10.9cde	12.4bcd	10.2cde	11.7bc
	Envoy	15.7ab	13.8abc	13.2abc	11.1cde	13.4ab
	Wafia	14.0abc	13.0abc	10.3cde	8.1e	11.3c
	Mean	13.9a	12.5ab	11.9b	9.2c	

Means within columns followed by different lowercase letters are significantly different

Table 8. Response of PEG 6000 induced drought stress on the seedling dry weight (mg) of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Seedling dry weight (mg)	**DZ19-2**	14.8abc	16.4ab	12.3c-f	7.8j	12.8a
	Lucilla	12.1d-g	11.1e-h	9.6g-j	8.2ij	10.2c
	DZ19-1	12.7cde	11.3d-h	12.3c-f	8.2ij	11.1bc
	Envoy	13.9bcd	9.9f-j	9.4hij	8.9hij	10.5bc
	Wafia	16.8a	10.6e-i	11.0e-h	8.3ij	11.7ab
	Mean	14.1a	11.9b	10.9b	8.3c	

Means within columns followed by different lowercase letters are significantly different

Table 9. Effect of PEG 6000 induced drought stress on the relative water content of wheat genotypes

	Genotypes	Levels of stress (MPa)				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Relative water content (%)	**DZ19-2**	0.86 ab	0.72 b-e	0.55 e-h	0.51 f-h	0.66
	Lucilla	0.97 a	0.70 b-g	0.67 b-h	0.55 e-h	0.72
	DZ19-1	0.76 b-d	0.62 c-h	0.57 d-h	0.52 f-h	0.62
	Envoy	0.82 a-c	0.73 b-e	0.48 h	0.47 h	0.62
	Wafia	0.71 b-f	0.71 b-f	0.51 f-h	0.50gh	0.60
	Mean	0.82 a	0.70 ab	0.56 b	0.51 b	

Means within columns followed by different lowercase letters are significantly different

The average seed vigour decreased statistically with the increasing levels of drought stress. When the average seed vigour among genotypes was examined, the highest germination was observed in Envoy genotype, and the lowest was in

DZ19-1, which was statistically similar to DZ19-2, Wafia, and Lucilla genotypes (Table 10). The size of seed is positively correlated with the seed vigor, and larger sized seeds have a tendency to produce more vigorous seedlings (Ries and Everson, 1973). It was pointed out that bolder seeds produced vigorous seedlings, taller plants with greater tillering, and higher dry matter as compared to smaller seeds under drought stress (Westoby *et al.,* 1996).

Table 10. Response of PEG 6000 induced drought stress on the seed vigour (%) of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Seed vigour (%)	**DZ19-2**	75.00	83.33	83.33	58.33	75.00 b
	Lucilla	91.67	85.00	75.00	73.33	81.25 b
	DZ19-1	88.33	90.00	80.00	61.67	80.00 b
	Envoy	98.33	93.33	85.00	80.00	89.17 a
	Wafia	98.33	88.33	78.33	58.33	80.83 b
	Mean	90.33 a	88.00 a	80.33 b	66.23 c	

Means within columns followed by different lowercase letters are significantly different

Proline

Proline is one of the most important metabolites which acts as cytosolute different types of environmental stresses like drought, high temperature, nutrient deficiency, exposure of heavy metals, and high acidity induced to accumulate proline for adaptation of plants (Oncel *et al*., 2000; Chen and Murata, 2002; Yasar *et al*., 2006; Wang, 2012).

The increased proline content under drought helps for osmotic adjustment. Genotypes, PEG-6000 induced stresses, and their interaction were statistically significant at 1% level (Tables 1 & 11). At increased PEG concentrations, the amount of proline increased compared to the control condition. However, the DZ19-1genotype accumulated the highest amount of proline (2.19 micromol/g) under control condition, and maintained the similar trends at all stress levels, indicating it as a drought tolerant genotype. The genotype Envoy produced the lowest proline content under stress conditions (Table 11). Plants under drought produce compatible solutes to maintain cell turgor, which results in reduced osmotic stress in plants (Silva *et al*., 2010). One of the important compatible solutes is proline, which acts as a protectant to defend cell membrane and also as an ROS scavenger that could provide energy for growth and survivability under stress condition (Sankar *et al*., 2007; Rezayian *et al*.,2018).

Chlorophyll a

The chlorophyll a (*Chl a*) content was significantly influenced by the genotypes and drought stress, and their interaction between drought stress and genotype. The values of *Chl a* decreased with the increase of drought stresses, and the lowest values were recorded at the severe drought stress in all the genotypes. Nevertheless, the genotypes DZ19-2 and DZ19-1 showed the highest amount of *Chl a* (0.90 and 0.83 mg/g, respectively) under control conditions well as under severe drought condition (Table 12). Chl content is known as an index of photosynthates for the evaluation of source (Zobayed *et al*., 2007), therefore decrease of *Chl a* can be considered as a non-stoma limiting factor in the drought stress conditions (Khayatnezhad and Gholamin, 2012). Stress can cause a change in chlorophyll content of plant leaves, and thus a change of photosynthetic function (Qian *et al*., 2003). Manivannan *et al*. (2007) concluded that *Chl a*, *Chl b*, and the total chlorophyll contents in sunflower varieties are considerable reduced due to drought stress.

Table 11. Response of PEG 6000-induced drought stress on the proline content of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Proline (micromol/g)	**DZ19-2**	0.81 e	1.34 de	2.40 ab	2.31 ab	1.72 b
	Lucilla	1.36 de	1.20 de	1.21 de	1.60 cd	1.35 cd
	DZ19-1	2.19 bc	2.67 ab	2.95 a	2.44 ab	2.56 a
	Envoy	1.00 de	0.95 de	1.27 de	1.16 de	1.10 d
	Wafia	1.01 de	1.26 de	0.96 de	2.72 ab	1.49 bc
	Mean	1.27 c	1.49 bc	1.76 ab	2.05 a	

Means within columns followed by different lowercase letters are significantly different

Chlorophyll-b

Drought stress had remarkable differential influence on the chlorophyll b (*Chl b*) content in the shoots. The *Chl b* was also significantly (1%) influenced by the interaction between PEG-6000 and genotypes (Table 1 & 13). DZ19-2 genotype accumulated the highest amount of *Chl b* in control condition (0.47 mg/g), and the lowest in drought stress of -0.6 MPa (0.21 mg/g). However, the DZ19-1 genotype showed the minimum resistance as against the drought stress of both -0.4 and -0.6 MPa. The minor changes in chlorophyll content and the stability of chlorophyll a/b ratio allow to conclude that the pigment apparatus is comparatively resistant to drought in wheat cultivars (Nikolaeva *et al*.,2010). Decreased or unchanged chlorophyll level during drought stress has been reported in many species, depending on the duration and severity of drought stress (Hu *et al*., 2007; Xu *et al*., 2020).

Total chylorophyll

Chlorophyll fluorescent is used as a stress tolerant index to study the effects of different kinds of stresses (Zobayed *et al*., 2007).Total chlorophyll content was remarkably influenced by the wheat genotypes; PEG-6000 induced drought stress, and their interaction (Table 1 & 14). In this study, the DZ19-2 genotype had the highest amount of chlorophyll under control. Although, the genotype Envoy protect its chlorophyll content as against the increased drought stress levels (like DZ19-2), and it was also statistically similar to DZ19-2 and

Envoy. The decrease in chlorophyll content under drought stress has been considered a typical symptom of oxidative stress, and Pigment photo-oxidation and chlorophyll degradation are responsible for diminishing the chlorophyll content in drought stress, and is considered as a typical symptom of oxidative stress (Sharif and Mohammadkhani, 2016).A similar dependence of chlorophyll content in dehydrated leaves was previously observed in barley seedlings by Pshibytko *et al*. (2004). The chlorophyll content in the non-stress treatment is higher than that of the drought stress treatments (Xu *et al*., 2020).

Table 12. Response of PEG 6000 induced drought stress on the chlorophyll a of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Chlorophyll a (mg/g)	**DZ19-2**	0.90 a	0.59 b-d	0.52 d	0.51 d-f	0.63 a
	Lucilla	0.55 cd	0.41 g	0.39 g	0.25 h	0.40 d
	DZ19-1	0.83 a	0.63 bc	0.30 h	0.42 fg	0.55 b
	Envoy	0.68 b	0.51 de	0.67 b	0.43 e-g	0.57 b
	Wafia	0.65 b	0.41 g	0.42 fg	0.40 g	0.47 c
	Mean	0.72 a	0.51 b	0.46 c	0.40 d	0.52

Means within columns followed by different lowercase letters are significantly different

Table 13. Response of PEG 6000 induced drought stress on the chlorophyll b of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Chlorophyll b (mg/g)	**DZ19-2**	0.47 a	0.28 c-e	0.22 e-ı	0.21 e-ı	0.29 a
	Lucilla	0.24 d-h	0.19 g-ı	0.20 f-ı	0.20 g-ı	0.21 b
	DZ19-1	0.33 bc	0.25 d-g	0.16 ı	0.17 ı	0.23 b
	Envoy	0.36 b	0.30 b-d	0.27 c-f	0.17 ı	0.27 a
	Wafia	0.21 e-ı	0.17 hı	0.20 e-ı	0.19 g-ı	0.20 b
	Mean	0.32 a	0.24 a	0.21 bc	0.19 c	

Means within columns followed by different lowercase letters are significantly different

Carotenoid

Genotypes, PEG-6000 induced drought stresses, and their interaction were found significant at 1% level on the carotenoid content. Differential responses among the genotypes regarding carotenoid content were recorded under control and PEG induced drought stress conditions. The carotenoid content gradually decreased with increasing drought stress from 0 (Control) to -0.6 MPa. However, at the highest level of drought stress (-0.6 MPa), the genotype DZ19-2 recorded the highest carotenoid content (0.17 mg/g), which was statistically identical with Envoy, DZ19-1 and Wafia genotypes. In previous reports, Shmat'ko and Shvedova (1977) revealed that drought resistant wheat varieties exhibited a minor or no change in the pigment content compared to drought susceptible varieties.

The result differs from those of Wang (2003), who reported that the carotenoid content in leaves of winter wheat increased under drought stress.

Table 14. Response of PEG 6000 induced drought stress on the total chlorophyll of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Total chlorophll(mg/g)	**DZ19-2**	1.72 a	1.13 de	0.93 f	0.89 fg	1.17 a
	Lucilla	1.01 ef	0.74 gh	0.74 h	0.54 j	0.76 d
	DZ19-1	1.50 b	1.13 de	0.58 ij	0.73 h	0.99 b
	Envoy	1.30 c	1.38 bc	1.23 cd	0.75 gh	1.16 a
	Wafia	1.10 de	0.73 hi	0.78 gh	0.73 h	0.83 c
	Mean	1.33 a	1.02 b	0.85 c	0.73 d	0.98

Means within columns followed by different lowercase letters are significantly different

Table 15. Response of PEG 6000 induced drought stress on the carotenoid content of wheat genotypes

	Genotypes	**Levels of stress (MPa)**				
		0 (control)	**-0.2**	**-0.4**	**-0.6**	**Mean**
Carotenoid (mg/g)	**DZ19-2**	0.36 a	0.26 cd	0.19 fg	0.17 f-h	0.25 a
	Lucilla	0.21 ef	0.15 hi	0.14 hi	0.10 j	0.15 c
	DZ19-1	0.33 ab	0.25 cd	0.11 ij	0.14 hi	0.21 b
	Envoy	0.25 cd	0.16 gh	0.29 bc	0.15 g-i	0.21 b
	Wafia	0.24 de	0.14 hi	0.16 gh	0.14 hi	0.17 c
	Mean	0.28 a	0.19 b	0.18 b	0.14 c	

Means within columns followed by different lowercase letters are significantly different

CONCLUSIONS

Water stress significantly reduced the germination, seedling growth and physiological properties in all wheat genotypes, and the genotype DZ19-1 showed the best performance against the drought stress. The proline level was increased due to drought stress, and the highest proline levels were observed in the DZ19-1 genotype. Among the tested genotypes, DZ19-1 can be treated as a drought tolerant genotype due to performing most excellent growth during seedling stage under drought conditions.

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EFFECT OF THE VEGETATION CYCLE ON TOTAL PHENOLIC AND FLAVONOID COMPOUNDS IN *Hypericum perforatum L.* AND *Melissa officinalis L.* COLLECTED IN MONTENEGRO

SUMMARY

Effect of vegetation cycle on total phenolic content (TPC) and total flavonoid content (TFC) in 80% methanolic and 50% ethanoic extracts in wild growing *Hypericum perforatum* L. and *Melissa officinalis* L. from different habitats in Montenegro was analyzed.

It was found that the type of extraction solvent and the altitude of selected habitats affect the value of the detected TPC and TFC. The highest TPC and TFC values were found in 80% methanolic extract of H1 locality in the month of July (16.66 mgGAE/gDW and 6.91 mgQE/gDW) as well as of M1 locality in the month of August (41.66 mgGAE/gDW and 11.07 mgQE/gDW) while 50% ethanolic extracts showed lower TPC and TFC content.

The TPC and TFC values of *H. perforatum* L. extracts were found to be highly variable in quantity and their dynamics was found irregular, while for the *M. officinalis* L. extracts the TPC and TFC were found to change in quantity in a regular manner during the plants vegetation cycle with the peak in the flowering stage (August), while the lowest concentration of these bioactive substances was found during fruiting stage (September) which is of the great value for the harvesting time recommendation.

Keywords: total phenolic content, total flavonoids content, *Hypericum* L., *Melissa* L.

INTRODUCTION

Plants are producing a multitude of bioactive compounds (Dixon, 2001), among them polyphenols like phenolics and flavonoids, that have been a limitless source of experimental analysis (Sujana *et al*., 2013) and which also have numerous different functions (Halliwell 2006, Albayrak *et al*., 2010, Sarbu *et al*., 2019, Obložinský *et al*., 2006). Phenolics possess antioxidant, antiproliferative, anticancer and anti-inflammatory activities, as well as antibacterial, antiviral and

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antifungal potential (Halliwell, 2006), while flavonoids are considered to have antithrombotic, antineoplastic and anti-hypertensive activities (Benayad *et al.*, 2014, Sarbu *et al.*, 2019) as well as antilipoxygenase activity (Obložinský *et al.*, 2006) among the others.

Phenolics and flavonoids, which are responsible for the biological activities of *Hypericum perforatum* L., can often be found in the leaves and flowers of a plant (Zobayed et al., 2006) and the disposal model of some flavonoids in plants reproductive complex during the flowering stage was also studied in a related *Hypericum* species (Mártonfi, 2006). *H. perforatum* L. is well recognized when it comes to flavonoids content (quercetin and kaempferol at the first place) (Nahrstedt and Butterweck, 2010) which are found in its leaves as glycosides located in special compartments of the cells of epidermis, and which are responsible for UV-protection (Germ, *et al*., 2010).

Leaves from *Melissa officinalis* L. contain flavonoids (quercitrin, rhamnocitrin, luteolin), as well as polyphenolics (rosmarinic acid, caffeic acid and protocatechuic acid) (Sofowora, *et al.,* 2013). The biological action of its extracts is primarily connected, similar to other plants, to the phenolics, flavonoids and terpenoids content (Škrovánková, *et al*., 2012).

In the literature there are several studies about phytochemical content, antimicrobial, antimutagenic and cytotoxic activity of essential oils from plants growing in Montenegro (Šćepanović *et al*., 2019, Damjanović-Vratnica *et al*., 2015, Bošković, *et al*., 2018, Božović *et al*., 2015, 2018; Artini *et la*., 2018, Tadić *et al*., 2017, Stešević *et al*., 2016, Vuković-Gačić *et al.*, 2006), while the effect of vegetation cycle on chemical content and antibacterial activity of essential oil was investigated by Damjanović-Vratnica *et al*., (2011) for the *Satureja montana* L. species.

The main goal of this study is to present dynamics of total phenolic and total flavonoids content during vegetation cycle in two different plant species – *H. perforatum* L. and *M. officinalis* L. wild-growing in Montenegro.

MATERIAL AND METHODS

Plant material

Wild-growing plants of *H. perforatum* L. and *M. officinalis* L. were collected in Montenegro in localities H1 (Ljubišnja), H2 (Vijenac) (Table 1.a) and M1 (Zenica), M2 (Stari Bar) (Table 1.b) respectively. The plant material was collected by hand from July to September 2014. The plant material was determined in the laboratory of the Study program for Biology, Faculty of Natural Sciences and Mathematics, University of Montenegro, and genus and species of the plant were identified and confirmed.

a)Aparatus

For the purpose of extraction water bath from the manufacturer Vims elektrik was used, and after the extraction, sand bath and automated rotor-vacuum evaporator IKA rv 10 were used for removing dissolvent from the extract.

Table 1. Localities description for *H. perforatum* L. (a) and *M. officinalis* L. (b).

a.

No.	Locality	Coordinates	Altitude
1.	Ljubišnja / H1	43°17'48"N 19°05'58"E	1509m
2.	Vijenac / H2	43°21'03"N 19°27'21"E	1373m

b.

No.	Locality	Coordinates	Altitude
1.	Zenica / M1	43°17'29"N 19°21'03"E	846m
2.	Stari Bar / M2	42°05'46"N 19°08'09"E	178m

All measurements were performed on a UV-VIS spectrophotometer CECIL CE 2021 in quartz cuvettes which were 10mm wide and with 83% light transmitting ability at 200nm.

b)Chemicals and reagents

Folin-Ciocalteu reagent was obtained from a producor named ALDRICH Chemistry. Solutions of 7,5% $NaHCO_3$, 2,5% $AlCl_3xH_2O$, and 10% ($Na(CH_3COO)*3H_2O$) were freshly prepared. Gallic acid and quercetin were used as reference substances.

Preparation of extracts

Hydroalcoholic extraction was done according to a modified method of Fialová *et al*., (2008). Namely, to the 0.10g of powdered drug (dried herba of *H. pefroratum L.* or dried folium of *M. officinalis* L.) 15ml of a) 50% ethanol and b) 80% methanol was added in a glass flask, and it was left to boil in a water bath for 30 min. Filtrate was poured into a measuring flask where the dilution was done. For the purpose of total phenolics determination filtrate was diluted 5 times, and for the purpose of total flavonoids determination, it was diluted 10 times.

Total phenolic content (TPC)

The TPC were determined according to the Habila et al., (2010) by using Folin-Ciocalteau assay. Namely, in 1.5 ml of Folin-Ciocalteau working solution was added 1.5 ml of $NaHCO_3$ and 200 μl of examinating samples. The samples were incubated at room temperature for 30 min. Then, absorbance was measured spectrophotometrically at 765 nm. The results for TPC concentration were expressed as milligrams of gallic acid equivalents per gram of dry weight (mgGAE/gDW). All the analyses were repeated 6 times.

Total flavonoids content (TFC)

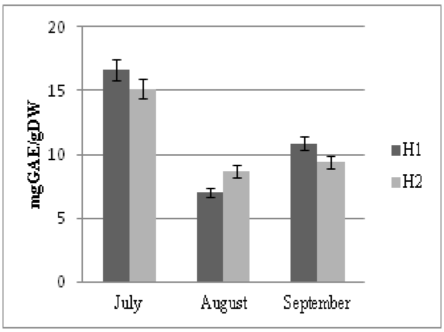
The TFC were determined spectrophotometrically by $AlCl_3$ method, according to modified assay described in Zou, *et al*., (2004). 1ml 2.5% $AlCl_3x6H_2O$ was mixed with 1ml of NaOH and left for the reaction for 5 min. Following this, 2ml of 10% (Na (CH_3COO) $*3H_2O$) and 6ml of 70% ethanol and 1ml of examinating sample were added. After the incubation of 30 min on the room temperature, the absorbance was measured at 420 nm. TFC was expressed as mg quercetin per gram of dry weight (mgQE/gDW). All the analyses were repeated 6 times.

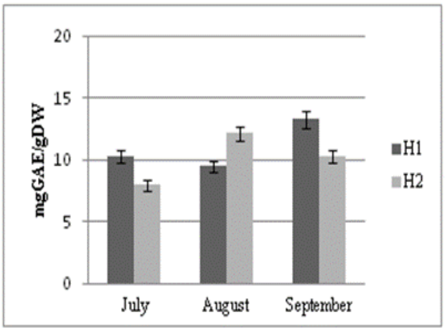
RESULTS AND DISCUSSION

Phenolics and flavonoids are bearers of plants biological activities (Olech, *et al*., 2012, Božin, *et al*., 2013). In this paper the variation in TPC and TFC of the wild-growing *H. perforatum* L. and *M. officinalis* L. from several different localities and different habitats in Montenegro, during their phenological cycle was investigated.

During the observation and analyses of TPC and TFC, in the dried herb (*herbae*) of wild-growing *H. perforatum* L., which were obtained during three consecutive months (July, August, September) for 80% methanolic and 50% ethanolic extracts **(Figure 1. and 2.),** a stable regularity in the dynamics of these bioactive substances during the vegetation period was not found.

In terms of 80% methanolic extracts **(Figure1.a)** the peak of TPC was found in July (16.66 mgGAE/gDW - for the H1 locality), while 50% ethanolic extract showed the peak of TPC in September (13.5 mgGAE/gDW - for the H1 locality) **(Figure 1.b).**



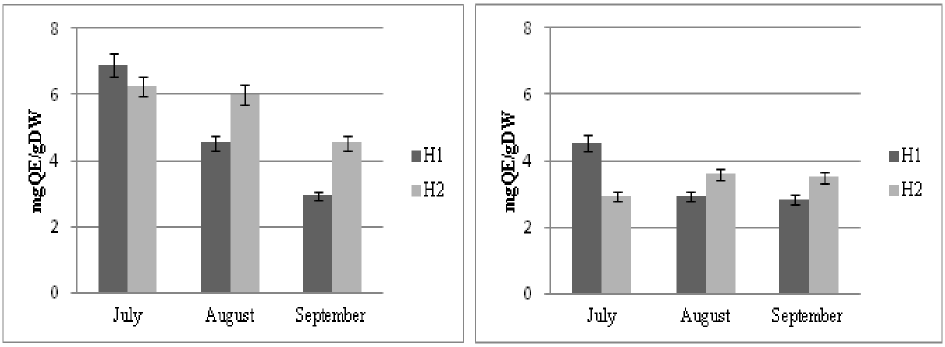


a) **b)**

Figure 1.a) Dynamics of total phenolic content in 80% methanolic hydroalcoholic extract of the wild-growing *H. perforatum* L. *herba* from the locality of H1 (Ljubišnja) i H2 (Vijenac). **b)**Dynamics of total phenolic content in 50% ethanolic hydroalcoholic extract of the wild-growing *H. perforatum* L. *herba* from the locality of H1 (Ljubišnja) i H2 (Vijenac).

When it comes to TFC in 80% methanolic extracts of *H. perforatum* L. **(Figure 2.a)** their peak was found in July (6.91 mgQE/gDW - for the H1 locality) and the 50% ethanolic **(Figure 2.b)** extract also showed the peak of TFC in July (4.54 mgQE/gDW - for the H1 locality).

Present data for TPC values were lower than in Sarikurkcu *et al*., (2020) and it seems that this is the case due to the different extraction type. This investigation also showed significantly lower TPC and TFC data than Tausevski *et al.*, (2019) found in flowering stage samples collected in Northern Macedonia.



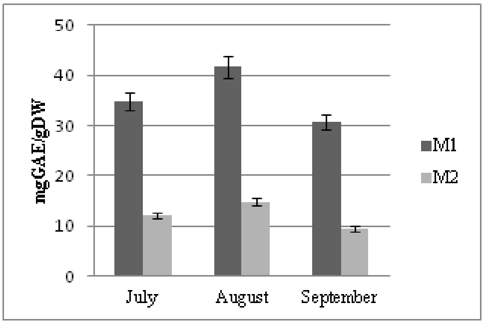
a) **b)**

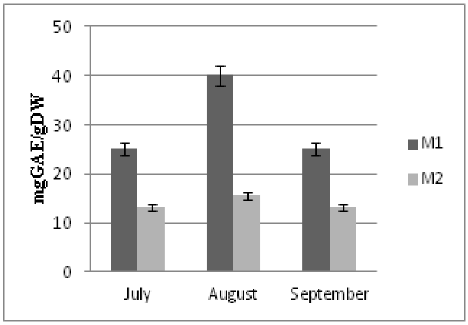
Figure 2.a) Dynamics of total flavonoid content in 80% methanolic hydroalcoholic extract of the wild-growing *H. perforatum* L. herba from the locality of H1 (Ljubišnja) i H2 (Vijenac).**b)** Dynamics of total flavonoid content in 50% ethanolic hydroalcoholic extract of the wild-growing *H. perforatum* L. herbae from the locality of H1 (Ljubišnja) i H2 (Vijenac).

The TPC and TFC values were found to be highly variable in quantity and their dynamics was found irregular unlike in Kazlauskas and Bagdonaite (2004) and Couceiro *et al.,* (2006). We also take into consideration data obtained by Toker, (2009) and Ciraki, *et al*. (2013) for *Hypericum triquetrifolium* Turra species, which is known for its higher content of hypericin when compared with *H. perforatum* L. (Al-Snafi, 2018). The research of Toker, (2009) encompassed three (vegetative, full flowering and mature fruiting stage) and of Ciraki,*et al.* (2013) five developmental stages (vegetative stage, floral budding stage, full flowering stage, fresh fruiting stage and mature fruiting stage) and both researchers concluded that a peak of bioactive substances concentration was in full flowering stage, which is not the case in this research.

During the monitoring of the content of TPC and TFC, in the dried leaf (*folium*) of wild-growing *M. officinalis* L. during three consecutive months (July, August, September) for 80% methanolic and 50% ethanolic extracts **(Figure 3. and 4.),** the maximum value of these bioactive substances was detected in the month of August during the flowering period (for 80% methanolic extract on the locality M1 TPC = 41,66 mgGAE/gDW and TFC = 11,07 mgQE/gDW **(Figure 3.a and Figure 4.a)** and for 50% ethanolic extracts on the locality M1 TPC = 40.15 mgGAE/gDW and TFC= 8,42 mgQE/gDW **(Figure 3.b and Figure 4.b),** while the lowest values were found in the month of September

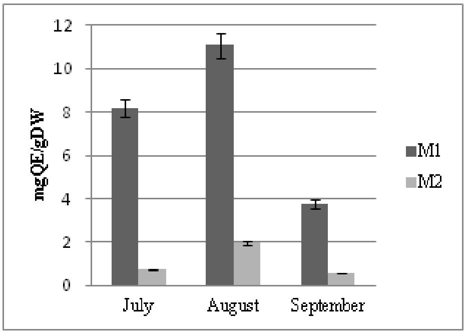
This range fits the value from Moacă *et al*., (2018) who found that TPC in the *M. officinalis* L. leaves extract was 32.76 mg GAE/g dry material, as well as Rehan *et al*., (2014). A higher TPC equal to 227.6 mg GAE/gDW was calculated for the hydroalcoholic extract from *M. officinalis* L. leaves (Moradi, *et al*., 2016) while the TFC of 12.5±2.11 mg/g as reported by Moradi *et al*., (2016), was in accordance with present data.

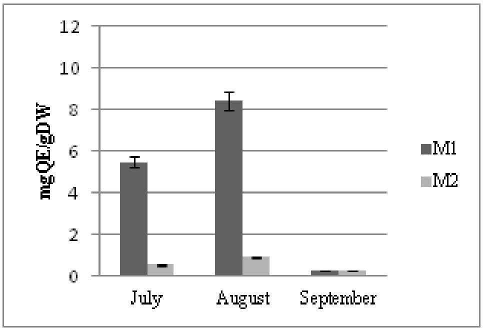




a) **b)**

Figure 3.a) Dynamics of total phenolic in 80% methanolic hydroalchoholic extract of dried wild-growing *M. officinalis* L. *folium* from the localities M1 (Zenica) and M2 (Stari Bar). **b)** Dynamics of total phenolic in 50% ethanolic hydroalchoholic extract of dried wild-growing *M. officinalis* L. *folium* from the localities M1 (Zenica) and M2 (Stari Bar).





a) **b)**

Figure 4.a Dynamics of total flavonoids in 80% methanolic hydroalchoholic extract of dried wild-growing *M. officinalis* L. *folium* from the localities M1 (Zenica) and M2 (Stari Bar).**b** Dynamics of total flavonoids in 50% ethanolic hydroalchoholic extract of dried wild-growing *M. officinalis* L. *folium* from the localities M1 (Zenica) and M2 (Stari Bar).

Total polyphenols in spray-dried extract before and after hydrodistillation was found the highest in *M. officinalis* L. among *Lamiaceae* family species, along with *Origanum vulgare* (Grigore *et al*. 2019).

For the *M. officinalis* L. species, the TPC and TFC were found to change in quantity in a regular manner during the plants vegetation cycle with the peak in the flowering stage (August), while the lowest concentration of these bioactive substances was found during fruiting stage (September), which is in a good agreement with the results of Saeb *et al*., (2011) who did the research on the plant material collected in India during three different plants development stages (vegetative growth, flowering stage and after flowering). Similarly, the amount of rosmarinic acid (%) was studied in samples of *M. officinalis* L. leaves in dependence of the plant ontogenetic phase at harvest time (Tóth *et al*., 2003). Statistically non-significant variability of this phenolics acid was found. Nevertheless, maximum content was found in the full flowering stage (3.91 %) and minimum content in the stage just before flowering (3.50 %).

For the both of afore mentioned species, 80% methanolic extracts had higher concentrations of investigated bioactive substances than the 50% ethanolic extracts, which suggests 80% methanol being a better solvent for this purpose. The variation of TPC values according to the solvent used for the extraction is also described in the literature (Moacă *et al*., 2018). We should also take into account that content of bioactive substances in plants is changed according to climate, environmental conditions, different habitat and locality (Stefanović, *et al*., 2018). For instance, the methanolic extract gained from the aerial parts of *M. officinalis* L. wild-growing in Romania had a TPC of 22 mg GAE/g extract (Armatu *et al*., 2010) and those from Bulgaria *(herbae*) a TPC of 48.86 mg GAE/100 g dry weight (Atanassova *et al*., 2011). These findings reinforce present results on TPC in *M. officinalis* L. leaf extracts which highly differs depending on a habitat, especially altitude of the selected localities, for example TPC values for the M1 locality (846m) (August) was 41.66 mgGAE/gDW while for the M2 locality (178m) was (August) 14.77 mgGAE/gDW.

CONCLUSIONS

In this study variation of TPC and TFC during three consecutive months of vegetation cycle was presented for the two wild-growing plant species: *H. perforatum* L. and *M. officinalis* L. The results of this investigation point towards the recommendation of collecting plant material of *M. officinalis* L. in Montenegro during the flowering phase (month of August) in which there is the peak of TPC and TFC, which is not the case with *H. perforatum* L.

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MAPPING HOT SPOTS OF SOIL MICROBIOME USING GIS TECHNOLOGY

SUMMARY

Virgin forests are unique ecosystems, which can be used as etalon for basic monitoring investigation. The paper presents original results of research. The aim of this study was long term investigation of soil microbiome in primeval forest ecosystems of Carpathian Biosphere Reserve, namely the structure of microbial communities, the number of major ecological-functional groups and determining hot spots.

The Hotspots mapping is realized through GIS (Geographical Information System) technology. It was found that the structure and functional activity of soil microbiome change with altitude. Rebuilding structure of soil microbiome was fixed at altitude 555m; 776m; 1040m. The soil at altitude of 1,040 meters above sea level was characterized by minimum content of ammonifiers.

At the altitude of 555 meters content of ammonifiers increased at six times, which indicates accumulation of organic matter in the soil. Similar changes occurred with the number of bacteria which are using mineral forms of nitrogen for their nutrition. Their maximum quantity was in the soil of biotope disposed at altitude of 555 meters above sea level. After 10 years, fluctuations of soil microbiota at different altitudes were the same. Long term monitoring during 2008-2018 years allowed determining hot spots in structural successions of soil microbiome.

***Keywords**:* Soil, microbiome, primeval beech forest, hotspots, GIS.

INTRODUCTION

A primeval beech forests are the ecosystems which were created during phylocoeno-genesis in corresponding to soil-climatic conditions and landscapes.

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Autotrophic and heterotrophic elements of primeval forest ecosystems and pedosphere have no indications of such anthropogenic influence which could change their natural status and sylvagenesis. Hence, they function as homeostasis ecosystems (Stoyko and Kopach, 2012). As etalon ecosystems, they better combine above resistance and stability with high productivity of biomass. Therefore, the virgin forests reliably indicate the direction of restoration of disturbed ecosystems (Hamor *et al.*, 2008; Debeljak, 2006).

Virgin forests are essential for the conservation of biological and genetic diversity. They reserve the relict and endemic species of flora and fauna. The study of primeval forest is a unique opportunity to explore the natural structure, diversity and genetic structure of unmodified forest and ecosystem dynamical processes and relationships that occur in them under the influence of ecological factors (Symochko & Kalinichenko, 2017). Despite of the intensive exploitation of forests in the last ten centuries, its area decreased by 3.5 times, and virgin forest ecosystems which have special value remained only in the Carpathian Mountains.

The few remnants of natural forests which could be potentially investigated are not larger than 50-100 ha, while continuous forest areas of more than 1,000 ha are very rare. In the Transcarpathian region of Ukraine (south-west), the CBR (Carpathian Biosphere Reserve) offers a unique opportunity for studying the biodiversity and natural processes of virgin or primeval forest ecosystems, i.e. forests that have never been significantly modified by human activity. The region covers an area of about 53,650 ha and became part of the World Network of Biospheres Reserves of UNESCO in 1992. However, it should be noted that the attention of researchers focused mainly on studies of flora and fauna biodiversity (Commarmot *et al.,* 2005; Commarmot *et al.,* 2013) and almost never directed to the ecological study of soil microbial communities. Biotic factors, such as host genotypes, developmental stages and abiotic factors, such as temperature, soil pH, seasonal variation, and the presence of rhizospheric deposits, act as chemical signals for microbes and influence the microbiome community structure and function (Walker *et al*., 2007; Rout and Southworth, 2013; Minz, *et al*., 2013). However, the extent to which both abiotic and biotic factors contribute to microbial communities is not fully understood (Turner *et al*., 2013; Zolla *et al*., 2013). Forests represent one of the largest and most important ecosystems on Earth, covering more than 40 million km^2 and representing 30% of the total global land area (Keenan *et al*., 2015). Primeval forests are ideal ecosystems to study the interaction of bacteria, fungi, and archaea with their abiotic environment (Grayston and Rennenberg, 2006). Virgin forests are essential for the conservation of biological and genetic diversity. They reserve the relict and endemic species of flora and fauna. The study of primeval forest is a unique opportunity to explore the natural structure, diversity and genetic structure of unmodified forest and ecosystem dynamical processes and relationships that occur in them under the influence of ecological factors. Moreover, since most European forest stands have been managed for centuries (Bengtsson *et al*., 2000), very little is known about the diversity, ecology, and distribution of soil microorganisms in natural, undisturbed forest ecosystems in Europe (Baldrian, 2017; Symochko *et al.*, 2015). Soil microorganisms have been largely ignored by conservation efforts. However, their role in biogeochemical processes, their diversity and abundance, and their potential as repositories of valuable genetic information and metabolic products make them as important as animals and plants to the biosphere and human welfare. Study of authentic soil microbiota creates the necessary prerequisites for the conservation of microbial diversity and forming the base of the eco-microbiological monitoring (Patyka and Symochko, 2013). The primeval forests as etalon ecosystems better combine above resistance and stability with high productivity biomass (Symochko and Hamuda, 2015). In the Transcarpathian region of Ukraine the Carpathian Biosphere Reserve offers a unique opportunity for studying the biodiversity and natural processes of primeval forest ecosystems, i.e. forests that have never been significantly modified by human activity. Due to this fact, the purpose of the research was to determine the number of different ecological-trophic groups of soil microorganisms, to estimate successional processes of authentic soil microbiota and determine hot spots of soil microbiome during long-term microbial monitoring.

MATERIAL AND METHODS

Description of sites and soil sampling

Materials of research were soil samples, which had been collected from natural ecosystems: virgin forests of Shyrokoluzhansky massif of the Carpathian Biosphere Reserve (CBR). The total area of the Shyrokoluzhansky massif is about 15,033 ha. The massif consists of two contiguous areas (foresters): Uholka and Shyrokyi Lug. It lies within the Krasnyanskyi physical-geographic area of the Middle mountain-Polonyny region and Uholka physical-geographic area of the Low Mountain-Rocky region. It is located between the rivers Tereblya and Teresva.

The massif is separated by the mountain range Krasna from the Mokryanka river valley and lies within the Duklyanska, Prokuletska, Rakhiv and Maramorosh tectonic zones. The Duklyanska zone covers the north eastern part of the massif and is represented by sandy and clay-sandy flysch. The southwestern part of the massif is occupied with the formations of the Prokuletska zone, which is represented by massive diverse-grained sandstones. The southern part of the massif is made up of the Maramorosh rocky zone sediments, which are represented by cretaceous sediments, palaeogene sandstones, gridstones, aleurolites, marlstones and argillites, and also small-grained greenish-grey flysch with some stratums of grey small-grained sandstones. The soils are very stony, mostly midloamy with good water and air penetration ability. Climate conditions change from mild-warm to cold. The massif belongs to three different climatic zones with annual average temperatures ranging from 0°C to +7°C, and annual average precipitation varying between

1,000 mm and 1,500 mm. The sum of active temperatures changes with the altitude from 800 °C to 2,300 °C. The temperature in January elevates from -3°C to -10°C., and in July from +12°C to +17°C.

Researches were conducted from 2008 to 2018 years. Sampling was carried out in depth of 0-25 cm at different altitudes from 555m to 1040m (Table 1). The soil sampling was carried out by standard methods (ISO 10381-6 : 1993). All samples were prepared using the unified procedure: they were air dried and grounded to < 3 mm in size; visible plant and mesofauna residues were removed. Experiments were performed in fivefold repetition. Studies of soils were carried out at the Scientific Research and Educational Centre of Molecular Microbiology and the Immunology of Mucous Membranes (Uzhhorod National University), Research Laboratory Monitoring of Water and Terrestrial Ecosystems of department entomology and biodiversity conservation (Uzhhorod National University) and in Laboratory of Microbial Ecology (Institute of Agroecology and Environmental Management, Kyiv Agrarian Academy of Sciences of Ukraine). The research was carried out within the framework of the complex project "Eco-microbiological monitoring of various types ecosystems of the Carpathian region" №0116U003331 (state registration number).

Microbiological analyses of soil

Microbiological study of soil was performed in sterile conditions following the standard protocols (Tepper *et al.*, 2004; Shyrobokov, 2011; Goldman and Green, 2015). The method of serial dilution was used to obtain the suspension where microorganisms titre were 10^{-3} CFU/ml. - 10^{-5} CFU/ml. 100 μl (CFU-Colony Forming Units) of the soil suspension was evenly distributed on the surface of the medium with a sterile spatula. For the study we used the following media: Meat peptone agar, Agar-Agar, Eshbi agar, Soil agar, Starch agar in 4 repetitions. Petri dishes with study material were incubated in the thermostat at 29-37ºC for 48-72 hours in aerobic conditions. The colonies which grew in these media were calculated on the assumption that one colony is formed from one vital cell. The results of measuring the number of microorganisms grown on the nutrient media were expressed in colony-forming units (CFU) per 1 g of dry soil. For this purpose, we determined the moisture of the soil samples for the experiments using the thermostat-gravimetric analysis and recalculated the obtained number of colonies taking into consideration the coefficient of moisture and solution of the soil suspension. The inoculations were repeated three times, the obtained data were analysed using mathematical statistics, calculating the confidence interval in the number of microorganisms.

Using GIS Technology on Hot Spots analyses

Spatial database development involved conversion of the data on spreadsheet to GIS shape file in ArcGIS 10.4 software. The shape file was further linked to elevation and other data. The coordinates were collected by GPS, the Coordinate System ETRS 1989 UTM.

The mapping process and hotspots analysis is carrying out by Geographical Systems Information (GIS) technology through ArcGIS 10.4.1 program. As it is known the ArcGIS analyses are very helpfully not only showing the hotspots but also creating a clear visual view of the distribution of the elements in the region. In our case the hotspots analyses have been carrying out using two techniques: (1) Spatial Statistic, Hot Spot Analyses (Getis-Ord Gi*); and (2) IDW (Inverse Distance Weighted). The Satellite image is taken from Base Map.

For the detailed analyses there were built separate thematic maps for each element as: Ammonifiers, Oligotrophes, Pedotrophes and Bacteria using mineral form of Nitrogen. These thematic hotspots maps are built using the IDW method (Inverse Distance weighted) (Fig.1 and Fig.2).

The final General Map presented on Fig.3 shows a comparative Hotspots analyses for the year 2008 and 2018 in the form of graphics bar.

Table 1. Characteristics of the soil samples

Nr.	**Vegetation**	**Coordinates**	**Altitude above sea level, m**
1	*Fagetum (silvaticae)*	48°17.663′ 23°44.389′	800
2	*Fagetum (silvaticae)*	48°17.659′ 23°45.523′	910
3	*Fagetum (silvaticae)*	48°19.349′ 23°45.628′	1010
4	*Fagetum (silvaticae)*	48°20.126′ 23°45.390′	655
5	*Fagetum (silvaticae)*	48°20.069′ 23°44.026′	650
6	*Fagetum (silvaticae)*	48°20.595′ 23°45.115′	1020
7	*Fagetum (silvaticae)*	48°21.292′ 23°44.595′	700
8	*Fageto (sylvaticae) Abietum (albae)*	48°21.817′ 23°45.557′	885
9	*Fagetum (silvaticae)*	48°21.805′ 23°44.529′	1040
10	*Fagetum (silvaticae)*	48°18.454′ 23°43.223′	773
11	*Abieto (albae) Piceeto (abietis) Fagetum (silvaticae)*	48°19.203′ 23°43.658′	776
12	*Fagetum (silvaticae)*	48°20.226′ 23°43.498′	683
13	*Fagetum (silvaticae)*	48°19.928′ 23°42.879′	800
14	*Fagetum (silvaticae)*	48°19.832′ 23°42.119′	844
15	*Fagetum (silvaticae)*	48°20.980′ 23°41.826′	970
16	*Fagetum (silvaticae)*	48°21.089′ 23°43.399′	645
17	*Fagetum (silvaticae)*	48°18.673′ 23°44.389′	555
18	*Fagetum (silvaticae)*	48°21.568′ 23°43.422′	925
19	*Fagetum (silvaticae)*	48°21.730′ 23°41.997′	890
20	*Fagetum (silvaticae)*	48°19.455′ 23°44.547′	770

Statistical analysis of data

The results of the experimental studies were statistically analyzed using the Microsoft Excel program package. Results were expressed as means (±) standard deviation (SD) and (SSD_{05}) smallest significant differences of experiments conducted in quadruplicating. The level of significance selected for the study was $P < 0.05$ (Bailey, 1995).

RESULTS AND DISCUSSION

Soil microorganisms are responsible for most biological transformations and drive the development of stable and labile pools of carbon, nitrogen and other nutrients, which facilitate the subsequent establishment of plant communities. Soil microbiome as a part of forest ecosystems plays an important role in sustainable development of forestry. Each of microbial niches has specific properties and, consequently, a specific bacterial community. Biocenotic relations of trophic and topical types are decisive in edaphotope shaping of different type of ecosystems. Studies of the soil were taken from primeval ecosystems revealed general regularities of distribution of main ecological-functional groups of microorganisms, their population dynamics in different habitats. The most favourable conditions for the development and functioning of microorganisms were in an edaphotops which were located at an altitude of 555-776 meters above sea level. It is highly connected to local temperature and water regime, as well as reserves of nutrients (organic origin) in the soil (Table 2).

As shown in Table 2, at the altitude of 555 meters content of ammonifiers was at six times higher and amounted to 6.24 million CFU/gr.d.s., what indicating a significant enrichment of soil organic matter of plant origin. The similar changes in the bacteria content, in the case of bacteria that used mineral nitrogen were observed. The maximum number of these microorganisms - 4.32 million CFU/gr.d.s. was in the soil at the altitude of 555 meters above sea level (Figure 1). At the highest point of sampling (1040 m.) their number was 2.65 times lower. Succession, dynamic changes of microbial communities of soil related primarily from the impact of abiotic factors such as temperature and humidity.

Rebuilding the functional structure of soil microbial cenosis due to the influence of exogenous factors, as evidenced not only by changing the number of specific ecological-trophic groups of soil microorganisms (Symochko, 2020; O'brien *et al*, 2016; Demyanyuk *et al*, 2020), but also from direction of microbiological processes in soil of virgin ecosystems. It should be noted that at altitudes of 776 meters above sea level, significant changes occur in the structure of microbial community.

The content of pedotrophes and oligotrophes in the structure of soil microbiome increased. The changes in the structure of soil microbiome indicate the realization of structural and functional successions and the presence of hot spots at these altitudes. Because bacteria inhabit small niches, the properties of their immediate environment rather than the mean soil properties affect the local bacterial community.

Long term investigations showed significant changes in the structure of soil microbiome, increased in twice the quantity of oligotrophic $4{,}97*10^6$ CFU/gr.d.s. (2008); $8.98*10^6$ CFU/gr.d.s. (2018) and pedotrophic bacteria $7.89*10^6$

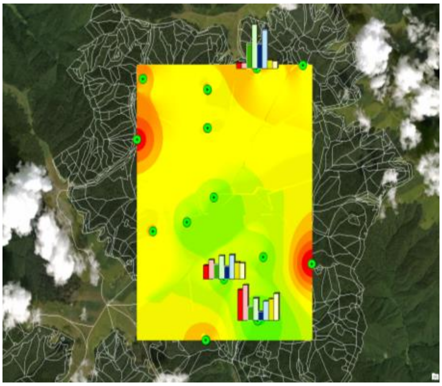
CFU/gr.d.s. (2008); 4.95*10^6 CFU/gr.d.s. (2018). Number of ammonifiers wasn't changed significantly.

Table 2. Soil microbiome of primeval forest ecosystems (2008 - 2018) (CFU/gr.d.s.)

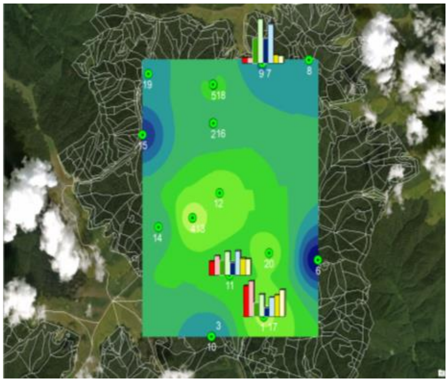
№	Altitude above sea level m	Number of soil microorganisms (CFU-colony forming units/ per 1 gram of dry soil)							
		Ammonifiers *10^6		Oligotrophes *10^6		Pedotrophes *10^6		Bacteria using mineral forms of nitrogen *10^6	
		2008	2018	2008	2018	2008	2018	2008	2018
1	800	2,77±0,03	2,90±0,05	3,53±0,03	6,72±0,04	2,78±0,01	6,03±0,01	3,45±0,01	3,04±0,01
2	910	1,64±0,02	1,88±0,03	3,68±0,01	4,90±0,03	2,96±0,04	6,45±0,03	2,18±0,04	2,90±0,02
3	1010	1,15±0,05	1,22±0,02	4,45±0,02	8,32±0,02	4,68±0,02	7,33±0,02	2,12± 0,03	2,34± 0,05
4	655	4,10±0,04	4,89±0,01	2,65±0,06	3,48±0,04	1,85±0,05	4,55±0,05	3,52±0,05	3,54±0,03
5	650	4,12±0,01	4,66±0,02	2,44±0,01	3,44±0,01	1,82±0,02	4,46±0,02	3,52±0,02	3,88±0,02
6	1020	1,13±0,01	1,33±0,05	4,90±0,01	8,56±0,03	4,42±0,01	7,77±0,04	1,85±0,01	1,97±0,01
7	700	3,12±0,02	3,67±0,02	2,87±0,02	5,02±0,02	2,35±0,01	4,56±0,01	3,95±0,07	4,56±0,03
8	885	2,56±0,08	2,64±0,08	3,38±0,07	4,39±0,06	2,68±0,01	3,70±0,03	2,84±0,02	2,84±0,02
9	1040	1,07±0,03	1,15±0,02	4,97±0,02	8,98±0,01	4,95±0,02	7,89±0,01	1,63±0,01	1,45±0,03
10	773	2,84±0,01	3,84±0,04	2,98±0,03	4,65±0,02	2,45±0,03	4,75±0,02	3,44±0,04	3,53±0,02
11	776	2,80±0,03	3,91±0,03	3,00±0,04	4,76±0,03	2,55±0,04	4,90±0,01	3,40±0,03	3,26±0,05
12	683	4,03±0,03	4,77±0,07	2,78±0,01	4,11±0,05	1,96±0,02	4,43±0,02	3,50±0,01	4,72±0,01
13	850	2,96±0,07	3,68±0,03	3,79±0,01	6,96±0,01	2,90±0,02	6,88±0,03	3,12±0,01	3,12±0,03
14	844	2,78±0,08	3,23±0,08	3,55±0,02	4,23±0,03	2,35±0,01	6,62±0,01	2,86±0,02	3,04±0,01
15	970	1,33±0,02	1,33±0,02	3,72±0,03	4,78±0,02	2,84±0,08	7,32±0,08	2,33±0,04	2,33±0,04
16	645	4,30±0,02	5,30±0,06	2,64±0,03	3,67±0,03	1,85±0,09	3,99±0,03	3,64±0,06	4,74±0,02
17	555	6,24±0,03	7,13±0,05	2,65±0,07	4,50±0,02	1,77±0,05	3,85±0,05	4,32±0,01	5,43±0,02
18	925	1,7±0,01	2,07±0,03	3,69±0,05	7,97±0,03	2,63±0,02	5,52±0,02	2,68±0,01	2,89±0,01
19	890	2,34±0,01	2,56±0,01	3,34±0,01	7,81±0,01	2,46±0,06	6,80±0,06	2,96±0,03	2,71±0,03
20	770	3,90±0,04	3,97±0,09	2,85±0,02	5,12±0,04	2,11±0,04	5,41±0,03	3,26±0,02	3,18±0,04

Changes in the structure of soil microbiome can be caused by two reasons: the influence of external factors and the availability of resources. Resource availability is also likely to be a fundamental driver of microbial succession, but the limiting resources and environmental factors regulating succession will be more complex given the far greater physiological diversity contained within microbial communities and the breadth of environments in which succession can occur. In autotrophic succession, nutrients and light are likely to be the primary resources limiting biomass accumulation. Long term monitoring of authentic soil microbiome allowed determining hot spots (Figure 1). Estimating the size of hotspots and the proportion of the total soil volume that they represent is a major challenge in soil microbial ecology.

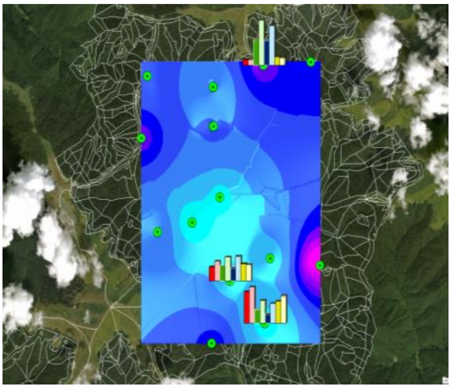
The occurrence of hotspots in soil, which is the most heterogeneous and complex component of the biosphere (Young and Crawford, 2004), is a result of soil development. The increasing variability of soil properties is a key characteristic of soil formation, structuring the environment. Such properties include local density and pore volume, soil acidity and redox potential, organic matter and nutrient contents, microbial biomass and composition of microbial communities, and enzyme activities. The heterogeneity of the soil environment is responsible for huge diversity not only of microorganisms but also of various processes ongoing at close distances that would be not possible in a homogeneous system (Kuzyakov and Blagodatskaya, 2015). The range of hotspot sizes is very broad, individual microbial cells are insufficient to be accepted as hotspots because they functions are not relevant on the higher scales.



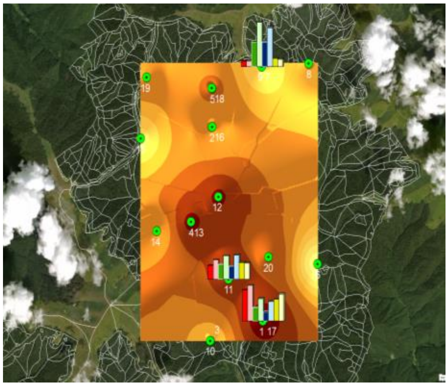
(a) - Ammonifiers



(b) - Oligotrophes



(c) - Pedotrophes



(d) - Bacteria

Figure 1: IDW GIS Hotspots Map (a) Ammonifiers; (b) Oligotrophes; (c) Pedotrophes; (d) Bacteria using mineral forms of nitrogen.

However, in the earliest stages of autotrophic succession, heterotrophs may also be in relatively high abundance, utilizing trace levels of available carbon (Okabe *et al*., 2007; Roeselers *et al*., 2007).

During endogenous heterotrophic succession, labile substrates will be consumed first, supporting copiotrophic microbial taxa that are later replaced by more oligotrophic taxa that metabolize the remaining, more recalcitrant, organic C pools in the later stages of succession (Rui *et al*., 2009).

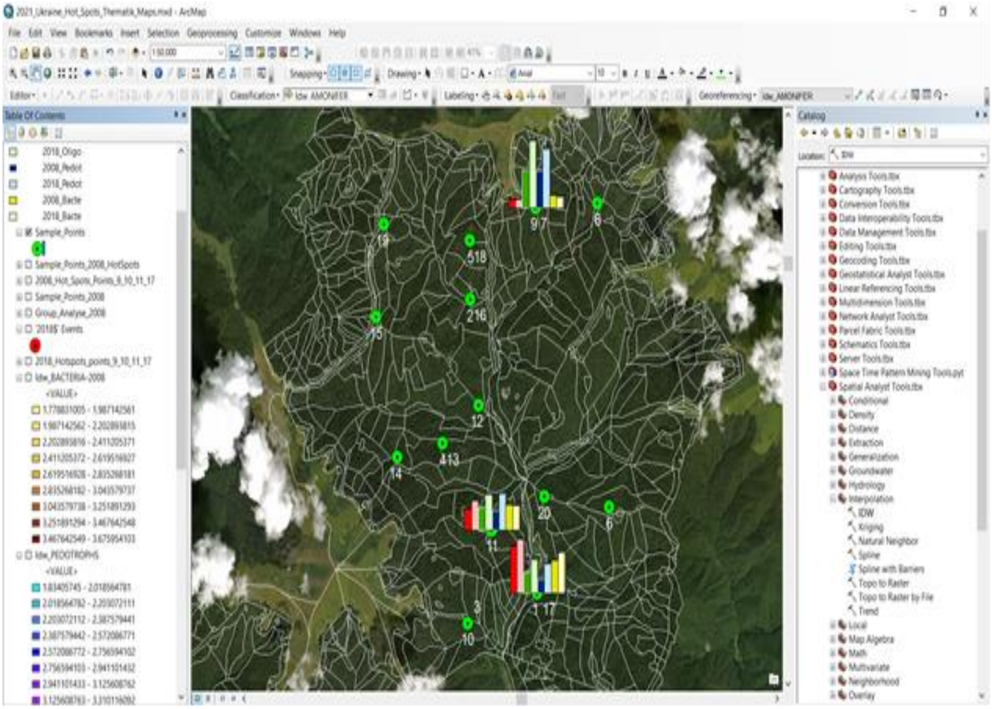


Figure 2: Hot Spot Analyses (Getis-Ord Gi*). A Comparative Map 2008 – 2018
(a) Ammonifiers; (b) Oligotrophes; (c) Pedotrophes; (d) Bacteria

Endogenous heterotrophic succession cause increasing biomass of oligotrophic bacteria and decreasing phylogenetic diversity. Diversity is indicate, how changed microbial communities during succession (Symochko, 2020). It should be noted that at altitudes of 555, 776, 1040 meters above sea level, significant changes occur in the structure of microbial community (Figure 2). The content of pedotrophes and oligotrophes in the soil increases.

Instead, the number of ammonifiers and bacteria using mineral forms of nitrogen is decreasing. At the altitude 555 meters above sea level content of ammonifiers was $6.24*10^6$ CFU/gr.d.s.(2008); $7.13*10^6$ CFU/gr.d.s. (2018) and bacteria using mineral forms of nitrogen $4.32*10^6$ CFU/gr.d.s.(2008); $5.43*10^6$ CFU/gr.d.s. (2018) , at the altitude 1040m content of these microorganisms decreased and number of ammonifiers was $1.07*10^6$ CFU/gr.d.s. (2008); $1.15*10^6$ CFU/gr.d.s. (2018) , bacteria using mineral forms of nitrogen - $1.63*10^6$ CFU/gr.d.s. (2008); $1.45*10^6$ CFU/gr.d.s. (2018).

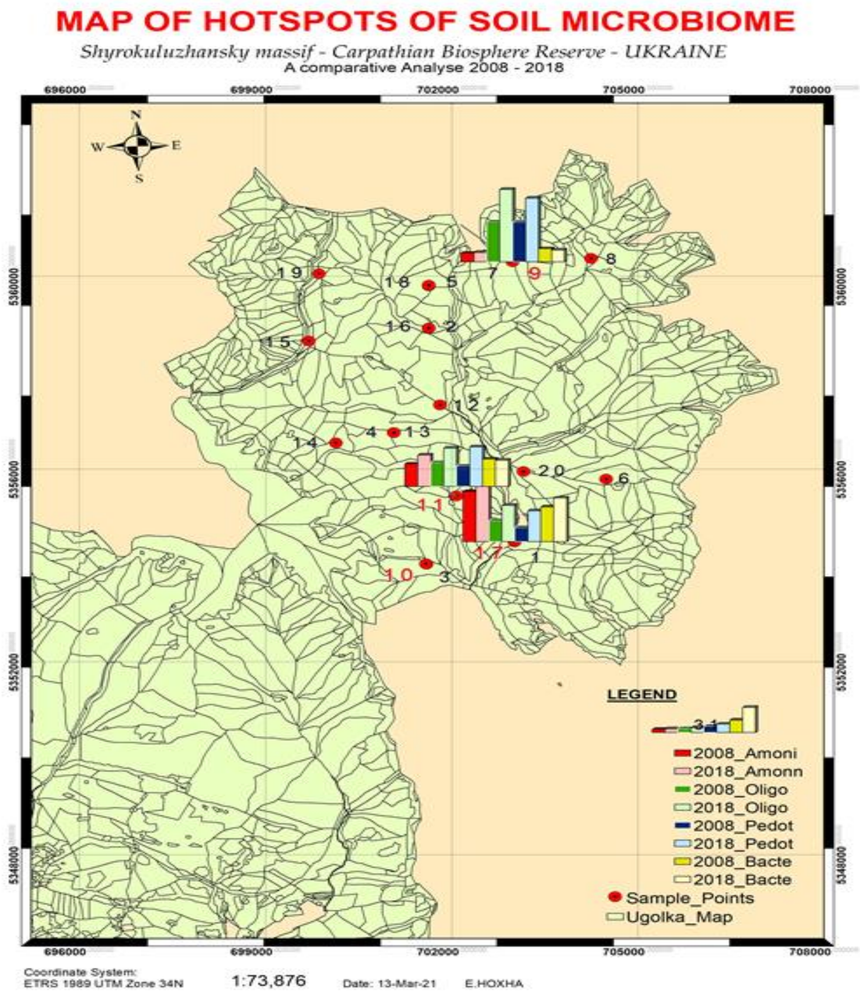


Figure 3: GIS Map of the Hotspots of soil microbiome of Shyrokoluzhansky massif in Carpathian Biosphere Reserve in Ukraine.
Comparative Map 2008 – 2018

The changes in the structure of soil microbiome indicate the realization of structural and functional successions and the presence of hot spots at these altitudes. For linking of the presence of bacteria or their activity to soil properties, it is important that soil is a complex of microniches with heterogeneous physicochemical properties on various scales. Because bacteria inhabit small niches, the properties of their immediate environment rather than the mean soil properties affect the local bacterial community. This spatial heterogeneity has been shown to result in the heterogeneity of bacterial communities on small scales. Furthermore, local dispersal limitations can also remarkably influence the bacterial community composition (O'Brien *et al*., 2016). Considering the high level of spatial variation of forest C stocks on the same scale, the occurrence of individual taxa in forest soil may actually be highly variable on a small scale and may differ among activity hot spots (Martiny et al., 2011; Kuzyakov and Blagodatskaya, 2015). The same dynamic of different functional groups of soil microorganisms were saved for 10 years (Figure 3).

In 2018 were fixed hot spots at the same altitudes 555, 776, 1040 meters above sea level. This is necessary to identify the mechanisms and factors affecting hotspot origin, formation and functioning. Our long-term investigations confirmed that the hotspots formed 10 years ago saved and continue to function.

CONCLUSIONS

Soil microbial community changed along successional time, but it showed significant difference at altitudes 555, 776, 1040 meters above sea level, which indicate hotspots in edophotopes at this altitude. Consequently, the number of representatives of major ecological-trophic groups of the soil microorganisms varies depending on the altitude of forest's biotopes disposition above sea level.

The number of ammonifiers, and bacteria that use mineral nitrogen decreased with the altitude increasing, the number of oligotrophes and pedotrophes gradually was increasing. Endogenous heterotrophic succession caused increasing biomass of oligotrophic and pedotrophic bacteria.

After 10 years, fluctuations of microbial cenosis at different altitudes were the same. Monitoring study database has both theoretical and practical value and can be used for creation of necessary measures to preserve authentic microbial communities, and to implement environmental principles of sustainable forestry. ArcGIS is an appropriate tool to make Hotspots map and different analyses.

Mapping of soil microbiome in primeval beech forest can be used as additional tools for conservation of unique, authentic soil microbiota.

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INFLUENCE OF STORAGE ON POMOLOGICAL AND SENSORY CHARACTERISTICS OF HAZELNUT FRUIT

SUMMARY

The research aims to study the changes in pomological and sensory characteristics of hazelnuts fruits before and after NA cold storage for five months. The paper analyzes fruits of hazelnut cultivars grown in agroecological conditions of north-western Bosnia and Herzegovina, in the area of municipality Kostajnica. The fruits were collected in the period of full maturity, in the 2020 year, and the following cultivars were analyzed: Istrian Long, Istrian Round, Romische Zellernuss and Cosford. The first analysis of fruits was performed after harvest, and the second analysis was executed after the storage of fruits in the shell. Fruit characteristics, studied on both period, were fruit weight, kernel weight, fruit length, fruit width, fruit thickness, kernel length, kernel width, kernel thickness, shell thickness, fruit shape, texture, color, taste, kernel bitterness, kernel sweetness, hardness, crunchiness, presence of double fruits, blank nuts, fruit roundness index and kernel percentage.

The results of the research show that there are significant differences in the values of the examined parameters before and after storage of fruit hazelnut. The biggest changes are recorded precisely on the shrinkage in the storage process and changes in the taste of stored hazelnuts. These results show the need to more investigate storage systems and optimal storage conditions for each variety.

Keywords: *Corylus avellana* L., fruit quality, drying, NA storage

INTRODUCTION

Hazelnut, as a nut fruit species, has a significant demand on the world market, primarily in the confectionery industry, and recently as a raw material for the production of cold-pressed oils and for the needs of the cosmetics industry. The growth in demand for hazelnuts in recent years has led to their deficit in the

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world market and a significant increase in price, which has further influenced the emergence of expansion in terms of raising new hazelnut plantations around the world. A drastic increase in the area under hazel is also recorded in the Western Balkans. So, the current areas under hazel in Serbia are 3,964 ha (Statistical office of the Republic of Serbia, 2017), in Croatia 5,530 ha (Croatian Bureau of statistics, 2019), while for Bosnia and Herzegovina (B&H) there are no data related to areas under hazel. Zavišić *et al*. (2020) state that during 2019, only in Republic of Srpska were produced 168,990 seedlings of hazelnut, which is 15 times more than in 2016, and also a large amount of planting material is imported from other countries.

Old and introduced cultivars are grown in newly planted orchards of hazelnut. Hazelnuts are grown in B&H exhibit different production characteristics that are closely related to agroecological conditions. Čmelik and Mališević (1996) state that in the hazelnut orchard planted on pseudogley soil in the Posavina area, there is a disturbance of the growth and yield of plants and the presence of fruits with poor production characteristics, especially in dry years. On the other hand, Ilić *et al*. (2017) state that hazelnut fruits in 13 cultivated varieties at several different locations from the Banja Luka region showed good morphometric and qualitative characteristics, which was confirmed by other researchers from the Western Balkan (Mitrović *et al*., 2009; Solar and Štampar, 2011; Skender *et al*., 2019).

The process of hazelnut production is extremely important for preserving the quality of the fruit and its aroma and taste until it is placed on the market, either for consumer use or as a raw material in the confectionery industry. Untimely harvesting, cleaning, drying and storage can significantly affect the loss of fruit quality of this kernel species. Drying before storing fruits is especially important because if it is not performed properly due to changes in temperature and light, lipid molecules are released that form free fatty acids, which further leads to changes and oxidation of oil (Turan, 2018).

In recent years, hazelnut producers in Bosnia and Herzegovina have marketed their hazelnuts mainly in local markets and pastry shops. With the increase in production, there has been a certain saturation of the market because there are no large domestic buyers of hazelnuts in our area, while foreign buyers have requirements that most producers do not meet, primarily due to the larger size of the fruit. In such conditions, there is a need to store fruits until they find their way on the market. Thus, growers are forced to store hazelnuts in improvised ways, ie in storage that do not fully meet the required norms. Controlled atmospheric conditions have a positive impact on the quality and nutritional value of hazelnuts (Markuszewski and Kopytowski, 2015). The same authors concluded that the dry matter content of hazelnuts doubles and the fruit weight decreases after three months of storage in a NA chamber compared to harvest. In the warehouse, there are changes in the sensory properties of the fruit, which is confirmed by De Santis *et al*. (2009), and especially emphasize the change in taste and crunchiness of the fruit. Miljkovic (2018) states that properly stored hazelnuts can be stored for up to 40 months without loss of weight and quality.

The aim of this research is to study the quality of hazelnuts at harvest and after storage in order for producers to give recommendations on the possibility of storage period without declining their quality.

MATERIAL AND METHODS

The research was done during 2020 and 2021 in the experimental orchard and cold storage "Strigo" in the area of municipality of Kostajnica on four cultivars of hazelnuts - Istrian Long, Istrian Round, Romische Zellernuss and Cosford. The fruits are harvested in September in the optimal period for harvesting. For each cultivar, 100 fruits were sampled, and they are divided into two groups. On the first group of fruits, analyzes were performed immediately after harvest (30 fruits for pomological analyzes and 20 fruits for sensory analysis of which 10 fruits of the samples was subjected to roasting for 20 minutes at temperature 115 ºC (IPBGR, 2008), (Gorenje, EC5111SG, Slovenia). The second group of fruits was placed in storage at a temperature of 7-8 ºC and at a relative humidity of 70%. The fruits were stored for five months after which the analyzes were repeated. Before storage, the fruits were dried in a dryer for three days at temperature 25-30 ºC.

From pomological characteristics of the fruit were monitored: length, width and thickness of fruit, length, width and thickness of kernel, shell thickness by movable scale (Unior, No 270, Slovenia, measuring range 0-150 mm), fruit and kernel weight by analytical balance (KERN & Sohn Gmbh, Germany, measuring range 0-600 ± 0.01 g), kernel percentage was calculated using the formula: kernel percentage (%) = (kernel weight/fruit weight) × 100). The index of fruit roundness was calculated according to the formula: fruit roundness index = (thickness + width) / (2 × length). Thus, were observed the presence of blank fruits and fruits with two kernels. Sensory analysis of fruits was performed using Bioversity Descriptors for hazelnut (IPBGR, 2008), and the monitored characteristics are fruit shape, texture, color, taste, kernel bitterness, kernel sweetness, hardness, and crunchiness of the fruit. The evaluation of sensory characteristics was performed by respondents of different ages and genders. Statistical data processing was performed on the basis of descriptive analysis with appropriate data for all measured properties. Examination and comparison of the observed properties were performed by calculating the mean values and standard error.

RESULTS AND DISCUSSION

The results of the research of the observed hazelnut cultivars from the Kostajnica area showed different values of the analyzed parameters before and after fruit storage.

The average values of pomological characteristics of the fruit in the examined hazelnut cultivars at harvest period are presented in Table 1.

Table 1. Average values of pomological characteristics of fruits of examined hazelnut cultivars at harvest period

Harvest period	Cultivars			
	Romische Zellernuss	Istrian Long	Istrian Round	Cosford
Measurements	Mean SE	Mean SE	Mean SE	Mean SE
Nut weight (g)	3.10 ± 0.10	4.04 ± 0.11	2.54 ± 0.08	2.18 ± 0.04
Kernel weight (g)	1.48 ± 0.04	1.82 ± 0.04	1.21 ± 0.03	1.10 ± 0.02
Nut lenght (mm)	19.28 ± 0.19	25.69 ± 0.20	19.29 ± 0.18	23.72 ± 0.15
Nut width (mm)	22.15 ± 0.33	20.70 ± 0.16	18.78 ± 0.17	15.58 ± 0.20
Nut thickness (mm)	19.16 ± 0.19	17.74 ± 0.13	16.76 ± 0.16	13.68 ± 0.14
Kernel lenght (mm)	13.89 ± 0.25	20.10 ± 0.20	14.87 ± 0.20	19.21 ± 0.12
Kernel width (mm)	16.88 ± 0.27	14.63 ± 0.27	14.71 ± 0.22	11.35 ± 0.12
Kernel thickness (mm)	14.86 ± 0.35	12.46 ± 0.22	12.75 ± 0.23	10.32 ± 0.15
Shell thickness (mm)	1.39 ± 0.04	1.97 ± 0.34	1.56 ± 0.03	1.23 ± 0.02
Presence od dual fruits	None	Presence	Presence	None
Blank nuts (%)	13.33	6.66	6.66	3.33
Nut shape index	1.07	0.74	0.92	0.61
Kernel percentage (%)	45.87	45.73	46.66	50.45

Prior to fruits storage, the highest average weight of fruits and kernels had Istrian Long (4.04 and 1.82 g), and smallest Cosford (2.18 and 1.10 g). In relation to the research conducted by Ilić *et al*. (2017) were observed lower nut weight and higher kernel weight in Istrian Long, as well as higher nut and kernel weight in Romische Zellernuss. According to measurements performed by Solar and Štampar (2011), a lower nut weight was recorded in Istrian Long, and a higher one in Romische Zellernuss. Skender et al. (2019) record a significantly lower nuts and kernel weight for Istrian Long in the Bihać area (2.41 g) and approximately the same Romische Zellernuss (3.04 g). The highest nut length was recorded in Istrian Long (25.69 mm), and the smallest in Romische Zellernuss (19.28 mm). The largest width and thickness of nuts was recorded in Romische Zellernuss (22.15 and 19.16 mm), and the smallest in Cosford (15.58 and 13.68 mm). According to research conducted by Ilić *et al*. (2017) slightly less similar or slightly smaller fruit dimensions were recorded in Istrian Long and Romische Zellernuss, while fruits were significantly larger compared to the Solar and Štampar research (2011). The largest kernel length was recorded in Istrian Long (20.10 mm) and the smallest in Romische Zellernuss (13.89 mm). The largest width and thickness of the nut was recorded in Romische Zelernuss (16.88 and 14.86 mm) and the smallest in Cosford (11.35 and 10.32 mm). The highest shell thickness was recorded in Istrian Long (1.97 mm) and the lowest in Cosford

(1.23 mm). In relation to the research conducted by Ilić *et al*. (2017) much higher values for shell thickness were recorded.

Table 2. Average values of pomological characteristics of fruits of examined hazelnut cultivars after storage

After storage	Cultivars			
	Romische Zellernuss	Istrian Long	Istrian Round	Cosford
Measurements	Mean SE	Mean SE	Mean SE	Mean SE
Nut weight (g)	2.69 ± 0.10	4.01 ± 0.10	2.25 ± 0.07	1.97± 0.04
Kernel weight (g)	1.22 ± 0.07	1.73 ± 0.06	1.03 ± 0.02	0.98 ± 0.03
Nut lenght (mm)	18.60 ± 0.27	25.89 ±0.38	19.13 ± 0.17	22.86 ± 0.28
Nut width (mm)	22.11 ± 0.16	20.57 ± 0.24	18.10 ± 0.21	14.44 ± 0.25
Nut thickness (mm)	19.48 ± 0.22	17.71 ± 0.18	16.45 ± 0.23	11.99 ± 0.22
Kernel lenght (mm)	13.66 ± 0.31	19.98 ± 0.16	14.85 ± 0.27	16.89 ± 0.38
Kernel width (mm)	16.75 ± 0.22	14.47 ± 0.17	14.46 ± 0.22	11.48 ± 0.19
Kernel thickness (mm)	14.98 ± 0.15	12.07 ± 0.21	12.70 ± 0.19	9.75 ± 0.20
Shell thickness (mm)	1.52 ± 0.04	1.79 ± 0.05	1.55 ± 0.04	1.18 ± 0.02
Presence od dual fruits	None	Presence	None	None
Blank nuts (%)	16.66	3.33	-	9.99
Nut shape index	1.11	0.73	0.90	0.57
Kernel percentage (%)	45.35	43.14	45.77	49.74

The presence of dual nuts was recorded in Istrian Long and Istrian Round. The highest presence of blank nuts was recorded in Romische Zellernuss (13.33%), and the lowest in Cosford (6.66%). In relation to the research conducted by Miletić *et al*. (2002) it's recorded a slightly higher number of blank nuts in Romische Zellernuss and approximately the same number in Istrian Long. The highest nut shape index was recorded in Romische Zellernuss (1.07), and the lowest in Cosford (0.61). The values of the nut shape index are approximately equal to the values recorded by Solar and Štampar (2011), and significantly higher for Romische Zellernuss and approximately equal for Istrian Long compared to the research conducted by Miletić *et al*. (2002). The highest kernel percentage was recorded for Cosford 50.45%, while the other three cultivars had an approximately uniform kernel percentage ranging from 45.73 for Istrian Long to 46.66% for Istrian Round. The values of kernel percentage in Romische Zellernuss and Istrian Long are significantly higher compared to the research conducted by Ilić *et al*. (2017), while compared to the research Solar and Štampar (2011) they are higher by approximately 3% for both cultivars.

The average values of pomological characteristics of the fruit in the examined hazelnut cultivars after storage are presented in Tab. 2.

Table 3. Sensory analyzes of fruits of examined hazelnut cultivars at harvest period

Harvest period		Cultivars			
		Romische Zellernuss	Istrian Long	Istrian Round	Cosford
Sensory characteristics of nuts based on the descriptor Bioversity international					
Shape		Oblate	Long subcylndrical	Globular	Long subcylndrical
Texture	before roasting	Medium corky	Smooth	Lightly corky	Smooth
	after roasting	Medium corky	Lightly corky	Lightly corky	Smooth
Colour	before roasting	Brown	Brown	Brown	Lightly brown
	after roasting	Brown	Brown	Dark brown	Dark brown
Sensory characteristics of kernels based on sensory tests performed with subjects					
Taste*	before roasting	Very good (6/10)	Very good (7/10)	Very good (6/10)	Very good (6/10)
	after roasting	Satisfactory (7/10)	Very good (5/10)	Very good (6/10)	Satisfactory (5/10)
Kernel bitterness*	before roasting	Yes (5/10)	No (1/10)	No (1/10)	No (4/10)
	after roasting	Yes (5/10)	No (4/10)	No (2/10)	Yes (6/10)
Kernel sweetness*	before roasting	Yes (5/10)	Yes (6/10)	Yes (8/10)	Yes (5/10)
	after roasting	No (3/10)	Yes (6/10)	Yes (8/10)	No (1/10)
Hardness*	before roasting	Yes (6/10)	Yes (9/10)	Yes (9/10)	Yes (6/10)
	after roasting	Yes (8/10)	Yes (8/10)	Yes (7/10)	Yes (6/10)
Crunchiness*	before roasting	Yes (6/10)	Yes (5/10)	Yes (5/10)	Yes (7/10)
	after roasting	Yes (10/10)	Yes (9/10)	Yes (8/10)	Yes (10/10)

* with the marked parameters the numerical assessment of the respondents from the total number is presented

The texture and color characteristics did not change much according to the treatment. In terms of taste, the cultivars showed quite the same taste before roasting according to the respondents while after roasting the taste quality was worse in the cultivars Romische Zellernuss and Cosford. Fruits with more pronounced bitterness of kernel were found in Romische Zellernuss during both observations, while bitterness was least pronounced in Istrian Round. In all cultivars there was an increase in bitterness of kernel after roasting. In terms of the sweet taste of the kernel, Istrian Round stood out in particular while the kernel of Cosford proved to be the worst. The fruits of all cultivars have, as expected, shown higher hardness and crunchiness after roasting.

Estimates of sensory characteristics of the fruit of the examined hazelnut cultivars after storage are presented in Tab. 4.

After five months of fruit storage, the largest changes were registered in fruit weight and thus in kernel weight, which affected the change in kernel percentage. Namely, in all observed cultivars was recorded shrinkage of fruits in the storage process, where is the largest magnitude of shrinkage of fruit and kernel recorded in Romische Zellernuss and the smallest one in Istrian Long.

Markuszewski and Kopytowski (2015) also confirm the weight loss of nuts in the storage process. Cultivar Cosford maintained the best kernel percentage in the storage process, while the largest difference of this parameter of stored fruits in relation to the harvest was recorded in Istrian Long. By analyzing individual parameters of fruit dimensions does not record significant differences between fruits analyzed in different period. Different values of fruit dimensions as well as different presence of dual fruits and blank nuts at harvest and after storage can be related to different representative group of fruits sampled for two time periods of analysis.

Estimates of sensory characteristics of the fruit of the examined hazelnut cultivars at harvest period are presented in the Tab. 3.

Table 4. Sensory analyzes of fruits of examined hazelnut cultivars after storage

After storage		Cultivars			
		Romische Zellernuss	Istrian Long	Istrian Round	Cosford
Sensory characteristics of nuts based on the descriptor Bioversity international					
Shape		Oblate	Long subcylndrical	Globular	Long subcylndrical
Texture	before roasting	Medium corky	Lightly corky	Lightly corky	Smooth
	after roasting	Medium corky	Lightly corky	Lightly corky	Smooth
Colour	before roasting	Brown	Brown	Dark brown	Brown
	after roasting	Dark brown	Brown	Dark brown	Dark brown
Sensory characteristics of kernels based on sensory tests performed with subjects					
Taste*	before roasting	Very good Yes (5/10)	Satisfactory (5/10)	Very good (5/10)	Very good (6/10)
	after roasting	Very good Yes (5/10)	Very good (5/10)	Very good (5/10)	Satisfactory (5/10)
Kernel bitterness*	before roasting	Yes (7/10)	No (3/10)	No (2/10)	Yes (5/10)
	after roasting	No (1/10)	No (4/10)	No (2/10)	Yes (5/10)
Kernel sweetness*	before roasting	No (2/10)	No (4/10)	No (6/10)	No (4/10)
	after roasting	Yes (7/10)	No (3/10)	Yes (6/10)	No (2/10)
Hardness*	before roasting	Yes (6/10)	Yes (6/10)	Yes (6/10)	Yes (6/10)
	after roasting	Yes (7/10)	Yes (8/10)	Yes (7/10)	Yes (6/10)
Crunchiness*	before roasting	Yes (5/10)	Yes (6/10)	Yes (7/10)	Yes (7/10)
	after roasting	Yes (7/10)	Yes (7/10)	Yes (6/10)	Yes (8/10)

* with the marked parameters the numerical assessment of the respondents from the total number is presented

The data show that the stored fruits had a worse taste in the opinion of the respondents, especially Romische Zellernuss after roasting and Istrian Long cultivar before roasting. There was an increase in the bitterness of the fruit in storage process in all cultivars before roasting, while in Romische Zellernuss the bitterness is reduced after roasting. In the process of storage, there is a decrease in the sweetness of the fruits of the observed cultivars, except for Romische

Zellernuss, which has a more intense sweetness after storage. The decrease in fruit hardness during storage was expressed in Istrian Long and Istrian Round, while the crunchiness of stored fruits was less present in the other two observed cultivars. The loss of taste and reduction of fruit crunchiness after storage of hazelnuts are also indicated by De Santis *et al.* (2009).

CONCLUSIONS

The results indicate different storage capacities of the examined hazelnut cultivars in the observed period in the chamber. Regardless of the fact that Istrian Long had the largest difference in kernel percentage between harvested fruits and stored fruits, this cultivar had the smallest magnitude of fruit shrinkage during storage, but also the largest fruits that maintained good hardness during storage. Cosford, in addition to the acceptable magnitude of fruit shrinkage, maintained good taste and crunchiness of the fruit in both treatments, before and after roasting. The fruits of Istrian Round had the largest sweetness and the least bitterness, which was least affected by the roasting process compared to other observed varieties. Considering that this is a preliminary research in the field of storage of this fruit species in the region of Republic of Srpska, the obtained results indicate the need for more detailed analyzes of storage systems and conditions for each cultivar separately and their impact on fruit quality. Also, the results of this research indicated the need for study the aroma and blanching (skinning of pelicule) of kernels before and after storage which are a very important features of the hazelnut kernel in the confectionery industry.

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QUANTIFYING LANDSCAPE FRAGMENTATION VIA EFFECTIVE MESH SIZE LANDSCAPE METRIC: CASE OF ALBANIA

SUMMARY

The assessment of landscape fragmentation (LF) in developing regions is vital to support sustainable decision making in managing the accelerating territorial transformations. Albania is a case where the territorial development processes exhibit extensive transformation rates of land use and land cover (LULC). Lack of measurements for the existing situation and the ongoing high rates of LULC alterations are alerting to act. First, for assessing the current fragmentation caused primarily by the road network and land use change, and second, for highlighting regions of significant importance for biodiversity protection. This study aims to address LF assessment by developing an analytical workflow of consecutive steps utilizing QGIS software. Furthermore, we aim to identify the existing degree of LF in Albania through quantitative results after defining key fragmenting geometries. Effective mesh size (*meff*) is selected as the landscape metric to be used in quantifying the assessment of LF in four levels. The materials of our study rely on open access geospatial data like, CORINE Land Cover, open street map, and digital elevation model, which are utilized as raw data of the analytical processes. At this stage the method is flexible enough to be applied in other developing regions. The results derived from *meff* calculation highlighted the extended influence of LF phenomenon, mainly caused by transportation infrastructure and agricultural areas. We push forward this method as a rapid quantitative landscape assessment technique to deliver reliable graphical and statistical results, which are of assistance to institutions responsible of decision-making processes in spatial planning and management in Albania and beyond.

Keywords: Albania, CORINE Land Cover, Effective mesh size, Landscape fragmentation, QGIS

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INTRODUCTION

Landscape fragmentation (LF) is considered as one of the most prominent issues regarding territorial development having negative impact on biodiversity, ecosystems, and quality of life at global, regional, and local levels (Jaeger *et al*., 2011; Ibáñez *et al*., 2014). Recent studies have shown the correlation between LF and consequences like land loss and flooding (Lam *et al*., 2018; Wu *et al*., 2019). The transformation dynamics of land use and land cover (LULC) have direct effect on landscape fragmentation and the shrinkage of natural landscape patches (Sharma *et al*., 2017). These alterations in LULC may lead to snowballing effects like soil erosion at basin level (Spalevic *el at*., 2017).

In order to safeguard the regional and global consequences of the local LULC transformation processes, the scientific community have highlighted the importance of monitoring the LULC transformation dynamics (Lambin and Geist, 2008). Current literature includes various indicators and metrics that have been developed for LF assessment (Llausàs and Nogué, 2012; Frazier and Kedron, 2017). Yet, there is no definitive agreement among scholars about the most effective tools for LF assessment (Almenar *et al*., 2019). However, some of them have been successfully integrated into decision making processes in land use planning.

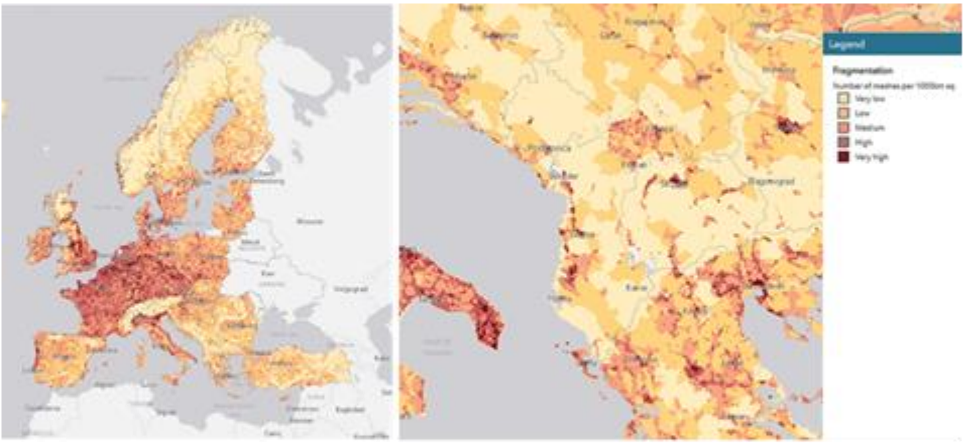

Figure 1: Fragmentation pressure of urban and transport infrastructure expansion in EEA report (EEA, 2018), where Albania is classified among countries with lowest LF degree

The effective mesh size (*meff*) method provides useful and informative data for quantifying LF as a significant input for local and regional planning (Girvetz *et al*., 2008). Despite statistical data, there are produced maps that visualize the LF’s spatial distribution, therefore making simpler the identification of habitat areas that are threatened by fragmentation phenomena. *Meff* method has been used in different regions, such as California, France, Germany, and Switzerland. While in developing countries where the landscape transformation rates are accelerating, LF assessment studies are still rare. European Environment Agency (EEA) reports that the fragmentation pressure of urban and transport infrastructure expansion (EEA, 2018) is less in developing European regions compared to the developed countries (see Figure 1).

In this study we bring the case of Albania, a developing country within the Mediterranean basin, with a dynamic structure of natural lands that reflect the unsustainable territorial development (Mullaj *et al*., 2017). Land reclamation programs during the socialist regime between 1945 and 1989 had a great impact on the natural lands and systems (Danermark, 1993; Rugg, 1994; Lusho and Papa, 1998). On the other hand, the post-socialist transition period (1990-ongoing) marks tremendous land use change in the rural, peri-urban and urban lands (Aliaj, 2003; Pojani, 2010). In recent years, the aspiration for EU membership has directed the governmental instances to take actions over sensitive issues in regional and global level, and among them are the sustainable territorial development and biodiversity conservation (Mele, 2017).

There are just a few studies on landscape development issues for the Albanian context. The most tangible ones have been conducted by Hysa and Türer Başkaya (2017) who have initiated an evaluation of LF in Albania with a specific focus on broad-leaved forest surfaces due to their dominant biodiversity and ecological values. This study relied only on the distance between broad-leave forested patches. However, other significant components of LF processes are not considered. Thus, more comprehensive frameworks are needed, which consider not only the fragmented landscapes but also the fragmenting agents.

The main objective of this study is to propose a practical and comprehensive GIS-based workflow for assessing the LF in Albania based on *meff* methodology. Furthermore, we tend to promote the utility of open source tools like QGIS, and freely accessible LULC data which can support similar researches in developing countries as the financial support to scientific research is very limited. Finally, we aim to highlight the importance of LF assessment for developing countries like Albania, and to motivate future studies at regional level like the Western Balkans countries. This is important as the region shares many unique trans-boundary landscapes which must be managed collaboratively.

MATERIAL AND METHODS

This empirical study aims to achieve quantitative results on LF in Albania through effective mesh size methodology applied by Girvetz *et al*. (2008). The evaluation is approached in 4 hierarchical stages, to see the impact of each of the fragmenting elements on the *meff* value at national scale. The main tool to conduct this study is QGIS 3.4. software. The raw materials rely on the open source data like CORINE Land Cove (CLC), open street map (OSM), and digital elevation model (DEM) (see Table 1).

CLC data are used as a medium for landscape fragmentation assessment, by reclassifying the land cover classes according to the methodology approached in this paper. OSM open source data provides the main spatial information about the road network geometries, which are the core fragmenting agents during the first two levels of LF assessment (FG1 and FG2). Consequently, OSM layer is attached to the CLC reclassification, using QGIS overlay techniques to classify four fragmenting geometries that will be the input material for *meff* assessment.

Table 1. Raw materials and the respective sources

Material	**Type**	**Description**	**Source**
CORINE Land Cover	vector	CORINE Land Cover is an inventory of 44 land cover classes. It uses a Minimum Mapping Unit of 25 ha and a minimum width of 100 m for linear features*.	Copernicus Portal**
OSM (open street map)	vector	OSM data is a free source. It provides data derived from the Open Street Map Project, such as transport infrastructure***.	OSM/Geofabric****
DEM	raster	Digital Elevation Model is a representation of terrains surface in 3D. We used the EU-DEM v1.1 data.	Copernicus Portal

* The description of CLC has been referred to Copernicus Portal descriptive sections.
** https://land.copernicus.eu/pan-european/corine-land-cover
*** Geofabrik website, Open Street Map section.
**** http://download.geofabrik.de/europe/albania.html

Study area

Albania is a small developing country in the south-eastern Europe, in Balkan Region (see Figure 2), with a dynamic background in its territorial development history. It has a unique natural setting character which is rich in landscape diversity and climate conditions (Mullaj *et al*., 2017). Even though Albania is a small country covering not more than 0.3% of the total area of European territory, it has more than 30% of flora and fauna found in Europe (Mullaj *et al*., 2017). This fact highlights the importance of landscape conservation and the weight of landscape interventions' consequences in Albanian territory at regional level.

The landscape of Albania is quite fragmented (Mullaj *et al*., 2017), testifying the inadequate territorial development history because of the conflicting and unwise political managements of the territory. The first threshold of land cover transformation was during communist regime with a polarized urban development, and agricultural and industrial expansion through deforestation, affecting thoroughly the landscape quality and natural capital (Rugg, 1994; Naka *et al*., 2002). Afterwards, the post-socialist period was confronted with chaotic land management because of the uncontrolled urban expansion since the 1990s (Cungu and Swinnen, 1998; Pojani, 2010). Since then, urbanization has been associated with relatively large transportation infrastructure projects, and other infrastructural mega projects such as hydropower plants and Trans-Adriatic Pipeline (TAP). These projects are considered negative pressures to the landscape and the surrounding habitat (Dervishi and Hysa , 2018).

The attempts of national instances to preserve and maintain natural resources have been increasing recently; some of the actions are Territorial and Administrative Reform, the law on freezing all construction permits in national level during 2014-2016 period (Mele, 2017; Hysa and Türer Başkaya, 2018), and the evidence from the State of Environment Report (SoER) that government recently has been giving priority to the re-evaluation and expansion of protected areas (Dervishi and Hysa, 2018).

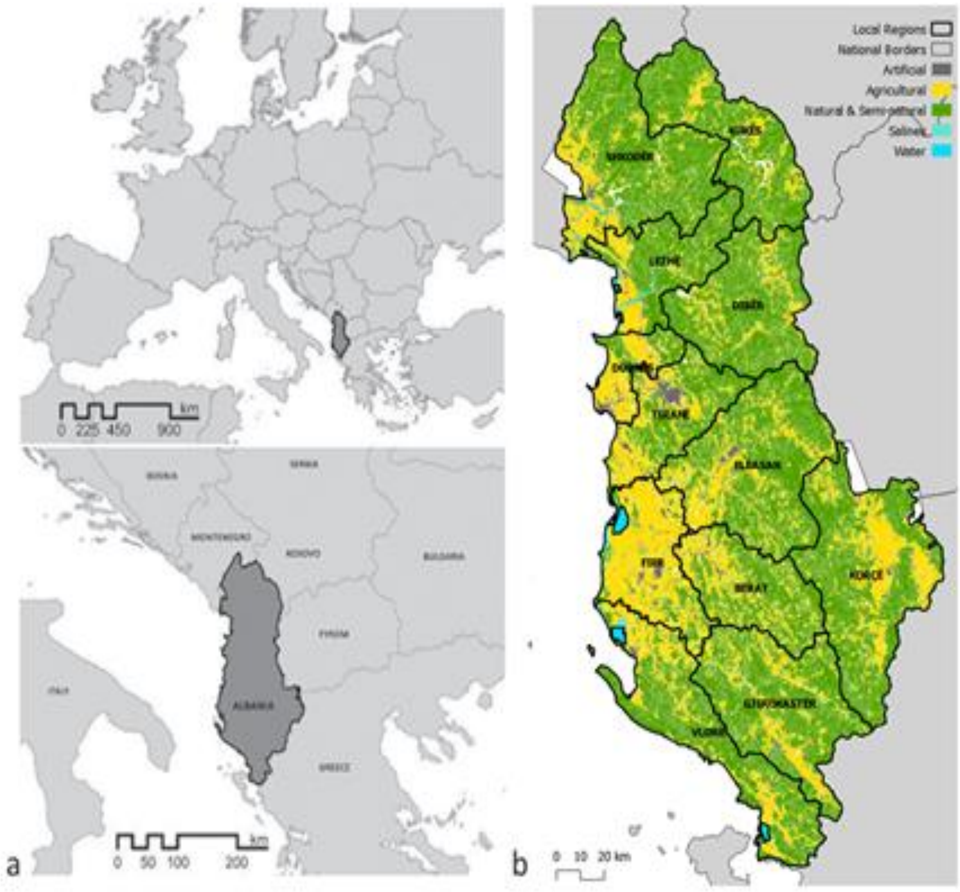

Figure 2: Albania within Europe (a) and the administrative regions and the main land cover classes (CLC-2018) (b)

The attempts of national instances to preserve and maintain natural resources have been increasing recently; some of the actions are Territorial and Administrative Reform, the law on freezing all construction permits in national level during 2014-2016 period (Mele, 2017; Hysa and Türer Başkaya, 2018), and the evidence from the State of Environment Report (SoER) that government recently has been giving priority to the re-evaluation and expansion of protected areas (Dervishi and Hysa, 2018). Additionally, Albania is a collaborating member country of EEA which is beneficial and favourable for improving environmental and biodiversity conservation actions.

However, the only assessment on landscape dynamics is the CLC data being regularly produced in a 6 years interval. Initiating a LF assessment in Albania, Hysa and Türer Başkaya (2017) have conducted a study utilizing Matrix Green Toolbox, and CLC data, to investigate the connect-ability of fragmented patches through edge to edge links, with a focus in the broad-leaved surfaces. They propose the consideration of environmental criteria in the decision-making process of TAR in similar geographies like Albania, as a substantial mediator that could lead to a sustainable management of cross-border natural landscapes, thus reducing the LF impact in country and regional level (Hysa and Türer Başkaya, 2018).

The results from the EEA report (2018) demonstrate that the fragmentation pressure in Albania is very low relative to the regional level (see Figure 1). It is of importance to mention that this evaluation has considered as fragmentation agents only transportation infrastructure network, and urbanized areas. Lekaj *et al*. (2019) have concluded that from 2000-2018 agricultural and wetland areas decreased by 3529.5 ha due to anthropogenic factors referring to the LULC analysis of ultramafic areas in Albania. Similar studies bring out the LF phenomenon in Albania at specific layers/context mainly caused by human development. Thus, the inclusion of other anthropogenic activities like agriculture must be considered as fragmenting agents.

Meff size as a method for Landscape Fragmentation assessment

Effective mesh size (*meff*) is a landscape metric first proposed by Jaeger (2000). It is defined as an expression of the probability that any two locations in the landscape are connected and not separated by barriers (such as roads, railways, rivers, etc) (Jaeger, 2000; 2007). It can also be interpreted as the average size of the area that an animal placed randomly in the landscape will be able to access without crossing barriers (Jaeger, 2002). Thus, *meff* measures landscape both inter-connectivity between patches and intra-connectivity within patch (Girvetz *et al*., 2008; EEA,2018), according to Equation 1.

$$m = \frac{1}{A_t} \sum_{i=1}^{n} A_i^2 \qquad \text{(Equation 1)}$$

where n is the number of patches, A_1 to A_n represent the patch sizes from patch 1 to patch n, and A_t is the total area of the region investigated.

The method by Girvetz *et al*. (2008) was referred to in this paper due to the inclusiveness of fragmenting elements in *meff* calculation, which leads to a more realistic LF value. They categorize the fragmenting geometries in 4 hierarchical levels; each higher level of fragmentation geometry builds on the previous one (see Table 2.). The first two levels rely on the road network which have direct ecological effect on living communities (Trombulak and Frissell, 2000).

Table 2 illustrates the set of fragmenting elements considered in this study. Some classes of CLC and OSM were excluded considering the compliance of the elements to each fragmentation geometry. For instance, for FG2-minor roads dataset it was excluded the ‘bridleway’, ‘footway’, and ‘path’ classes because of their natural character; for FG3-agricultural areas dataset the excluded classes are clc244 ‘agro-forestry’, clc231 ‘pastures’, and clc223 ‘olive groves’ since we did not consider them as artificial barriers. Regarding the FG4 elements, it was revised the minimum height for alpine areas by considering the Albanian geomorphology and territorial character. In the case of Albania, the mountainous geography reaches the highest peak with Korabi Mountain at 2751 meters. Thus, we decided to revise the limit for alpine lands from 3000 m (Girvetz *et al.*, 2008) to 2000 m.

Table 2. Summary table of fragmenting elements used to define each fragmentation geometry (after Girvetz *et al.*, 2008).

	Fragmenting elements	OSM and CLC input layers
FG1	Highways	osm: highways
	major roads	osm: motorway, motorway link; primary, primary link; secondary, secondary link; tertiary, tertiary link; trunk, trunk link
	railroads	osm: railroads
	urbanized areas	clc: 111, 112, 121, 122, 123, 124, 131, 132, 133
FG2	FG1 and minor roads	osm: cycleway, living street, pedestrian, residential, service, steps
FG3	FG2 and agricultural areas	clc: 211, 212, 221, 222, 242, 243
FG4	FG3 and lakes, major rivers, alpine areas	Lakes, major rivers, alpine areas above 2000m

Workflow of the study

The work process for LF assessment in this study is entirely conducted through QGIS 3.4 software and organized in 4 sequential phases; adjustment of base material (A), classification of FG layers (B), calculation of area and *meff* values (C), and a concluding step to visualize the results of *meff* for each FG layer (D) (Table 3.1 and Table 3.2). The Table 3 illustrates each step utilized in QGIS through specific toolbox commands, and the input and output layers extracted during the FG1 process phases. The steps are hierarchically applied, following the logic of adding geometries over the earlier one (see Table 2).

Table 3.1The workflow for assessing the Level 1 of LF (FG1) in QGIS

		Step/Reason	Input Layer	Toolbox	Output Layer
A	1	Fixing the geometries to avoid errors in processing.	CLC2018	Fix geometries	CLC_fixed geometries
	2	Unifying CRS from 4043 to3035	CLC2018	Export	CLC_2018
	3	Prepare base material to be used in FG classification.	CLC2018	Clip -Fix geometries	CLC_clip

Table 3.2 The workflow for assessing the Level 1 of LF (FG1) in QGIS

		Step/Reason	Input Layer	Toolbox	Output Layer
B	4	Select the land cover classes in *clcAlb_clipped* layer that will be included in *FG1_natural* layer.	CLC2018-FG1	Select by expression /dissolve	CLC2018-FG1-natural
	5	Import OSM roads layer for Albania. *Fix_clipped_roads* to select the roads included in FG1.	OSM-Roads	Export/clip /select by expression	CLC2018-FG1-roads
	6	Dissolve the layer and then add the buffer value (7.5m) for it.	CLC2018-FG1	Dissolve - buffer	CLC2018-FG1
	7	Clip OSM railways layer to *study area borders* layer.	OSM-Railways	Export/ clip/ buffer	CLC2018-FG1-rail
	8	Merge roads and railways into *FG1_transport*. Dissolve and buffer (7.5m) the output layer.	CLC2018-FG1	Merge /Dissolve/ buffer	CLC2018-FG1-transport
	9	Fragmentation of FG1 natural layer by FG1 transport layer.	CLC2018-FG1	Fragscape–Split	CLC2018-FG1-frag.
C	10	Extracting the 'Area' for each fragmented patch.	CLC2018-FG1	Field calculator	
	11	Removing the patches smaller than 25ha**	CLC2018-FG1	Extract by attributes	
	12	Extracting the Sum=Total Area (Area_Total).	CLC2018-FG1	Basic statistics	Statistics html file
	13	Calculating Meff individual: 'Area' * 'Area'/ 'Area Total'	CLC2018-FG1	Field Calcuclator	Meff_ind
	14	Extracting the Sum=Meff Total (Meff_total).	CLC2018-FG1	Basic statistics	Statistics html file
D	15	Export the *Fragmentation_FG1_natural* layer after meff calculations, as *FG1_meff*.	CLC2018-FG1	Export–save features	CLC2018-FG1-meff
	16	Apply the categorization of patches according to *meff* value.	CLC2018-FG1_meff	Symbology categorized	Map

** This is due to the MMU of CLC data, considering the smaller patches as accidental during splitting operation.

RESULTS AND DISCUSSION

This research paper provides evidence of LF level through empirical results which are derived from *meff* calculation. The objective is to have a complementary work to achieve a more inclusive database for LF assessment in Albania. The results derived from this study display a total *meff* value decreasing when fragmentation geometries are added hierarchically, from FG1 to FG4.

According to the results presented in Table 4, the *total meff* value for FG1 is 184727ha, FG2 is 101628 ha, FG3 is 90102 ha, and FG4 is 27898 ha. LF increases with the decreasing of *total meff* value, respectively from FG1 to FG2 decreases by 44.9%, from FG2 to FG3 by 6.3%, from FG3 to FG4 by 33%, and in total from FG1to FG4 by 84.9% (see Figure 3b). Number of patches is another indicator of LF degree for each level of FG. The number of patches increases from FG1 to FG2 by 591 patches, from FG2 to FG3 by 62 patches, and from FG3 to FG4 by 573 patches (see Figure 3a).

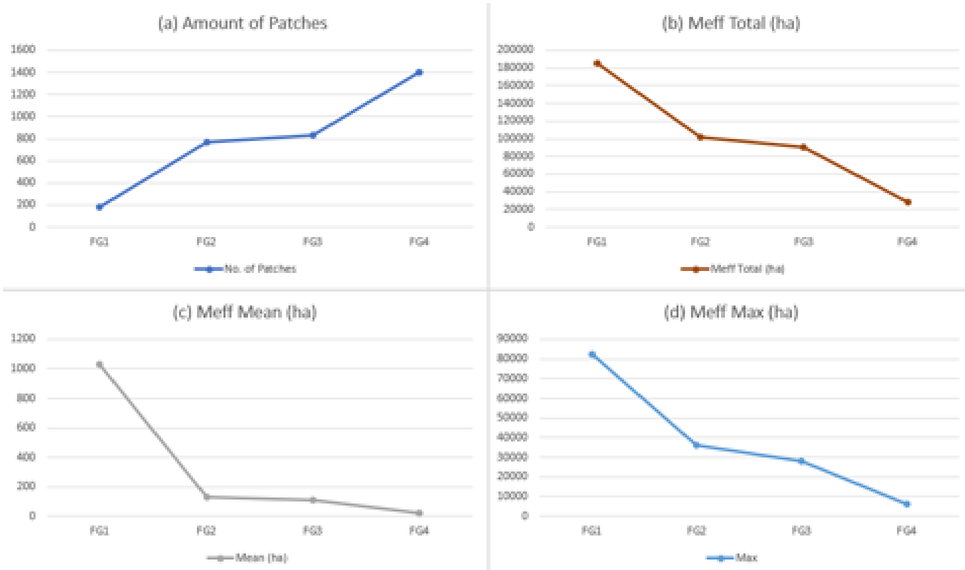

Figure 3: Meff values: (a) Amount of Patches, (b) Meff Total, (c) Meff Mean, (d) Meff Max.

These values highlight the highest impact of level 1 and 2 of urbanized and transportation network fragmenting geometries (FG1 and FG2), followed by natural features included in level 4 (FG4). On the other hand, agricultural areas seem to have the lowest impact in fragmentation of the landscape, although they reduce a considerable amount of the 'Total Area' by 7008 ha (see Table 4). This can be due to their proximity to urbanized areas which already have been calculated in FG1 and FG2, minimizing the impact of agricultural surface.

Comparing 'Mean' and 'Max' values it is noticed that the fragmentation of landscape into small and isolated patches is higher when transportation infrastructure layer is added in FG1 and FG2 (see Figure 3c and 3d); mean value is the average *meff* value relative to the number of total patches and gives an overall idea of the fragmentation degree; maximum value is the highest *meff* value (the largest and less fragmented patch).

Natural features seem to have approximately the same impact as transportation networks and urban settlements. Considering the geographic context of Albanian territory, predominant natural elements such as lakes, high mountains and rivers are mainly concentrated in the Eastern part of the country (see Figure 4). This region is less urbanized compared to the western part of the country. Therefore, FG4 *meff* value increases significantly.

Table 4. Statistics for Fragmenting Geometries.

	No. of Patches	Area Total (km2)	Mean (ha)	Max	Median	total *meff* (ha)
FG1	179	27695	1032	82363	0.013	184727
FG2	770	27417	131	35967	0.007	101628
FG3	832	20409	108	27998	0.003	90102
FG4	1405	18748	19	5935	0.005	27898

Visual representation of *meff* values for each individual patch contributes for more tangible results, by adjusting the LF spatially within the study area (Figure 4). The western part of Albania is highly fragmented, concentrated more in between the central and southern Albania. The main reason is urbanization, transportation infrastructure and industrial development. While the LF being present in the eastern part of Albania is caused by natural features amplification/dominance when calculating FG4.

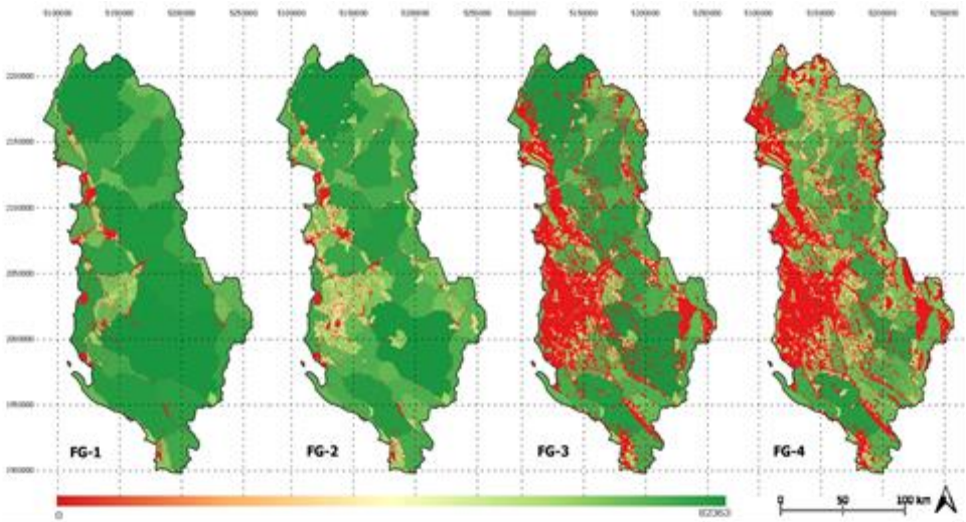

Figure 4: Landscape fragmentation maps for FG-1, FG-2, FG-3, and FG4 according to *meff* results (utilized by QGIS 3.4). *meff* results for each fragmented patch are represented in the legend.

Figure 5 presents the box plot of landscape fragmentation at four levels as a statistical support to the visual information delivered in Figure 4. The *meff* values are represented in logarithmic values to make the interpretation of the results clearer. According to the box plot, there is a continuous decrease in the upper bound of *meff* values while the FG level increases. The upper bound of *meff* values for FG4 is about ten times smaller than the upper bound of FG1. The mean values follow a similar decreasing trend. On the other hand, the lower bound of *meff* values remains the same at minimum values for all fragmentation levels.

The results of upper and lower bounds are supporting the assumption that the increase in fragmenting elements (from FG1 towards FG4, see Table 2.) leads to a decrease of *meff* values. The only unexpected situation is related with the results of the third quartile of FG4. There is a slight increase in the median value and the upper bound of the third quartile of FG4 compared to FG3. The reason behind that could be related with the fragmenting geometries introduced at level 4 (FG4).

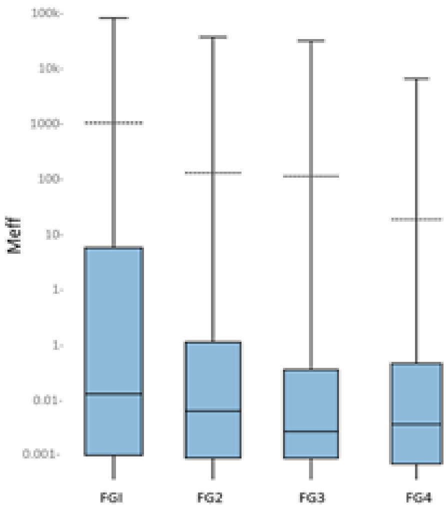

Figure 5: Box plot of landscape fragmentation in Albania represented in logarithmic *meff* values of FG1, FG2, FG3, and FG4.

In the method presented in our work, FG4 is the level in which the water surfaces and alpine lands are introduced as fragmenting agents (see Table 2). Figure 5 implies that the alpine lands (altitude above 2000m) consist of a considerable number of natural areas of small size (low *meff* value). Thus, their reclassification as fragmenting elements result in a slight increase in third quartile of *meff* values for FG4.

The results presented in Figure 6, rely on the spatial distribution of *meff* results per local district as shown in Figure 2. The graph illustrates fragmentation values for each level FG1, FG2, FG3, and FG4 for all districts (Figure 6). Human activity in agriculture and industry is mostly situated in the western Albania region (coastal region). It has greater expansion of national transport arteries, and other human settings.

Districts found in the coastal western and central regions like Lezhe, Durres, Tirane, Fier, and Vlore reflect the highest LF indicated in *meff* values. Durres, Fier, Lezhe and Tirane are the most fragmented regions when compared to other districts. The industrial activity in Albania is mainly concentrated in Durres, Fier, Tirana which is the capital city. Vlore is less fragmented as a result of a minimized human impact in its territory. The uncontrolled urban development in developing counties is accepted to have significant negative impact on natural environments (Parsipour *et al*., 2019).

In the same line, Elbasan, Berat, and Gjirokaster have considerable human activity but are less intense when compared to abovementioned industrial zones. The Eastern part has a predominance of mountainous terrain and the major economic activity for its residents is agriculture. Diber, Kukes and Korce are eastern regions, where Korce seems to be less fragmented, and Kukes is highly fragmented due to natural features and highland characteristics within its boundaries.

Shkoder district is situated in the north-west of Albania. It reflects highest *meff* values in four levels that have been calculated in this inquiry. The lowest *meff* value is noticed in FG4 and this is because of the abundance of natural features like lakes, rivers, and alpine areas. While the records for Berat reports the highest fluctuation of *meff* value among four levels of the analysis. This is due to the vast and diverse amount of natural features in its territory, which lead to high *meff* values at FG1 and significantly low at FG4.

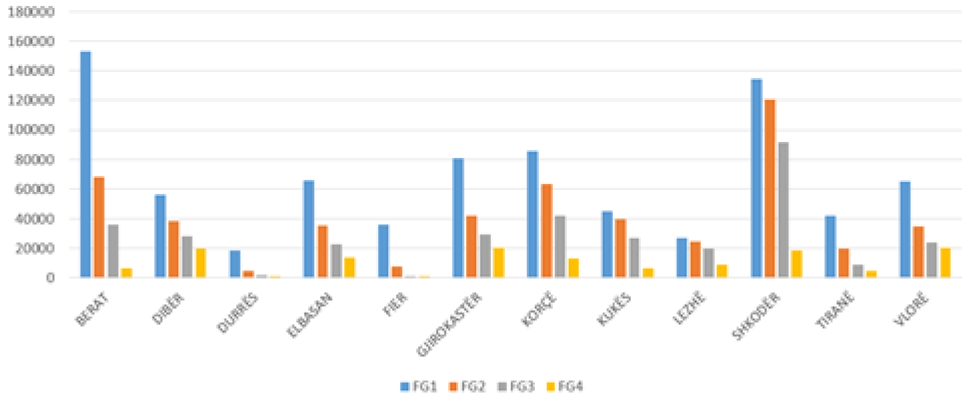

Figure 6: Landscape fragmentation in Albania by administrative regions represented in absolute values of FG1, FG2, FG3, and FG4

Our results confirm the fact that Albanian territory holds a considerable level of LF. The empirical data derived from *meff* calculation supplies evidence that LF in Albania is higher than presented in the EEA report. The method of four calculated levels of fragmentation geometries (FG) intends to analyze how each category reflects the total value of *meff*. Transportation infrastructure results to have the highest impact due to an uncontrolled urban expansion that occurred after the 90s (post-socialist period); as the consequences of urban development in the period between 1990 and 2000 have been irreversible. The transportation network not only fragment the natural lands but also elevate wildfire ignition probability and the human activities interfere to wildland vegetated surfaces (Hysa and Spalevic, 2020).

Similarly, FG4 elements have the same influence in LF degree as transportation networks. Their barrier effect is characterized by surface expansion dividing and at the same time distancing the surrounding habitats from each other. In the case of Albania their impact is very prominent due to its mountainous terrain and richness in blue infrastructure.

LF in Albania has also a considerable impact at regional level, since it has more than 30% of flora and fauna found in Europe (Mullaj *et al*., 2017). This fact addresses LF to be significantly integrated in decision-making actions related to environmental and territorial development programs. LULC dynamic developments such as urban expansion, new infrastructure projects, and agricultural activity are future events that can occur as a result of population growth, consumption, migration, etc.

The results of this inquiry can guide the responsible instances to consider the barrier effect for each category of intervention and foster projects with connectivity character to minimize fragmentation of the territory and improve the minimal requirements of endemic fauna and flora communities. The case of 'Long-Term Defragmentation Programme' in the Netherlands (Nunes *et al*., 2005; van der Grift, 2005) is a vital reference for guiding concrete actions to mitigate the LF. The results are relevant for regional level comparative studies, to promote studies related to landscape degradation and LF causing irreversible loss to biotic communities. Thus, this study can also motivate future studies comparing between countries in the Western Balkans Region and in the Mediterranean Basin.

Future works can elaborate downscaling of the method presented here to the local and metropolitan scale. This can lead to geospatial and statistical results for each smaller spatial unit to determine best future scenarios for a less fragmented territory at a gradient of spatial scales. The downscaling is crucial for better understanding the impact of LULC transformation on the ecosystem services provided by the interconnectivity of blue-green infrastructure at the metropolitan and urban scales (Deslauriers *et al*., 2019; Hysa, 2021).

CONCLUSIONS

This study presented a rapid and practical workflow for LF assessment at landscape scale. The study area consists of the territory of Albania. The raw data rely on various open source geospatial data providing information about the LULC, transportation network and geomorphology (DEM) of the study area. LF analysis is based on the effective mesh size (*meff*) landscape metric. Our results show that the natural landscapes in Albania face considerable levels of LF, refuting the report by European Environment Agency on LF at continent scale. The main causes are both anthropogenic (transportation network, urbanized areas, and agricultural lands) and natural (major rivers, lakes, alpine lands).

The method defines a hierarchical workflow of four stages. At each stage there are unique sets of fragmenting geometries which are expanding by additive elements like, main transportation routes, secondary roads, agricultural lands, and dominant natural features. According to our results, secondary road network and dominant natural elements have the highest impact in landscape fragmentation. Their capillary structure split the landscape patches into smaller patches and elevate the LF levels in Albania. Since Albania is a developing country where considerable investments in transportation network are still to come, it is vital that the decision-making bodies take in consideration the LF concerns while making new plans. This approach must be applied by other developing countries in the region of Western Balkans as they share unique trans-boundary eco-regions that holds values at continental scale.

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ANTIMICROBIAL ACTIVITY OF GINGER (*Zingiber officinale*) AND ROSEMARY (*Rosmarinus officinalis*) ESSENTIAL OILS

SUMMARY

New advances in the food industry are directed towards exploiting natural resources. Nowadays, essential oils and their antimicrobial activities are the subject of many researches. Their possible use as natural food additives is particularly prominent. This study analyzed the influence of ginger and rosemary oil on the growth of pure bacterial culture using the disk diffusion method. *Escherichia coli, Staphylococcus aureus* and *Salmonella spp*. were used as test organisms for antimicrobial susceptibility testing.

The results showed that both types of oil inhibit bacterial growth, although inhibition rate varies between different bacterial species. It certainly depends on the type of plant used for oil extraction. Study has shown that ginger and rosemary oil can potentially be used in treating diseases caused by these bacteria.

Keywords: antimicrobial activity; ginger (*Zingiber officinale* Roscoe), human pathogens, rosemary (*Rosmarinus officinalis* L.).

INTRODUCTION

In the last decades, the interest in essential oils has been increased (Chouhan *et al*., 2017). They have been used for centuries in natural medicine (Boesl and Saarinen, 2016), mainly due to the large number of biologically active phytochemicals in their composition. Many essential oils are claimed to have antimicrobial activity and are used for the prevention and treatment of many infectious diseases as alternative medicines (Stea *et al*., 2014). The food industry

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offers a wide range of products that have antimicrobial activity. Preventing microbial proliferation and protecting food from oxidation is a burning issue. Therefore researches have been directed towards essential oils and plant extracts, as potential natural additives in food (Bellou *et al*., 2016). Fresh and dry roots of ginger (*Zingiber officinale* Roscoe) contain essential oil (ginger oil) and oleoresin (ginger extract). The benefits of ginger oil mainly come from mono- and sespe-terpenoids such as neral, geranium, 1,8-cineol, α-zingiberen, β-bisabolene and β-sesquiphellandrene (Shirooye *et al*., 2016). It also contains a-pinen, B-pinen, stone, linalool, borneol, y-terpineol, nerol, geranol and geranyl acetate (Ekundayo *et al*., 1988). Ginger oil consists of 90% sesquiterpenes, which are responsible for anti-inflammatory, antibacterial and other medicinal properties. Research has shown that rosemary (*Rosmarinus officinalis* L.) is rich in volatile oils, flavonoids and phenolic acids, which are characterized by antiseptic and antiinflammatory effect (Haida *et al*., 2015). The leaf extract showed an extremely high antioxidant activity (Monino *et al*., 2008).

Many higher plant species have been used for spice and medical application; however, recent researches have addressed the aplication of essential oils and spice extracts in control of diseases caused by pathogens (Cui *et al*., 2018; Teles *et al*., 2019) and improvement of food safety. Antunes *et al*. (2012) found that essential oils inhibit synthesis of macromolecules in pathogenic bacteria. Taking into account the expansed amount of antibiotic-resistant microbes, it is necessairy to find novel antipathogenic agents and assess their ability to protect food products from spoilage (Wang *et al*., 2020).

Although the impact of ginger and rosemary oils in the suppression of human pathogen`s growth is well documented, only a few reports in Bosnia and Herzegovina have addressed the influence of essentials oils on human pathogens (Dzaferovic *et al*., 2019; Gavric *et al*., 2018). Thus, the main focus of this paper was to determine the impact of ginger and rosemary essential oils on three human pathogens.

MATERIAL AND METHODS

The study analyzed the effect of ginger and rosemary oil on the growth of bacteria from the *Enterobacteriaceae* (*Escherichia coli* and *Salmonella spp*.). and the *Staphylococcaceae* family (*Staphylococcus aureus*). Oil extraction from rosemary and ginger leaves and root was carried out in the Microbiology Laboratory at the Faculty of Agriculture and Food Sciences in Sarajevo. The oils were obtained by distillation technique using Clevenger apparatus. Antimicrobial susceptibility testing of essential oils on the growth of *S. aureus*, *E. coli*, and *Salmonella spp*., was done using the disk diffusion method. This method is based on the diffusion of oil through a filter paper applied on the surface of the sown substrate. Mueller-Hinton's (MH) agar was used as the experimental medium. 18-hour cultures of *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *Salmonella spp.* were used for the experiment, which were seeded in a nutrient medium and incubated at 37°C. Petri dishes with MH medium were inoculated with 0.1 ml of bacterial suspension. Filter paper sterile disks with a 6 mm diameter were placed on the surface of prepared Petri dishes using aseptic technique. Each disc was then immersed with 10 µl of essential oil. The experiment was performed in six replications. Petri dishes were incubated for 24 h at 37 ºC. The results were examined after 24 hours by measuring the diameter of inhibition zones. Afterward, the effect of the essential oils was determined. Statistical analysis was done using ANOVA and Bivariate Pearson Correlation test in SPSS v24 programme.

RESULTS AND DISCUSSION

Research was done in order to analyze the antimicrobial activity of ginger and rosemary oil on three human pathogens using the disk diffusion method. The results have been established using the standard values for the inhibition zones of gentamicin and chloramphenicol antibiotics for *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella spp.* (Table 1).

Table 1: Standard values for the inhibion zones of gentamicin and chloramphenicol antibiotics for *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* spp.

Human pathogens	Antibiotics	Inhibition zone (mm)		
		S	I	R
Escherichia coli	Gentamicin	≥15	13-14	≤12
	Chloramphenicol	≥18	13-17	≤12
Staphylococcus aureus	Gentamicin	≥15	13-14	≤12
	Chloramphenicol	≥18	13-17	≤12
Salmonella spp.	Gentamicin	≥17	13-16	≤14
	Chloramphenicol	≥17	/	≤17

Legend: S – sensitive; I – moderately sensitive; R – resistant

For *E. coli*, ginger oil has an average inhibition zone of 23.67 mm and therefore belongs to S category (sensitive) compared to both types of antibiotics. Correspondingly with the results, ginger oil shows a strong antimicrobial and therapeutic effect. Rosemary also shows an inhibitory effect on *E. coli* whose inhibition zones diameter has an average value of 17.33 mm (Table 2), and therefore belongs to S category (sensitive) in comparison with gentamicin antibiotic. *E. coli* is sensitive to gentamicin and correspondingly the rosemary oil has a very therapeutic effect. In comparison with the chloramphenicol, this oil belongs to the I category (moderately sensitive) which leads to the conclusion that *E. coli* is sensitive to rosemary oil and has the same effect as the aforementioned antibiotic in an increased dosage. Ginger essential oil has a strong inhibitory effect on *S. aureus* with an average value of the inhibitory zone of 23.17 mm, which is included in category S (sensitive) for both types of antibiotics.

This oil has a strong potential to inhibit the growth of *S. aureus* and act therapeutically.

Table 2. Impact of ginger and rosemary essential oil on growth of *Escherichia coli, Staphylococcus aureus and Salmonella spp.*

Human pathogen	Essential oil	Inhibition zone (mm)						
		Discs						AVG ±SD
		1	2	3	4	5	6	
E. coli	Ginger	20	22	25	28	22	25	23.67±2.87
	Rosemary	17	23	12	23	12	17	17.33±4.93
S. aureus	Ginger	20	18	24	26	26	25	23.17±3.37
	Rosemary	20	20	21	23	15	10	18.17±4.79
Salmonella spp.	Ginger	20	19	25	28	23	24	23.17±3.31
	Rosemary	20	25	20	19	20	26	21.67±3.01

Legend: AVG – average; SD - standard deviation

Table 3. Comparison of differences in the antimicrobial activity of ginger and rosemary essential oils between three human pathogens

ANOVA		Sum of Squares	df	Mean Square	F	Sig.
E. coli	between groups	120.333	1	120.333	7.398	.022
	within groups	162.667	10	16.267		
	total	283.000	11			
S. aureus	between groups	75.000	1	75.000	4.369	.063
	within groups	171.667	10	17.167		
	total	246.667	11			
Salmonella spp.	between groups	6.750	1	6.750	.674	.431
	within groups	100.167	10	10.017		
	total	106.917	11			

Rosemary oil has an average inhibition zone of 18.17 mm (Table 2) and therefore belongs to category S (sensitive) when it comes to both types of antibiotics. This indicates the sensitivity of *S. aureus* to this essential oil, and we can assume that it can be used in therapy.

Ginger oil has a strong inhibitory effect on *Salmonella* spp., with its average value of 23.17 mm (Table 2), which defines it as S (sensitive) compared to both types of antibiotics. This oil has a strong potential to inhibit the growth of *Salmonella* spp. and act therapeutically. Rosemary oil with an average 21.67 mm of inhibition zone belongs to the S (sensitive) category when it comes to both types of antibiotics, meaning *Salmonella* spp. is sensitive to this oil and will act in therapy. Using the ANOVA test, we compared the antimicrobial activity of ginger and rosemary oil among three bacterial species tested (Table 3). According to the obtained results ginger and rosemary oil have a statistically different effect only on *E.coli* (p=0.022<0.05). For *S.aureus* and *Salmonella spp*., ginger and rosemary oil did not show any significant difference in antimicrobial activity (*S.aureus* p=0.063>0.05, *Salmonella spp.* p=0.431>0.05).

Table 4. Results of the correlation test

Correlations							
		Ginger	Rosemary	Ginger	Rosemary	Ginger	Rosemary
		E. coli		*S. aureus*		*Salmonella*	
Ginger *E.coli*	Pearson Correlation	1	.249	.646	.135	.910*	-.108
	Sig. (2-tailed)		.634	.165	.798	.012	.839
	N	6	6	6	6	6	6
Rosemary *E.coli*	Pearson Correlation	.249	1	-.389	.353	-.053	.279
	Sig. (2-tailed)	.634		.445	.493	.920	.593
	N	6	6	6	6	6	6
Ginger *S.aureus*	Pearson Correlation	.646	-.389	1	-.287	.857*	-.348
	Sig. (2-tailed)	.165	.445		.582	.029	.499
	N	6	6	6	6	6	6
Rosemary *S.aureus*	Pearson Correlation	.135	.353	-.287	1	.111	-.619
	Sig. (2-tailed)	.798	.493	.582		.834	.190
	N	6	6	6	6	6	6
Ginger *Salmonella*	Pearson Correlation	.910*	-.053	.857*	.111	1	-.415
	Sig. (2-tailed)	.012	.920	.029	.834		.414
	N	6	6	6	6	6	6
Rosemary *Salmonella*	Pearson Correlation	-.108	.279	-.348	-.619	-.415	1
	Sig. (2-tailed)	.839	.593	.499	.190	.414	
	N	6	6	6	6	6	6

*. Correlation is significant at the 0.05 level (2-tailed).

Correlation can take on any value in the range [-1, 1]. The sign of the correlation coefficient indicates the direction of the relationship, while the magnitude of the correlation (how close it is to -1 or +1) indicates the strength of the relationship. According to the correlation results (Table 4), there is a strong positive correlation (r= +.910*) between the effect of the ginger oil against

Escherichia coli and *Salmonella spp.* Also, the effect of ginger oil on *S. aureus* and *Salmonella spp*. have a strong positive correlation as well (r=+.857*).

Several researches have addressed the mechanisms of action of essential oils and their compounds. Gutierrez *et al*. (2008) found that monoterpenes and phenols exist in rosemary essential oil are responsible for antimicrobial activity. Rosemary essential oil is more effective against *E. coli* and *S. aureus* compared to *Pseudomonas* spp. (De Martino *et al*., 2009), which is in accordance with our results. Furthermore, rosemary essential oil showed more expressed antibacterial activity against *Salmonella* spp. compared with *E. coli*. Similar observation was noticed by Ouwehand *et al*. (2010). Genena *et al*. (2008) found that Gram-positive *S. aureus* was more sensitive to rosemary essential oil, which is in accordance with our results. Ojeda-Sana *et al*. (2013) reported that the cell wall structure of Gram-negative bacteria blocks the absorption of components of oils, which explain the resistance of these bacteria.

In previous studies, ginger essential oil also showed high efficiency in the suppression of the growth of pathogenic bacteria. Hanan *et al*. (2016) found that an increase of concentration of ginger essential oil has an inhibitory effect on the growth of *Salmonella typhimurium, E. coli, S. aureus,* and *Bacillus cereus*. As shown previously, the antimicrobial activity of ginger essential oil may be due to gingerols and phenolic components (Mascolo *et al*., 1989). In contrast, Indue *et al*. (2006) reported the absence of antimicrobial activity of ginger extracts against *E.coli* and *Salmonella spp*.

CONCLUSIONS

The disk diffusion method confirmed that ginger and rosemary oil has antimicrobial activity and that in certain conditions they prevent the growth of some human pathogens. Ginger oil showed to be more effective on the growth of *E. coli* compared to rosemary oil. Results for antimicrobial activity of ginger oil did not show any major differences between different bacterial species tested. This confirms that ginger oil has a very strong antimicrobial and therapeutic effect. Based on the results, rosemary oil has pronounced antimicrobial activity and strong therapeutic effect, especially when it comes to *Salmonella spp* and *S. aureus* while for *E. coli* it has to be used in a higher dosage than standard to demonstrate its therapeutic effect.

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EFFECT OF MAGNESIUM AND SULFUR FERTILIZER ON YIELD, DISEASES AND DISORDERS OF SEED POTATO

SUMMARY

A study was undertaken to find out the effects of two major nutrient elements such as Magnesium (Mg) and Sulfur (S) on plant growth, tuber, and tuber disease incidence of potato (*Solanum tuberosum* L.). Data were recorded on stem number/ten hills, plant height, canopy coverage, tuber number per ten plants, tuber weight per plot and incidence of diseases such as common scab (*Streptomyces scabis*), dry rot (*Fusarium sp.*) and soft rot (*Erwinia carotovora*) of potato. The doses of nutrients were Mg @ 0, 10, 15, 20 kgha^{-1} and S @ 0, 10, 20, 30 kgha^{-1}. Every nutrient increased plant growth and tuber yield but decreased disease incidence considerably over control up to second highest dosage. The highest yield of 33.61 and 31.50 tha^{-1} were obtained with 15 kgha^{-1} Mg and 20 kgha^{-1} S. When the two elements were applied together at 15 kgha^{-1} Mg and 20 kgha^{-1} S independently, the highest tuber yield of 35.48 tha^{-1}, and the incidence of tuber diseases and disorders were also minimal. Based on findings of the present study it may be concluded that application of Mg, 21.11 kg and S, 4.28 kgha^{-1} along with 140 kg N ha^{-1} are optimum for maximum plant growth, tuber yield and minimize incidence of disease and disorders of potato tuber.

Keywords: Magnesium, sulfur, potato, yield, quality.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most widely cultivated horticultural crops in the world and holds the 4th largest crop by global production volume (Marcomini et al., 2019). Now, it is cultivated and consumed more than

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160 countries worldwide (Andre et al., 2014). On the other hand, it ranks next to rice and wheat in terms of production and internal demand in Bangladesh (Rubayet et al., 2017). It contains significant amount of Vitamin-B, C and minerals. The yield of the crop in Bangladesh is very low compared to other potato growing counties. The average yield of potato in Bangladesh is 19.6 tha^{-1} in 2014-15 which is much below the potential productivity of the crop (Anon, 2016). Many factors are responsible for low yield of potato such as seed quality, soil fertility, irrigation facilities, nutrient management, pest infestation, etc. (Iqbal et al., 2019). One of the majors constrains for potato cultivation is unavailability of quality and healthy seed tubers. Diseases and disorders are important for qualitative and quantitative tuber losses (Hossain et al., 2004). Among the diseases, tuber-borne diseases and disorders such as soft rot (*Erwinia* spp.), dry rot (*Fusarium* spp.), common scab (*Streptomyces scabies*) skin spot, black heart, heat injury, secondary growth, greening, etc., play important role in reduction of seed quality. Potato crops require a balanced fertilization for plant growth, and both yield and quality of tubers (Koch et al., 2020). One of the most important constrains of higher tuber yield is the lack of adequate balanced fertilizer application. Since 1980, farmers were using only NPK-fertilizers, but now they are applying S and Zn along with NPK. Many researchers are in opinion that application of minor nutrients such as S, Mg, Zn, B in addition to essential major elements can play a good role in increasing the yield of potato (Sharma *et al*., 2011).

Plants are deficit to Mg in the soil having low pH, sandy in nature and highly leached soil with low Cation Exchange Capacity. The most common symptom of Mg deficiency was observed in the potato field is plants showed interveinal chlorosis on the older leaves. Magnesium is an important constituent of chlorophyll molecule, therefore, essential for photosynthesis. Magnesium increase NPK uptake and thereby increase yield and promotes uptake and translocation of phosphorus (Guo *et al*., 2016). With NPK fertilizers supplemental Mg can increase yields and have tremendous effects on potato quality and net returns, even when soil test Mg levels seem adequate. Magnesium is also important in the activity of a large number of enzyme systems in plants, systems that are particularly important in the metabolism of carbohydrates. Magnesium plays a vital role in the adsorption of other nutrients especially phosphorous, potassium and calcium, acts as a catalyst, activators and co-factors in several enzymatic activities and participates in active protein and carbohydrate metabolism. Potatoes are highly sensitive to Mg nutrient deficiency than other crops. Mg deficiency is one of the most important factors for poor growth of potato in the peat and sandy soils. Mg deficiencies may be corrected through the use of magnesium sulfate (Epsom salts or Kieserite) in fertilizers.

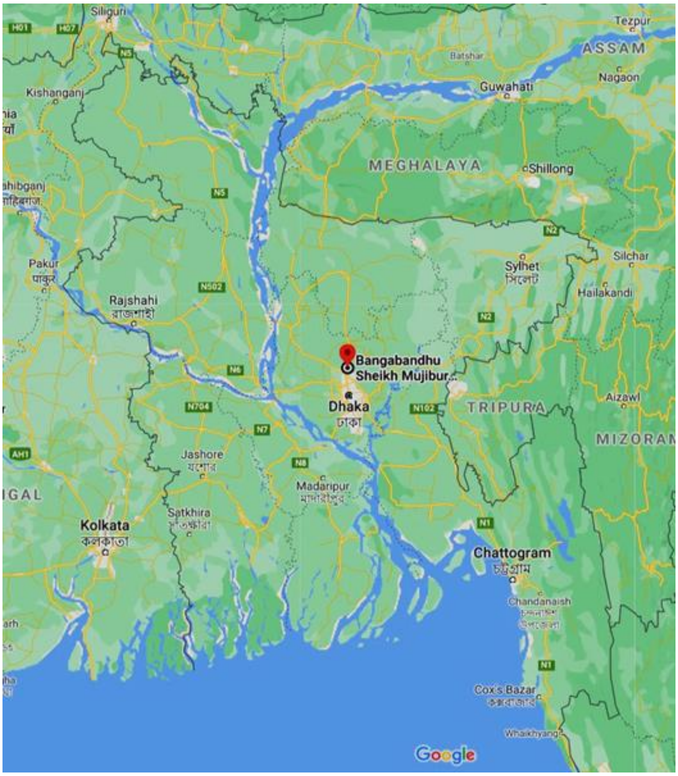
Sharma et al. (2011) observed that sulfur application in potato showed significant influence on quality and yield. The highest tuber yield, large size and medium size tuber yield, dry matter content, specific gravity, sugar content and starch content were found with application of sulfur. Sulfur helps to minimize the diseases, for instance common and powdery scab. This effect may be due to a reduction in the soil pH where elemental sulfur is used. However, a programme of foliar S, can also reduce infection (Brierley *et al*., 2008). Sulfur can reduce common scab, late blight, stem canker of potato. In additionally, Sulfur can balance other nutrients and make the environment less favorable for the pathogen. Applications of elemental S to soil would control potato scab. It appears that the mechanism for control comes from the reduction in soil pH. Elemental sulfur can aid in reducing the infection levels of common scab caused by the *S. scabies*. Best effects come from applying sulfur to the soil in a readily available form at planting. The application of sulfur significantly reduces the infection of *Rhizoctonia solani* and *Streptomyces scabies* and increase tuber yield. It may elucidate S-induced resistance mechanisms in plants (Hanna *et al*., 2006). Above discussion indicates that application of Mg and S may help in increasing production and quality of potato. Hence, to address these problems the study was undertaken with the major objective to investigate the effects of applying different levels of magnesium and sulfur on tuber yield and quality of seed potato.

MATERIALS AND METHODS

The experiment was conducted at Bangabandhu Sheikh Mujibur Rahman Agricultural University farm, Gazipur, Bangladesh (Map 1) during 2014-2016 to find out effect of Magnesium (Mg) and Sulfur (S) on plant growth, tuber yield and tuber health. Mg was applied at 0, 10, 15 and 20 $kgha^{-1}$, which were designated as Mg_0, Mg_{10}, Mg_{15} and Mg_{20}. Sulfur was applied at 0, 10, 20, 30 $kgha^{-1}$, which were designated as S_0, S_{10}, S_{20} and S_{30}. In this experiment the treatment combinations were Mg_0S_0, Mg_0S_{10}, Mg_0S_{20}, Mg_0S_{30}, $Mg_{10}S_0$, $Mg_{10}S_{10}$, $Mg_{10}S_{20}$, $Mg_{10}S_{30}$, $Mg_{15}S_0$, $Mg_{15}S_{10}$, $Mg_{15}S_{20}$, $Mg_{15}S_{30}$, $Mg_{20}S_0$, $Mg_{20}S_{10}$, $Mg_{20}S_{20}$ and $Mg_{20}S_{30}$. Magnesium as magnesium chlorite ($MgCl_2.6H_2O$) and Sulfur as gypsum ($CaSO_4{\cdot}2H_2O$) were used in the experiment.

Experimental site: The soil of the experimental field belongs to Salna series under the Agro Ecological Zone AEZ-28, Madhupur Tract (24.05^0 N latitude and 90.16^0 E longitude) at an elevation of 8.4 m above the sea level. The texture of the soil was silty clay in surface layer and silty clay loam in subsurface layer. The results of soil test reveal that the soil contains 1.4% organic matter, magnesium 1.05 mE/100 g soil, sulfur 2.29 ppm and pH 6.7-6.9. Land of the experimental plots was prepared in the month of November using a tractor driven harrow and disk plough followed by laddering to obtain a good tilth. Weeds and other debris were removed. Before ploughing cowdung based compost was applied at 10 tha^{-1}. A blank dosage of N, P, K, Zn and B was used at 140-35-140-4-1.5 $kgha^{-1}$and applied as urea, TSP, MOP, zinc oxide and boric acid, respectively. One half dosage of NK and other nutrients were mixed with soil in furrows at the time of plantation of seed tuber. After final land preparation, experimental unit plots were prepared. The unit plot size was 2.25 m × 2.40 m. Plot to plot and block to block distance was 1 m. The experiment was laid out in

4 × 4 factorial design in randomized complete block with three replications. The potato seed tubers of variety Diamant (basic class) were collected from Bangladesh Agricultural Development Corporation (BADC). Seed tubers were kept under defused light for sprouting. Sprouted tubers were planted in 5-7 cm deep furrows in 3rd week of November. The planting spacing was 60 cm row to row and 15 cm tuber to tuber. Four lines were accommodated in each unit plot. Standard cultivation operations were followed to grow potato (Anon, 2008). First irrigation was applied one week after planting which was continued for several times as per requirement. After 20 days of planting weeding was done. To control the fungal diseases especially late blight, Dithane M-45 was applied at the rate of 2.5 $kgha^{-1}$ and to control aphids, Admire was applied at the rate of $1Lha^{-1}$. Both the pesticides were applied as foliar spray for six times at 10 days interval starting from 20 days of planting.



Map 1. Location of study area (24°02'15.1" N, 90°23'53.1" E, https://goo.gl/maps/ivZHT7iRS4j23wUq7)

Data recording

Plant growth parameters: At 45 days after planting of seed tuber under different experiments, 15 cm × 60 cm area was selected randomly and data on canopy coverage was recorded and expressed in percentage. At 60 days after planting 10 plants were selected randomly from each plot and data on plant growth attributes viz. number of stems per 10 hills and at 60 days after planting plant height was recorded. Stem killing was done on 80^{th} day of after planting to avoid spread of viruses and for hardening of tuber skin. After ten days of stem killing potato tubers were harvested manually and care was taken to avoid tuber injuries at harvest. Seven days after air drying healthy tubers were sorted out and graded into four grades on the basis of tuber size. Grading of tuber was done following a standard grading system based on size according to Bangladesh Agricultural Development Corporation (BADC) the only Government's seed producing agency in Bangladesh (Anon., 2008). The grades were undersize (20 – 28 mm), grade A (28 – 41 mm), grade B (41 – 56 mm) and oversize (>56– 60 mm). Data on plant growth and tuber yield attributes in terms of stem number per ten plants, plant height, canopy coverage, number of tubers per ten plants and tuber yield per unit plot were recorded.

Data on disease: Data on diseases and disorders were taken after 7 days of harvest and expressed percentage (w/w) based on per plot yield of potato. The disease was identified based on visible symptoms. Whenever necessary, the identification was confirmed by laboratory tests. The data on incidence (%) of common scab, soft rot and dry rot were recorded. Data on different disease incidence were computed using a standard formula shown below:

$$\%\ \text{incidence} = (\text{Weight of infected tuber/Weight of total tuber}) \times 100$$

Statistical analysis: Data were analyzed statistically using Statistix 10 analytical software (https://www.statistix.com/) for proper interpretation of results. The data recorded on different parameters were subjected to analysis of variance (ANOVA) and the means were compared using least significant difference (LSD) test at 5% level of significance. Graphs and figures were prepared as and when necessary for proper presentation of the data.

RESULTS AND DISCUSSION

Stem number per 10 hills

Main effect of Mg: The highest number of 53.3 stems per 10 hills was recorded at 10 kg Mg ha^{-1} (Mg_{10}), which was statistically similar to 15 kg (Mg_{15}) and 20 kg Mg ha^{-1} (Mg_{20}) but significantly higher compared to control. The lowest number of 47.5 stems per 10 hills was produced under control (Mg_0) (Fig. 1).

Main effect of sulfur: The highest number of 53.3 stems per hill was found at S_{20}, which was statistically similar to S_{10} and S_0. The lowest stem number of 48.3 per 10 hills was observed in S_{30}, which was statistically similar to S_0 and S_{10} (Fig. 2).

Interaction effect: The highest number of 56.7 stems per 10 hills was produced in treatment combination $Mg_{10}S_{20}$, which was statistically similar to $Mg_{20}S_{10}$, $Mg_{10}S_0$, $Mg_{10}S_{30}$, $Mg_{15}S_{10}$, $Mg_{15}S_{20}$, $Mg_{20}S_{20}$, Mg_0S_0, Mg_0S_{10}, Mg_0S_{20}, $Mg_{10}S_{10}$, $Mg_{15}S_0$, $Mg_{15}S_{30}$ and $Mg_{20}S_{30}$. The lowest stem number of 40.00 per 10 hills was recorded in Mg_0S_{30} which was statistically similar to $Mg_{20}S_0$ (Table 1).

Plant height

Main effect of magnesium: Significantly the highest plant height of 64.90 cm was recorded at 15 kg Mg ha^{-1}, which was statistically different from its other doses. The lowest plant height of 57.40 cm was produced in control (Fig. 1).

Main effect of sulfur: The highest plant height of 63.40 cm was found at 20 kg S ha^{-1}, which was statistically similar to 30 kg S ha^{-1}. The lowest plant height was observed in control (S_0) Application of caused significant increase in this parameter compared to control (Fig. 2).

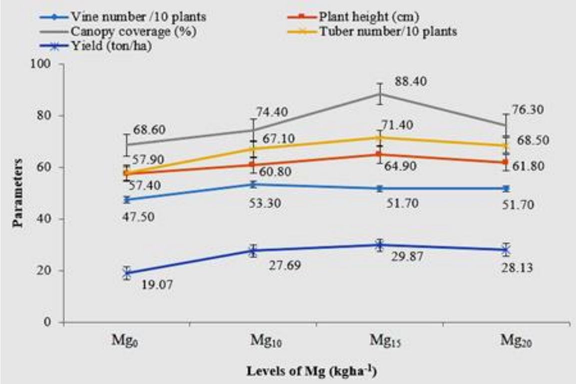


Fig 1. Effect of magnesium (Mg) on growth and yield attributes of potato at different levels under a constant level of NPKBZn [Mg_0 = No magnesium, Mg_{10} = 10 kg Mg ha^{-1}, Mg_{15} = 35 kg Mg ha^{-1}, Mg_{20} = 20 kg Mg ha^{-1}.]

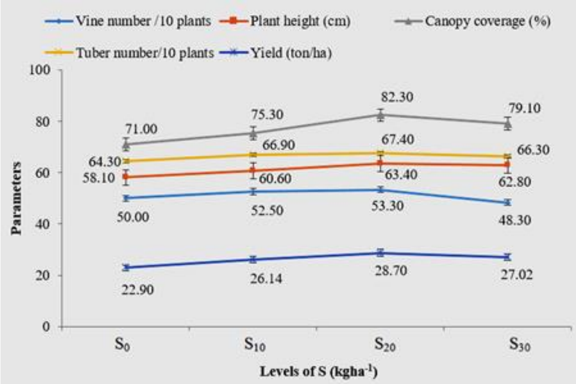


Fig 2. Effect of Sulfur (S) on growth and yield attributes of potato at different levels under a constant level of NPKBZn [S_0 = No sulfur, S_{10} = 10 kg S ha^{-1}, S_{20} = 20 kg S ha^{-1}, S_{30} = 30 kg S ha^{-1}.]

Interaction effect: Significantly the highest plant height of 69.0 cm was produced in $Mg_{15}S_{20}$ which was different from other treatment combinations. The lowest plant height of 54.3 cm was recorded in control (Mg_0S_0) (Table 1).

Table 1. Interaction effect of magnesium (Mg) and sulfur (S) on growth and yield attributes of potato at different levels under a constant level of NPKBZn

Level of Mg / Dose of S	Mg_0*	Mg_{10}	Mg_{15}	Mg_{20}
Stem number /10 plants				
S_0*	50.0 a[1]	53.3 ab	50.0 ab	46.7 bc
S_{10}	50.0 ab	50.0 ab	53.3 ab	56.7 a
S_{20}	50.0 ab	56.7 a	53.3 ab	53.3 ab
S_{30}	40.0 c	53.3 ab	50.0 ab	50.0 ab
Plant height (cm)				
S_0	54.3 k[1]	58.3 ij	61.0 efg	58.7 hij
S_{10}	57.3 j	60.0 ghi	63.7 cd	61.3 efg
S_{20}	57.3 j	63.0 cde	69.0 a	64.3 bc
S_{30}	60.7 fgh	61.7 defg	66.0 b	62.7 cdef
Canopy coverage (%)				
S_0	65.0 j[1]	67.0 i	82.0 de	70.0 h
S_{10}	66.7 ij	73.0 g	87.0 c	74.7 g
S_{20}	69.7 h	81.0 e	95.3 a	83.0 d
S_{30}	73.0 g	76.7 f	89.3 b	77.3 f
Tuber number/10 plants				
S_0	57.0 g[1]	63.8 f	68.4 cde	67.8 e
S_{10}	57.2 g	67.1 e	72.7 ab	70.6 bc
S_{20}	58.4 g	68.8 cde	74.4 a	68.2 de
S_{30}	58.9 g	68.8 cde	70.2 cd	67.2 e
Seed Potato Tuber Yield (ton/ha)				
S_0	16.41 j[1]	22.94 g	26.84 ef	25.39 f
S_{10}	18.30 i	28.26 de	29.83 bc	28.19 de
S_{20}	21.41 h	30.64 b	32.86 a	29.90 bc
S_{30}	20.15 h	28.93 cd	29.96 bc	29.04 cd

*Mg_0 = No magnesium, Mg_{10} = 10 kg Mg ha^{-1}, Mg_{15} = 15 kg Mg ha^{-1}, Mg_{20} = 20 kg Mg ha^{-1}. *S_0 = No sulfur, S_{10} = 10 kg S ha^{-1}, S_{20} = 20 kg S ha^{-1}, S_{30} = 30 kg S ha^{-1}. [1]Figures under the same parameter within row and column are averages of three replications and having a common letter(s) do not differ significantly (*P= 0.05*) by LSD test.

Canopy coverage

Main effect of magnesium: The maximum of 88.4% canopy coverage was recorded at 15 Kg Mg ha^{-1} (Mg_{15}), which was statistically different from other treatments. The minimum canopy coverage of 68.6% in control was also statistically different from other treatments (Fig. 1).

Main effect of sulfur: The maximum canopy coverage of 82.3% was recorded at 20 Kg S ha^{-1} (S_{20}) and the minimum canopy coverage of 71.0% were found in control, which was statistically different from other treatments (Fig. 2).

Interaction effect: The canopy coverage under different treatment combination of Mg and S ranged from 95.3% to 65.0%. The highest canopy coverage of 95.3% was found at $Mg_{15}S_{20}$, which was not significantly different from other treatment combinations. The lowest canopy coverage of 65.0% was found under control, which was statistically similar to Mg_0S_{10} (66.7%) (Table 1).

Tuber per ten plants

Main effect of magnesium: The minimum tuber number of 57.9 per 10 plants was recorded from control, which was significantly increased due to application of Mg at different levels. The maximum average number of 71.4 tubers per 10 plants was found at Mg_{15} (Fig. 1).

Main effect of sulfur: The maximum average number of 67.4 tubers 10 per plants was observed at S_{20}, which was statistically similar to S_{10}. The minimum tuber number of 64.3% per 10 plants was recorded under control (Fig. 2).

Interaction effect of Mg and S: The maximum average number of 74.4 tubers per 10 plants was obtained from $Mg_{15}S_{20}$, which was similar to $Mg_{15}S_{10}$. The minimum number of 57.0 tubers per 10 plants was found under control, which was statistically similar to Mg_0S_{10}, Mg_0S_{20} and Mg_0S_{30} (Table 1).

Seed Potato Tuber Yield (tha^{-1})

Main effect of magnesium: The lowest tuber yield of 19.07 tha^{-1} was produced in control plot, which was increased to 29.87 tha^{-1}. The highest yield per plot of 29.87 tha^{-1} was produced at Mg_{15} (Fig.1).

Main effect of sulfur: The significantly highest yield was obtained with served 28.70 tha^{-1} at 20 kg S ha^{-1} (S_{20}) and the lowest yield per plot was found 22.90 tha^{-1} under control, which was significantly different from other treatments (Fig. 2).

Interaction effect of Mg and S: The average highest yield of 32.86 tha^{-1} was found at $Mg_{15}S_{20}$, which was different from other treatment combinations and the lowest yield per plant was 16.41 tha^{-1} under control, which was statistically similar to Mg_0S_{10} and Mg_0S_{30} (Table 1).

Grading of healthy tubers: Healthy tubers were graded into four grades based on their size. These were Grade-A (diameter 28-41 mm), grade-B (diameter 41-55 mm), undersize (diameter 20-28 mm) and oversize (diameter 55-60 mm). Occurrence of tuber under each grade was expressed as percentage of total healthy tubers. Occurrence of grade-B tubers was maximal followed by grade-A, undersize and oversize tuber (Fig. 3).

Grade A (diameter 28-41 mm)

Main effect of magnesium: The highest grade-A seed tuber of 27.48% was recorded at 20 $kgha^{-1}$, which was statistically similar to 10 $kgha^{-1}$. The lowest Grade-A seed was produced at control (Mg_0) (Fig. 3).

Main effect of sulfur: The highest occurrence of grade A seed tuber of 27.41% was found at 30 kg S ha^{-1} which was statistically similar to 20 kg S ha^{-1}. The lowest occurrence Grade A seed was observed in control (S_0) which was statistically similar with 10 kg S ha^{-1}

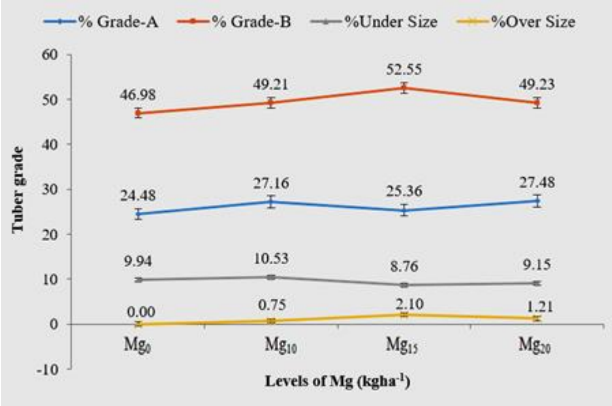


Fig 3. Main effect of Mg on occurrence of different grades of tubers under a constant level of NPKBZn [Mg_0 = No magnesium, Mg_{10} = 10 kg Mg ha^{-1}, Mg_{15} = 15 kg Mg ha^{-1}, Mg_{20} = 20 kg Mg ha^{-1}]

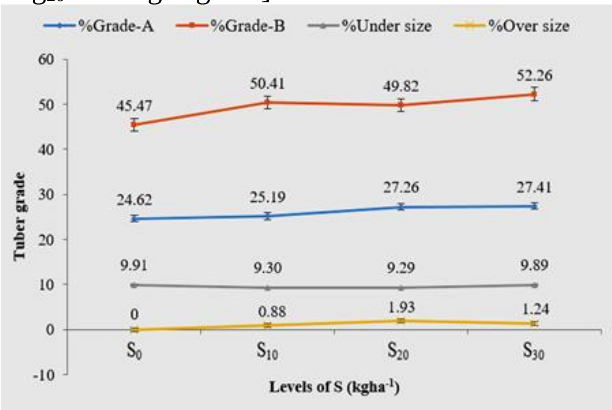


Fig 4. Main effect of S on occurrence of different grades of tubers under a constant level of NPKBZn [S_0 = No sulfur, S_{10} = 10 kg S ha^{-1}, S_{20} = 20 kg S ha^{-1}, S_{30} = 30 kg S ha^{-1}]

Interaction effect of Mg and S: The highest-Grade A seed tuber (29.41%) was produced in $Mg_{10}S_{20}$ which was statistically similar with $Mg_{10}S_{30}$, Mg_0S_{30}, $Mg_{20}S_{10}$, $Mg_{20}S_0$, $Mg_{20}S_{30}$ and $Mg_{20}S_{20}$. The lowest Grade A seed tuber (20.18%) was recorded in control (Mg_0S_0) which was statistically similar with Mg_0S_{10} (Table 2).

Grade B (diameter 41-55 mm)

Main effect of magnesium: The highest grade-B seed was found 52.55% at 15 kg Mg ha^{-1} (Mg_{15}), which was significantly different from other treatments. The lowest grade-B of 46.98% was recorded from control significantly higher compared to other treatments (Fig. 3)

Main effect of sulfur: The main effect was significant. The highest occurrence of 52.26% grade-B tubers was recorded at 30 kg ha^{-1} (S_{30}) and the lowest occurrence of 45.47% grade-B tubers was observed under control, which was significantly higher from other treatment combinations

Table 2. Interaction effect of magnesium (Mg) and sulfur (S) on tuber grade size at different levels under a constant level of NPKBZn

Level of Mg / Dose of S	Mg_0*	Mg_{10}	Mg_{15}	Mg_{20}
% Grade-A (28-41mm)				
S_0*	20.18 g[1]	25.70 cde	25.04 de	27.55 abc
S_{10}	22.44 fg	24.46 ef	26.17 cde	27.7 abc
S_{20}	26.77 bcde	29.41 a	25.72 cde	27.13 abcd
S_{30}	28.55 ab	29.07 ab	24.5 ef	27.52 abc
% Grade-B (41-55 mm)				
S_0	45.42 fg[1]	43.14 g	49.12 cde	44.2 g
S_{10}	48.03 ef	51.3 bcd	52.07 bc	50.26 cde
S_{20}	45.44 fg	48.62 de	53.82 ab	51.4 bcd
S_{30}	49.02 cde	53.77 ab	55.19 a	51.07 bcde
% Under size (20-28mm)				
S_0	7.93 e[1]	13.36 a	9.13 cde	9.2 cde
S_{10}	8.67 de	11.01 b	8.88 de	8.64 de
S_{20}	10.57 bc	8.87 de	8.57 de	9.14 cde
S_{30}	12.59 a	8.89 de	8.44 de	9.62 bcd
% Over size (>55-60mm)				
S_0	0 c[1]	0 c	0 c	0 c
S_{10}	0 c	0 c	2.74 b	0.80 c
S_{20}	0 c	0 c	4.51 a	3.19 ab
S_{30}	0 c	3.00 b	1.13 c	0.83 c

*Mg_0 = No magnesium, Mg_{10} = 10 kg Mg ha^{-1}, Mg_{15} = 15 kg Mg ha^{-1}, Mg_{20} = 20 kg Mg ha-1, *S_0 = No sulfur, S_{10} = 10 kg S ha^{-1}, S_{20} = 20 kg S ha^{-1}, S_{30} = 30 kg S ha^{-1}. [1]Figures under the same parameter within row and column are averages of three replications and having a common letter(s) do not differ significantly (*P= 0.05*) by LSD test.

Interaction effect: Interaction effect of Mg and S on grade-B tubers was significant. The highest grade-B 55.19% seed tubers were found at $Mg_{15}S_{30}$, which was statistically similar to $Mg_{15}S_{20}$ and $Mg_{10}S_{30}$. The lowest occurrence of 43.14% grade-B seed tuber was found at $Mg_{10}S_0$, which was statistically similar to $Mg_{20}S_0$, Mg_0S_0 and Mg_0S_{20} (Table 2).

Undersize (diameter 20-28 mm)

Main effect of magnesium: The highest occurrence of only 10.53% undersize seed tuber was produced due to application of Mg at 10 kg ha^{-1}, which was statistically similar to control (gha^{-1}). The lowest occurrence of 8.76% undersize seed tuber was recorded in 15 kg Mg ha^{-1} which was statistically similar to 20 kg Mg ha^{-1} (Fig. 3).

Main effect of sulfur: No significant different was observed in under size tuber due to sulfur. The highest under size seed tuber (9.91%) was observed in control (S_0) and the lowest under size seed tuber (9.29%) was in 20 kg S ha^{-1} (Fig. 4).

Interaction effect: The highest under size seed was found in $Mg_{10}S_0$ (13.36%) which was statistically similar with Mg_0S_{30}. On the other hand, the lowest healthy under size seed was recorded in control Mg_0S_0 (7.93%) which was statistically similar with $Mg_{15}S_{30}$, $Mg_{15}S_{20}$, $Mg_{20}S_{10}$, Mg_0S_{10}, $Mg_{10}S_{20}$, $Mg_{15}S_{10}$, $Mg_{10}S_{30}$, $Mg_{15}S_0$, $Mg_{20}S_{20}$ and $Mg_{20}S_0$ (Table 2).

Oversize (diameter >55-60 mm)

Main effect of magnesium: Oversize tubers were not produced in absence of Mg (control) and lowest level of the element. The maximum occurrence of 2.10% tubers was recorded at 15 kg Mg ha^{-1} of the element (Fig. 3).

Main effect of sulfur: The highest occurrence of only 1.93% oversize seed tuber was recorded at 20 kg ha^{-1} (S_{20}) and there was no oversize seed tuber was observed under control (Fig. 4).

Interaction effect: The highest occurrence of only 4.51% oversize seed tuber was found at $Mg_{15}S_{20}$, which was statistically similar to $Mg_{20}S_{20}$. Oversize seed tuber was not formed at the treatment combinations $Mg_{20}S_0$, $Mg_{15}S_0$, $Mg_{10}S_{20}$, $Mg_{10}S_{10}$, $Mg_{10}S_0$, Mg_0S_{30}, Mg_0S_{20}, Mg_0S_{10} and Mg_0S_0 which were statistically similar with $Mg_{20}S_{10}$, $Mg_{20}S_{30}$, $Mg_{15}S_{30}$ (Table 2).

Incidence of diseases: Like experiment 1, three important potato diseases were recorded from harvested tubers. The diseases were common scab, soft rot and dry rot.

Incidence of common scab

Main effect of Mg: Main effect of Mg on common scab was significant. The highest common scab incidence was 7.01% (w/w) was under control (Mg_0). Application of Mg caused significant decrease in disease incidence showing the lowest common scab incidence of 5.10% at Mg_{10} (Fig. 5).

Main effect of sulfur: The main effect of S on common scab was also significant. The highest incidence of 9.66% (w/w) of common scab was found under control (S_0). Application of S reduced the disease incidence significantly over control (S_0). The lowest incidence of 3.33% common scab disease was recorded under S_{30}, which was statistically similar to S_{20} (Fig. 6).

Incidence of soft rot and dry rot: The main effect of Mg and S on incidence of soft rot and dry rot was very low. The incidence soft rot and dry rot under Mg ranged 0.87-2.76% and 0.25-0.82%, respectively. Their incidence under S ranged 1.17-2.34% and 0.37-0.73%, respectively (Fig. 5 and 6).

Interaction effect of Mg and S on total disease incidence: The maximum total disease incidence was recorded under control (Mg_0S_0). Combined application of Mg and S caused significant decrease in disease incidence over control. The decreasing tendency incidence continued up to $Mg_{15}S_{20}$ (Fig. 7).

Incidence of disorders: Different disorders such as secondary growth and greening were found in harvested potato tubers, but their incidence was negligible (Fig. 8). So the details are not given.

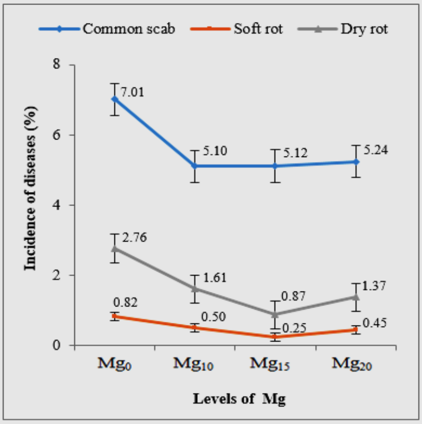


Figure 5. Incidence of common scab, soft rot and dry rot diseases at different levels of magnesium

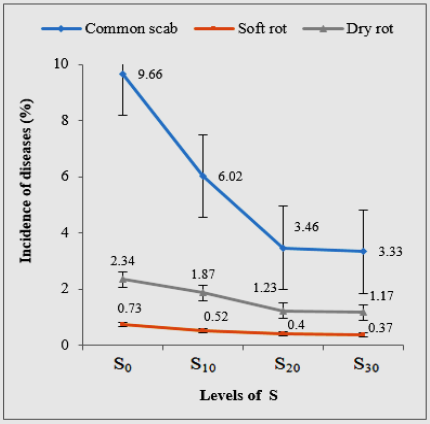


Figure 6. Incidence of common scab, soft rot and dry rot diseases at different levels of sulfur

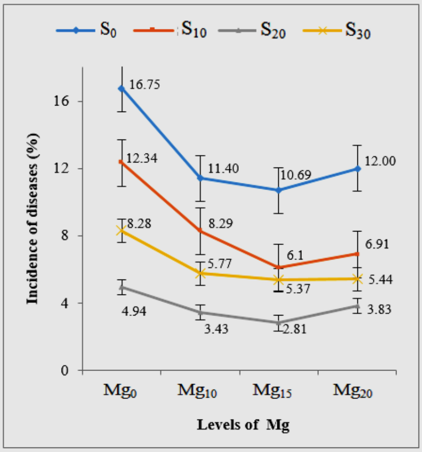


Figure 7. Interaction effect of Mg and S on incidence of total diseases

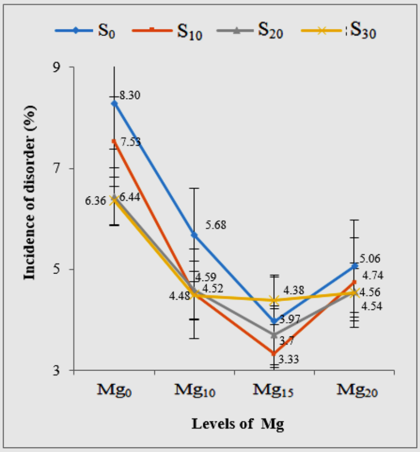


Figure 8. Interaction effect of Mg and S on incidence of total disorders

Estimation of optimum level of magnesium and sulfur

Regression analysis was done and optimum and economic dose of fertilizer were calculated using the formula Y = -b/2c from the response curve (Gomez and Gomez, 1984). Dobermann et al. (2000) stated that the optimal rate of fertilizer application to a crop is that rate which produces the maximum economic returns at the minimum cost, and this can be derived from a nutrient response curve. The large and significant R^2 value in case of Mg and S of regression indicates that the quadratic response fitted the data. Response curve shows that yield increased with the increasing of nutrients at certain level and thereafter yield was decreased. Results presented in Fig 9 shows that potato tuber yield increased with increasing level of Magnesium to a certain limit and then decreased with further increase of nutrients level. But the increment of yield was prominent in case of Mg and the highest yield (29.87 tha^{-1}) was obtained from 15 kg Mg ha^{-1}. Magnesium has distinct effect on the yield. But further application of Mg yield began to decrease. It was indicating the detrimental effect of over fertilization. The reason might be medium Mg status in soil. From the regression equations optimum dose of Magnesium fertilizer is 15.28 kg ha^{-1} for better yield. In case of sulfur, potato tuber yield was also increased with increasing level of sulfur fertilizer to a certain limit and then decreased with further increase of nutrients level (Fig. 20). The highest yield (28.70 tha^{-1}) was obtained from 20 kg S ha^{-1}. But further application of S yield began to decrease. The reason might be medium S status in soil. The regression equations suggested that optimum dose of sulfur fertilizer is 21.11 kg ha^{-1} for better seed tuber yield (Table 3).

The results of present experiment suggest that growth parameters, yield and yield components of potato are responded positively to Mg and S fertilizers either applied as sole or in combination. The average tuber weight, healthy seed tuber and total tuber yield was positively and significantly influenced by different levels of Mg and S fertilizer and their interaction. The highest tuber yield, average tuber weight, number of tubers per plant and number of stem per hill were obtained from application of Mg and S fertilizer in combination at the rate of 15 Kg Mg ha^{-1} and 20 Kg S ha^{-1}. Other researchers also found positive effect of Mg and S fertilizer on growth and yield. Talukder et al. (2009) tested 5 levels of magnesium viz., 0, 5, 10, 15, and 20 kg ha^{-1} to observe its effects on potato and to find out the optimum and economic dose of Mg for potato.

Table 3. Regression equation and Optimum dose of Magnesium and Sulfur fertilizer

Nutrients levels ($kgha^{-1}$)	Seed potato tuber yield (tha^{-1})	Yield increased over control (%)	Regression equation and R^2 value	Optimum dose of fertilizer (kg ha^{-1})
Magnesium Levels				
Mg_0	19.07	-	$y = -0.0443x^2 + 1.3538x + 18.999$ $R^2 = 0.9927$	15.28
Mg_{10}	27.69	45.24		
Mg_{15}	29.87	56.67		
Mg_{20}	28.13	47.53		
Sulfur Levels				
S_0	22.90	-	$y = -0.0123x^2 + 0.5193x + 22.717$ $R^2 = 0.9646$	21.11
S_{10}	26.14	14.19		
S_{20}	28.70	25.37		
S_{30}	27.02	18.01		

Mg_0 = 0 kg Mg ha^{-1}, Mg_{10} = 10 kg Mg ha^{-1}, Mg_{15} = 15 kg Mg ha^{-1}, Mg_{20} = 20 kg Mg ha^{-1}. S_0 = 0 kg S ha^{-1}, S_{10} = 10 kg S ha^{-1}, S_{20} = 20 kg S ha^{-1}, S_{30} =30 kg S ha^{-1}.

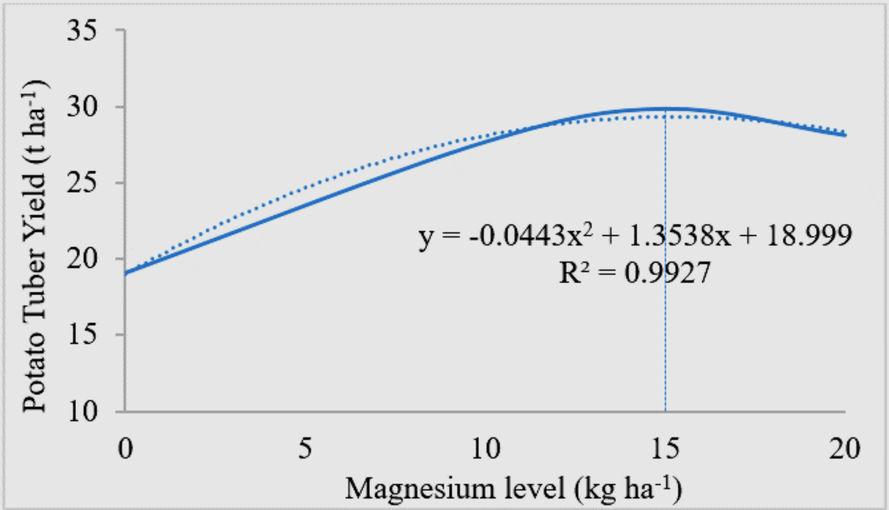


Figure 9. Relationship between magnesium levels with potato tuber yield

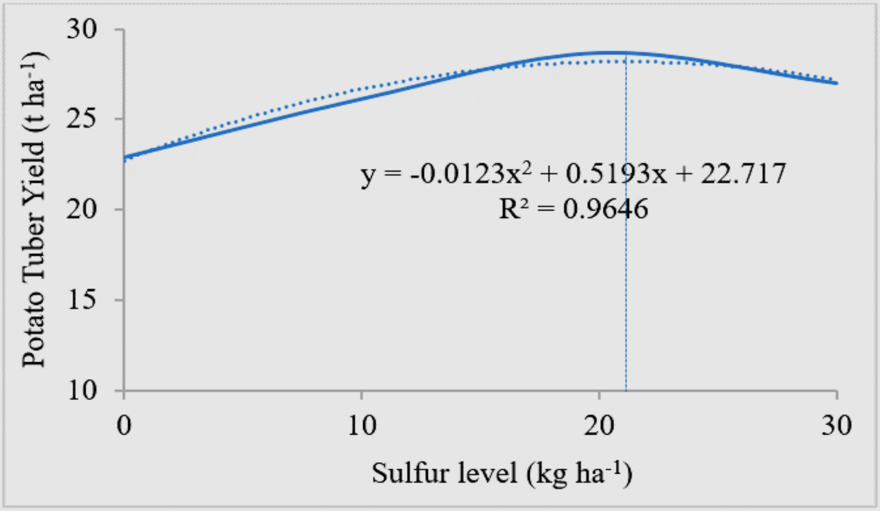


Figure 10. Relationship between sulfur levels with potato tuber yield

Tuber yield tended to decrease with increasing rate of Mg beyond 10 kg ha^{-1}. They suggested that maximum tuber yield (30.32 tha^{-1}) could be obtained at 13 kg ha^{-1} of Mg. Draycott and Allison (1998) recommended for potato to apply 15-20 kg Mg ha^{-1} to obtain higher tuber yield. The result of present study is very close to their findings. Sharma et al. (2011) showed that sulfur application in potato had significant influence on quality and yield. Highest tuber yield, large size and medium size tuber yield, dry matter content, specific gravity, sugar content and starch content were increased with increasing dose of sulfur.

Kristufek et al. (2000) observed that control of common scab is mainly caused by different soil amendments and breeding for disease resistance. One of the best methods for combating scab is the use of acid producing fertilizers, especially those that contain liberal amounts of sulfate of ammonia. Hanna et al. (2006) also found that the application of S can reduce bacterial and fungal diseases in potatoes at the rate of 25 kg S ha^{-1}. Soil characteristics greatly affect by the severity of potato scab in soils with pH 7. The use of acidifying fertilizers such as ammonium sulfate or diammonium phosphate or applications of sulfur that reduce the soil pH, can induce control of common scab disease (Wiechel and Crump, 2010). The results of present experiment partially support the findings of Kristufek et al. (2000) and Wiechel and Crump (2010). In present experiment, aggregate tuber yields increased quadratically with increasing Mg application rates up to 15 kg Mg ha_{-1}, reaching a plateau thereafter, yield was decreasing at the application of 20 kg Mg ha_{-1}. Similar result was found in case of sulfur application rates. Tuber yield increased quadratically with increasing sulfur application rates up to 20 kg S ha_{-1}, reaching a plateau thereafter, yield was decreasing at the application of 30 kg S ha_{-1}. Similar results also reported by Talukder et al. (2009); Barczak et al. (2013).

CONCLUSIONS

The present study revealed that, the incidence of common scab, soft rot and dry rot diseases of seed tuber reduces in application of Mg and S at the rate of 15 kg Mg ha^{-1} and 20 kg S ha^{-1}. The higher tuber yield was also obtained from this combination of Mg and S. However, regression analysis suggested that the maximum yield may obtain at a combination of Mg and S at the rates of 15.28 kg Mg ha^{-1} and 21.11 kg S ha^{-1} to the study area and other similar areas having alluvial soil. Therefore, it is suggested that farmers should apply 15.28 kg Mg ha^{-1} and 21.11 kg S ha^{-1} along with standard dose of NPK to obtain maximum yield and disease-free seed tuber of potato.

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SKADAR LAKE CULTURAL LANDSCAPE AND ARCHITECTURAL HERITAGE
Potential for the Development of Fisheries and Rural Tourism

SUMMARY

The settlement of Vranjina is situated on a slope in the eastern part of the island of the same name, on the northern belt of Skadar Lake in Montenegro. With its exceptional historical and cultural values, protected natural heritage and partly preserved architectural heritage of vernacular architecture, this settlement is just one of many examples in the Skadar Lake National Park, with specific potential for rural tourism development. The Skadar Lake basin is rich in diverse ambience, which is reflected in organically developed landscapes and associative cultural landscapes through the Charter on the International Recognition of Cultural Landscapes. The results of the research, based on years of urban and architectural research in the Skadar Lake area by the authors, indicate the possibility of reviving the tradition of fishing, as an important branch of agriculture, as a contribution not only to sustainable agriculture, but also to protection and valorization of architectural heritage and traditional fishermen's houses on the Skadar Lake.

Keywords: cultural landscape, rural tourism, Skadar Lake, fisheries, sustainable agriculture, heritage

INTRODUCTION

Revitalization of the villages and sustainable valorization of the settlements of Skadar Lake, along with the preservation of the landscape are the basis for the development of rural tourism in this region of Montenegro. The cultural landscape of Skadar Lake and its surroundings formed by a significant number of real properties has not been valorized, which has resulted in permanent devastation and degradation. The non-valorized segment of cultural heritage comprises cultural landscapes, rural ensembles, groups and structures, fortifications - the system of Austro-Hungarian and other fortifications and structures of old mills, bridges and roads.

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Stanojevic and Vasic (1975), in the book History of Montenegro (Book Three, From the Beginning of the 16th to the End of the 17th Century, Volume One), describe Skadar Lake from the Ottoman Empire as the most important economic basin of Montenegro: "…The second and most important economic basin of Skadar Lake, unusually rich in fish, and mostly in bleak, which has been exported in large quantities to Venice and other Italian cities since the beginning of the 16th century… At an earlier time, 400-500 boats made of hollowed out logs could be seen on the lake. Endless flocks of sea ducks, which were believed to be heralds of a good catch, would simply cover the sky, like black clouds." These citations precisely speak of the fishing tradition which, in addition to agriculture in fertile fields that were not so flooded at the time, brought significant income to the local population, which would be an example of sustainable agricultural development today.

In order to preserve the cultural landscape, authentic biodiversity and landscape, either by bringing back the traditional boats or introducing alternative modes of lake transport without the use of fossil fuels in the area of Skadar Lake National Park (Lazarević, 2016), preconditions would be created for the development of ecological rural tourism which will attract environmentally aware tourists, lovers of tradition and culture, especially after the COVID-19 pandemic.

Therefore, this paper points out all the potentials and limitations for the development of rural tourism through the activation of fisheries, as an important traditional branch of agriculture, protection of biodiversity and cultural landscape, and restoration of architectural heritage in order to create necessary preconditions for a comprehensive development of Skadar Lake.

MATERIAL AND METHODS

An exceptional group of cultural settlements comprises traditional folk architecture, old uninhabited or sparsely populated and neglected fishing villages situated on the Lake shores - Radus, Krnjice, Poseljani, Karuc, Dodosi, and other once inhabited villages where people used to live on fishing and untangle their nets. Therefore, it must not be allowed for the cultural goods which were previously recognized and placed under protection by the Law to be treated as the settlement of Vranjina which was placed under protection by the Law on 9 January 1979, when its significance was accepted, and which was soon after left to urbanization pressures, uncontrolled development and incompliance (Gakovic, 2013). Precisely using the example of the settlement of Vranjina, as a settlement protected by the Law on Protection of Cultural Heritage for more than 30 years, this paper will point out the potentials for the development of fisheries and rural tourism and all the limitations mostly caused by human interventions in previous decades. The villages around the lake suffer loss in their old look and beauty, traditional shape and function every day. This was especially intensified after the earthquake in 1979, when entire villages with old houses, local churches and other buildings were destroyed in this area. The reconstruction of the damaged villages was carried out relatively quickly, although spontaneously, without any professional indicators and controls, so in many cases it was not possible to take into account the preservation of old national values found on the damaged buildings.

In order to better explain the scientific issue, through a presentation of the historical genesis of the settlement, both primary and secondary sources were extensively reserached and reviewed, but also the graphic drawings by the authors of the paper are presented. The methodology relies on analytical research of extensive material within the sub-topic of the paper: Functional spatial potentials and limitations of houses on the Skadar Lake for the development of traditional fisheries and rural tourism and links with modern sustainable development strategies with an emphasis on sustainable agriculture.

RESULTS AND DISCUSSION

Historical Genesis and Urban Planning Layout of the Settlement of Vranjina

The age of the settlement cannot be reliably determined. There are no clear indications and evidence of the existence of a settlement in the pre-Ottoman period, which is partly a consequence of the physical and geographical characteristics (Figure 1) of the island which provides no strong basis for living, other than its suitabile location for fishing.

According to records, the oldest building on the island was the Vranje Monastery, that is the Monastery of St. Nicholas of Vranje built in 1223 by Bishop Hilarion (Radusinovic, 1964).

Figure 1. The Skadar Lake Geological Map Board (Spatial Purpose Plan National Parc Skadar Lake, RZUP, 1999)

Figure 2. Urban Genesis of the Island of Vranjina,
drawings by Vukota Vukotic (1998)

The majority of inhabitants came to the island during the Turkish rule. The reasons were very heterogeneous: escape from blood feuds, exile from the fraternity, fight against the Turks ... which led to a unique example of a community with so many represented fraternities and tribes. Then, approximately 350 years ago, the first houses were built after the island had been bought by the Turks ("families from the Pocek, Dapcevic, Gazivoda, Buskovic, Pajovic, Sjekloca...brotherhoods") (Radusinovic, 1964).

The settlement itself is of compact type, which is imposed both by the terrain and a routine way of life of the locals. The small bay which rises abruptly into the hill after approximately 30 meters conditioned the seemingly spontaneous arrangement of houses, and there is some truth in this, nevertheless. The houses are mostly placed perpendicular to the isohypses, which maximally saves space and scarce arable land (Figure 2). There are also houses that are parallel to the isohypses - this is usually the case with ground floor houses.

There is no spatial accent, if the settlement itself is not taken as such, or a particular centre of the settlement, as in other Skadar settlements - around the springs or cisterns ("*gustijerna*" – private cistern for the collection of stormwaters). Everything is so intertwined and interconnected with nature that only the bay can be regarded as the settlement centre, with boats which were once the only means of transport or "freight animals", through waterways cut into European Bladdernut and Water Chestnut (thick wetland vegetation).

The building itself, using the slope of the terrain and placed directly on the isohypses, is predestinated to be on "two storeys" (on two floors) where, due to the terrain characteristics (Figure 3), the ground floor is much smaller than the first floor which is also the core of the house, the space where people live their lives, despite the limitations imposed by nature, for the purpose of its survival.

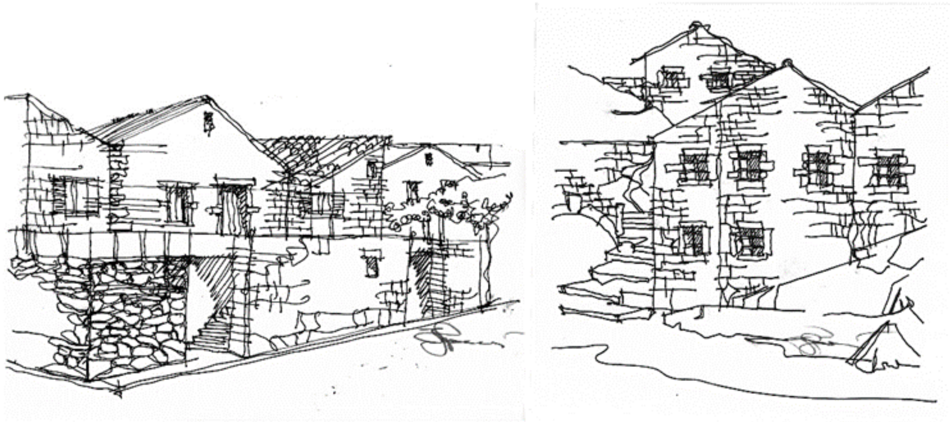

Figure 3**.** Two-storey house, Vranjina, drawings by S.G. Popovic (1978)

The causes for the settlement of the island significantly influenced the way of living; the house itself with narrow openings was a real small fortress, if necessary, and the compactness of the settlement made it possible to provide common resistance. Locals fished in groups in order to be close at hand in case of an attack on an open lake. The catch was divided into equal parts, assistance was jointly provided to the poor and everyone acted jointly against the Turks (Radusinovic, 1964).

All this indicates the possibility of reviving the tradition of fishing, as an important branch of agriculture, in response not only to sustainable agricultural development, but also to the protection and valorization of architectural heritage, traditional fishing houses on the Skadar Lake. The revival of the villages and settlements of the Skadar Lake, along with the preservation of the cultural landscape, is the basis for the development of rural tourism in this region of Montenegro. With the need to introduce alternative forms of lake transport without the use of fossil fuels and in order to preserve the cultural landscape of the Skadar Lake National Park, preconditions would be created for the development of ecological rural tourism which will attract environmentally aware tourists, lovers of tradition, especially after the COVID-19 pandemic.

Functional Spatial Potentials and Limitations of Houses on the Skadar Lake for the Development of Traditional Fisheries and Rural Tourism

As for the functional layout, the course of evolution is very noticeable. Starting from a single space in which all existential functions are performed, which characterizes the older buildings, via the addition of a new room with a separate entrance to the original space, in order to "improve living standards" as it would be referred to today (Figure 4), to the most widespread type of houses in the settlement of Vranjina - a house with two rooms, a kitchen or "cook house" with a fireplace ("bell-shaped oven"), as the center of the house, and a bedroom or "chamber, room" which, although mostly larger in size, serves only to meet the need specified in its very name (Figure 4).

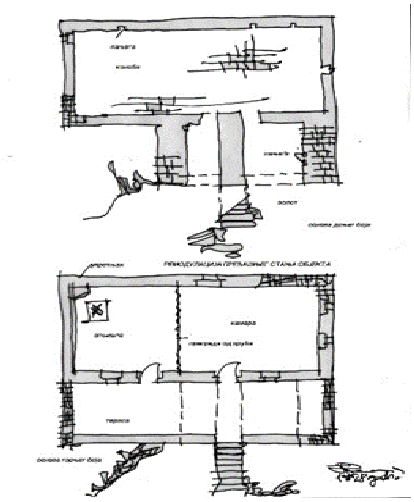

Figure 4. House, typology 1930, drawings by S. G. Popovic (1978)

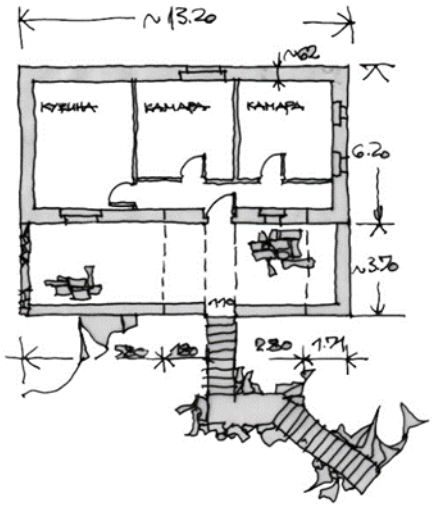

Figure 5. House, typology 1960, drawings by S. G. Popovic (1978)

Later on, houses are characterized by somewhat more complex content. The number of rooms is increasing. In most cases an entrance hall is added to separate the kitchen and the bedroom; it is also used for storing everyday clothing and footwear and adding a new part of the house to the existing one, with a change of use of rooms (Figure 5).

Storey buildings (Figure 6) retain the same characteristics on the first floor, while the ground floor, as a much smaller area due to terrain constraints, is mainly used for "economic needs", livestock accommodation, fishing tools (Figure 7), storage and drying of food.

The functions of a kitchen or "cook house" are not only to prepare and consume meals; it is also used for the entire daily life of a family. This is because, in addition to two or three beds which are usually there, not including those that may be in the kitchen, items of value that the family owns are kept and exhibited there, starting from pictures of sons and daughters, who mostly live in the city, to various dinner sets, handicrafts and valuables.

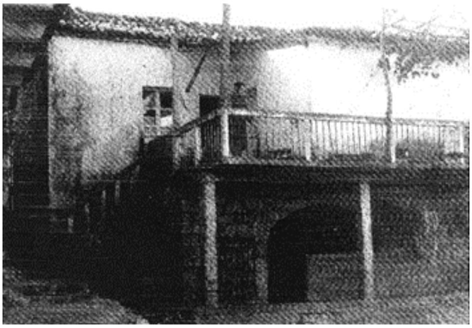

Figure 6. House on a storage room, photo by S.G.Popovic, 1978

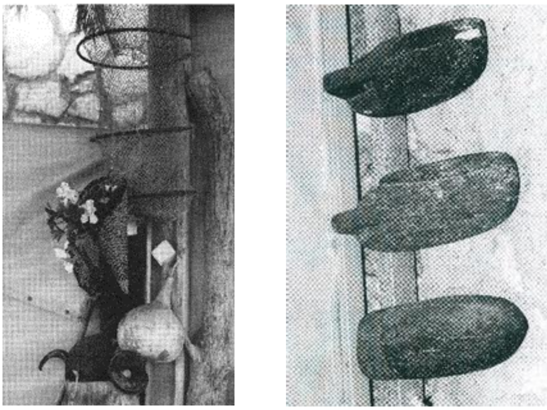

Figure7**.** Interior of a chamber (picture to the left) and molds for overshoes (picture to the right), photo by S.G.Popovic, 1978

Basement, pantry, storage for boats - all these are examples of using the room under the above-mentioned primary parts of the building. The room is as wide as the building, though considerably narrower, and its function depends on the occupation of family members.

Due to the presence of a large number of people from different tribes and fraternities, during its genesis the settlement developed no cultural specificity that would characterize a certain way of life or belonging to a special ethnological background.

Each local brought certain habits and peculiarities, and the influence of the proximity of the Coast (Figure 8) is indisputable.

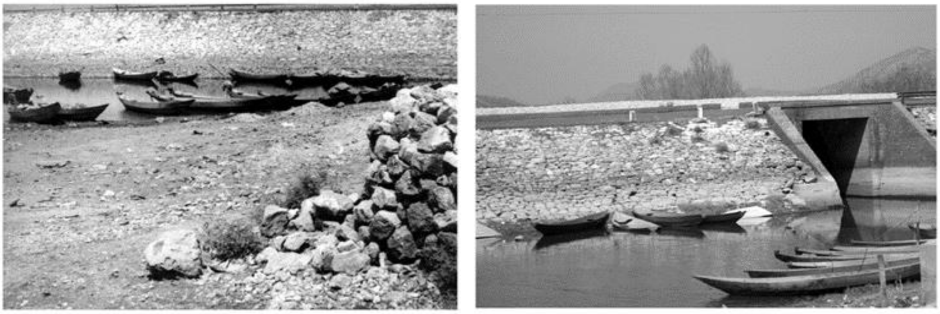

Figure 8. Indigenous boat for transporting bulky goods, 7 m long, 1 m wide and 0.5 m high, photos by S.G.Popovic, 1978

These facts indicate all the limitations of the settlement of Vranjina when it comes to a longer stay in order to meet the needs of housing, but also the advantages of the geographical location of the settlement on the road between the Capital City and the Coast.

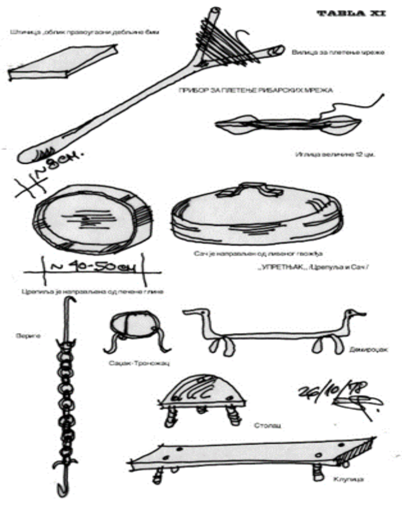

Figure 9. Tools for making fishing nets, chains, pottery, drawing by S.G. Popovic

On the other hand, these spatial and functional limitations of houses can be a potential for quick and easy revitalization of settlements and revival of rural tourism with traditional fishing (Figure 9).

Architecture is an eternal human concern and passion, the culmination of which is experienced exactly today. In an era of dynamism, hectic life, digitalization and rapid socio-economic changes, people seem to lose self-confidence and nostalgically turn to the past seeking solace in it. The same goes for architecture which, in the conditions of today's inhumanity, neglect of the built and natural heritage and neglect of traditions, tries to find its meaning in looking back.

However, looking back to what preceded us, certain things are noticed which made the common name "house" invaluable from an architectural point of view as well. Dilapidated, indistinctive, unattractive dwellings offer a real wealth of elements, gained through many years of experience through the life of a building and people in it.

Humanity, harmony, proportions, harmony with nature - that is why it was worth turning back. The configuration of the terrain, vegetation, natural conditions, tradition and mentality of the people, local customs and culture - all this is unconsciously embedded in an inconspicuous stone shape to which the farmers or peasants are connected more and more intimately than the city residents are connected to their architectural apartments equipped with all the wonders of white ware and comfort of living. This is because it is the home where they "came into the world, where they feast and mourn and from where they will be taken out dead."

This retrospective, compared to the present time, confirms the values that are the input for the development of rural tourism, the importance of preserving the cultural landscape and landscape, the need of tourists or visitors for a strong connection with nature and the natural. In addition, this retrospective is supported by the determination of the State, as a declared ecological State, to take care of the natural and built cultural heritage, as well as the adopted development strategies of Montenegro.

Montenegro's Strategic Commitments to Develop Sustainable Agriculture and Food Value Chain as a Basis for the Development of Sustainable Rural Tourism

The National Strategy for Sustainable Development until 2030 was adopted in 2016 (Ministry of Sustainable Development and Tourism, Government of Montenegro, 2016), while the Smart Specialization Strategy until 2024 recognized agriculture (with an emphasis on the protection of endangered and endemic plant and animal species) as one of the three priority vertical strategic areas (sustainable agriculture and the food value chain), along with sustainable and health tourism and energy and sustainable environment, which indicates the additional importance of the development of rural tourism in the upcoming years.

Agriculture is a sector that plays a multiple role in the development of society and economy of Montenegro. Its economic importance is reflected in high contribution to GDP creation (more than 7%, while accounting for about 2% at the EU level). There is even greater share of agriculture in the employment of the labour force, since almost one fourth of the total number of employed people in Montenegro are engaged in family farms. In addition, agriculture plays other important roles as well: it constitutes the basis for the food value chain (food industry and related sectors); it contributes to the development of tourism; it encourages the development of many other sectors (production of equipment, machinery and packaging material, transport and numerous services); it is crucial in the sustainable development and mitigation of the depopulation of rural areas; it contributes to the fight against poverty in rural areas and it is an important factor in preserving tradition and the overall cultural heritage of the Montenegrin villages (Ministry of Science, Government of Montenegro, 2019, Smart Specialization Strategy of Montenegro 2019-2024)

The contribution of tourism to rural development is important if the local population participates in its development. It is also a means of protecting the environment, economic and cultural and historical traditions, local culture, etc. (Gasic *et al.,* 2015).

Agriculture is marked by high complementarity with other priority sectors, especially with tourism as the driver of all types of agritourism and health tourism through the presentation of traditional gastronomy in the context of the tourist offer. Also, the sustainable development of the sector mitigates the negative demographic trends, contributes to the balance in territorial development, enables the inclusion of different groups and contributes to the mitigation of the consequences of climate change (Ministry of Science, Government of Montenegro, 2019, Smart Specialization Strategy of Montenegro 2019-2024)

Historically, definition of the term *rural* meant something located outside the city walls. Viewed from the economic aspect, it can be said that *rural* means a territory used for food production, while according to the sociological aspect *rural* means an environment characterized by strong backwardness in relation to technological and cultural development, which is more noticeable in urban areas (Milic, 2011).

On the one hand, rural development plays a significant role from the aspect of socio-economic development of each country, while on the other hand, rural development has a significant role from the aspect of environmental protection as an environment in which the population of a country lives. This indicates the need for strategic care for the preservation of traditional architecture and systematic and planned renovation of houses on the Skadar Lake, with previous detailed architectural research, in order to develop rural tourism and include this valuable UNESCO-recognized area of the National Park for the purpose of completing the tourist offer throughout the territory of Montenegro and creating the preconditions for the development of cross-border cooperation in this field with Albania (development of fishery and aquaculture products, in a traditional architectural house of the Skadar Lake). Rural areas directly depend both on the environmental resources and natural landscape that provides great opportunities for rural and urban population.

Rural tourism creates conditions for satisfying the needs of an increasing number of tourists that seek a healthy life-style and experiences that imply pleasure in nature, traditional cuisine, hospitality of husbandries/households in rural areas, enjoyment in tradition and preserved customs and other authentic experiences (Ministry of Sustainable Development and Tourism, The Program of Rural Tourism Development of Montenegro, 2019).

Adequate protection and care of cultural heritage and landscape are key to the further development of cultural tourism in the rural areas, especially to their connection with peri-urban and urban heritage. Settlement patterns and its dichotomy with its cultural and environmental peculiarity have a direct impact on an agrarian landscape and further development of rural tourism (Fikfak, A., Popovic, G.S. & Kosanovic, S., 2015 and Konjar, M., Kosanović, S., Popović, S.G. and Fikfak, A., 2018).

Given the additional confrontation with the crisis during the COVID-19 pandemic, in the upcoming period special attention should be paid to the management of rural cultural tourism in response to the recovery from the pandemic, considering that numerous studies already confirm that tourist demand will be focused on escape from the urban and salvation in the rural areas. There are many discussion and researches on the topic of rural development as economy driver for tourism sector in post COVID-19 recovery of countries, mainly analysed for territory of China, giving that the recovery of the entire economy, including tourism, is evident only in the territory of Asia, while the rest of the world is still struggling with one of the biggest global crises (UNWTO, 2020).

Cultural Landscape Preservation as Part of the Rural Tourism Strategy

The concept of a cultural landscape is a variable and multifaceted category which has been differently defined and investigated over the last decades in a number of natural, social and humanistic sciences. The value of cultural landscape (Figure 10) is based on authenticity and autochthony, representativeness (relict, endemism and rarity), biological and geological diversity, abundance of natural phenomena and processes, functional uniqueness, age, aesthetic value and conservation, sustainable development in a global context.

At the same time, landscape is a perfect complex of living environment and territorial arrangement, and it is equivalent to a set of functions of general interest and in different fields (culture, ecology, environment, society); it is also an important economic resource whose sustainable management can directly create new jobs and indirectly affect the improvement of living standards and the achievement and promotion of human rights. Cultural landscape is vital for a more human life of people and sustainable development of the society as a whole (Ministry of Sustainable Development and Tourism, Strategy for the Development of Cultural Tourism of Montenegro with an action plan until 2023, 2018). Today's concept of cultural landscape includes the characteristics of natural area, as well as the forms enforced on physical space due to human activities, the physical structure of space and cultural order. This symbolic appropriation of territory transforms the physical environment into a cultural landscape, which is by definition building culture out of a certain territory (Munarriz, 2007).

Figure 10. Landscape of the Skadar Lake, photo by S.G. Popović, 2000

Figure 11**.** Landscape of Vranjine settlement, photo by S.G. Popović, 1978

Inconsistency in terminology can be noticed in the literature belonging to different scientific disciplines, so that in parallel with the term *cultural landscape*, the terms *cultivated landscape, anthropogenic landscape*, can also be found, while some researchers make a connection with the Marxist concept of ‘humanized’ nature. The modern notion of cultural landscape indicates a great diversity of physical and associative relations, the most important of which is the relation of the population (inhabitants) towards the territory they inhabit and its natural elements.

It is impossible to separate the cultural landscape from the architectural environment (Figure 11 and Figure 12). On the contrary, insisting on an integrated approach contributes to the growing importance of the whole area, the authentic values of the man-made landscape, as well as other categories of cultural heritage as a resource for sustainable development. Landscape development reflects the development of civilization, its level, social organization and climatic characteristics as the most important precondition for the survival of ecosystems (Figure 13).

Figure 12. Karuc fishing village, photo by S.G. Popovic, 2000

Figure 13. Vranjina Settlement, drawings by S.G. Popovic, 2006

CONCLUSIONS

Research by the former Ministry of Science, conducted for the purpose of developing the Smart Specialization Strategy, identified approximately 40 companies engaged in the processing of meat, dairy products, vegetables and fruits, herbs and aromatic plants and mushrooms, production of honey, olive oil, potato and cereals, fisheries and aquaculture (Ministry of Science, Government of Montenegro, 2019). However, the exact number of fishing companies is unknown. A cluster of ponds has been identified; however traditional fishermen remain in the shadow of development. It is precisely through the development of rural tourism that the settlement of Vranjine, as well as a number of other settlement structures and villages on the Skadar Lake, including the most famous fishing village of Karuc, can be renewed and traditional fishing can be revived, giving local residents a chance to earn income by strengthening the economy of the whole country.

In 2011, the National Location Study of Vranjina with Lesendro was developed, which defines these sites as follows: Relying, together with Virpazar, on the basic infrastructural direction of Podgorica-Adriatic Sea, Vranjina has a significant development chance. Formed on the island, as a fishing settlement of a compact type, Vranjina is today connected to the mainland, but it still remains an attractive settlement, with interesting potentials.

Located on the island of the same name, with an extremely interesting settlement structure directly leaning on the Lake, Vranjina is planned as one of the entry points to the Park, from the direction of Podgorica. In addition to the settlement itself, specific values are the monastery complex, the National Park building and the island fortress Lesendro. The picturesque ambience and the convenience of the traffic position are the basis for the development of transit and excursion tourism, through the construction of well-designed hospitality and trade facilities, related to the main activity of the settlement - fishing. The planned accommodation capacities are envisaged within the settlement itself, then in the Monastery dormitory and on the site next to the existing building of the National Park. The specific building of the Lesendro fortress is intended for events and hospitality activities (Gakovic, 2013).

Rural tourism is an interaction of agricultural production, presentation of tradition, traditional gastronomy and tourist services, in a word, the use of existing resources. Rural tourism is recognised in the Spatial Plan for the Special Purpose of the National Park Skadar Lake (Ministry of Sustainable Development and Tourism, 2018), but further steps have yet to be identified. Therefore, the development of rural tourism should be based on the principles of sustainable development, through the revitalization of existing, traditional buildings or heritage, to which a new purpose is given – the tourist one. Rural tourism (especially farm tourism) is therefore an essential part of the overall, sustainable development (Ministry of Agriculture and Rural Development of Montenegro, Program for the Development of Agriculture and Rural Areas in Montenegro under IPARD II -2014-2020).

In order to create infrastructural preconditions for the development of rural tourism, it is necessary to:

- Revalorize the overall architectural heritage and provide adequate protection, all in accordance with the Law on Protection of Cultural Heritage of Montenegro;

- Carry out a research on the impact of climate change on floods, and organize coastal areas with a cascading profile that will ensure the usability of platforms in front of houses during frequent floods; and,

- Carry out a detailed research, bio-mapping and engineering with the aim of preserving biodiversity and economic valorisation of the biological potential of the autochthonous flora and fauna of the Skadar Lake.

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MEDICINAL PLANTS FAM. *ASTERACEAE* FROM BJELASICA MOUNTAIN USED IN FOLK AND SCIENTIFIC MEDICINE

SUMMARY

The family *Asteraceae* (*Compositae*, sunflower family) is one of the most numerous families, with about 1,000 genera and more than 20,000 species distributed throughout the world. They are mostly herbaceous plants, but there are also shrubs, and less often trees and vines. This family also includes many medicinal species.

Geographical position, climatic conditions have enabled the rich biodiversity on the mountain Bjelasica in Montenegro, which is spread over an area of 620 km². The geological base, which is mainly composed of silicate rocks, also affects the fact that not all recorded species are equally represented in Bjelasica. Some species are found in large and others in small quantities.

This research covers 15 species from the fam. Asteraceae that are used in medicine. Shoots are used from: *Achillea milefolium* L., *Antennaria dioica* (L) Gaertn, *Bellis perennis* L. *Centaurea cyanus* L. *Cichorium intybus* L. *Matricaria chamomilla* L., *Tussilago farfara* L., *Petasites hybridus* L., *Taraxacum officinale* Weber, *Artemisia absinthium* L., *Solidago virguarea* L. Underground organs are used from: *Carlina acaulis L., Cichorium intybus L., Inula helenium L., Petasites hybridus L., Teraxacum officinale* Weber and *Telekia speciosa (Schreb.)* Baumg. *Arctium lappa* L.

Keywords: Bjelasica, medicinal plants, *Asteraceae*, Montenegro.

INTRODUCTION

The examined medicinal plants of the Asteraceae family are characterized by a whole range of vegetation forms. This includes annuals (*Centaurea cyanus, Matricaria chamomilla,)*, biennials (*Arctium lapa*), herbaceous perennials (Achillea *millefolium, Anternnaria dioica, Bellis perennis, Carlina acaulis,*

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Cichorium intybus, Inula helenium, Petasites hybridus, Taraxacum officinalis, Telekia speciosa, *Tusilago farfara*, *Solidago virgurea*) and perennial subshrub species (*Artemisia absinthium*).

The plants have underground organs in a shape of a crawling rhizome (*Achillea millefolium*, *Anternnaria dioica*), wooden rhizome (*Artemisia absinthium*), branched tuberous rhizome (*Inula helenium, Solidago virg*urea), short and knotted rhizome (*Solidago virgurea*), thick and spindle-shaped rhizome (*Carlina acaulis*) and spindle root (*Arctium lapa*, *Centaurea cyanus, Cichorium intybus, Matricaria chamomilla*).

Depending on the stem height, these medicinal plants can have no stem *Carlina acaulis,* short stem*: Taraxacum officinalis* (2-10 cm), *Bellis perennis* (about 15 cm), *Anternnaria dioica* (about 20 cm), *Tusilago farfara* (about 30 cm.) i *Petasites hybridus* (about 40cm). Somewhat higher stems are found in *Matricaria chamomilla* (about 60 cm), *Centaurea cyanus* (about 70 cm), *Achillea millefolium* (about 80 cm). Tall stems are found in *Telekia speciose* (90-200cm), *Artemisia absinthium* (120 cm) *Cichorium intybus*, (120cm) and *Inula helenium* (150 cm). Reproductive organs are round inflorescences bearing achene, one-seeded fruit.

The widespread distribution of the common dandelion, that is, *Taraxacum officinale*, along with its ability to tolerate a wide range of environmental conditions, makes this plant a good candidate as biological monitor of environmental metal contamination (Giacomino et al. 2016)

The study of the traditional uses of plants in the Ancona district, in the Marche region, Central Italy. The ethnobotanical data concern medicinal (122 species), the study increases present-day. knowledge of the traditional local uses of plants in the Marche region, medicinal and food uses, and of ethnobotanical aspects as a whole, , in terms of which will allow many of these uses to be preserved in the future (Lucchetti et al. 2019).

Abandonment or misuse (excessive grazing, fertilization) has led to the degradation of meadow vegetation and major changes in floristic composition and the extinction of many species. Such negative influences lead to qualitative and quantitative changes in meadow vegetation (Aćić, 2019)

In the work "Conspectus Florae Montenegrinnae" Rohlen (1942) published all the data on the distribution of medicinal plants in Bjelasica.

According to Pulević (1965), close to 300 plants are used in Montenegro as, to a greater or lesser extent, in the pharmaceutical industry and in folk medicine. Lakušić (1966) and Lakušić and Milojević (1972) gave their contribution to the study of the flora of Bjelasica. Medicinal plants of Bjelasica were the subject of graduate (Veljović; 2001) and Master's theses (Balijagić; 2009). Balijagic with associates (2009) published the Atlas of Medicinal Plants of the Bijelo Polje Region.

Organic production LAB in combination with the collection of wild plants and forest fruits can be a significant direction of development for many small family farms in hilly and mountainous areas (Stepanović and Radanović, 2011).

MATERIAL AND METHODS

Mountain of Bjelasica (Figure 7) occupies an area in the central-continental part of the Montenegro, between 42° and 43° north latitude and 19° and 20° east longitude, having circular shape with 30 km in diameter. This area belongs municipalities of Andrijevica, Berane, Bijelo Polje, Mojkovac and Kolašin and it's borders are determined by the rivers Lim and Tara, and from the north, rivers Ljuboviđa and Lepenica. Along with these riverbeds, there are also roads that connect the mentioned municipalities and, in some way, determine the borders of Bjelasica in relation to other mountains.

Figure 1. : Mountain Bjelasica
(Source: http://bjelasica.wifeo.com/srpski.php)

Bjelasica is a distinctly hilly and mountainous area. The dale of Tara and Lim up to 900m altitude has the characteristics of a temperate continental climate, up to 1300m it has the characteristics of humid continental climate and above that, alpine climate.

Thanks to the geological base, which is mainly composed of silicate rocks of volcanic origin, the relief of Bjelasica is divided by a dense network of watercourses, tributaries of the Tara and Lim and characterized by very easy passability and accessibility to the highest mountain peaks, great wealth of vegetation, abundance of running waters and extremely good potential for hiking.

Plants of family *Asteraceae* were determined on site or in the laboratory. For the determination of plants on the spot, we used illustrated bilinar (Horvatić et al., 1954) and Medicinal plants with a key for determination (Jančić, 2001). For determination in the laboratory, we used the Flora of SR Serbia Volume VII (Sarić et al., 1989), Systematic of Medicinal Plants (Živanović and Pavlović, 1999), Guide to the World of Medicinal Plants (Tasić et al., 2009) and Atlas of Medicinal Plants of the Bijelo Polje Region (Balijagić et al., 2009).

The abbreviation BJ (2020) used in this paper represents the initial letters of the surname and name of Balijagić Jasmina. All pictures except the map are from private collection of Jasmina Balijagić.

RESULTS AND DISCUSSION

Acchillea milefolium L., Yarrow Verbena (Sarić et al., 1989) (Fig.1). It was found in the following localities: Jelovica, Biogradska gora, Mušovića rijeka, Paljevine, Tusta, Šiška, Gnjionik, Crni Vrh (Lakušić, 1966), Andrijevica (Pulević, 1965), Bjelasica (Lakušić and Milojević, 1972), Vinicka, Buče, Lubnice, Kršara (Lukić et al., 1992), Brzava, Buče, Majstorovina, Kordelj, Strmenica (Balijagić, 2009). Katun Rasova, Glavaca, Dolac (BJ et al., 2020). Cosmopolitan plant on Bjelasica. It grows on dry to moderately moist meadows, along roads, on rocky outcrops, forest glades, in katuns from the foothill to the alpine zone. The herbaceous above-ground part of the plant *(Millefolii herba)* is used, less often only the inflorescence *(Millefolii flos)* or only the leaf *(Millefolii folium)* in folk medicine and the pharmaceutical industry.

Antennaria diocia (L) Gaertn. Cudweed (Pulević V., 1965). It was found in the following localities: Zekova Glava, Troglav, Kosa Bjelasice (Lakušić R., 1966). It grows on meadows, pastures and rocky places in the alpine zone above 1600m altitude. Inflorescences *(Antennariae flos)* and the above-ground part of the plant *(Antennariae herba)* are used in the form of tea in folk medicine.

Arctium lappa L. Burdock, Coltsfoots, Butterbur (Sarić M. Et al., 1989). It was found at the following localities: Bjelasica (Lakušić R. And Milojević B., 1972), Ravna Rijeka (Balijagić J., 2009), Lubnice (Balijagić J., et al. 2020). It grows on rudines, next to roads, unused pastures on the foothills. Moderately distributed on the mountain Bjelasica. The root *(Bardanae radix)* is used in the folk medicine.

Figure 1. *Acchillea milefolium* L.

Figure 2. *Artemisia absinthium L.*

Artemisia absinthium L. Wormwood (Pulević V., 1965) (Fig.2). It was found in the following localities: Vratilo – Kolašin (Rohlena J., 1942), Berane area, Andrijevica area, villages between Berane and Bijelo Polje (Lakušić R. And Milojević B., 1971), Buče (Lukić P. Et al. 1992), Brzava, Bubanje (Balijagić, 2009), Berane, Trebaljevo (BJ et al., 2020). It grows along roadsides on sandy, rocky limestone terrains, in sunny and dry places up to altitude of 1600m. It is less represented on the mountain Bjelasica due to the lack of limestone land. The herbaceous above-ground parts of the plant *(Absinthii herba or Abshintii summitas)* are used in folk medicine, and less and less often in scientific medicine.

Bellis perennis L. Daisy (Pulević V., 1965) (Fig.3). It was found at the following localities: Murgaš (Lakušić R., 1966), Bjelasica (Lakušić R. And Milojević B., 1972), Femića krš (Balijagić, 2009), Dolac, Crni Vrh (BJ et al., 2020). Commonly found on meadows and pastures up to altitude of 1700m. The inflorescence *(Bellidis flos)* is used, less often only the leaf *(Bellidis folium)* or the whole plant above-ground, without roots *(Bellidis herba)* as a folk remedy.

Carlina acaulis L. Silver thistle (Pulević V., 1965) (Fig.4). It was found in the following localities: Andrijevica (Rohlena J., 1942), National Park „Biogradska gora" (Veljović Đ., 2001), Kordelj, Rasova (BJ, 2020). Habitat: grows on sunny, mountain meadows. Quite widespread up to altitude of 2000m. It is an indicator of a healthy environment. The root *(Carlinae radix)* is used in folk medicine.

Figure 3. *Bellis perennis* L.

Figure 4. *Carlina acaulis* L.

Figure 5. *Centaurea cyanus* L.

Centaurea cyanus L. Cornflower (Pulević V., 1965). It was found at the following localities: Bjelasica (Lakušić and Milojević, 1972), Brzava (Balijagić, 2009), Crni Vrh (BJ, 2020). It grows on rocky, sunny terrains and in cultivated areas. Calciphilic species. It is poorly represented on the mountain Bjelasica. Lingual and adjacent tubular flowers without pappuses *(Cyani flos sine calycibus)* are used in folk medicine.

Cichorium intybus L. Chicory (Pulević V., 1965). It was found at the following localities: Bjelasica (Lakušić R. and Milojević B., 1972), National Park

„Biogradska gora" (Veljović Đ., 2001), Brzava, Buče, Lubnice (Balijagić, 2009), Trbaljevo, Kurikuće, CrniVrh, Dolac (BJ et al., 2020). It grows in sunny places along roads, on meadows, abandoned places. Widespread species, up to altitude of 1200 m. Medicinal uses include the root *(Cichorii radix)*, less often the above-ground part of the plant *(Cichorii herba)* or only the flower *(Cichorii flos)*. It's used more in folk than in scientific medicine.

Inula helenium L. Elecampane (Pulević V.,1965). It was found at the following localities: near Kolašin (Pulević, 1965), Bjelasica (Lakušić and Milojević, 1972), Biogradsko jezero (BJ et al., 2020). It grows on moist meadows, along forest edges, on the banks of streams and rivers. A rare species that is highly sought after. Rhizome with roots *(Helenii rhizoma or Inulae, or Enulae radix)* is used in folk medicine and as a medicinal raw material in the pharmaceutical industry.

Matricaria chamomilla L. Chamomile, (Sarić M. et al., 1989). It was found in the following localities: Kolašin (Rohlena J., 1942), Andrijevica, Kolašin (Pulević V., 1965), Bjelasica (Lakušić R. and Milojević B., 1972), National Park „Biogradska gora" (Veljović Đ., 2001; Vuković A., 2001), Majstorovina (Balijagić 2010), Lubnice, Glavaca, Trebaljevo (BJ et al., 2020). It grows on warm and dry terrains, mostly in the alpine zone. The chamomile flowers *(Chamomillae flos)* are used internally and externally, in folk medicine and as a medicinal raw material in the pharmaceutical industry.

Petasites hybridus L. Butterbur (Sarić M. et al., 1989). It was found in the following localities: upper basin of Tara and Lim (Blečić V., 1958), National Park „Biogradska gora" (Veljović D., 2001), Brzava, Ravna Rijeka (Balijagić, 2009), Lubnice, Kurikuće (BJ et al., 2020). It grows in moist meadows, along the banks of streams and rivers up to altitude of 1100 m. The leaf *(Petasitidis folium)* is used in folk medicine.

Figure 6. *Taraxacum officinale* Weber

Taraxacum officinale Weber. Dandelion (Pulević V., 1965). It was found in the following localities: Jelovica, Šiška, Jarčeve strane, Troglav, Bjelilo, Otaševo lice (Lakušić R. 1966), Bjelasica (Lakušić R. and Milojević B., 1972), Buče (Lukić P. et al. 1992), National Park „Biogradska gora" (Veljović Đ., 2001), Majstorovina, Brzava, Prijelozi (Balijagić 2009), Lubnice, Kurikuće (BJ et al 2020). Widespread in mesophilic meadows in the subalpine and alpine zone. The root *(Taraxaci radix)* is used in folk medicine.

Table 1. Officinal usage and specific parts used

Species	Plant parts used in medicine				Officinality	Action and usage
	folium	flos	herba	*Radix/ rhizoma*		
Achillea milefolium L.	*Milefolii folium*	*Millefolii flos*	*Millefolii herba*		Helv VII, BHP19 90, ÖAB 9, Jug.II, Komisija E.	Antiphlogistic, stomachic, carminative, cholagogue, choleretic, antispasmodic. It is used in disorders of the digestive organs. Externally in skin inflammatory processes and mucous membranes, for rinsing wounds, burns.
Antennaria dioica (L) Gaertn.		*Antennariae flos*	*Antennariae herba*		Belg V, PFX, Komisija E.	In the treatment of diseases of the bile and bile ducts. In folk medicine for cough and chronic bronchitis.
Arctium lappa L.				*Bardanae radix*	PF X, DAC 86, BHP 83,	Diuretic action and oxygen properties. Used mainly against itchy skin.
Artemisia absinthium L			*Absinthii herba* - *Absinthii summitas*		DAB 10, Helv VII, ÖAB 90, BHP 83, Ph Eur. 6.0	Carminative, cholagogue, choleretic, antispasmodic. In the treatment of bile and biliary tract diseases. In folk medicine for stomach disorders and mild menstrual problems.
Bellis perennis L	*Bellidis folium*	*Bellidis flos*	*Bellidis herba*		Hager.	It has an astringent anti-inflationary effect. It is used internally for coughs and inflammation, to relieve diarrhea, liver and kidney dysfunction.

Carlina acaulis L				*Carlinae radix*	EB 6.	It has antimicrobial, antifungal, diuretic, diaphoretic and antispasmodic effects. In spasms in the digestive tract.
Centaurea cyanus L.		*Cyani flos sine calycibus*			Komisija E, Ph Eur. VI, DAB 10, Helv VII, PF X, EB 6. ÖAB 90, BHP 83.	As a diuretic and choleretic remedy.
Cichorium intybus L.		*Cichorii flos*	*Cichorii herba*	*Cichorii radix*	PF VII, Ital V, Ph Eur. 6.0, Komisija E.	In the treatment of diseases of the liver, bile and bile ducts. Cholagogue diuretic and as a stomachic.
Inula helenium L				*Helenii rhizoma* - *Enulae radix*	PF X, EB 6, BHP 90; Ned.5, Pol.III	Expectorant, bactericidal, holagogenic, diuretic remedy. It is used for dry cough, bronchitis, pneumonia.
Matricaria chamomilla L		*Chamomillae flos*			Helv. VI.	In disorders of the digestive organs. Externally in inflammatory processes on the skin and mucous membranes, for rinsing wounds, burns
Petasites hybridus L.	*Petasitidis folium*			*Petasitidis rhizoma*	HAB 1, HPUS. 88, Komisija E.	Rhizome is used in adjunctive therapy for the treatment of spasmolytic pain in the kidney and also respiratory diseases
Taraxacum officinale Weber	*Taraxaci folium*			*Taraxaci radix*	Ph Eur 6.0, BHP 90, ÖAB 90,	Bitter drug and choleretic. It acts as a diuretic, laxative, antirheumatic.
Telekia speciosa (Schreb.)				*Telekiae radix*	PF X, EB 6, BHP 90; Ned.5, Pol.III	

Tussilago farfara L	*Farfarae folium*	*Farfarae flos*			Helv VII, EB 6, PF VIII, DAB 10, BHP 9',	For cough, asthma and bronchitis.
Solidago virguarea L.			*virgaureae herba*		Ph.Eur.60, BHP83, PF X.	In the treatment of urological diseases, prostate hypertrophy, gout. Externally - for various skin inflammations and eczema.

Telekia speciosa (Schreb.) Baumg. Yellow Ox-eye (Sarić M. et al., 1989). It was found in the following localities: National Park „Biogradska gora" (Veljović Đ., 2001), Majstorovina, Jelovica (Balijagić, 2009), Kurikuće (BJ et al., 2020). Moderately distributed species, grows in beech and fir forests, along the banks of clear mountain streams and springs, up to altitude of 1600 m. In folk medicine, roots are used (*Telekiae radix*).

Tussilago farfara L. Coltsfoot (Pulević V., 1965). It was found in the following localities: the valley of the river Bistrica and its tributaries, the valley of the Mušović river, the valley of Lim and Tara and their tributaries (Lakušić R. and Milojević B., 1972), Lubnica (Lukić P. et al., 1992), National Park „Biogradska gora" (Veljović Đ., 2001; Vuković A., 2001), Kordelj, Majstorovina, Brzava, Strmenica, Bubanje (Balijagić, 2009), Buče, Crni Vrh (BJ et al., 2020). It belongs to the first spring species. It grows in bare, moist, rocky, open and sunny habitats. It spreads up to altitude of 1500 m. Moderately distributed. The leaf *(Farfarae folium)* and the flower *(Farfarae flos)* are used more in the folk than in the scientific medicine.

Figure 7. *Solidago virguarea* L.

Solidago virguarea L. woundwort (Sarić M. et al. 1989). It is widespread on Bjelasica. It was found at the following sites: Bardov do, Rasova (Balijagić et al, 2009). It occurs mainly in forests and on different substrates, in open places in the highlands. It is represented on Bjelasica in large quantities. It is used (Solidaginis virgaureae herba) in scientific and folk medicine.

Orientation quantities of *Achillea milefolium L.* are found in Bjelasica in large quantities (Lakušić and Milojević et al., 1992; Balijagić, 2009). The situation on the ground indicates that its representation has not changed even during our survey in 2020. According to Lakušić and Milojević (1972), Lukić et al., (1992), Balijagić (2009) and based on our research (2020) *Taraxacum officinale*, Weberi is also represented in large quantities. Based on the previous and our research from 2009 to 2020, *Solidago virguarea L.* is represented in large quantities.

The most common species in Bjelasica in 2020 is *Bellis perennis L.* as well as the species *Cichorium intybus L.* To a lesser extent (Lakušić and Milojević, 1972; Lukić et al., 1992; Balijagić (2009), the species *Artemisia abshintium L.* is represented and during our visit (2020) we found and recorded it on limestone soils. The calcified species *Centaurea cyanaus L.* is also poorly represented. *Matricaria chamomillia L.* and *Telekia speciosa* (Schreb.) Baumg are moderately represented on Bjelasica, while *Inula helenium L.* is a rare species that is in great demand.

CONCLUSIONS

Not all recorded species are equally represented in Bjelasica. Some species are found in large and some in small quantities.

The flower of seven species is used in folk and scientific medicine (*Achillea milefolium* L., *Antennaria dioica* (L) Gaertn, *Bellis perennis* L. *Centaurea cyanus* L. *Cichorium intybus* L. *Matricaria chamomilla* L., *Tussilago farfara* L.).

The leaf is used from five species (*Achillea milefolium* L. *Bellis perennis* L., *Petasites hybridus* L., *Taraxacum officinale* Weber, *Tussilago farfara* L.)

The aboveground part (herba) is used by five species (*Achillea milefolium* L. *Antennaria dioica* (L) Gaertn., *Artemisia absinthium* L. *Bellis perennis* L. *Cichorium intybus* L.)

Underground organs are used by seven species (*Carlina acaulis* L., *Cichorium intybus* L., *Inula helenium* L., *Petasites hybridus* L., *Taraxacum officinale* Weber, *Telekia speciosa* (Schreb.) Baumg.

Above-ground and underground parts are used by one species (*Cichorium intybus* L.)

Based on our research in 2020 and research from previous years, there have been no significant changes in the disturbance of the number of natural populations of the studied species.

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In memoriam

Prof. dr Stanka Filipović
(1939-2021)

Naša draga i poštovana koleginica, prof. dr Stanka Filipović, jedna od najznačajnijih naučnica u Crnoj Gori, preminula je 8. marta 2021. godine u Podgorici. U ime kolektiva Biotehničkog fakulteta, a kao njenoj studentkinji i bliskoj saradnici, pripala mi je čast da izrazim visoko poštovanje i zahvalnost za njen rad i izuzetan doprinos u oblasti hemijskih nauka.

Stanka P. Filipović rođena je 03.06.1939. godine u Prekornici – Cetinje. Prirodno-matematički fakultet Univerziteta u Beogradu, grupa hemija, završila je 1964. godine. Specijalističke studije iz Sanitarne hemije završila je 1974. godine, a stažirala na medicinskim – specijalizovanim i naučnim institucijama u Beogradu, Portsmoutu, Zagrebu, Ljubljani i Sarajevu. Odbranila je doktorsku disertaciju 1983. godine na Prirodno-matematičkom fakultetu Univerziteta u Beogradu, i stekla zvanje doktora hemijskih nauka. Godine 1994. imenovana je za eksperta Saveznog ministarstva za nauku, tehnologiju i razvoj u oblasti zaštite životne sredine, i eksperta za sanitarne deponije od strane MMF-a. U zvanje redovnog profesora izabrana je 1995. godine na Metalurško-tehnološkom fakultetu Univerziteta Crne Gore, a naučnog savjetnika 1996. na Medicinskom institutu Univerziteta Crne Gore.

U oblasti ekotoksikologije slatkih voda usavršavala se na Univerzitetu u Mičigenu (School of Public Health, Environmental and Industrial Health; School of Natural Resources; Atmospheric and Oceanic Science; Water Resources and Engineering and Great Lakes and Marine Waters Center), kao i toksikološkoj laboratoriji na Univerzitetu Kalifornije u San Francisku. U cilju proučavanja mikrozagađivača u jezerskim ekosistemima boravila na Univerzitetima u Milanu i Firenci.

Učestvovala je na brojnim seminarima, konferencijama i kongresima u zemlji i inostranstvu (SAD, Kina, Japan, Italija, Francuska, Španija, Njemačka, Danska, Malta i Albanija) u oblasti hemije voda, zaštite životne sredine, speleologije i ekotoksikologije.

Samostalno i u koautorstvu objavila je preko stotinu naučnih radova – najviše sa ciljem proučavanja kvaliteta slatkih prirodnih voda (fizičko-hemijski, biohemijski, hidrohemijski, geohemijski i ekotoksikološki aspekti), transformacija toksikanata i geohemijskih veza površinskih i podzemnih voda. Učestvovala je u izradi velikog broja stručnih elaborata i studija, Bila je član brojnih naučnih i stručnih asocijacija među kojima i Evropskog odbora za zaštitu voda.

Tokom bogate četrdesetdvogodišnje karijere, u periodu od 1964. do 1988. godine, radila je kao medicinski hemičar-analitičar, specijalista sanitarne hemije, šef Odsjeka za vode i načelnik Odjeljenja Sanitarne hemije i ekotoksikologije u Republičkom zavodu za zdravstvenu zaštitu i medicinska istraživanja u Titogradu. Od 1984. do 1985. godine bila je načelnik Vojne hemijsko-radiološko-toksikološke laboratorije u Crnoj Gori. Bila je angažovana i na Građevinskom fakultetu u Beogradu, i Institutu za hidrotehniku Građevinskog fakulteta u Sarajevu. Od 2000. godine do odlaska u penziju 2005. g. radila je u Centru za zemljište i melioracije Biotehničkog instituta Univerziteta Crne Gore, baveći se istraživanjem uticaja kvaliteta vode za navodnjavanje na zemljište i biljku.

Profesorka Filipović je dala veliki doprinos u obrazovanju, stručnom osposobljavanju i naučnom usavršavanju brojnih generacija učenika, studenata i istraživača. Od početka svoje profesionalne karijere obavljala je nastavne aktivnosti počevši od Gimnazije "Slobodan Škerović" i Više vojne akademije, te Srednje medicinske škole Medicinskog zavoda u Titogradu do angažmana kao predavača na Građevinskom fakultetu, Prirodno-matematičkom fakultetu, Metalurško-tehnološkom fakultetu i Medicinskom institutu/fakultetu Univerziteta Crne Gore (predmeti iz oblasti metoda prečišćavanja pitkih i otpadnih voda, hemije prirodnih voda i propagacije zagađivača).

Bila je vodeći istraživač mnogih domaćih i stranih projekata, uglavnom vezanih za zaštitu životne sredine. Od posebnog značaja je njeno učešće u projektu "Limnological investigations of Skadar Lake" koji je realizovao Biološki zavod u Titogradu zajedno sa dvije prestižne međunarodne naučno-istraživačke institucije – Smithsonian Institution, Washington D.C. i University of Wisconsin-Milwaukee Center for Great Lakes Studies, u periodu 1970–1976. g. Rezultati ovih prvih opsežnih limnoloških istraživanja su publikovani u monografiji "The Biota and Limnology of Lake Skadar" (G. S. Karaman and A. M. Beeton (Eds.), University Veljko Vlahović, Institute of Biological and Medicine Research Titograd, Montenegro, Yugoslavia, 1981). U saradnji sa istraživačima drugih profila, doprinijela je i primijenjenim istraživanjima – razvijanju pilot postrojenja za poboljšanje prečišćavanja pijaće vode, rješenja za brojne simulacione laboratorijske oglede, kao i nadgradnju ekspertnih sistema pijaćih voda.

Radila je sa velikim entuzijazmom čak i u poznim godinama. Njen rad je bio visoko cijenjen od strane eminentnih naučnika iz zemlje i inostranstva. Posebno je bila ponosna na Zahvalnicu za doprinos u odvodnjavanju Cetinjskog polja, koju je dobila 1985. godine od Opštine Cetinje; Diplomu za doprinos razvoju speleologije od Speleološkog Saveza Bosne i Hercegovine – 1986. godine i Jubilarnu povelju Jugoslovenskog društva za zaštitu voda – 1996. godine u Beogradu.

Životnu priču Stanke Filipović (rođene Banović), ponosne i znamenite Crnogorke, sa njenim ličnim uspjesima i postignućima, porodičnom srećom i tugom, objavili su mr Trivo Zolak i Olivera Kukić u prvoj knjizi edicije "Čudesna moć žene", još 1999. godine.

Naša draga profesorica i koleginica Stanka, njegovala je tradicionalne vrijednosti, a istovremeno pratila zahtjeve modernog društva. Bila je spremna da prihvati izazove – u nauci i generalno u životu. Voljela je i osjećala ljude i prirodu. Pored pomoći i podrške koju je nesebično pružala svima, umjela je da nas razveseli i uvijek je bilo prijatno u njenom društvu. Sa posebnim smislom za humor, znala je i da uputi kritiku na duhovit način. Radovala se našim životnim i profesionalnim uspjesima. Veze koje su nas spajale, nisu ni sada prekinute, jer će nastaviti da živi u našem sjećanju.

Velika nam je čast što je prof. dr Stanka Filipović dio svog radnog vijeka provela na Biotehničkom fakultetu. Neka joj je vječna slava i hvala za sve, a posebno za nemjerljiv doprinos u oblasti proučavanja sastava i kvaliteta voda Crne Gore i zaštite životne sredine.

Dr Ana Topalović

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- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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