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CONTENT

Hellstedt, M. (2020): Detection of odour from animal production in Finland. Agriculture and Forestry, 66 (1): 07-14.

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Maarit HELLSTEDT [1]

DETECTION OF ODOUR FROM ANIMAL PRODUCTION IN FINLAND

SUMMARY

Agriculture is the most significant source of ammonia emissions. These emissions cause, for example, odour problems, which are a local nuisance to neighborhoods, and acidification. Traditionally, animal production farms have been situated in rural areas far from densely populated areas. However, as urban areas grow, the urban population is moving nearer to these farms. Problems arise when farms need to grow or change their production and their neighbors oppose this due to the potential for odour. Thus, odour annoyance must be taken into account when providing environmental permissions for animal production units. Two different estimation methods are used in Finland. The first is a curve based on the number of livestock units in the production unit. The second, which is still under development, is a model based on animal- and production-dependent odour factors, the prevailing wind direction and the topography of the area. However, both methods have deficiencies. When the production unit has already been established, prevailing odour can be measured with an olfactometric method based on the odour sensations of a panel of people with different sensitivities to odour annoyance. The olfactometric method can also be used to estimate the effect of different production technologies on odour. Examples of applications of the olfactometric method are presented.

Keywords: Animal production, odour, annoyance, measurement.

INTRODUCTION

Agriculture is the most significant source of Ammonia emission that causes e.g. odour problems. Animal production farms have traditionally been situated in the rural areas well apart from densely populated urban areas. As urban areas grow, new transition zones where farms, urban population and e.g. recreation seek for their own space and how to fit together. Problems arise when farms need to grow or change their production and neighbors oppose the environmental permit due to expected odour annoyance. Problems also arise where cultivated fields are situated near urban areas and the farmer wants to use manure as fertilizer to the fields (Hellstedt and Haapala, 2014). Animal production farms applying environmental permission often face an odour emission challenge.

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Odour annoyance has to be taken into account on the environmental permissions for animal production units.

There are two different estimation methods used in Finland. The first simple one is a curve that is based on the number of livestock units in the production unit. The other one, still under development, is a model that is based on animal and production dependent odour factors, prevailing wind directions and topography of the area. Both of these methods have deficiencies.

There are, however, methods available for reducing the odor level of animal production. Directive on industrial emissions (European Parliament and Council, 2010) defines Best Available Techniques (BAT) which are feasible for reducing environmental emissions. A Reference Document (Santonja et al. 2017) is updated on a regular basis where the available BAT technologies are listed. The challenge for the farmer is to prove that the techniques planned to be used have the odour reducing effect expected. There are available equipment to measure the components causing odour. These mainly on research purposes developed measuring methods are slow and expensive. Practical methods with affordable equipment and straightforward calculations are needed.

The main source of odour in animal farms is manure. The odour increases steeply as the temperature of manure gets higher (Hügle and Andree, 2001). The effect is steeper with pig manure than with cattle manure, Figure 1. Therefore techniques which cool down the inside air in production units could reduce odour emission especially from swineries. In the inside air of an animal houses dust particles and odour particles are of the same size and interlocked. Consequently, high-pressure spraying that both cools the air down but also reduces dust could also reduce the odour.

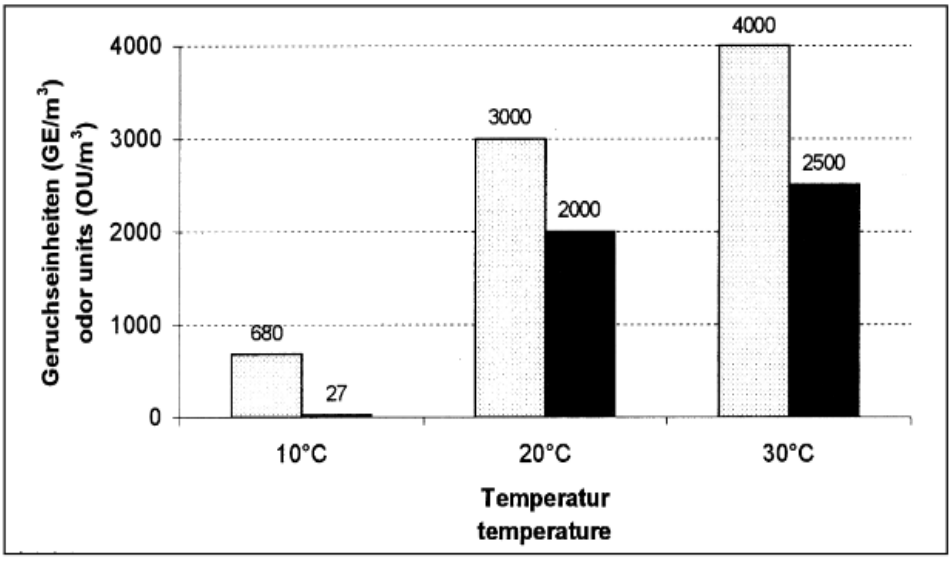

Figure 1. Odour concentration (OU/m3) of swine- (white) and cattle (black) slurry at different temperatures (Hügle and Andree, 2001).

Recent research projects have aimed to create practical methods to measure the annoyance of odour from a production unit in practical situations and to prove the effect of different new environmental technologies on the odour. In this paper results from three different practical measurements are reported.

MATERIAL AND METHODS

The prevailing odour from an established production unit was detected from two different farms, a broiler farm and a swinery, near an urban area using an odour panel, Figure 2. The sensitivity of panelists was tested individually according a specific procedure (Pen-Test). Applying the protocol suggested by Brand et al. (2011) and more detailed described in Hellstedt and Haapala (2014) the panelists approached the source of odour from different directions defined according to the prevailing wind direction in the area, Figure 3.

Measurements to estimate the effect of different production technologies on odour were performed in a new swinery that has invested in different environmental technologies. The farm produces ca. 3000 fattening pigs annually.

The technology inside the production unit tested was a high-pressure water spraying system. The high-pressure water spraying is originally designed to cool down the inside air in the swinery. But it also reduces dust that otherwise would be carried through ventilation. Two compartments of the swinery were used. One compartment had the technology on and the other one off. Odour measurements were done at three different locations along the central aisle of the compartment.

Pellon Biosampo™ was used to treat manure on the swinery. The treatment had a notable effect on the odour level of the slurry. To measure the effect, a test was done. Raw slurry was taken directly from the buffer storage before the treatment plant and the treated liquid fraction was taken from the last treatment tank. Both the spread liquids were fresh. The characterization of the materials is shown in table 1.

Broadcast spreader with a splash plate was used for spreading both the raw slurry and the treated liquid fraction. The spreading area was about 1 ha. The weather was sunny, temperature + 12 °C and wind speed ca. 5 m/s. Odour measurement were done at a corner of the spreading area under the prevailing wind.

Table 1. The nutrient and dry matter content of the raw slurry and treated liquid fraction.

	N_{tot} g/kg	N_{sol} g/kg	P g/kg	K g/kg	Total solids %
Raw slurry	4.1	3.1	0.57	2.4	2.85
Treated liquid fraction	2.9	2.2	0.24	1.8	1.27

Figure 2. Persons of the odour panel approaching the source of odour.

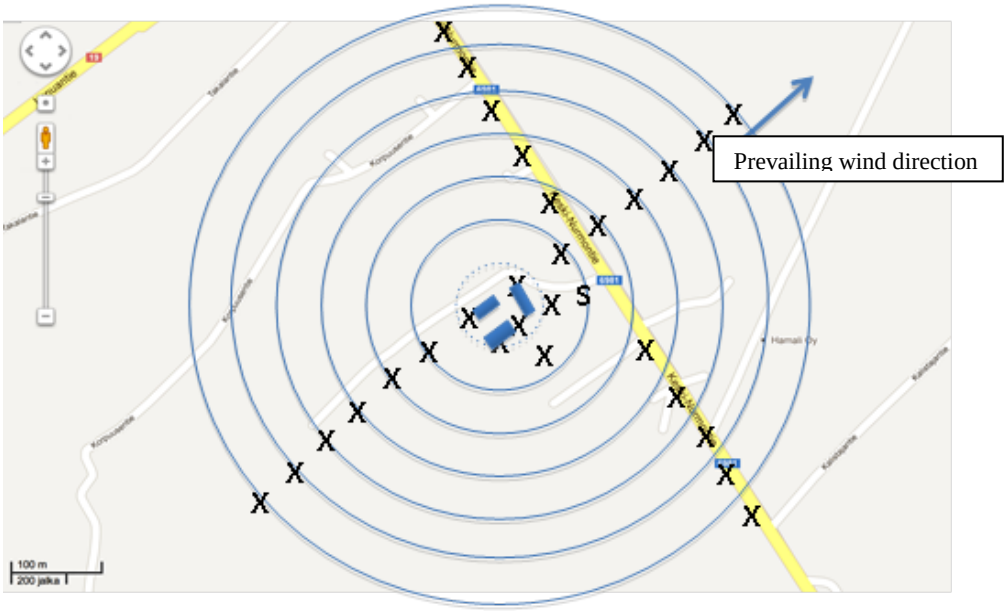

Figure 3. Principle of the odour panel method, x stands for the different locations were the odour measurements were carried out.

In all these cases odour emission was measured with an olfactometric method that is based on odour sensation of a person, Figure 4a. A Nasal Ranger™ field olfactometer was used. The odour was measured as odour

concentration, expressing the amount of dilution needed to make the air odourless, Figure 4b. Also the character of the odour was assessed in most of the cases.

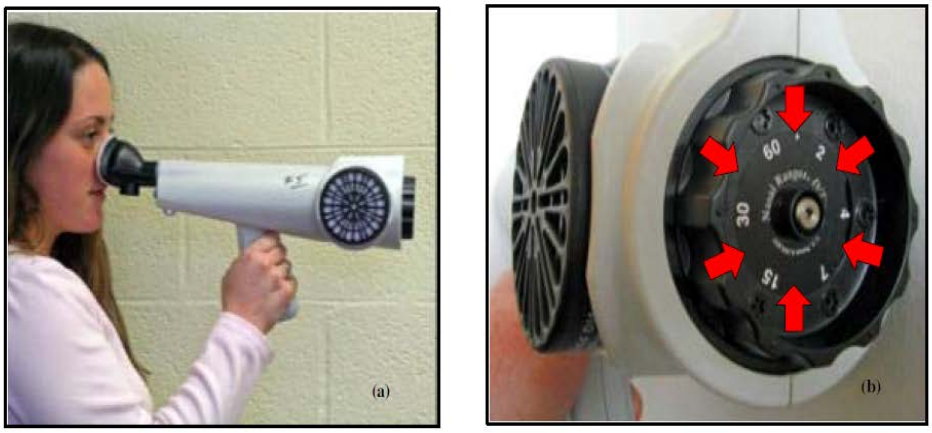

Figure 4. (a) The field olfactometer in use. (b) The dilution dial located at the air intake of the unit, which is unseen by the odour assessor during use (100% active carbon-filtered air blank positions are marked with arrows). Nasal Ranger (2013).

RESULTS AND DISCUSSION

The results of the odour panel show that the odour from the farm was found stronger on areas which are situated downwind from the production unit, Figure 5. Odour was also found slightly at intervals in other directions because the wind was quite low and the odour fall down all around the farm. The forest area next to the swinery seems to prevent the odour to spread into that direction. The odour from a broiler farm was found less annoying than that from a fattening pig farm.

According to the results the high-pressure water sprayer reduces odour as it cools down the inside air in the swinery. The reduction of odour level measured is significant (p>67%) during the spraying, Figure 6. Until now these sprayers have been sold to farms on the basis of animal welfare. Because of its effect on odour the investment on the high-pressure sprayer is more beneficial. The amount of water used in spraying is so small that the floors do not get wet. Therefore, there will be no extra water running to the slurry gutters.

The measured odour annoyance from spreading of the treated liquid fraction was significantly lower than that of the raw slurry.

The initial reading of the olfactometer at the start of spreading was 60 (maximum reading) for both the treated liquid fraction and the raw slurry. The odour of the treated liquid fraction vanished in about 30 minutes while for the odour from raw slurry the time was the double, about 60 minutes, Figure 7.

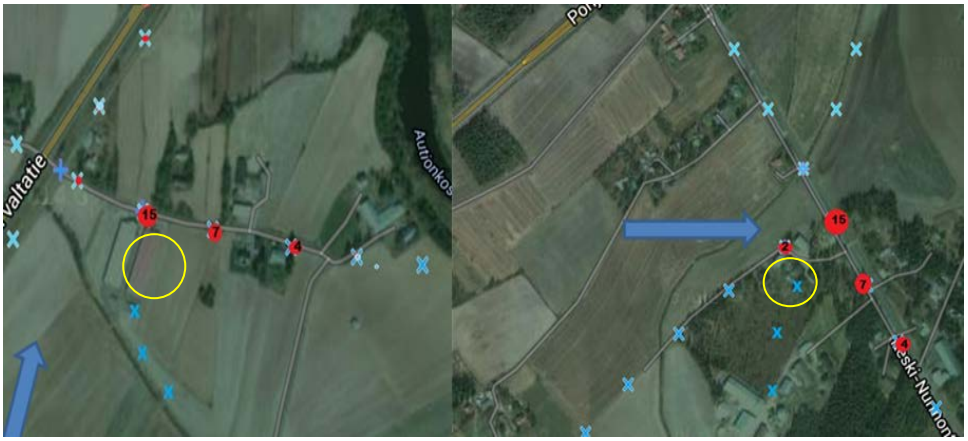

Figure 5. Odour emission detected by the panelists from the broiler farm (left) and from the swinery (right). The source of odour is inside the yellow circle. The size and the number inside the red dot indicate the average odour concentration according to the reading of the dial plate of the olfactometer. Red dot without a number indicates odour concentration less than 2.

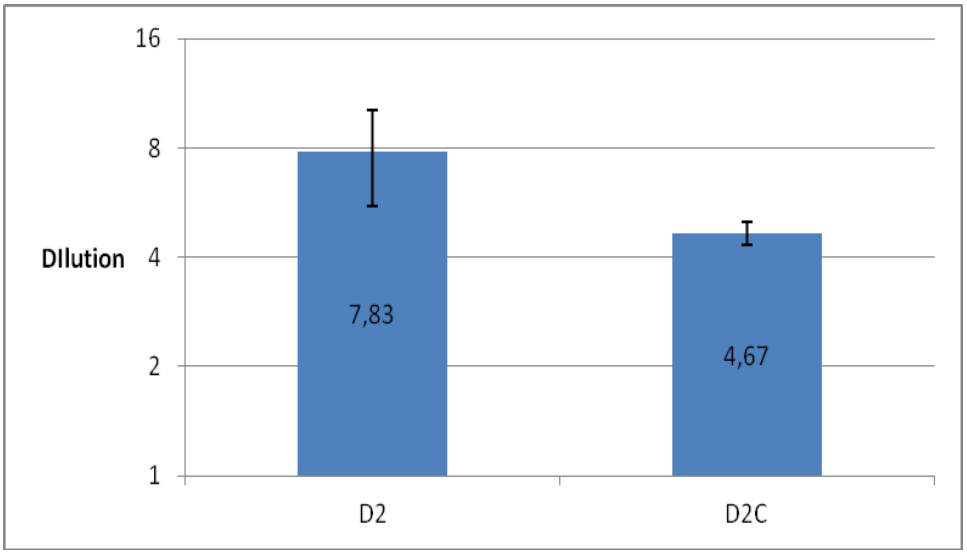

Figure 6. Odour reduction of high-pressure water spraying in a swinery. D2=without spraying, D2C=with spraying.

Also the character of the odour for the treated liquid fraction was less annoying. According to the assessment of the test persons the odour was more like the odour of soil or wet straw than that of manure.

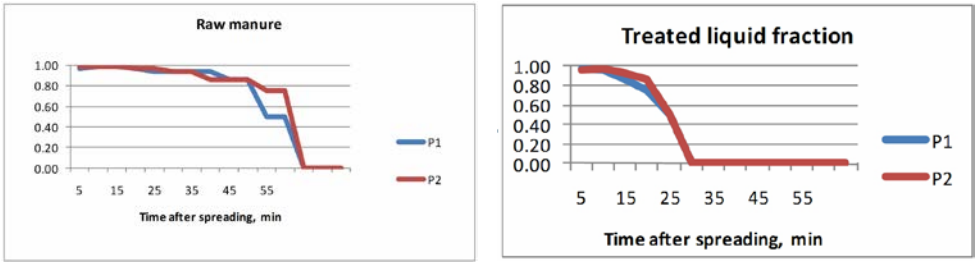

Figure 7. Reduction of odour after manure spreading. Raw slurry (left) and treated liquid fraction (right). Calculated as relative odor where initial odour is 1.0. P1 and P2 are the different observers.

CONCLUSIONS

The results on the use of portable field olfactometer on detecting odour on practical situations are positive and give valuable information on the effect of different technologies. The measurements performed so far are a good basis for further studies. More data is needed of the sensibility of the method to different weather conditions as they change between experiments and makes it difficult to compare the results of different techniques with each other. Also the number of panelists needed for different setups has to be studied further. Due to the disease risks of visitors the number of panelists used in measurements inside the production units has to be minimized. Besides that there has been also the difficulty of recruiting valid persons to the panels.

Also further studies are needed to gather wider and deeper evidence of the effect of different technologies on odour and to calculate the economics of the use these technologies.

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Xynias, N. I., Tasios, E. I., Korpetis, G. E., Pankou C., Avdikos, I., Mavromatis, G. A. (2020): Effect of the 1BL.1RS wheat-rye chromosomal translocation on yield potential in bread wheat. . Agriculture and Forestry, 66 (1): 15-21.

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EFFECT OF THE 1BL.1RS WHEAT-RYE CHROMOSOMAL TRANSLOCATION ON YIELD POTENTIAL IN BREAD WHEAT

SUMMARY

To investigate the effect of 1BL.1RS wheat-rye chromosomal translocation on bread wheat yield, four and six commercial cultivars with and without translocation, respectively, were evaluated in the field. In addition to yield, three agronomic traits were measured: days to heading, plant height and 1000 kernel weight. Experiments were performed in two different locations (one cold and one dry) for three years at each location, and four replications were conducted. Despite observed differences between the genotypes, translocation was not found to produce an advantage. Even though the two cultivars did not differ, the one without translocation was ranked first and the one with translocation was ranked second. The cultivar Kavkaz/Cgn, one of the donors for translocation, was ranked eighth, and the fourth cultivar with translocation was ranked last. Based on these results, we studied the genotypes in each location to determine whether translocation produced any adaptation effect. The results revealed that three of the four cultivars with translocation performed well in the cold environment, and only one performed well in the dry area. One of the cultivars without translocation exceeded the yield of the cultivars with translocation. In the dry area, five of the non-translocated cultivars performed as well as the translocated ones. Thus, it could be concluded that translocation had no obvious positive effect on yield. Similar results were recorded for the other three traits. Further research with more environments and more sophisticated analysis is needed to achieve more accurate conclusions.

Keywords: fixed model, analysis of variance, adaptation, yield, days to heading, plant height, 1000 grains weight.

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INTRODUCTION

The continuous deterioration of the environment, the increasingly declining agricultural area and the simultaneous increase in human food needs have created a stifling environment in agricultural research. For these reasons, the plant breeders have turned to the search for new germplasm to overcome the problem (Fehr, 1987). An interesting case is the use and integration of new genes in high yielding varieties. The 1BL.1RS wheat-rye chromosomal translocation carrying valuable genes appears as an interesting alternative (Tang et al., 2009). According to literature the presence of the translocation offers to the host cultivar high yield potential (Kim et al., 2004; Xynias et al., 2007), mainly due to increased seed weight and production under drought conditions (Ehdaie et al., 2003). Furthermore wide adaption (Rajaram et al., 1983; Schlegel and Meinel, 1994), the resistance to stress conditions (Bartos and Bares, 1971; Zeller, 1973; Moreno-Sevilla et al. 1995; Karelov et al. 2018) and the increased plant regeneration rate (Rabinovich, 1998) are three more valuable traits which can be transferred to new cultivars via the translocation.

The translocation was transferred to modern wheat cultivars though the Russian cultivar Kavkaz (Weng et al., 2007). The 1BL. 1RS translocation, despite the positive genes it also carries some other genes, reducing the bread making quality of the host cultivar (Graybosch et al., 1993; Fenn et al., 1994). However, their expression is influenced by the genetic background of the host cultivar (Rabinovich, 1998; Dimitrijevic et al., 2008) rendering the production of new germplasm with good end product quality, despite the presence of the translocation feasible.

The aim of the present study was to investigate the effect of the 1BL.1RS wheat-rye chromosomal translocation on yield performance and three other traits in bread wheat cultivars carrying the translocation compared to cultivars without the translocation under low input. conditions.

MATERIAL AND METHODS

a. Plant material

For the purpose of the study nine hellenic bread wheat cultivars (Acheron, Elissavet, Orfeas, Apolonia, Acheloos, Vergina, Doerani, Nestos and Strymonas) that were developed at the Cereal Institute of Thessaloniki (Anonymous, 2016) and the Russian cultivar Kavkaz/Cgn, one of the donors of the 1BL.1RS wheat-rye chromosome translocation (Xynias et al., 2006; Weng, 2007) were used. Three of the hellenic cultivars were found to carry the 1BL.1RS wheat-rye chromosome translocation (cvs. Acheron, Elissavet and Orfeas) whereas the other six cultivars, were lacking the specific translocation (Xynias et al., 2006; Peros et al., 2015).

b. Method

The experiments were established for three successive years 2015-16 to 2017-18 in the main Farm of the School of Agricultural Sciences, in Florina (40°46´ N, 21°22´E, 707 m asl), representing a cold and wet environment and in

the main farm of Plant Breeding and Genetic Resources Institute, Hellenic Agricultural Organization-"Demeter" in Thermi (40°32´ N, 23°00´E, 15 m asl), representing a marginal dry environment Table 1). Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P205 at 80 and 40 kg ha-1, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary.

Table 1. Rainfall and temperature regimes in the environments of Florina and Thermi (Thessaloniki).

Crop.	Florina				Thermi			
Season*	Rainfall (mm)		Temperature (^{0}C)		Rainfall (mm)		Temperature (^{0}C)	
	Total	Critical**	Min	Max	Total	Critical**	Min	Max
2015-16	526.8	292.4	-14.6	35	294.9	188.4	-8	38
2016-17	423	193.2	-21.8	37.6	385	98***	-10	40
2017-18	921	232.4	-9	34.2	482.3	153.6	-4	36

*From November till July, **In March, April, May, ***only 15.8 mm rainfall from the second half of March till the end of the first half in May

Randomised complete blocks field design statistical analysis for each location and combined over cropping seasons was made (Steel and Torrie, 1960). The fixed model was used, in which the years were the random and the evaluated genotypes the fixed variable (McIntosh, 1983). The plots were consisted of five rows (plot area 3m^2) of which the three inner were threshed (harvest area 1.8m^2). Except yield, three more traits influencing yield potential were studied: i. e. days to heading, maturity height and 1000 kernel weight were studied. Comparisons were performed between the aforementioned traits to reveal how they affect each other (Steel and Torrie, 1960).The means were compared according to the L.S.D. method. The data obtained were analyzed statistically with Mstat-C (Freed and Eisensmith, 1986).

RESULTS AND DISCUSSION

The across testing environments combined analysis of variance produced significant genotypic effects for all traits studied (Table 2). The expected significant environment effect was observed for all traits. Except in the effect of the environment which accounted 11.6% of the yield total sum of squares - TSS (as compared to 6.9% and 58% for genotypes and genotype x environment interaction - GEI effects respectively), the values on the other traits were the ones expected. Thus, the environment accounted for 93.6% of the earliness to heading TSS (as compared to 1.2 and 4.4% for genotypes and GEI effects respectively), 84.4% of the plant height at maturity TSS (as compared to 7.0% and 3.5% for genotypes and GEI effects respectively) and 73.3% of the 1000 kernel weight TSS (as compared to 4.7 and 11.6% for genotypes and GEI effects respectively).

Similar results were reported in bread wheat (Yan et al., 2000) and in durum wheat (Agorastos and Goulas, 2005). A highly significant GEI effect was observed for grain yield and for all other correlated traits.

Table 2. Over environments analysis of variance for yield, days to heading, maturity height and 1000 kernel weight in 10 bread wheat varieties.

Source	Degrees of freedom	Mean squares			
		Yield	Heading	Maturity height	1000 kernel weight
Environments (E)	5	**	**	**	**
Cultivars (G)	9	**	**	**	**
GE interaction	45	**	**	**	**
CV		17.01	1.68	5.61	6.72
TSS environments		11.6	93.6	84.4	73.3
TSS cultivars		6.9	1.2	7.0	4.7
TSS GE interaction		58.0	4.4	3.5	11.6

Table 3. Ranking for yield (g/plot), days to heading, maturity height (in cm) and 1000 kernel weight (g) of the evaluated bread wheat varieties.

a/a	Cultivar	Yield	Heading	Maturity height	1000 kernel weight
1	Acheron*	CD	DE	CD	A
2	Elissavet*	A	C	CD	E
3	Kavkaz/Cgn*	D	C	B	D
4	Orfeas*	D	BC	B	E
5	Apollonia	A	D	C	D
6	Acheloos	AB	BC	A	BC
7	Yecora E	CD	E	E	CD
8	Doerani	BC	B	B	AB
9	Nestos	BC	A	D	BCD
10	Strymonas	D	C	B	CD
	LSD	54.95	1.508	2.641	1.373

(*) Cultivars carrying the 1BL.1RS wheat-rye chromosomal translocation
Cultivars followed by different letters are significantly different at p=0.05 level

The ranking of the examined bread wheat cultivars for each individual trait is presented in Table 3. It becomes evident from this table that there is no general effect of the presence of the translocation in all traits. Only cultivar Elissavet performed well in yield but with no differences from cultivars Appolonia and Acheloos, which did equally well. The other three cultivars carrying the translocation were ranked last. In days to heading only Aheron was equally early with cultivar Yecora E, which is one of the earliest cultivars under the hellenic climatic conditions.

No differentiation was noticed between the rest of the cultivars, carrying the translocation or not. Cultivar Acheloos was the most vigorous one, whereas cvs Kavkaz/Cgn and Orfeas were equally vigorous with cultivars Doerani and Strymonas. In 1000 kernel weight, cv. Acheron performed equally well with cv. Doerani, whereas cvs Elissavet and Orfeas were ranked last.

Table 4. Over years analysis of variance for yield, at each location, of the bread wheat cultivars studied.

Source	Degrees of freedom	Mean squares	
		Florina	Thermi
Years (y)	2	ns	**
Varieties (V)	9	**	**
Y x V	18	**	**
CV		17.35	16.66
TSS	Years	1.2	22.3
	Cultivars	19.9	19.3
	Interaction	54.1	37.9

Table 5. Ranking for yield (g/plot) at each location, of the bread wheat cultivars studied.

a/a	Cultivar	Florina	Thermi
1	Acheron*	A	CD
2	Elissavet*	A	AB
3	Kavkaz/Cgn*	A	E
4	Orfeas*	E	BCD
5	Apollonia	AB	A
6	Acheloos	A	ABC
7	Yecora E	DE	AB
8	Doerani	BC	AB
9	Nestos	CD	A
10	Strymonas	BC	D
	LSD	79.56	77.02

(*) Cultivars carrying the 1bl.1rs wheat-rye chromosomal translocation
Cultivars followed by different letters are significantly different at p=0.05 level

A second issue that was attempted was to study the genotypes in each location separately in order to find out whether there was any adaptation effect of the translocation. In this analysis, the differences in yield between the genotypes tested were significant in both locations (Table 4). The expected significant year effect was observed only in Thermi. This could be interpreted by the similarity in weather conditions in the location of Florina during all testing years. The ranking of the cultivars studied revealed that three of four of them carrying the translocation performed well in the cold environment (Table 5). Only one cultivar

without the translocation exceeded in yield the aforementioned cultivars carrying the translocation. These results support the view that there must be some advantage of the translocation in cold environments, but this cannot be generalized since one cultivar carrying the translocation was ranked last. In the dry area, five of the non-translocated cultivars were ranked in higher places. Only one of the cultivars carrying the translocation performed equally well with the translocated one.

The above results suggest that there is not any positive effect of the presence of the translocation on yielding potential of the host cultivar. Only in the cold area of Florina there was some advantage of the translocation. In the dry area only one cultivar with the translocation performed well. This does not agree with the results of Hoffmann (2008), who stated that the translocation offers resistance to drought to the host cultivar. One could presume from the performance of the evaluated germplasm that the presence of the translocation is not enough to ensure any yielding advantage. Probably, the genetic background of the host cultivar is essential for the translocation to express its valuable properties. The same was concluded by Lisova *et al*., (2005) who studied the biotic resistance of cultivars carrying the translocation, by Lazaridou *et al*., (2014) who studied the of the effect of the translocation on the androgenic response and Xynias *et al*. (2018) who studied the effect of the translocation on bread making quality.

CONCLUSIONS

Summarizing the data discussed applicable to the particular set of bread wheat genotypes evaluated under the range of environments, no effect of the presence of the translocation was observed. However, since the fixed model was used these conclusions apply only to the varieties and environments studied. Further research, applying the random model, is needed to draw general conclusions.

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INFLUENCE OF CITRATES NANOPARTICLES ON MORPHOLOGICAL TRAITS OF BACTERIAL CELLS PSEUDOMONAS SYRINGAE PV. ATROFACIENS

SUMMARY

Electron microscope investigations have revealed disintegration of the cell wall of *Pseudomonas syringae* pv. *atrofaciens* and subsequent killing of bacterial cells after application of 1% solutions of the citrates of Ag-Cu nanoparticles, the complex Co-Cu-Zn-Fe-Mn-Mo-Mg (Avatar-1) and iodine-selenium. In field conditions, it was revealed that pre-sowing treatment of wheat seeds with the aforementioned 1% solutions significantly inhibited the development of basal glume rot in cereals with artificial infection of *Pseudomonas syringae* pv. *atrofaciens*. Consequently, reduction of the typical symptoms of basal glume rot of cereals and the percentage of isolated cells of *Pseudomonas syringae* pv. *atrofaciens* D13 were detected in wheat plants upon pre-sowing treatment of wheat seeds by experimental biocides. These results can be explained by structural changes in the bacterial cells of the pathogen that lead to reduction of viable forms of bacteria due to damage of both the cell wall and internal contents.

Keywords: Wheat, bacterial diseases, *Pseudomonas syringae*, nanoparticles.

INTRODUCTION

The soft wheat, due to its unique composition and high nutritional quality, is a widespread food crop in many countries of the globe. Wheat grains contain vitamins, minerals, protein, starch, fiber, etc. Thus, the protein content of wheat grain can range from 10% to 18% of the total dry matter. The selection aimed at improving the qualitative composition of the protein allowed the creation of new

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varieties with high protein content in the grain and improved amino acid composition. However, unfortunately, new varieties had undesirable characteristics, such as reduced yields and susceptibility to diseases and pests (Šramkováa, 2009; Kalinichenko et al., 2017).

One of the phytopathogenic microorganisms that cause significant loss of yield and quality is *Pseudomonas syringae* pv. *atrofaciens* - causal agent of basal glume rot of cereals. This disease under favorable meteorological conditions for its development can reach a considerable spreading and infect up to 80% of plants. The consequence of bacterial disease is the deterioration of the physical, technological and biochemical properties of the grain, reducing the content of glutenin and gliadin increasing the content of gluten of poor quality. In this case the following symptoms are observed: lesions of the lower part of the cover scales (maybe and upper), spots of various organs of plants. Brown, beige or black elongated blotches and streaks on different organs of diseased plants are observed throughout grain-filling period. With a significant defeat, the ear deforms and develops a succulent grain with a darker embryo (Gvozdyak et al., 2011).

So-called biocides, different in chemical composition, have a wide range of bactericidal properties. Among them compounds of silver, copper and halogens, in particular iodine. It is known that silver compounds interact with thiol groups of membrane bound enzymes, which plays an essential role in the inactivation of bacteria, causes the release of K^+ – ions from microorganism cells, inhibits the cell division, damages the cell membrane, precipitates in vacuoles and the cell wall in the form of granules, interacts with components of DNA, but the exact mechanism of action of these compounds is unknown. Halogens are oxidizers thiol groups to disulfides, sulfoxides or disulfoxides; they inhibit the synthesis of DNA. The compounds that contain chlorine or iodine more often are applied on practice as antiseptics and disinfectants. The antimicrobial action of iodine is fast, even at low concentrations. Its target is the key groups of proteins and amino acids, nucleotides and fatty acids, which leads to cell death (McDonnell and Russell, 1999).

At the same time, progress in science and the development of nanotechnologies has enabled the increase of the efficiency of these and other biogenic elements in times. Application of the nanoscale materials leads to a damage of the cell surface, changing in permeability of the membrane and death of the bacterial cell (Ahmada, 2017). Due to chelation by aqueous solution the citrate and small sizes (less than 100 nm), the chelates of the nanoparticles of biogenic elements ease penetrate through the cell membrane and begin to rapidly act at the molecular level.

Therefore, the aim of our work was to study the bactericidal properties of various biocides: the chelated nanoscale metals in citrated form and biologically active iodine that effect on the morphological characteristics of bacterial cell, in particular, *P. syringae* pv. *atrofaciens* D13, which was isolated from wheat and rated as aggressive (aggressiveness 4 points).

MATERIAL AND METHODS

For research citrates of nanoscale metals were used: 1% solution of Ag + Cu nano aqua citrate - from the preparation "Shumerske sriblo" with concentration of nanoparticles Ag 250 ml/L and with the concentration of nanoparticles Cu 250 mg/L and citric acid (Nanomaterials and nanotechnology Ltd, Ukraine); 1% solution composition of nanoparticles Co+Cu+Zn+Fe+Mn+Mo+Mg, that is prepared from micro fertilizer Avatar-1 (organic), with composition of nanocomponents: Co – 0,0001–0,0025%; Cu – 0,01–0,08%; Zn – 0,001–0,007%; Fe – 0,0015–0,008%; Mn – 0,0005–0,005%; Mo – 0,00001–0,0025%; Mg – 0,01–0,08% and citric acids – 0,5–10 mg/L, purified water (Avatar Ltd, Ukraine) and a preparation of biologically active iodine in a complex with 1% solution selenium BP I-Se (preparation "Jodis-concentrate plus Se"). BP I-Se consists of purified water enriched with polyatomic ions I, 80 mg/dm^3, Se citrate, intended as a biologically active food additive (SPC «Iodis», Ukraine).

Bacterial culture *P. syringae* pv. *atrofaciens* D13 was isolated from clay-brown spot-strokes on a leaf-sheath of wheat, that was collected in the Dnipropetrovsk region. Isolation of bacteria from plant samples, inoculation and cultivation of them on solid medium and preparation of a bacterial suspension were performed according to generally accepted techniques (Patyka et al., 2016, 2017).

The morphological signs of bacterial cells for short-term effects of the test compounds, which were 15 minutes, were investigated using a transmission electron microscope JEOL JSM 1400 on the TEM sample support metal mesh 'grids'.

The field experiments have been performed with Pecherianka variety spring wheat plants. The plants of *Triticum aestivum* L. have been grown in field conditions on research areas (area 50 m^2) of Zabolotny Institute of Microbiology and Virology of NASU. The following scheme has been used for experiments: 1 – control (water); 2 – pre-sowing treatment of seed with 1% solution of Ag + Cu; 3 – pre-sowing treatment with 1% solution micro fertilizer Avatar-1 (organic); 4 – pre-sowing treatment of seed with 1% solution BP I-Se.

Artificial inoculation of Pecherianka variety spring wheat plants in the boot stage was carried out by bacterial suspension of the strain of *P. syringae* pv. *atrofaciens* D13 (causal agent of basal glume rot of cereals). Density of the suspension was1×10^9 CFU/ml. Artificial inoculation was done by injecting a bacterial suspension into a stem in 10-fold repeatability on each of the variants. 14 days after bacterial infection, typical visual manifestations of disease symptoms have been evaluated in conditional units (number of spots, strokes, total area of damage) in comparison with control (without treatment) and experimental plants (pre-sowing treatment condition). 4-point of the scale have been used for estimated of the disease symptoms.

RESULTS AND DISCUSSION

Researches were showed a decrease in the degree of visual display of bacterial disease at artificial inoculation with the causative agent of basal glume rot of cereals - *P. syringae* pv. *atrofaciens* D13 in condition pre-sowing treatment of wheat seed with solutions of nanosized metal citrates (1% Ag-Cu) and 0.1% of Avatar-1 (Co + Cu + Zn + Fe + Mn + Mo + Mg) and 1% solution of biologically active iodine in the composition of I-Se composition.

In variants with pre-sowing treatment of wheat seeds by nanoparticles I-Se, the manifestation of artificial infection decreased and amounted to 0.9 number, compared to control (without treatment) – 2.2 number (Fig. 1).

Fig. 1. Symptoms of a disease at artificial inoculation *Pseudomonas syringae* pv. *atrofaciens* D13 (control).

To check the biocides effectiveness, bacterial strains isolation from artificial inoculated of plants have been performed. After bacteriological analysis of the pieces of the infected tissue, colonies were selected and accounted, which according to morphological properties are similar to *Pseudomonas syringae*. Then the oxidase activity test was performed, as well the oxidase-positive reaction of micro agglutination on glass with antiserum to the strains *P. syringae* pv. *atrofaciens.*

It has been demonstrated, that 12% of the isolated bacteria of *P. syringae* pv. *atrofaciens* and 2.2 conditional units on a scale typical visual manifestations of bacterial disease of wheat was corresponded (Table 1). In other variants of the experiment, up to 10% of these bacteria were detected. In the variant with pre-sowing treatment of wheat seeds BP I-Se, where artificial infection was 0.9 number, found 4% of cells *P. syringae* pv. *atrofaciens.*

Consequently, in wheat plants, the effects of various treatment by investigated of biologically active substances showed a significant decrease in the percentage of cells *P. syringae* pv. *atrofaciens*. The next step in our analysis was to detect the effect of the substances investigated with biocidal properties on the morphology of *P. syringae* cells in the short-term effect of nanocitrates,

performed by electron microscopy. An electron microscopic image of the test substances at 1% concentration is visualization in Fig. 2 (A-I).

Table 1. Influence of pre-sowing treatment of wheat with biologically active substances on the percentage of the causative agent of basal glume rot of cereals in plant tissues

The variants of treatment	The content of *P. syringae* pv. *atrofaciens* D13 after reisolation (%)
Control (without treatment)	12
pre-sowing treatment of 1% Ag-Cu solution	10
pre-sowing treatment of 0,1% Avatar-1 solution (Co+Cu+Zn+Fe+Mn+Mo+Mg)	10
pre-sowing treatment of 1% BP I-Se solution	4

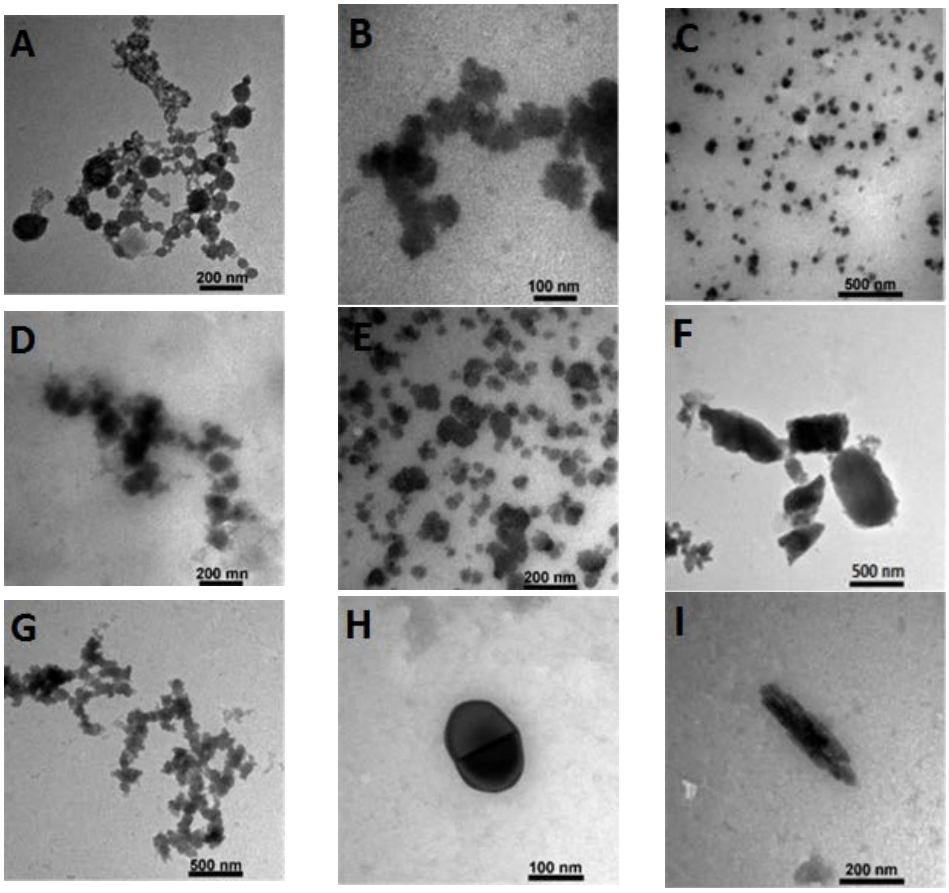

Fig. 2. Microphotographs of Trans missive electron microscopy of the substances investigated of biological activity substances with biocidal properties: Ag-Cu (A–C); Avatrar-1 (D–F); I-Se (G–I).

Compared to the native, intact cells of bacteria *P. syringae* (Fig. 3, A-E), morphological changes of the cell membranes and bacterial cells are observed for the action of test substances, the of which is clearly seen in the microphotographs obtained by Trans mission electron microscopy (Figs 4-6). It has been found destruction of the cell membrane, which have lead to loss of homeostasis, bacteriolysis and cell death as a result by exposed to bacterial cells of the components experimental 1% solutions (Avatar-1, Ag-Cu and BP I-Se). (Fig 4, A-H; Fig. 5, B-G; Fig.6, A-G).

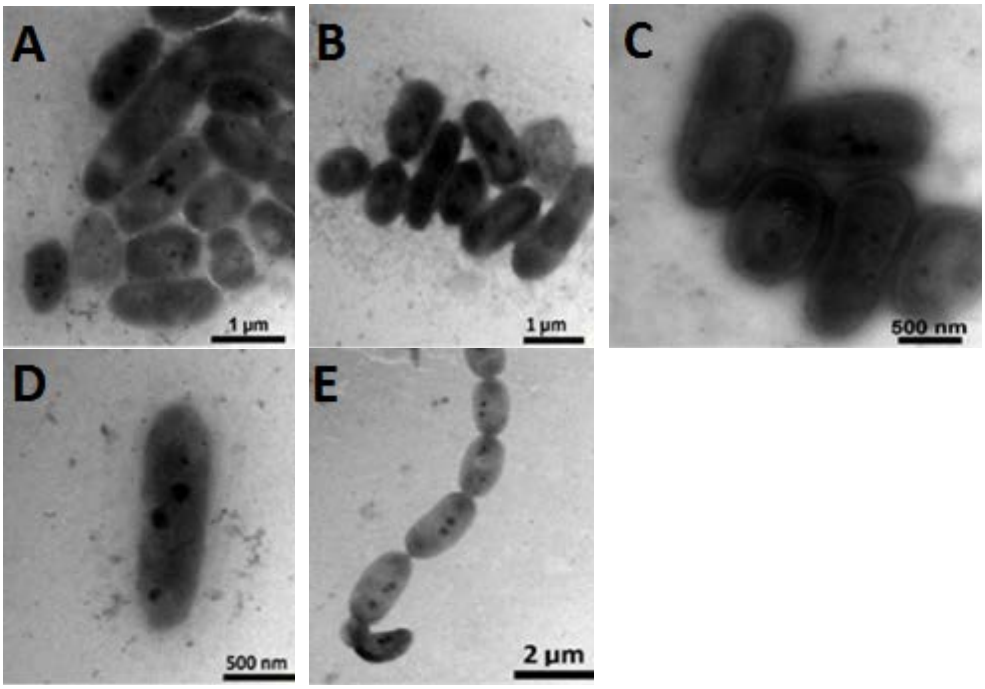

Fig. 3. Microphotographs of Trans missive electron microscopy of *Pseudomonas syringae* pv. *atrofaciens* D13 cells (A-E).

Reduction of basal glume rot of cereals the visual symptoms and the percentage of isolated *Pseudomonas syringae* pv. *atrofaciens* D13 cell was detected by pre-sowing treatment of wheat seeds by experimental biocides, have been attributable to explained by structural changes in the bacterial cells of the pathogen, which lead to a reduction of viable forms of bacteria due to damage of both, the cell wall and internal contents. Thus, the studied carboxylates of nanoparticles and the bio preparation of iodine-selenium have an actual bactericidal activity in a small concentration. Consequently, the effect of the studied biocides was caused the destruction of the cell wall, its cracking, which lead to the leakage of cytoplasmic content and subsequent cell lysis.

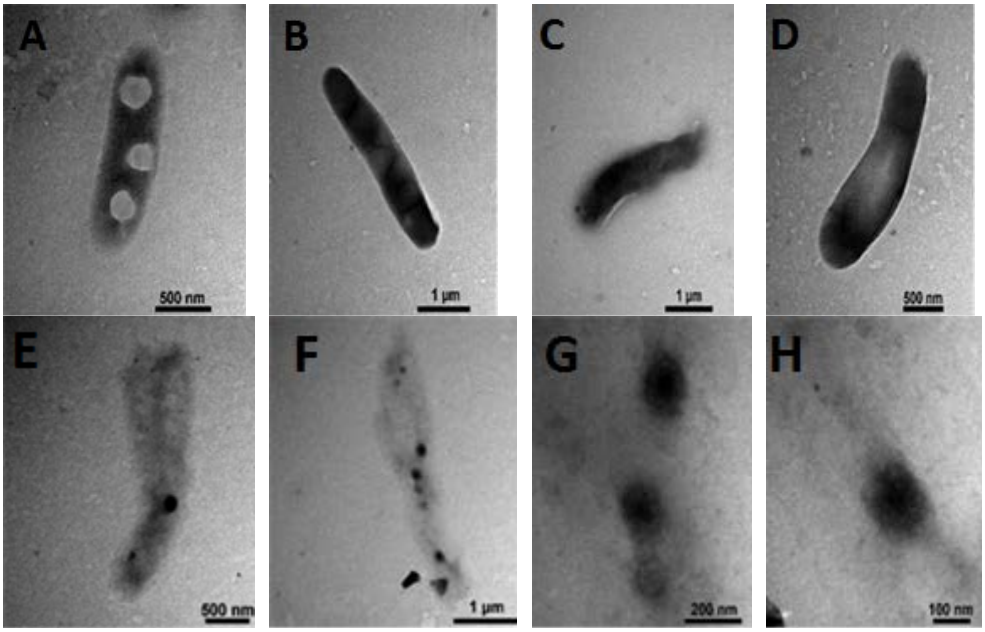

Fig.4. Microphotographs of Trans missive electron microscopy of *Pseudomonas syringae* pv. *atrofaciens* cells at action 1% solution of citrate nanocomposite of Ag-Cu (A-H).

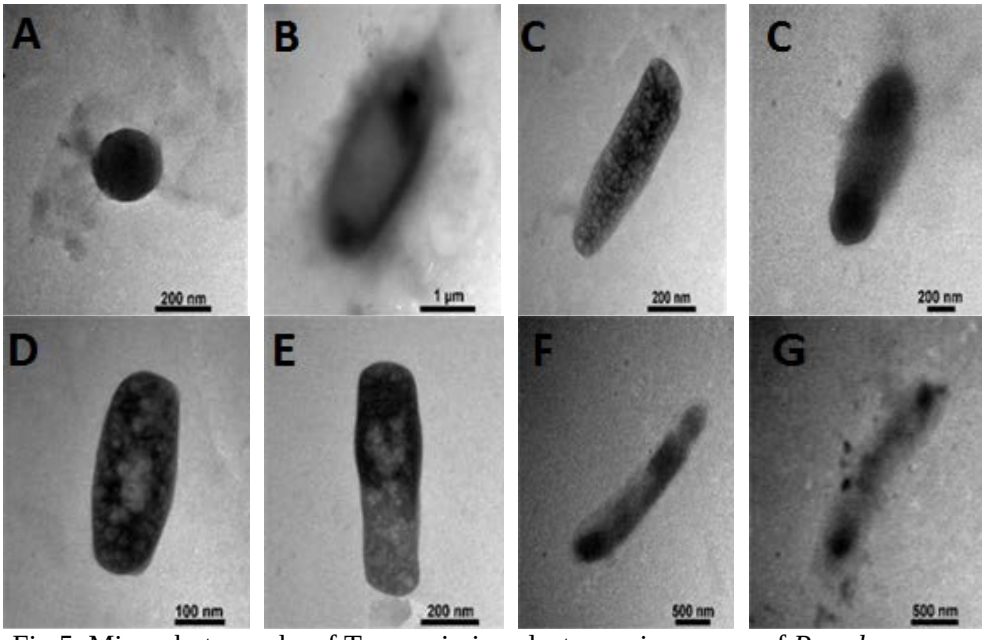

Fig.5. Microphotographs of Trans missive electron microscopy of *Pseudomonas syringae* pv. *atrofaciens* cells at actions 1% Avatar-1 solution (citrate of nanocomposite Co;Cu;Zn;Fe;Mn;Mo;Mg) (A-G).

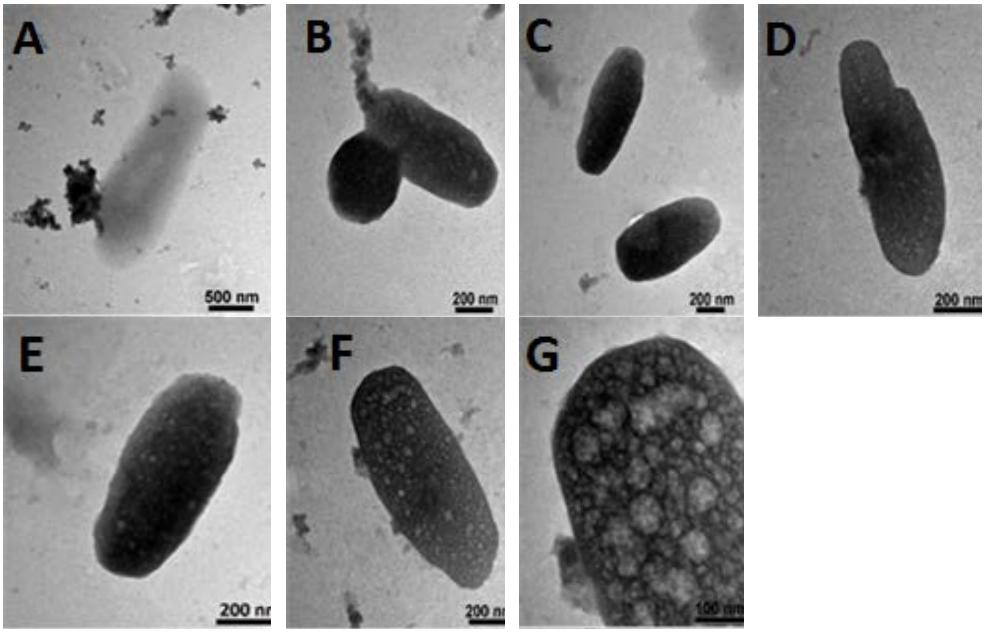

Fig.6. Microphotographs of Trans missive electron microscopy of *Pseudomonas syringae* pv. *atrofaciens* cells at action of BP I-Se (A-G).

Similar effects of experimental elements in the form of nanoparticles are given in Siddiqi K.S. et al. (2018), which shows that Ag NPs stops the development and multiplication of many bacteria by binding Ag/Ag^{+} to the biomolecules present in microbial cells. Due to its small size, Ag nanoparticles are diffused into the cell, destroying the cell wall. In the work of Ahmada A. et al. (2017) shows that chitosan-modified silver nanoparticles lead to the destruction of the bacterial membrane potential, inducing a high level of intracellular active forms of oxygen (AFO). In studies of antibacterial activity of nanoparticles of copper, cobalt, and nickel in the form of chelated nanoparticles, which is an intermediate form between nanoparticles and ions, it is assumed that their action is due to the binding of amino acids (DeAlba-Montero I. et al., 2017). Thus, the investigated forms of biocides are suitable as agents with bactericidal action for possible application in crop production, but further research is needed to find out the optimal parameters for their use for this purpose.

CONCLUSIONS

It was found out that during pre-seed treatment of seeds with 1% solutions of citrates of Ag-Cu nanoparticles, as well as the nanocomposite - Co; Cu; Zn; Fe; Mn; Mo; Mg (Avatar-1) and biopreparation of iodine-selenium there was a significant inhibition of the development of *Pseudomonas syringae* pv. *atrofaciens* cells with reducing their viability. With method Trans missive electron microscopy was shown, that solutions of citrates of Ag-Cu nanoparticles,

as well as the nanocomposite - Co; Cu; Zn; Fe; Mn; Mo; Mg (Avatar-1) and biopreparation of iodine-selenium have an actual bactericidal activity in a small concentration, that reduction of viable forms of bacteria due to damage to the cell wall.

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IDENTIFICATION OF SELECTION SIGNALS THROUGH HAPLOTYPE STRUCTURE IN GENOME OF DUAL-PURPOSE BREEDS

SUMMARY

The aim of this study was to analyse the selection signals by the integrated haplotype homozygosity score (iHS) in dual-purpose Slovak Spotted cattle in order to clarify the effects of natural or artificial selection. Overall, 85 animals genotyped by a high-density SNP array were included in the analysis. After quality control, 35995 autosomal loci with an average adjacent SNP spacing of 69.3 kb were included in the analysis. Then, haplotypes were reconstructed for each chromosome. Next, the R package rehh was used to compute the score based on a matrix of integrated extended haplotype homozygosity statistics for both ancestral and derived alleles. The average iHS score across the genome was 0.83. The selection signals in the genome had positive iHS values. A common iHS score (higher than 2.5) was chosen to indicate genomic regions with extreme iHS frequency due to outliers (according to the boxplot distribution). In each region with selection signals, quantitative trait loci (QTLs) were identified. For BTA s 3, 6, 8 and 21, signals were identified in regions of milk production. Marbling scores, QTLs and signals within BTA 12 were placed around QTLs for calving ease. Based on the results, we can conclude that the identified regions in the genome that are affected by positive selection correspond to the breeding goals for Slovak Spotted cattle.

Keywords: artificial selection, cattle, genomic region, homozygosity, iHS score.

INTRODUCTION

The bovine genome was one of the first among livestock sequenced. The reason could be the importance of cattle in human nutrition and their evolutionary position as representative of ruminants (Tellam et al., 2009). It is assumed that since cattle domestication have been selected by the human 800 breeds, due to various economic, social and religious reasons. Those breeds represent a significant part of the world heritage (Mason et al., 1998).

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Natural and artificial selection has led to the formation of cattle breeds which are specialized in particular directions. Several specific regions in the genome were under pressure due to selection and it is generally accepted that these genomic sequences or selection signatures, which control key phenotypes are involved in specific traits. Positive selection pressure can reduce or eliminate the negative allele frequency in the genome of offsprings (Biswas and Akey, 2006). Therefore, selection signals in populations could provide genomic information to simplify selection and provide information about history of selection (Akey et al., 2002; Willing et al., 2010).

Determination of selection signals can be accomplished using several methods. For this study, extended haplotype homozygosity (EHH) test (Sabeti et al., 2002) and integrated haplotype homozygosity score (iHS) (Voight, 2006) were chosen. EHH is used for identifying long haplotypes that carry a so-called core allele with high frequency within the population and detection of genomic regions that are candidates for having undergone recent selection (Sabeti et al., 2002). The iHS method compares EHH between ancestral and derived alleles within the population. The iHS test can avoid the impact of heterogeneous recombination rates across whole genome of cattle. This iHS method is best performed when the selected allele segregates at intermediate frequencies in the population (Zhang et al., 2017).

Recently, selection signals have been found in several local cattle breeds in Slovakia (Kasarda et al., 2015). Based on this, the aim of our study was to find selection signals in the genome of Slovak Spotted cattle by calculation of integrated haplotype homozygosity score (iHS). For each region with selection signal was identified quantitative traits loci and explore their potential functional genes in Slovak Spotted cattle using high-density single nucleotide polymorphism (SNP) genotyping data.

MATERIAL AND METHODS

For this study dataset of 85 Slovak spotted cattle were used to detect signatures of selection in the genome. These animals were genotyped by two platforms: Illumina BovineSNP50v2 BeadChip (sire 37) and ICBF International Dairy and Beef v3 (dams 48), respectively.

Standard quality control of genotyping data was performed to exclude individuals with >10% missing genotype across the autosomal loci, markers missing in >10% of individuals, and loci with minor allele frequency <0.01 using the PLINK software (Purcell et al., 2007). After quality control remained in dataset information about 35995 autosomal loci. Analysis of selection signals using iHS statistic was based on haplotype frequencies as specified Voight et al. (2006). The haplotypes were reconstructed for each autosome by using software fastPHASE (Sheet and Stephen, 2006). The iHS statistic reflects the structure of haplotype and indicates abnormal long haplotypes carrying the ancestral and derived allele (Qanbari et al., 2011). Standardized iHS was calculated as follows:

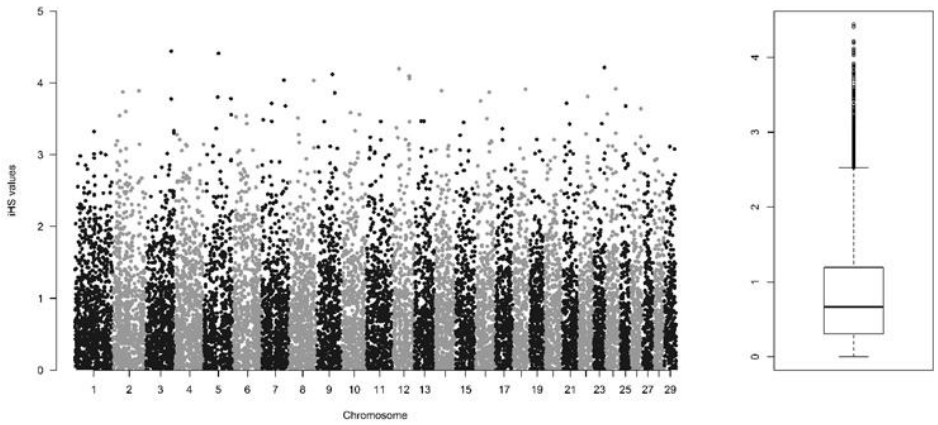
$$iHS = ln \frac{iHH_A}{iHH_D}$$

Where iHHA represents the integrated EHH score for ancestral allele and iHHD represents integrated EHH score for derived allele. The positive iHS values indicate higher homozygosity outlying the derived allele and negative values indicate higher homozygosity outlying the ancestral allele. This analysis was performed in the R software environment using package rehh (Gautier and Vitalis, 2012).

The genome-wide significance threshold of selected SNPs with positive value of iHS was determined by the outliers according the boxplot distribution and then all SNPs above the average of significance were assigned to the genomic QTL´s location according to the Bovine genome database (animalgenome.org). For identification of these genes the Genome data viewer was used (https://www.ncbi.nlm.nih.gov/genome/gdv/?org=bos-taurus).

RESULTS AND DISCUSSION

The selection signals in Slovak spotted cattle were detected based on the set of 35995 SNPs markers, with the average distance of 69.3 kb between adjacent SNPs. The average value of iHS was 0.83 ± 0.68. The highest iHS value was observed for marker located on chromosome 15 (8.03) in position 33.6 Mb. Throughout the entire genome, the lowest iHS (0.0001) was found for SNP on chromosome 5 in genomic position 115.5 Mb. The iHS method identified 15 regions in the genome of Slovak spotted cattle that were significantly affected by positive selection (Table 1). Genome-wide distribution of the iHS score was visualized to obtain the chromosomal distribution of selection signals (Figure 1).



A) B)

Figure 1. Genome-wide plot of the *iHS* score, A) the distribution of *iHS* values within the autosomes, B) the genome-wide significance threshold.

The genome-wide significance threshold of iHS score was set to 2.5, according to boxplot distribution, where outliers were identified as values over the maximum (Fig. 1B). Annotation of genomic regions with identified selection signals revealed several candidate genes, and those significant iHS genomic regions in Slovak Spotted cattle comprised 28 genes. BMPR1B genes were identified in the region on BTA6, Yao et al. (2018) demonstrated that by targeting BMPR1B the miR-125B regulates GC apoptosis in the yak ovary. In the region BTA21 CSK gene, affecting the immune system, was observed (Zimin et al., 2009). In the region on BTA3 the SCMH1 gene, upregulating the expression of several bone biomarkers, was found (Pei et al., 2019). The LPAR1 gene able to the response to tissue damage and infections agens was identified in the region on BTA6 (Zimin et al., 2009). In the region BTA8 PTGR1 gene which affects the prostaglandin reduce activity, was observed (Zimin et al., 2009). In the region BTA12 SOX21 gene affecting the hair follicle development, was obtained (Kiso et al., 2009). In the region BTA21 RHCG gene, able to regulate of pH, was found (Zimin et al., 2009). Using this analysis and by the describes QTL´s, we found that Slovak Spotted cattle were previously selected based on important traits for milk and beef production, muscle development, stature and reproduction (Table 1).

Schawanrzenbacher et al. (2012) detected the significant selection signals on BTA17 and 28 for Brown Swiss and on BTA14 observed SNP under strong selection in close neighbourhood to the DGAT1 K232A polymorphism, with strong effects on milk production traits. Iso-Touru et al. (2016) identified selection signals for Finnish Ayrshire and Eastern Finncattle on BTA 20, both breeds being used mainly to dairy production and the region on BTA21 harbored 44 different genes within Yakutian and Ukrainian Gray. Maiorano et al. (2018) observed the most significant signatures of selection for Gir cattle within dairy population on chromosome 16 (23 candidate genes) and within beef population on chromosome 6 (43 candidate genes).

The SLC24A4 gene located on BTA21 was common in the dairy and beef populations (Maiorano et al., 2018). Simianer et al. (2010) observed for Holstein, that candidate regions identified through the iHS test comprised genes involved in the biological processes such as anatomical structure development, muscle development, spermatogenesis and fertilisation and these findings are consistent also with Flori et al. (2009). Zhao et al. (2015) identified 80 significant regions with selection signals by iHS test for several commercial dairy and beef breeds, including Angus, Belgian Blue, Charolais, Hereford, Simmental cattle, Limousin and Holstein-Friesian. Kasarda et al. (2015) observed only seven regions with several genes (HGD, LOC101904412, DCAF15, EYA4, NUMA1, FTO, CA10) affected by selection in Slovak Pinzgau cattle. Zang et al. (2017) identified for Chinese Jinnan cattle QTL regions associated with meat and carcass traits on chromosome 8. These findings confirm that during the evolution of each breed has been performed selection for improvement of important phenotypic traits and to achieve breeding objectives.

Table 1. Genomic regions with significant iHS score in Slovak spotted cattle.

BTA	Position	iHS value	QTL´s
3	104999360-105064402	4.1	
6	31016787-31126062	2.81	carcass weight, body weight, calving ease
6	54456990-54554982	2.61	milk yield, milk fat yield, marbling score, calving ease
8	37192348-37451820	3.1	stillbirth, clinical mastitis, dystocia, calving ease, body weight (birth)
8	102530898-102664941	3.56	
8	102664941-103083474	2.8	udder swelling score, shear force
12	35324036-35454037	2.72	shear force, milk fat yield, body weight, structural soundness, height (mature)
12	68453584-68628611	3.11	milk fat yield, milk fat content, muscle iron content
12	68628611-68784880	3.6	muscle iron content
12	69874053-69925933	3.2	muscle iron content
12	69925933-69969069	2.87	muscle iron content
12	69969069-70000094	3.45	calving ease, daughter pregnancy rate
21	21090081-21442215	3.31	marbling score, milking speed, milk fat yield, body weight
21	34165847-34339514	3.1	shear force, somatic cell score, calving ease, body weight
21	36136065-36341943	2.93	somatic cell score, body weight

CONCLUSIONS

Studies of mapping selection signals in cattle provides an ideal opportunity to investigate how the artificial selection has influenced the variability and architecture of the bovine genome. In this study, several regions under strong selection pressure have been found by a genome-wide scan of iHS score in Slovak Spotted cattle.

This test showed that the breeding history of Slovak Spotted cattle has been subject to positive selection similarly as commercial dairy and beef breeds. Our analysis discovers genes under positive selection, which are related to beef production, immunity and reproduction and revealed quantitative traits loci under positive selection, which are reflecting the selection of the required traits according to the breeding objectives. These results confirm the importance of Slovak Spotted cattle as important dual-purpose genetic resource in Slovakia.

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LAND SUITABILITY ANALYSIS FOR EMERGING FRUIT CROPS IN CENTRAL PORTUGAL USING GIS

SUMMARY

Fruit production is an important component of agricultural production in Portugal, and it has a positive impact on the economy, especially in rural areas. In recent years, there has been increased investment in so-called 'emerging crops'. It is agreed that using the crops that are best suited to the soil and climate conditions as well as the socio-economic environment promotes sustainable use in rural areas. The objective of this study is to determine the suitability of different emerging fruit crops for cultivation in the Beira Baixa region based on analysis of soil and climate factors. The pistachio tree (*Pistacia vera* L.), strawberry tree (*Arbutus unedo* L.), almond tree (*Prunus dulcis* (Mill.) DA Webb) and walnut tree (*Juglans regia* L.) were checked against the biophysical criteria for cultivation. The results were processed using a geographic information system. Analysis was performed using the analytical hierarchy process (AHP). Thus, after dividing the problem into hierarchical levels of decision-making, a pairwise comparison of criteria was performed to evaluate the weights of these criteria based on a scale of importance. Then, the consistency of these operations was validated. The AHP was adequate for evaluation of fruit tree species' suitability since it enabled integration of several criteria, decision-making and problem resolution. It is essential to be aware of the suitability and resilience of new crops in order to meet the need to adapt to climate change.

Keywords: Multi-criteria evaluation, Land use suitability, Fruit cultivation, Analytic Hierarchy Process, GIS.

INTRODUCTION

The current trends indicate that are emerging a new consumers profile that are looking for an increased of variety, freshness and healthy options and they are also seeking a higher proportion of fresh and different produce in their eating choices (Campos & Madureira, 2019).

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The concept of sustainability agriculture indicate that is necessary to promote the accountable use of resources, such water and nutrients, and decreasing the use of pesticides in a way which considers the future needs and will not compromise the quality of the environment. Which indicates that is important to respect the agro climatic conditions and identify and promote the appropriate adaptation strategies of the crops.

The Beira Interior region of Portugal is characterized by soil and climate conditions favorable to peach and cherry production (Simões et al., 2015), representing the crops with more public investment and expansion (Lopes, Alberto, Luz, & Simões, 2018). It is the most productive region of these fruits in Portugal, containing 1640 ha of peach crops and 2230 ha of cherry crops (INE, 2014 in Ferreira et al., 2016), which represents 45% of the national area of peach production and 39% of the cherry production area. However, in the last five years that region is seeking new patterns and configuration of agrarian landscape, introducing the new fruit crops, through the foreign investments and benefiting from new irrigation infrastructures. The emerging crops that are identified in the aim of this study are the almond tree, pistachio tree, strawberry tree and walnut tree, distributed by different areas across the Beira Interior region.

Facing the trends of climate change is urgent to address agricultural adaptation more coherently and promote the suitability of plant, weather and soil conditions. Hence, that investigation identifies the suitable sites for the emergent crops production using a model based on multi-criteria spatial analysis AHP. The suitable areas for the emerging crops are determined by an evaluation of the climate, soil and topographical factor and the understanding of local biophysical restraints. In this kind of situation, many variables are involved and each one should be weighted according to their relative importance on the optimal growth conditions for crops through multicriteria evaluation and Geographic Information Systems.

Multicriteria spatial decision analysis has been widely applied in various studies in different fields, many of which are published and have been cited by many authors as processes of relevant decision making. This is the case of Kangas, et al. (2000) referring to the use of GIS in the decision-making process through the multicriteria analysis in the planning of forest resources conservation actions, allowing actions as directed by the determination of the priority areas. Quinta-Nova & Roque (2014) developed a model based on multicriteria spatial analysis AHP to determine the suitability levels for agroforestry uses of the sub region of Beira Interior Sul. The criteria used were the soil potential, slope and aspect. The authors note that this analysis identified the areas where the use of land should be subject to a conversion and/or a change of management.

This work was intended to identify the suitable areas which can be exploited for the fruit production with new crops in the Beira Baixa region, by the integration of several criteria. In this research, site suitability analysis was carried out using GIS and AHP as multicriteria decision analysis (MCDA) technique. It

is therefore essential to be aware of the suitability and resilience of new crops in order to meet the need to adapt to climate change.

MATERIAL AND METHODS

The Beira Baixa region is an administrative division in eastern Portugal and integrates the Beira Interior Region. The region covers an area of 4,614.6 km² and has a population of 84,046 inhabitants. The area includes four municipalities: Idanha-a-Nova, Penamacor, Vila Velha de Ródão and Castelo Branco. This territory is mainly occupied by forest and agroforestry uses (60.8 %) and agriculture (36.2 %).

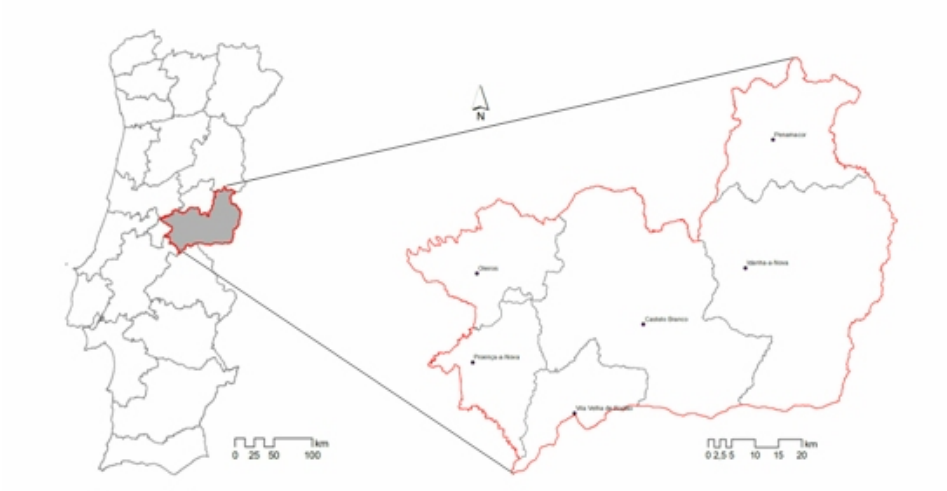

Figure 1. Study area location

The classification of the crops suitability resulted from the integration of a set of biophysical criteria based on the climate and soil requirements of crops and the optimal operating conditions associated with different uses. Geoprocessing and spatial analysis was performed to geographic data, namely soils, climate and elevation. In this study all the criteria (Table 1) are reflected in the corresponding GIS layers.

To characterizing the main climatic parameters that influence the crops studied in different stages and therefore its productive potential, are used the climate data of 7 meteorological stations around Beira Baixa region (Fundão, Castelo Branco, Portalegre, Coimbra, Guarda, Santarém in Portugal, and Caceres in Spain). In Table 2 are identified the stations nearest of the region and identified the main representative climatic parameters that are influence to crops ecological conditions. Was observed that the mean of total annual rainfall goes by 523 mm (Cáceres) until 882 mm (Guarda). During the blossom period the rain can damage fruit production, specially combining with low temperatures (with risk of frost). The mean monthly minimum temperatures registered during the blossom period

was around 3.5ºC and 6,8ºC and 11,2ºC of minimum in Castelo Branco. Generally, the mean relative humidity during the fruits growth with average of 62%, that parameter is important because influence the diseases risks of the crops and influence their capacity of evapotranspiration.

Table 1. Criteria considered in determining crop suitability

Criteria	Description
Mean annual temperature	Average of the 12 mean monthly temperatures (ºC)
Mean total annual rainfall	Total annual depth of precipitation from a given precipitation time series (mm)
Chilling hours	Sum of hours with temperature ≤ 7.2 °C (h)
Crop heat units	Influence of temperature on a crop's growth and development (h)
Mean relative humidity	Ratio of the actual amount of water vapour present in a volume of air at a given temperature to the maximum amount that the air could hold at that temperature (%)
Biogeography	Portugal biogeographic units and adapted species
Elevation	Height above the Earth's sea level (m)
Soil pH	Measure of the acidity or alkalinity of a soil
Soil Organic Matter	Fraction of the soil that consists of plant or animal tissue in various stages of decomposition (%)

Table 2. Mean annual and monthly values of climatic parameters (1971-2000)

Parameters		Fundão	Castelo Branco	Portalegre	Cáceres	Coimbra	Santarém	Guarda
Mean total annual rainfall (mm)		842.9	758.3	852.4	523	905	696	882
Mean total monthly rainfall (mm)	AprIl	78.5	58.1	78.4	39	84.8	65.7	83.1
	May	68.3	65.1	67.5	48	79.5	56.2	84.9
	Sept.	35.0	36.5	42.1	32	51.7	36.2	47.5
Mean monthly temperature (°C)	March	10.7	12.7	11.5	7.7	12.6	12.9	7.1
	April	12.0	13.1	12.3	9.6	13.9	14.1	8.0
	May	15.3	16.8	15.3	13.4	16.2	16.5	11.6
Mean monthly minimum temperatures (ºC)	March	5.2	7.5	7.6	1.4	6.9	7.3	3.3
	April	6.8	8.0	8.2	3.5	8.4	8.6	4.2
	May	9.6	11.2	10.6	7	10.8	10.5	7.4
Chilling hours		1150	1050	950	950	705	750	1350
Mean relative humidity (Jun-Aug)		51.7	53.7	61.3	52.0	77.3	75	65

The different layers corresponding to each criteria were classified in two suitability levels: low or medium suitability (1) and high suitability (2). After creating layers resulting from the reclassification in suitability levels, the general suitability for each crop was performed using a multicriteria decision analysis - the Analytic Hierarchy Process - AHP (Saaty, 1980).

The AHP decomposes a problem, question or decision, in all the variables that constitute it, in a scheme of criteria and subcriteria and then makes pairwise comparisons between them (Antunes, 2012). The comparison between criteria is made using a scale of 1 to 9, wherein 1 is equally preferred and 9 is highly preferred (Saaty, 1980). The AHP reverts comparisons on numerical values that can be processed and compared to the full extent of the problem. The AHP calculates the weight value for each criterion (wi) by taking the eigenvector corresponding to the largest eigenvalue of the matrix, and then normalizing the sum of the components to a unity. Pairwise comparison matrices were used with AHP software in order to value the selected factors and their classes.

The consistency of the matrix after obtaining the weight values is judged based on a consistency ratio CR. If CR < 0.10, the pairwise comparison matrix is considered to have acceptable consistency and the weight values calculated are considered valid and can be utilized. Finally the spatial data was superimposed to integrate all the factors in a single layer - the suitability map for each crop.

RESULTS AND DISCUSSION

The AHP analysis of the selected crops indicates that the climatic influence is determinant in the development of these species, since its weight in the AHP analysis varies between 62.43% for Walnut tree and 90.12% for Strawberry tree.

Table 3. Criteria weights and consistency ratios

Crop	Criteria weights	Consistency ratio
Pistachio tree	Mean total annual rainfall (25.08%); Chilling hours (25.08%); Crop heat units (25.08%); Mean relative humidity (10.97%); Elevation (4.60%); Soil Organic Matter (4.60%); Soil pH (4.60%)	0.008
Strawberry tree	Biogeography (47.86%), Mean total annual rainfall (21.13%); Mean annual temperature (21.13%); Soil Organic Matter (4.94%); Soil pH (4.94%)	0.021
Almond tree	Mean total annual rainfall (26.48%); Mean annual temperature (26.48%); Chilling hours (26.48%); Elevation (10.94%); Soil Organic Matter (4.81%); Soil pH (4.81%)	0.009
Walnut tree	Mean total annual rainfall (35.95%); Chilling hours (26.48%); Elevation (15.35%); Soil Organic Matter (6.38%); Soil pH (6.38%)	0.012

The analysis of the consistency ratio (CR) of the AHP showed that there was consistency in the pairwise comparison matrix, and thus, the weight values calculated could be considered valid (Table 3).

Based on the map analysis, about 75,235 hectares, corresponding to 16.4% of the total area available, are classified as highly suitable for almond tree, especially in the south east part of the region. Low winter and spring temperatures and very high summer temperatures inhibit growth and fruit set. On the other hand, very high summer temperatures when accompanied with low soil moisture can result in the shrinkage of almond. The needs of almond in cold (in order to break the dormancy of the buds) are 250-350 (and in some cases over 500) hours of exposure to temperature less than 7 °C (Alonso, et al. 2005).

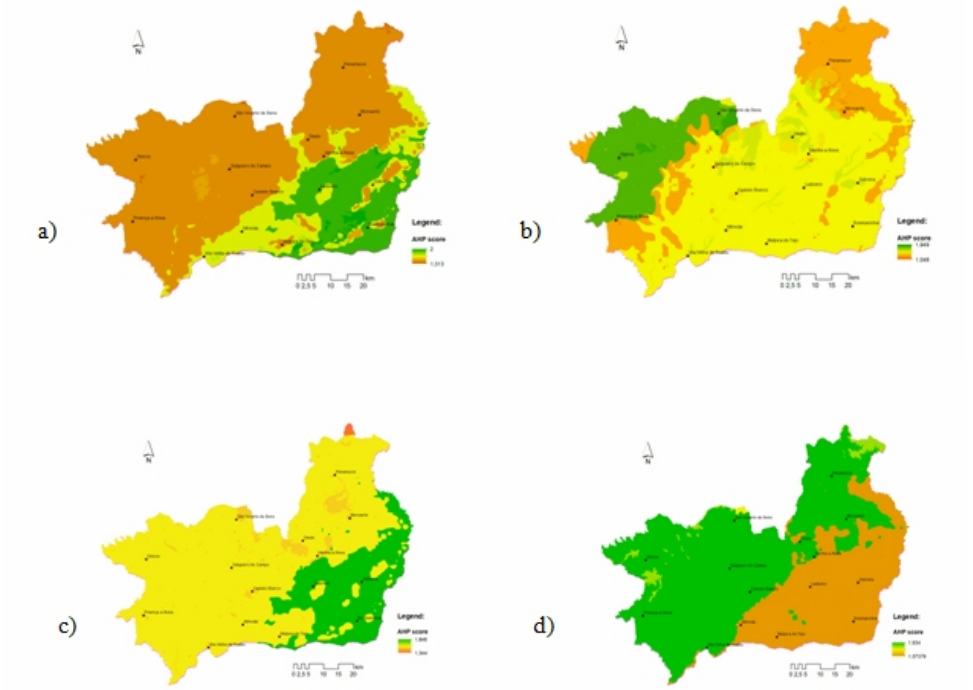

Figure 2. Crop suitability maps: a) Almond tree - Prunus dulcis; b) Strawberry tree - Arbutus unedo; c) Pistachio tree - Pistacia vera; d) Walnut tree - Juglans regia

Strawberry tree is one of the most common fleshy fruited species in the Mediterranean region. The area with high suitability to strawberry tree is 72,423 hectares, corresponding to 15.8% of the total area available, corresponding to northwest of the region, in an area with higher altitude (around 600-800 m) with more suitable climatic conditions and topographic adaptation.

The area with higher potential to pistachio tree is 72,679 hectares, corresponding to 15.9% of the total area available and overlaps the potential almond tree crop area, resulting from natural conditions, especially the climatic

influence. This plants are known as drought tolerant and are able to survive and even produce fairly yield with very little water (Ferguson et al., 2002). An annual rainfall of at least 300 to 450 mm has been reported as the optimum amount of precipitation for this crop (Goldhamer, 2005).

In the case of walnut tree, highlighting the total 288,983 hectares, corresponding to 63.2% of the total area available. Higher precipitation (minimum of 600 mm) during the winter and the temperature are the main parameters that compromise this crop, especially in the last case, during the opening of male flowers.

In Figure 2 we present the crop suitability maps resulting from the Analytical Hierarchy Process.

CONCLUSIONS

This methodological approach allowed us to assess the suitability of four emerging fruit crops (Almond tree Strawberry tree, Pistachio tree and Walnut tree) in the Beira Baixa region of Portugal. The AHP was based on a set of criteria contributing to a reflection on the adequacy of those crops for the climatic and soil characteristics of the region. The crops suitability maps thus obtained enable decision making. The main results obtained indicate that the methodology used, using AHP and GIS, could provide a guide map for decision makers in order to achieve better agriculture productions facing their ecological limitations. The results put on evidence the biophysical evaluation of territory and provide information at a local level that could be used by farmers to choose their crops.

For further study is recommend to select other factors, like irrigation facilities and socio-economic factors, and other parameters which influence the sustainable land use. However, further investigation is needed to integrate the impact of climate change in crops planning to assist in supporting future national strategies for agriculture.

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DISTRIBUTION AND TRANSMISSION OF PLUM POX VIRUS IN UKRAINE

SUMMARY

The most sensitive viral disease of the stone crops is Sharka, the causative agent of which is the plum pox virus (PPV, Plum Pox virus). PPV worldwide has a quarantine status. PPV is widespread in many regions of Ukraine and poses a serious problem to horticulture stone crops of our country. The purpose of our research was to analyze the distribution and the harmfulness of this pathogen, to describe the ways of transmission, to carry out research on the identification of aphids that were carriers of the disease and to characterize its molecular features and strains diversity in the territory of Ukraine. The samples were visually selected from the central and northern regions of Ukraine. Modern methods of molecular diagnostics were used such as: polymerase chain reaction with reverse transcription, sequencing. The phylogenetic analysis confirmed the identity of the strains and helped us made a comparative characterization of the samples to the already known strains. Depending on the strain, different kinds and varieties of plants could be damaged and crop losses could significantly vary. Therefore, it is important to determine the diversity of PPV strains and their similarities with other isolates. The result revealed high level of damage to stone crops in the territory of Ukraine, especially in Odessa and the Kiev region. Harmfulness and distribution of this disease increases every year. These researches are needed to find ways to fight this pathogen and stop the spread of a dangerous virus in Ukraine.

Keywords: Plum pox virus, distribution, strain diversity, PCR, phylogenetic analysis, Ukraine.

INTRODUCTION

Plum pox virus (Potyviridae, Potyvirus) is a harmful agent that causes a dangerous disease of stone crops. PPV is a quarantine object in most countries of the world. Plum pox virus has a wide range of host plants.

For the first time Plum pox virus was discovered in Bulgaria in 1915. The disease is spread from Bulgaria to the north and east (Atanassov, 1932). The

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disease caused by PPV was first described in Ukraine in 1966 and since then it has been spreading all over the country. Then in 1969 a minor outbreaks seen in Chernivtsi, Lviv, Zakarpattia, Ivano-Frankivsk and Vinnitsa regions. PPV is widespread in almost all regions and is a serious threat to horticulture of our country. The main method of fighting the disease is destruction of infected plants, which leads to significant economic losses.Other prevention of disease is a breeding resistant crops that are not susceptible to the virus. Genetically modified plums carrying the gene of capsid protein PPV, showed high resistance to PPV infection (James, 2006). The rate of spread of the virus in the garden depends on the distance between healthy trees and source of infection and the PPV transmission effectiveness depends on the sensitivity of culture and population density of aphids (Ratushnyak, 2002). The main route of transmission of the virus - with the help of aphids. The virus is persistently transmitted by about twenty different species of aphids: Aphis cracciora, A. gossypii, A. spiraecola, A. hederae, Muzus persicae, M. varians, Phorodon humuli (Subr, 2013). During non-persistent transmission, viruses are not transported through the membranes of the carrier and do not enter the membrane. The maximum ability aphids to transmit of Plum pox virus was recorded at temperatures of 20-23 ° C. The infectivity of the virus in the body of insects persists for no more than 4 hours (Garcia, 2007). The rate of spread of the virus in gardens depends on the distance between healthy trees and the source of the infection, and the effectiveness of aphids in the transmission of PPV depends on the sensitivity of the culture, the density of the aphid population and the period of virus entry. To establish phylogenetic relationship used approaches and methods of molecular phylogeny (Chirkov, 2016). In general, describing the divergence of PPV isolates circulating in Ukraine can be noted their high homologic nucleotide sequences capsid protein, regardless of region distribution or host plant. To date, nine strains of the virus have been isolated: PPV-An, PPV-C, PPV-CR, PPV-D, PPV-EA, PPV-M, PPV-Rec, PPV-T and PPV-W (Garcia, 2014). According to the literature data, strains of PPV such as Winona, Marcus, Dideron were detected in Ukraine (Mavrodieva, 2013).

The aim of our work is to study the distribution and the harmfulness PPV, to describe the ways of transmission, to carry out research on the identification of aphids that were carriers of the disease and to characterize its molecular features and strains diversity in the territory of Ukraine.

MATERIAL AND METHODS

Materials for research were samples of stone crops from private garden farms of Kyiv, Cherkasy, Odessa, Kharkiv, Ivano-Frankivsk, Vinnytsia regions (Fig 1).

Samples of leaves and fruits of plums, apricots, peaches, cherry plums, cherries, sweet cherries were taken with visual symptoms. Namely: chlorotic spots or rings, deformation of leaves and small rings on the fruit, sometimes with brown or reddish necrotic lesion.

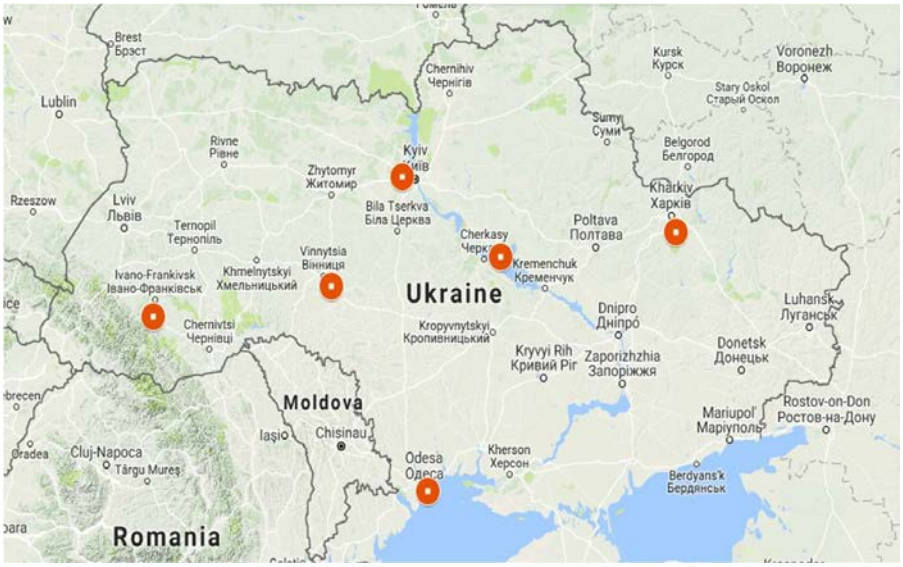

Fig. 1. Regions of monitoring distribution Plum pox virus in Ukraine

The following methods were used: visual diagnosis, total RNA purification, polymerase chain reaction with reverse transcription, sequencing, phylogenetic analysis (Cambra, 2006)

From the samples, total RNA was isolated using a set of RNeasy Plant Mini kit (Qiagen, UK) reagents. Subsequently, all specimens were diagnosed with RT-PCR, using the Thermo Scientific RevertAid Reverse Transcriptase reagents and primers P1, P2 (Wetzel, 1991) and primers for detecting D and M strains(Glasa, 2004). For detection of amplification products was used electrophoresis with 1.5% agarose gel (Sigma) in electrophoretic buffer TBE (89 mM TRIS borate and 2 mM EDTA, pH 8.3). The establishment of the nucleotide sequence of the capsid protein gene of Plum pox virus is carried out after amplification of this gene. The amplification products were cleansed using MinElute Gel Extraction Kit (Qiagen, UK). Amplified fragments were sequenced using Applied Biosistems 3730x1 DNA Analyzer, Big Dyeterminators, version 3.1. MEGA 6 software package was used for phylogenetic analysis.

RESULTS AND DISCUSSION

In the first stage samples of leaves and fruits of stone groups were selected in the spring-summer period from 2017 to 2019. Part of the specimens had characteristic symptoms of Plum pox virus, other part of it had asymptomatic infection or mixed. The classic symptoms of PPV on plums, apricots, cherries, peaches were very rare (Fig. 2,3).

The visual observation of symptoms of the lesion is a rather unreliable method of detecting and diagnosing viral infections, since the manifestation of the symptoms of viral lesion mainly depends on the interaction of the virus and the plant.

A B

Fig. 2. Symptoms caused by PPV: A - leaf deformation light green mosaic on plum, B - yellow rings and blotches, brown rings on fruit peach

A B

Fig. 3. Symptoms caused by PPV: A – aphid of Phorodon humuli; B - leaf deformation on plum and plant damage by aphids.

Often, the strains of the same virus can cause various symptoms on plants of the same species, as the symptoms of plants are influenced by the conditions of plant growth and the presence of a mixed infection, which is quite common in the case of viral disease. Therefore, the presence of a viral infection should be confirmed by specific methods of diagnosis of viral infections and the identification of viruses, in particular by methods of serological diagnostic. Further, all positive samples were diagnosed using molecular techniques RT-PCR (Fig.4).

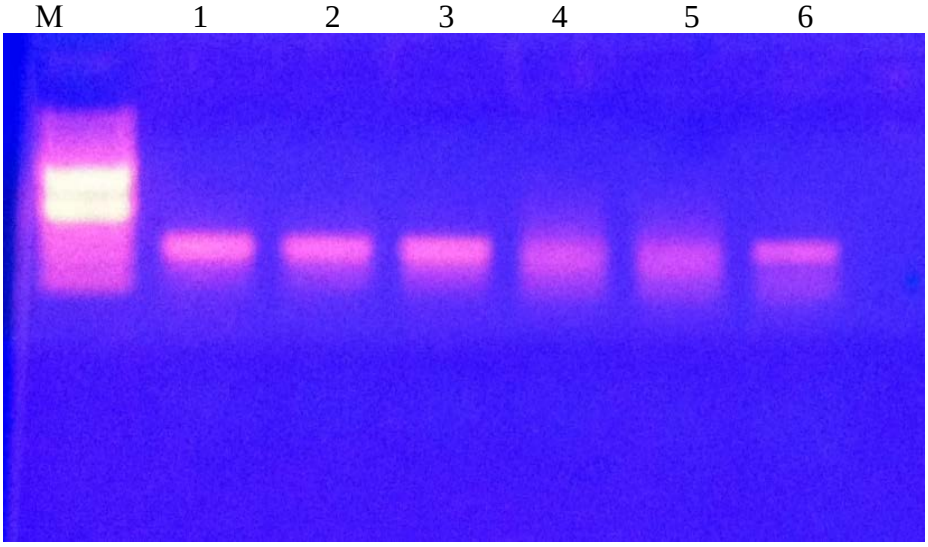

Fig. 4. Electrophoresis of products (243 bp) of RT-PCR of PPV in agarose gel with primers: M - markers, 1-2 - plum samples from Odessa region, 3- 4 peach sample from Odessa region. 5- sample apricots of the Kiev region; 6- positive control

Currently, polymerase chain reaction (PCR) is a promising and accurate method for detecting viral infection. The advantages of this diagnostic method are high sensitivity and ability to determine variety of strains of viruses. In the studied regions we detected strains and found that the most common was D strain (apricot, peach, plum), but M strain (apricot, peach) was rarely found, and in some cases we observed a co-infection. As a result, samples of the capsid protein gene of the Ukrainian isolates were sequenced. The genetic diversity of PPV has been established in different regions of Ukraine.

We comparatived Ukrainian isolates with the isolates of neighboring countries from the GenBank database (http://www.ncbi.nlm.nih.gov). According to the topology of Neighbor-Joining tree it was based on sequences of CP gene, Ukrainian isolates of PPV belonged to M and D strains. These nucleotide sequences of Ukrainian isolates of PPV together with the sequences of other PPV strains were used when constructing the phylogenetic tree. Partial nucleotide sequence of the CP gene of potato virus Y was used as the out group. (Fig.5). From this dendrograms, it can be argued that all Ukrainian isolates have high similarities, regardless of the region or the plants distribution within the host (Tab.1,2).

Methods and approaches for molecular phylogeny are used to establish the phylogenetic affinity of isolates of plum pox virus. To determine the phylogenetic relationships between different strains and isolates, the nucleotide sequences of the capsid protein genes are compared using methods for determining evolutionary distances.

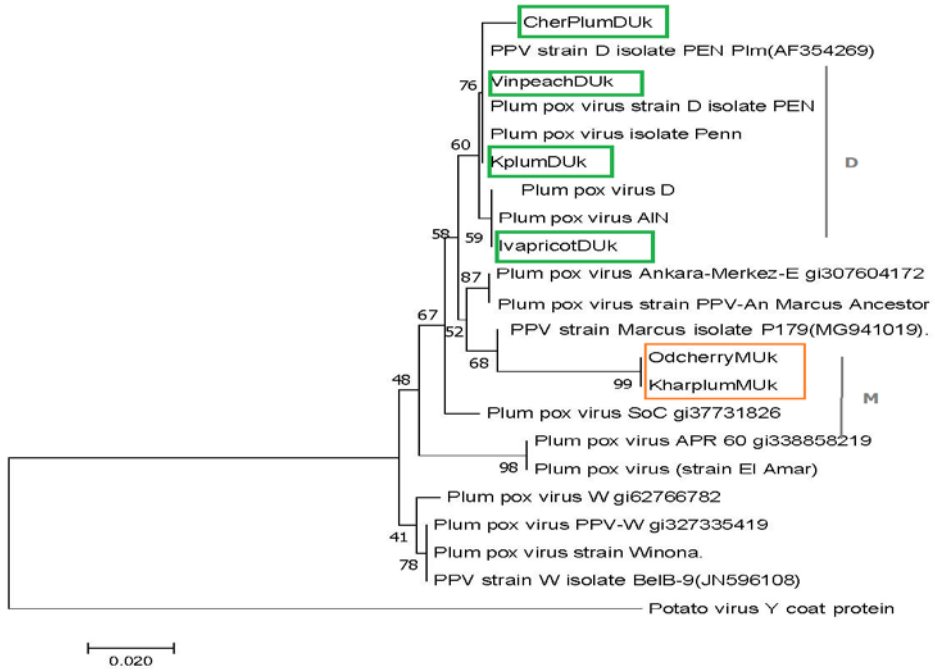

Fig. 5. Phylogenetic tree showing phylogenetic relationships among previously known strains/isolates of PPV and six Ukrainian isolates based on their partial nucleotide sequences of the coat protein gene. Hasegawa-Kishino-Yano model + Gamma distribution (HKY + G). Bootstrap values are shown next to the nodes.

To this end, the MEGA 6.0 program compared the PPV sequences obtained directly from our research and the GenBank international portal. Not only Ukrainian isolates were taken, but also isolated strains from other countries and continents. The aim was to calculate percentages of similarity (distance between sequences) of the sequences studied.

Using incomplete sequence of coat protein gene for the phylogenetic analysis of the Ukrainian isolates, we installed that PPV circulating in all regions of monitoring distribution, D strain of plum pox virus was the predominant one in all regions, whereas its M strain was rarely found and infected only in two regions. Ukrainian PPV isolates were highly 95-99% similar to previously described isolates of PPV from GenBank.

From this dendrograms, it can be argued that all Ukrainian isolates have high similarities, regardless of the region or the plants distribution within the host strains. Analyzing dendrograms we found that all samples belonged to D and M strains. In Cherkasy, Kharkiv, Ivano-Frankivsk Vinnytsia regions we found only D strain, and in Kyiv, Odessa regions, the presence M and D strains. Comparing PPV isolates from Ukraine, can be noted their high (99%) homogeneity of capsid protein nucleotide sequences, regardless of region distribution or host plant.

Table1 Comparison of Ukrainian PPV isolates by partial sequences of their CP gene, %

The name of the isolates	Odcherry MUk	Kharplum MUk	Vinpeach DUk	KplumD Uk	IVapricot DUk	Cherplum DUk
Odcherry MUk	-	98	95	96	95	97
Kharplum MUk	98	-	95	96	94	95
Vinpeach DUk	95	95	-	98	99	98
Kplum DUk	96	96	98	-	99	98
IVapricot DUk	95	94	99	99	-	99
Cherplum DUk	97	95	98	98	99	-

Table2 Comparison of Ukrainian PPV isolates with isolates from the GenBank database for nucleotide sequences of the part of CP gene, %

The name of the isolates	Plum pox virus strain D isolate PPVNJ MK208990 (Chine) Unpublished	Plum pox virus strain M isolate CY2 EF626558 (Cyprus) Unpublished	Plum pox virus PPV-D Ya1 LC375126 (Japan) (Maejia, 2020)	Plum pox virus isolate Pl45 EU734801 (Turkey) (Serçe, 2009)	Plum pox virus ElAmar DQ431465 (Turkey) (Glasa, 2006)
Odcherry MUk	97	99	96	96	97
Kharplum MUk	96	98	97	97	96
Vinpeach DUk	99	96	99	98	97
Kplum DUk	98,9	95	98,8	98	98,6
IVapricot DUk	99,5	96	99,7	98	97
Cherplum DUk	98	95	98	98	99

Analyzing the results of the research, it can be stated that the level of identity does not depend on the place of distribution and the host plant. For instance, apricot isolate of PPV collected in Ivano-Frankivsk region was most identical to Plum pox virus strain D isolate PPV NJ (Accession number MK208990) from Chine, Plum pox virus PPV-D Ya1 (Accession number LC375126) from Japan.

Analyzing the percent similarity sequences of the coat protein gene of Ukrainian isolates and isolates from GenBank, we can assume that due to

evolutionary processes, some strains may be reversed to related strains without changing their own strain.

CONCLUSIONS

In summary, Plum pox virus was detected in all investigated regions, the circulation of M and D strains. Results show high level of identity of coat protein gene sequences of Ukrainian isolates of PPV regardless of their sampling site or the host plant, region of distribution. The distribution and harmfulness of the disease increases, therefore it is important to determine genetic diversity of PPV strains, so that analyzing the development of spread of the virus in different regions of Ukraine and neighboring countries, to establish the origin and to predict the development of possible epidemics.

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HEAVY METAL CONTENT IN SOIL AND THEIR BIOACCUMULATION IN EARTHWORMS (*Lumbricus terrestris* L.)

SUMMARY

The aim of this study was to determine the concentrations of heavy metals (Cu, Fe, Zn, Pb, and Cd) in the soil and earthworm body (*Lumbricus terrestris*), as well to estimate Bioaccumulation Factor (BAF) in earthworm body. The soil and earthworm samples were taken three times, from March to June 2018, in six different locations in Kosovo (Mitrovicë, Kishnicë, Kastriot, Barilevë, Drenas and Lipjan) and were brought to the laboratory for heavy metal analysis. Concentrations of heavy metals in soil and earthworm samples were determined by an Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer brand model 1100 (Boston, MA, USA). Mean heavy metal concentration in soils were, 0.03-0.78mg/kg, 70.62-264.29mg/kg, 18.96-82.24mg/kg, 0.11-0.52 and 0.03-0.42mg/kg for Cu, Fe, Zn, Pb, and Cd respectively. The mean concentration range recorded in earthworms (*Lumbricus terrestris*) were 0.02-0.42mg/kg for Cu, 53.11-205.31mg/kg for Fe, 15.74-53.15mg/kg for Zn, 0.07-0.43mg/kg for Pb, and 0.01-0.37mg/kg for Cd.

Based on the results obtained it was shown that there are statistically significant differences of different levels of significance regarding the content of heavy metals according to locality (Factor A), substrate (Factor B), heavy metal (Factor C) and factor interactions (A*B), (A*C), (B*C), and (A*B*C). The accumulation of heavy metals in earthworm samples maintains the same profile as mean heavy metal concentration of the soil Fe>Zn>Pb>Cu>Cd. Since some organisms like reptiles, birds and some other vertebrates feed on earthworms, transfer of these metals across the food chain is most likely, and therefore this research will be useful for risk assessment by relevant institutions responsible for the monitoring and surveying of environmental pollution and food security and safety in Kosovo.

Keywords: heavy metals, bioaccumulation factor, earthworms, ANOVA.

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INTRODUCTION

One of the most important environmental problems in the world is the soils contamination by heavy metals in the industrial areas, and especially the contamination of the agricultural lands. Heavy metal can be defined as any metal with specific gravity higher than 4.00 g/cm3 and is toxic and poisonous even at low concentration (Duruibe et al., 2007). Heavy metals are considered serious pollutants because they are toxic and non-degradable (Nwuche and Ugoji, 2008). When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues. Animals that graze on such contaminated plants and drink from polluted waters, as well as marine lives that breed in heavy metal polluted waters also accumulate such metals in their tissues. Humans are in turn exposed to heavy metals by consuming contaminated plants and animals, and this has been known to result in various biochemical disorders (Duruibe et al., 2007).

The loading of ecosystems with heavy metals can be due to excessive fertilizer and pesticide use, irrigation, atmospheric deposition, and pollution by waste materi¬als (Aydinalp and Marinova, 2003).

Some authors reveals that lead (Pb) accumulates in the body organs (i.e., brain), which may lead to poisoning (plumbism) or even death. The gastrointestinal tract, kidneys, and central nervous system are also affected by the presence of lead, whereas regarding cadmium (Cd) the major threat to human health is chronic accumulation in the kidneys leading to kidney dysfunction (Wuana and Okieimen, 2011). According to the same authors copper in high doses can cause anaemia, liver and kidney damage, and stomach and intestinal irritation while zinc (Zn) is essential for human health and its shortages can cause birth defects. Most Zn is coming into the water resources during industrial activities, such as mining, coal, and waste combustion and steel processing. From water the Zn is entered in food chain through plants and finally arrives in the human body.

In recent years, earthworms have been widely used in the breakdown of a wide range of organic residues including sewage sludge, animal wastes, crop residues and industrial refuse in producing vermicomposts (Dominguez and Edwards, 1997; Kale, 1998).

Earthworms constitute an important group of soil organisms in terms of biomass, as food for many animals and in maintaining soil structure and fertility. Earthworms are located at the beginning of the consumer food chain and can transfer the contaminated food to their predators and ultimately to humans. According to some authors earthworms utilize a significant amount of soil organic matter for feeding, produce huge amounts of biogenic structures, and determine the activities of micro-organisms and other smaller invertebrates included in their ‘functional domains’ defined as the sum of biogenic structures that they have created in soil and the organisms that inhabit them (Lee, 1985). The use of earthworm as a bio-indicator for soil pollution was shown by Morgan and Morgan (1998). Stafford and Mc Grath (1986) also reported positive

correlation between earthworm and total soil Cu, Pb and Zn concentration from various metal contaminated sites.

Uba et al., (2009), carrying out a study on content of heavy metals in earthworms (*Lumbricus terrestris*) and associated soils in dump sites, confirms that earthworms accumulated some amount of heavy metals from dumpsite soils and levels of these metals accumulated in the earthworms tissues were less than 1 mg/kg for Cd, Cu, Pb and Mn while the ratio was higher than 1 mg/kg for Zn metal. The other authors, Bamgbose et al., (2000), in a study on the physico-chemical properties and heavy metal accumulation in contaminated and uncontaminated soil, shows that the concentration of heavy metals influences the pH and organic matter of the soil. Dumpsites usually contain various kinds and concentrations of heavy metals, which are dependent on the age, location and type of waste, (Ebong, et al., 2007).

In some regions and areas worldwide, especially around the mines where the industry is highly developed, soil pollution from heavy metal contamination has increased to the point that it endangers human life, and the reduction and eventual elimination of pollution in these areas is urgently more than necessary. A variety of physical, chemical and biological techniques have been proposed and implemented to achieve this aim. One of the methods is the usage of earthworms to clean up the soil from various pollutants, such as heavy metals, by the process of vermicomposting (Bianchin, 2009; Cheng-Kima et al., 2016). The term vermicomposting represents the process where earthworms ingest, grind and digest organic waste and finally convert it into a much finer, humified, microbially active material by the cooperative action of earthworms and microorganisms (Roshan Singh et al., 2014).

The indices of metal accumulation by organisms in their body tissue is known as bioaccumulation factor (BAF). It is the ratio of the level of metals in organisms to the soil substrate (Owagboriaye et al., 2015).

The aim of this work is to assess the level of some heavy metals in soil and earthworms as well to determine the bioaccumulation factor (BAF) of some heavy metals (Cu, Fe, Zn, Pb and Cd) in earthworm body with the total contents in the soils.

MATERIAL AND METHODS

The present study was conducted in the year of 2018 by collection of heavy metal contaminated soil and earthworm samples from five different sites in the Republic of Kosovo: 1) Mitrovicë 2) Kishnicë 3) Kastriot 4) Barilevë, 5) Drenas and 6) Lipjan. For our purposes the agricultural land was used. The main heavy metal source contaminating the environment and agricultural soils in Kosovo is the industry, which is mostly located in the eastern part of the country (Zogaj et al., 2014). The following heavy industry sites are found in this area: the ore-metallurgic combine “Trepça” in Mitrovica, the Kosovo Energetic Corporation in Obiliq (Kastriot), “Ferronikeli” in Drenas, the Battery Factory Ni-Cd “IBG-Gjilan”, „Cementorja“ Hani i Elezit.

Soil sampling

Soil samples were collected from agricultural land with a hand soil auger at least 10 meters away from the main stream and about 2 kg of soil was collected from top 5 cm layer at each site, after removal of surface vegetation and litter, in transparent polythene bags.

Earthworm sampling

Regarding earthworms (*Lumbricus terrestris*) they were collected by digging into the soil from the same sites and then placed in sample bottles and labeled. All the bags (soil and earthworm samples) were marked with regard to the sites from which the samples were collected. Since freshly collected soil were moist the first step in preparation of soil sample was to dry with air or oven-dry as soon as possible to halt all biological transformation activities (Ndubuisi et al. 2017).

Preparation and preservation of soil samples

The soil samples were spread out on a flat surface sheet of paper and left in the oven at the temperature of 105ºC for three days to fully dry all moisture present. After this step, the sieving of soil samples vas performed, in order to remove gravel, stones and plant debris. The materials that was able to pass through the 2mm mesh, as fine earth material, was selected and kept for analysis.

Preparation and preservation of earthworm samples

The earthworm samples were placed in Petri dishes, and then refrigerated for 24 hours in order to purge the soil in the gut. After that they were removed, rinsed slightly with de-ionized water and then frozen pending analysis (Bamgbose et al., 2000).

Physicochemical properties of the soil samples

The soil physicochemical properties were determined using the approved specific standard methods for each of them according to Miller et al., 2013. Soil acidity or pH value was measured in soil water solution 1:2 (Method S-2.40), organic matter with loss on ignition (Method S-9.20), nitrogen with Kjeldahl (Method S-8.10), phosphorus according to Olsen et al. (Method S-4.10), potassium, calcium and magnesium with Ammonium Acetat (Method S-5.10).

Heavy metal determination in soil and earthworm

Samples in the amount of 3 g each (soil and earthworm), were treated with a 10 ml concentration HNO3 and 10 ml concentration of H2 SO4 at a temperature of 400°C for over 1 hour (Latifi et al., 2017). Mineralized samples were mixed with distilled water and filtered with filter paper (Whatman No. 0.45 μm). The filtrate was set at 50 cm3 in a volumetric balloon and leveled up to the mark with distilled water. Concentrations of heavy metals in soil and earthworm samples were determined by an Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer brand model 1100 (Boston, MA, USA).

Estimation of Bioaccumulation Factor

Bioaccumulation factor (BAF) was calculated using the formula: BAF = Cbiota/Csubstrate, where Cbiota and Csubstrate are the total concentrations of heavy metals in earthworms and soil substrate respectively (Owagboriaye et al.,

2015). The higher the BAF value is means that the greater the amount of heavy metal is accumulated in the body of the earthworm and vice versa.

Statistical Analysis

The data was statistically analyzed by analysis of variance (ANOVA) using computer software MSTAT-C from the University of Michigan, USA. Analysis of variance (ANOVA) and LSD -test were used to assess the differences in metal concentrations in soil and in earthworms as well, whereas the level of significance for the differences was set at $p<0.05$ and $p<0.01$ as well. Interaction factors were calculated from the table of ANOVA which was according to split-split plot experimental design. Factor A represents Locality (Mitrovicë, Kishnicë, Kastriot, Barilevë, Drenas and Lipjan), Factor B represents substrate (soil and earthworm), and Factor C represents heavy metal (Cu, Fe, Zn, Pb and Cd). Interaction between factors were A*B (locality * substrate), A*C (substrate * heavy metal), B*C (substrate * heavy metal) and A*B*C (locality * substrate * heavy metal).

RESULTS AND DISCUSSION

Table 1 presents the results of the physicochemical properties of the soil samples from the localities where the experiment was conducted.

Table 1. Physicochemical properties of the soil samples

Locations	pH	Organic matter %	Nitrogen %	Nutritients mg/100g soil			
				P_20_5	K_20	Ca	Mg
Mitrovicë	6.69	3.15	0.17	10.9	14.5	178	16.4
Kishnicë	6.85	2.73	0.13	12.1	15.7	189	15.9
Kastriot	6.43	2.08	0.10	9.7	15.3	154	15.2
Barilevë	7.12	2.95	0.14	12.3	13.6	194	15.5
Drenas	7.51	2.76	0.11	9.2	12.3	206	15.6
Lipjan	7.09	2.84	0.08	3.9	14.8	197	16.1
Average	6.95	2.75	0.12	9.7	14.4	186	15.8

Table 2. Heavy metal content in soil (mg/kg)

Locality	Heavy metal (mg/kg)				
	Cu	Fe	Zn	Pb	Cd
Mitrovicë	0.38	264.29	82.24	0.23	0.42
Kishnicë	0.78	174.15	76.18	0.52	0.25
Kastriot	0.11	114.69	33.62	0.39	0.13
Barilevë	0.03	70.62	18.96	0.11	0.04
Drenas	0.08	213.64	28.47	0.14	0.12
Lipjan	0.04	152.44	19.50	0.12	0.03
Average	0.24	164.97	43.16	0.25	0.17

As it can be seen the average of pH is 7.24 (neutral) and the organic matter 2.75%. With regard to the content of Nitrogen, P_20_5, K_20, Ca and Mg the values are 0.12 %, 9.7, 14.4, 186 and 15.8 mg/100 g soil respectively.

The results obtained from the heavy metal content in the soil have shown that the values obtained for all the analyzed metals are below the permissible limit according to the critical limits for EU countries and Kosovo legislation in force.

The results of these study regarding heavy metal concentrations in soil and earthworm samples from the six study locations in Republic of Kosovo is presented in tables 2 and 4.

In table 3 there are threshold reference values for some heavy metals in soil in EU countries and in Kosovo (Zogaj et al., 2014).

Similar results for the level of heavy metals in the soils of Kosovo have also been reported by other authors and our data are consistent with those of these authors (Zogaj et al., 2014).

Table 3. Heavy metal limits according to EU and National Legislation

Metals	Threshold		
	EU	Kosovo	Germany
Pb	300	50	100
Zn	300	300	200
Cu	140	100	60
Cd	1-3	2	1.5

The copper content in the soil samples ranged from 0.03 to 0.78 mg/kg (average 0.24 mg/kg). Copper is classified as micronutrient and earthworms did not accumulate this metal too much. The content of this metal in the earthworm's bodies ranged from 0.02-0.42 mg/kg (average 0.14 mg/kg) and was lower than that of the soil (Tab. 4).

Table 4. Heavy metal content in earthworms (mg/kg)

Locality	Heavy metal (mg/kg)				
	Cu	Fe	Zn	Pb	Cd
Mitrovicë	0.23	205.31	53.15	0.18	0.37
Kishnicë	0.42	162.41	46.92	0.43	0.16
Kastriot	0.07	92.10	29.10	0.34	0.09
Barilevë	0.02	53.11	15.74	0.07	0.02
Drenas	0.05	185.17	22.59	0.10	0.07
Lipjan	0.03	105.81	16.34	0.08	0.01
Average	0.14	133.99	30.64	0.20	0.12

Iron (Fe) in the soil samples was determined in the range of 70.62 to 264.29 mg/kg (average 164.97 mg/kg) and in the earthworm's bodies from 53.11 to 205.31 mg/kg with an average of 133.99 mg/kg and these differences are statistically highly significant, for the $p > 0.01$ (Tab. 6). The zinc content in soil samples ranged from 18.96 to 82.24 mg/kg (average 43.16 mg/kg). Zinc, similar

to copper, is a microelement and its content in the earthworm's bodies was within limits 15.74-53.15 mg/kg (average 30.64 mg/kg). The range of lead content in soil was within 0.11 to 0.52 mg/kg with an average of 0.25 mg/kg (Tab. 2). The range of Pb content in the earthworm's bodies was 0.07-0.43 mg/kg with an average of 0.20 mg/kg (Tab. 4). Cadmium content in compost samples ranged from 0.03-0.42 mg/kg (average 0.17 mg/kg). The earthworms accumulated cadmium; the content of cadmium in the earthworm's bodies were lower (0.01-0.37 mg/kg) than in the soil.

These results has indicated that earthworm's bio-accumulate heavy metals in their tissue having the soil as its habitat, they feed on the debris of dead plants and animals which are components of the soil organic matter. The degree of bio-accumulation of these heavy metals by earthworms is dependent on the degree of accumulation in the soil. In our case the bioaccumulation of heavy metals in earthworm samples maintains the same profile as mean heavy metal concentration of the soil i.e. Fe>Zn>Pb>Cu>Cd.

By analyzing the obtained data regarding the bioaccumulation factor (BAF) of heavy metals in soil and earthworm's (Tab.5), it can be seen that the values are different for different heavy metals.

Table 5. Bioaccumulation factor of heavy metals in soil/earthworm

Locality	Heavy metal (mg/kg)				
	Cu	Fe	Zn	Pb	Cd
Mitrovicë	0.61	0.78	0.65	0.78	0.88
Kishnicë	0.54	0.93	0.62	0.83	0.64
Kastriot	0.64	0.80	0.87	0.87	0.69
Barilevë	0.67	0.75	0.83	0.64	0.50
Drenas	0.63	0.87	0.79	0.71	0.58
Lipjan	0.75	0.69	0.84	0.67	0.33
Average	0.64	0.80	0.77	0.75	0.60

The highest value of bioaccumulation factor, as average, was found to be for Fe (0.80 mg/kg) and the lowest one for Cd (0.60 mg/kg). Regarding other metals it was different and had these values of 0.77 mg/kg, 0.75 mg/kg and 0.65 mg/kg for Zn, Pb and Cu respectively. The bioaccumulation factor in all heavy metals was less than 1 mg/kg. This trend is similar to the result obtained by other authors (Agbaire & Emoyan, 2012, Uba et al., 2009). According to Ma et al., (1983), the amount of heavy metals accumulated within earthworm tissues is partly dependent on the absolute concentration of metal within a given soil and the physico-chemical interactions.

In recent years, many researchers (Marta Bożym, 2017, Shahmansouri et al., 2005, Gaganmeet and Hundal, 2015) have focused on heavy metals accumulation in earthworm's and their relationships with total and bioavailable fractions in soil, and reported that metals in earthworms were significantly related

to the concentration of heavy metals in a soil. Our results are in concordance with the results reported by these authors.

Based on the results of the analysis of variance (Tab. 6), it was shown that there are statistically significant differences of different levels of significance regarding the content of heavy metals according to locality (Factor A), substrate (Factor B), heavy metal (Factor C) and factor interactions (A*B), (AxC), (BxC), and (AxBxC).

Table 6. Heavy metal concentrations in soil and earthworms (ANOVA)

Locality (A)	Substrate (B)	Heavy metal mg/kg (C)					Average (A*B)	Average (A)
		Cu	Fe	Zn	Pb	Cd		
Mitrovicë	Earthworm	0.23	205.31	53.00	0.18	0.37	51.82	60.57**
	Soil	0.38	264.29	81.24	0.23	0.42	69.31	
	Average (A*C)	0.31	234.80	67.12	0.21	0.40		
Kishnicë	Earthworm	0.42	162.41	46.92	0.43	0.16	42.07	46.23
	Soil	0.78	174.15	76.18	0.52	0.25	50.38	
	Average (A*C)	0.60	168.28	61.55	0.48	0.21		
Kastriot	Earthworm	0.07	92.10	29.10	0.34	0.09	24.34	26.97
	Soil	0.11	114.69	32.62	0.39	0.13	29.59	
	Average (A*C)	0.09	103.40	30.86	0.37	0.11		
Barilevë	Earthworm	0.02	53.11	15.74	0.07	0.02	13.79	15.87**
	Soil	0.03	70.62	18.96	0.11	0.04	17.95	
	Average (A*C)	0.03	61.87	17.35	0.09	0.03		
Drenas	Earthworm	0.05	185.17	22.59	0.10	0.07	41.60	45.05
	Soil	0.08	213.64	28.47	0.14	0.15	48.50	
	Average (A*C)	0.07	199.41	25.53	0.12	0.11		
Lipjan	Earthworm	0.03	105.81	16.34	0.08	0.01	24.45	29.44
	Soil	0.04	152.44	19.50	0.12	0.03	34.43	
	Average (A*C)	0.04	129.13	17.92	0.10	0.02	Average B	
Average (B*C)	Earthworm	0.14	133.98	30.62	0.20	0.12	B1 33.01**	
	Soil	0.24	164.97	42.83	0.25	0.17	B2 41.69**	
Average (C)		0.19Ns	149.48 **	36.73	0.23Ns	0.15**	Average A*B*C	
FACTOR A B C AB AC BC ABC								
LSD	1 %	13.3438 5.9719 9.1075 16.7091 32.0536 14.3676 71.4261						
	5 %	9.9084 4.5368 6.9189 12.0825 21.1587 10.4887 38.9108						

Legend: Ns = No significant, p > 0.05 = significant, p > 0.01 = highly significant

As far as the localities (Factor A) are concerned, statistically significant differences have been observed, where the content of heavy metals as average during these surveys was higher in the locality of Mitrovicë (60.57 mg/kg), in relation to the Barilevë site where the content of heavy metals as average, regardless of the type of metal, was lowest (15.87 mg/kg). In this regard we can say that the locality Mitrovicë has been most polluted with heavy metals and that the observed differences are highly significant compared to the other localities. To our opinion this was expected given that in Mitrovica there is a metallurgical plant "Trepça" where zinc, iron and lead are produced and the pollution of soil, air and water is at a higher rate compared to other localities involved in these researches.

Statistically significant differences were also observed with respect to the substrate (Factor B). The content of heavy metals as average was higher in the analyzed soil samples (41.69 mg/kg), while the lowest in analyzed samples of earthworms (33.01 mg/kg) and the observed differences were very significant. Regarding the type of heavy metal (Factor C), regardless substrate (soil or earthworm's), statistically significant differences was also found (Average C). The highest content is found in Fe content (149.48 mg/kg) while the lowest in Cd (0.15 mg/kg) and the differences between them are highly significant. As a metal Fe is more widespread because Kosovo soils contain higher levels of Fe compared to other metals. In the locality of Drenas works Ferronickel combine where extraction of iron and nickel is done. This justifies the presence of Fe in larger quantities compared to the other metals. The average content of other heavy metals was 36.72 mg/kg, 0.23 mg/kg and 0.19 mg/kg for Zn, Pb and Cu respectively. Even among these elements, statistical differences of different levels of significance have been found.

With respect to factor interaction A*****B, A*****C, B*****C and A*****B*****C statistical significant differences of different levels of significances have been found as well (Tab. 6). Regarding interaction of the factors AxB (locality x substrate) there are many combinations, for instance if we compare heavy metal content in the soil at Drenas (48.50 mg/kg) with heavy metal content in Mitrovicë (69.31 mg/kg) these differences are statistically highly significant since the difference is higher than 16.7091 mg/kg which is at level of 1% of LSD (Tab. 6). In other case if we compare heavy metal concentration in soil in Lipjan (34.43 mg/kg) with that in the soil in Kastriot (29.59 mg/kg) we can see that there are no statistically significant differences since the difference is less than 12.0825 mg/kg (Tab. 6). The same comparison we can undertake at all interactions between factors we are interested to know and to confirm the level of significances.

CONCLUSIONS

Heavy metal pollution of the soil and Ecosystem has become something of a global concern and this is as a result of its adverse effect on living organisms when they come in contact with these metals. They tend to be toxic and of a detrimental effect, this can be seen as a result of its ability to bio-accumulate in

the body of living organisms thus interfering with the food chain. In conclusion, this study confirms that earthworms accumulated some amount of heavy metals from soils in investigated localities. The study also showed that the levels of these metals accumulated in the earthworm tissue were less than 1 mg/kg. This study therefore confirms the potentials of earthworms to accumulate heavy metals from soils and so can be used as a bioindicator for pollution studies. Since some organisms like reptiles, birds and some other vertebrates feed on earthworms, transfer of these metals across the food chain is most likely, and therefore this research will be useful for risk assessment by relevant institutions responsible for the monitoring and surveying of environmental pollution and food security and safety in Kosovo.

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BIOCHEMICAL RESPONSES OF TWO SUGARCANE VARIETIES TO WHITEFLY NEOMASKELLIA ANDROPOGONIS INFESTATION AND ITS CONTROL BY A NEW BUTENOLIDE INSECTICIDE, FLUPYRADIFURONE

SUMMARY

Sugarcane whitefly, *Neomaskellia andropogonis* Corbett (Homoptera: Aleyrodidae), is established in the sugarcane ecosystem of Khuzestan Province, and it is one of the most important pests of sugarcane. Two fields of the CP69-1062 and IRC99-02 varieties of sugarcane were selected for evaluation of their biochemical responses, including pigment content, nitrogen, total protein, phosphorus and soluble sugars, to *N. andropogonis* infestation. Also, the efficiency of a new butenolide insecticide, flupyradifurone , was assessed based on the population density of *N. andropogonis*.

The results indicate that infestation of *N. andropogonis* reduced the chlorophyll content, nitrogen and total protein of both examined varieties. In addition, flupyradifurone significantly decreased all life stages of *N. andropogonis* and the population density for 28 days after application the insecticide application. Furthermore, flupyradifurone did not adversely influence parasitic wasps ' activity in the parasitising nymphal stage of *N. andropogonis*. Based on our findings, the parasitism percentage reached 55.1% and 63.0% for the control and flupyradifurone-treated plots of IRC99-02, respectively, and 61.9% and 65.7% for the control and flupyradifurone-treated plots of CP69-1062, respectively.

Keywords: Control, field, insecticide, sugarcane, whitefly.

INTRODUCTION

Sugarcane (Saccharum species hybrids) is cultivated more in the tropics and subtropics and continues to be one of the most important industrial crops (James, 2004). Sugarcane is an important cash crop grown in Khuzestan region, Iran; So that more than 100,000 hectares of sugarcane are grown annually in this region (Nikpay & Goebel, 2016). Different types of insect pests infest sugarcane

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which is categorized according to geographical distribution, taxonomic category, feeding habit, damage rate, etc. (Leslie, 2004).

Several species of whiteflies attack sugarcane. In Pakistan, *Aleurolobus barodensis* (Maskell) was more abundant whitefly than *Neomaskellia andropogonis Corbett* (Homoptera: Aleyrodidae) (Inayatullah, 1984); in Thailand *A. barodensis* (Charernsom & Suasa-ard, 1989); and *A. barodensis* and *N. bergii* (Signoret) were reported from different states of India (Balikai et al., 1998; Vemuri et al., 2014). In Iran, *N. andropogonis* is the species of whitefly firstly reported by Askarianzadeh and Manzari (2006). In recent years, *N. andropogonis* has been considered as one of the important pests of sugarcane fields of Iran (Nikpay, 2017). The whitefly infested sugarcane stalks and leaves, sucking the sap and excretion of honeydew lead to sooty mold growth which reduces photosynthesis of sugarcane and result in more than 50% yield losses (Nikpay, 2017; Srikanth et al., 2012). The ratoon crop of sugarcane is more sensitive and show severe damage by this pest (Masih et al., 1990; Srikanth et al., 2012); since ratoon crops produce more stalks per hectare than plant crops, however the stalks are usually not as thick and the growth of ratoon crops are weaker than planted canes (Vock, 2000). Damage of sugarcane whitefly not only prevents plant growth, but also reduced photosynthesis and chlorophyll content in sugarcane leaves lead to decreases in sugar content (Srikanth et al., 2012).

There are different methods for controlling whiteflies in sugarcane fields. Chemical control has always been the most effective way to reduce population of sugarcane whitefly (Chaudhary & Jaipal, 2006; Koohzad-Mohammadi et al., 2017; Masih et al., 1990). Three insecticides have been evaluated by Masih et al. (1990) in Pakistan and Inexit 60EC was found to be more efficacious in controlling whitefly. Vijayaraghavan and Regupathy (2006) found thiamethoxam (200 g a.i) effectively reduced the population compared to dimethoate and imidacloprid. However, Bhavani and Rao (2013) findings indicated that the removal of infested leaves + spraying of imidacloprid was the most effective treatment for controlling *A. barodensis* population under filed conditions. In previous study, the toxicity of dinotefuran and deltamethrin were proved to be more than spiromesifen against *N. andropogonis*. However, spiromesifen had less adverse effects on pupal parasitism (Koohzad-Mohammadi et al., 2017).

Flupyradifurone (FPF), under the trade name Sivanto®, is a new butenolide insecticide with fast contact toxicity, and feeding efficacy suitable for controlling insect vectors of virus like whiteflies and aphids. It functions as a partial agonist of postsynaptic AChRs and binds to insect nicotinic acetylcholine receptors (Jeschke et al., 2015).

The present study was conducted to 1) evaluate sugarcane whitefly damage on some biochemical properties of CP69-1062 and IRC99-02 varieties of sugarcane, 2) assess the effects of a new butenolide insecticide, flupyradifurone, to slow down the population density of sugarcane whitefly, *N. andropogonis* and

to evaluate the efficacy of flupyradifurone on nymphal parasitism by parasitic wasps under natural field conditions.

MATERIAL AND METHODS

Sugarcane varieties: The experiments were carried out on two sugarcane varieties of CP69-1062 (Canal Point USA) and IRC99-02 (cross made in Cuba and selected in Iran) from October to November 2018. Two different fields with history of sugarcane whitefly infestation were chosen for the experiments. The fields were located at Salman Farsi Agro-industry farms (48°35'E, 31º8'S), Ahvaz, Iran. The conditions of the experiments were the same as described by (Koohzad-Mohammadi et al., 2017). Each experimental block (plot) consists of four rows, 15 meter long and 1.8 meter inter-row spaces (135-m2) in random places of each field. Before planting of sugarcane varieties, phosphorous fertilizer (Super phosphate triple/300 kg per hectare) was applied with a pneumatic fertilizer machine based on standard procedure of sugarcane nutrient treatments in Iran. Each sugarcane variety was planted as billets (50-70 cm and free from stalk borers infestation) and following planting of seed cane sets all furrows were treated with Atrazine and Sencor herbicides (2+2 kg per hectare) based on local recommendations as early post emergence application for suppressing of annual weeds. During the crop growth all weeds in experimental plots removed manually by hand.

Biochemical properties estimation in sugarcane leaves infested by *N. andropogonis*: The infested leaves of CP69-1062 and IRC99-02 varieties of sugarcane were collected from Salman Farsi farms that were contaminated with *N. andropogonis*. Un-infested leaves were collected from the pest-free fields as control to evaluate the impact of whitefly damage on the content of some biochemical properties of sugarcane leaves. The leaves containing about 150 to 200 nymphs of sugarcane whitefly were considered as infested leaves. For each biochemical property, four replicates including four infested or un-infested leaves of both varieties were collected. The leaves were put into a plastic bag and transferred to the lab. At first, the infested or un-infested leaves were gently washed with distilled water and air-dried.

Chlorophyll content: The Chlorophyll content of four randomly selected plants from each variety was measured by the method of (Lichtenthaler, 1987). Based on this method, 0.1 g fresh leaf tissue weighed and 10 ml acetone Merck 80% was added on the leaves. The leaves were well-rubbed and then 5 ml acetone was added so that the final volume was 15 ml. The sample was centrifuged (SIGMA-D-37520 Osterode, Germany) for 20 min at 4000 rpm. Then the supernatant was obtained to measure chlorophyll a, b, carotenoid and total chlorophyll. Spectral absorption apparatus, UV 2100 Spectrophotometer, (Chemito Instruments Pvt. Ltd., Braunschweig, Germany) was used to estimate chlorophyll a, b, and carotenoid at wavelengths of 664.5 nm, 647 nm and 452.5

nm, respectively. Acetone 80% was used as control (Blank). The pigments estimated in mg/g wet weight of a plant leaf.

Nitrogen and total protein content: The nitrogen content of infested and un-infested leaves was determined by steam distillation using Kjeldahl digestion procedure. Crude protein content was measured by multiplying nitrogen content by a plant factor. The amount of nitrogen and protein were measured in milligrams per gram (mg/g) (AOAC International, 2006).

Phosphorus: Sugarcane leaves were dried at 70^{o}C and analyzed for P content using UV 2100 Spectrophotometer (Chemito Instruments Pvt. Ltd., Braunschweig, Germany) at wavelength of 470 nm.

Soluble sugar: The soluble sugar content was determined by the method of (Dubois et al., 1956). The whitefly infested and un-infested leaves were in an oven at 70^{o}C and analyzed for soluble content using UV 2100 Spectrophotometer (Chemito Instruments Pvt. Ltd., Braunschweig, Germany) at wavelength of 490 nm.

Insecticide formulation: Flupyradifurone (FPF) under the trade name Sivanto® (200 SL, Bayer CropScience, Germany) is a new insecticide from butenolide class register for controlling major sucking pests like aphids and whiteflies. Sivanto is agonist of nicotinic acetylcholine receptors. It is systemically translocate acropetally in the xylem, and is translaminarily distributed into the adjacent plant cells (Jeschke et al., 2015).

Field experiments: Experiments were carried out in Randomized Complete Block Design with four blocks (plants) and three replications (leaves). Flupyradifurone was applied on 6-October 2018 as soon as the population of whitefly eggs was observed in the fields. Foliar spraying was conducted in the early morning by a 15-litre volume sprayer (Hardi International, England).

Flupyradifurone was sprayed at the concentration of 0.3 lit/ha and untreated plots (water only spray) were considered as control. Sampling was made randomly and for each treatment, a total of 12 leaves were taken from the fields. Sampling was conducted before starting Sivanto application and 3 days, 1, 2, 3 and 4 weeks after spraying. The number of egg, nymph, pupae and adults of *N. andropogonis* per leaf and the number of parasitized pupae were recorded under stereomicroscope (Wild M3c, Heerbrugg Switzerland). The parasitized pupae are characterized by circular exit holes indicating emergence of parasitoid wasps.

Statistical analysis: All data were checked for normality using non-parametric Kolmogorov-Smirnov tests at P = 0.01. For the biochemical properties, mean comparison between control and infested leaves and also the comparison between two varieties, CP69-1062 and IRC99-02 was performed with t-test at P = 0.01.

The mortality data was transformed to square root of arcsine to normalize the data, but non-transformed data are presented in figures. The data from the fields were analyzed using Two-Way Analysis of Variance, with treatment

(control and flupyradifurone) and exposure time as main effects. Differences among exposure times were separated using Tukey-Kramer honest test (HSD) and between control and flupyradifurone with student t-test at 0.05 significance levels using SPSS version 16 (SPSS, 2007).

RESULTS AND DISCUSSION

The results showed that in whitefly infested leaves, there was no significant difference in chlorophyll a content between CP69-1062 and IRC99-02 sugarcane varieties. The chlorophyll a content was 0.583 and 0.824 mg/g respectively. The chlorophyll a content in the control plots of IRC99-02 (1.48 mg/g) was significantly more than control of CP69-1062 (0.864 mg/g). Similar results were obtained in the chlorophyll b, carotenoid and total chlorophyll, and in all the greatest amount was recorded in un-infested leaves of IRC99-02 variety of sugarcane (Figure 1).

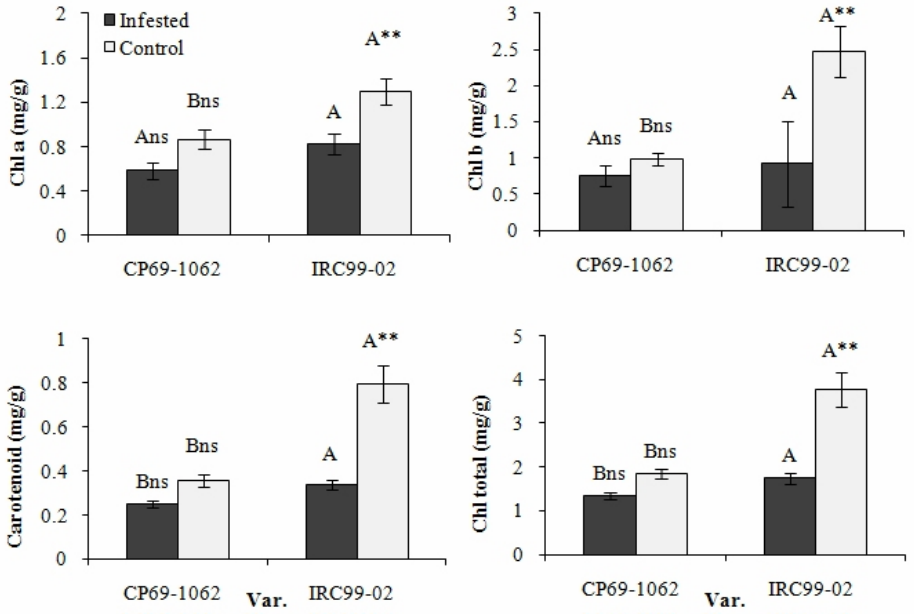

Figure 1: Mean content (± SE) of pigments in sugarcane leaves infested by *Neomaskellia andropogonis*

ns: there was no significant difference between the infested and control leaves in each variety separately with t-test; ** there was a significant difference ($P = 0.01$). Means followed by the same upper case letters between two varieties were not statistically significant at $P = 0.01$ with t-tes

There were not significant differences between varieties in nitrogen levels of the un-infested leaves, but it was significantly more than infested leaves. Comparable results were obtained for protein, and whitefly infestation significantly reduced the protein content of both varieties. In contrast, in both phosphorus and soluble sugar content, there were no significant differences neither between varieties nor infested and un-infested leaves (Figure 2).

It was evident that the population density of all life stages of sugarcane whitefly was considerably higher in control than flupyradifurone treated leaves of CP69-1062 variety of sugarcane. However, for flupyradifurone in most cases the population was almost zero. For nymphal stage, an increasing trend in control was observed on 20 October which seems nymphs' population build up was slower on CP69-1062 than IRC99-02 variety. The pupal population of control plots reached to its peak level of 156 pupae per leaf on 27 October 2017 (Figure 3).

It was apparent that three days after foliar application of flupyradifurone, the egg population decreased and reached to 534 and 225 eggs per leaf for control and treated leaves, respectively. The highest nymphal population was observed on control plots of IRC99-02 variety in 13-October, while the population was low in flupyradifurone treatment at all sampling dates. The pupal population increased dramatically and reached to 214 pupae per leaf in 20 October and remained stable until 3 November (Figure 4).

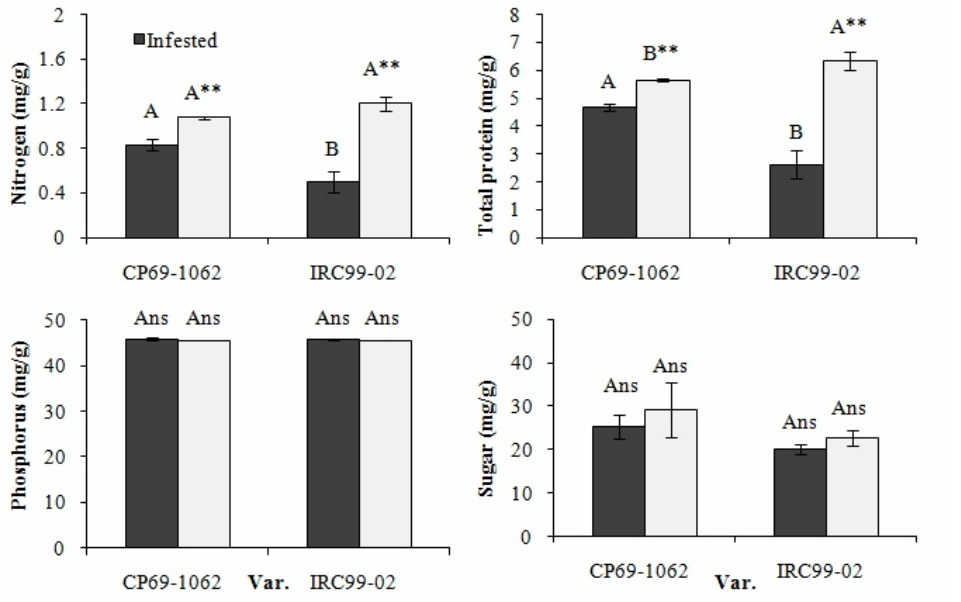

Figure 2: Mean (± SE) of nitrogen, total protein, phosphorus and soluble sugars in sugarcane leaves infested by *Neomaskellia andropogonis*

ns: there was no significant difference between the infested and control leaves in each variety separately with t-test; ** there was a significant difference (P = 0.01). Means followed by the same upper case letters between two varieties were not statistically significant at P = 0.01 with t-test.

Flupyradifurone is recommended to control whiteflies and aphids with excellent speed of action to quick feeding cessation (Jeschke *et al.*, 2015). In our study, the minimum population was recorded in flupyradifurone treated plots. This trend continued throughout the season, so that spraying flupyradifurone prevented whitefly population built up even after 28 days.

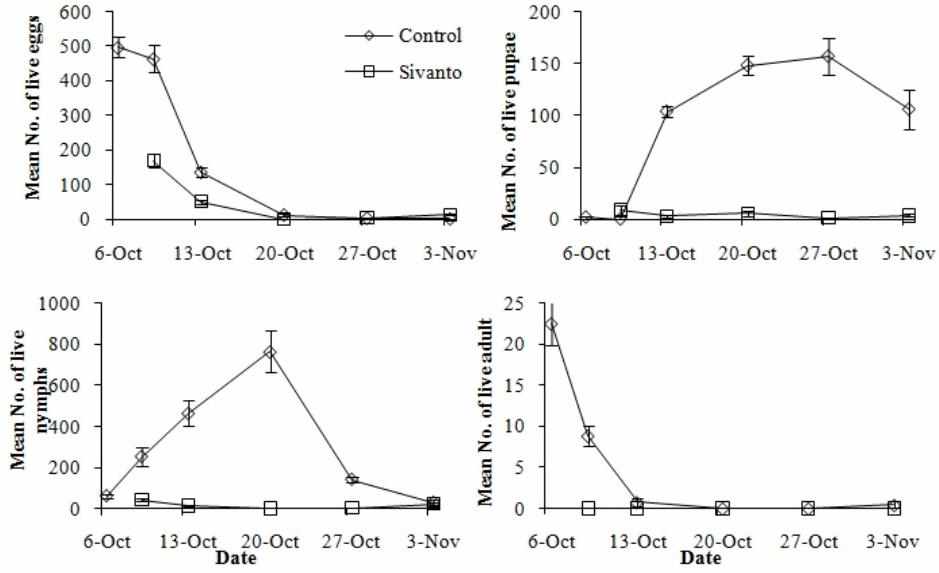

Figure 3: Population density of *Neomaskellia andropogonis* different life stages exposed to flupyradifurone on CP69-1062 variety of sugarcane.

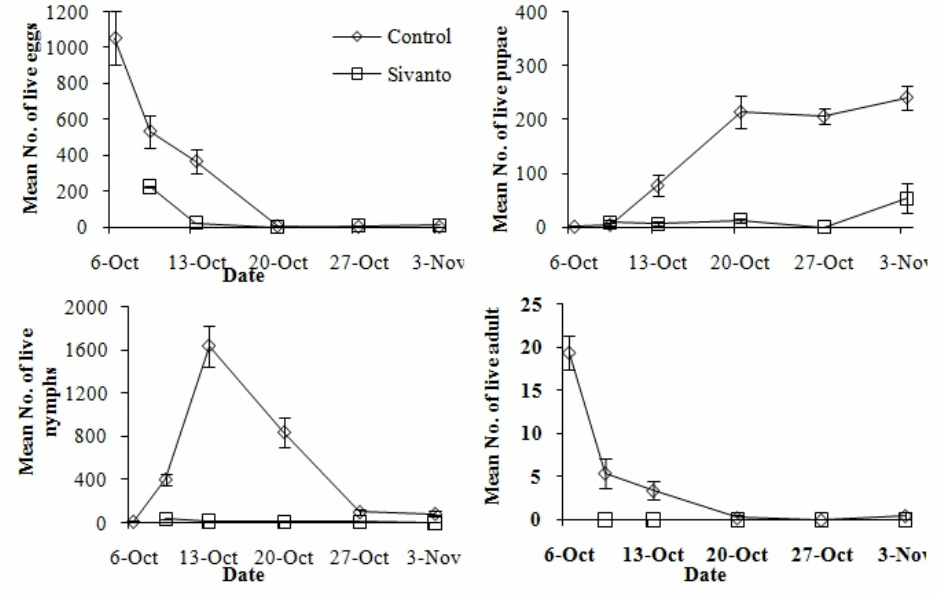

Figure 4: Population density of *Neomaskellia andropogonis* different life stages exposed to flupyradifurone on IRC99-02 variety of sugarcane.

Nauen et al. (2015) reported flupyradifurone had a prominent effectiveness against sucking pests such as aphid and whitefly species with antifeedant activity and suppression of honeydew excretion.Flupyradifurone indicated higher toxicity against *Lygus hesperus* Knight (Hemiptera: Miridae) when compared with other insecticides including sulfoxaflor, flonicamid, thiamethoxam, fenpropathrin, essential oils (rosemary and peppermint oils), and mineral oil in commercial strawberry fields in 2014 and 2015 (Joseph & Bolda, 2016).

Flupyradifurone provided control of whitefly eggs from 37.9% after 3 days to 100% after 14 days on CP69-1062 variety of sugarcane. Nymphal mortality was significantly higher (< 71%) in CP69-1062 variety treated with flupyradifurone than that of control. After 3 days of spraying flupyradifurone, 54.0% pupae were dead which increased to 90.0% after 21 days. Generally, adult mortality was 100% in leaves treated with flupyradifurone (Figure 5).

Mortality percentage for all life stages of *N. andropogonis* was almost zero in control plots of IRC99-02 variety. The percentage of egg mortality was 32.3% after 3 days of flupyradifurone application but 90% mortality achieved after 7 days. The mortality of whitefly nymphs was recorded 68.5% after 3 days of the insecticide foliar application and significantly increased to 94% after 7 days. Mortality percentage of pupae was 51.0% after 3 days of flupyradifurone foliar application and increased significantly to 89.9% after 21 days. However, the efficacy of flupyradifurone declined 28 days after spraying on IRC99-02 variety of sugarcane. Complete mortality of *N. andropogonis* adults were recorded on flupyradifurone treated plots (Figure 6).

In both tested varieties, the nymphal parasitism percentage increased on 21st day of sampling in control and flupyradifurone plots, which can be due to two reasons: 1. the delayed appearance of parasitic wasps, or 2. absence of nymphs in the fields for parasitism. There was a significant difference between control and flupyradifurone in both varieties on 21st day. However, the nymphal parasitism was raised over time after 28 days of the insecticide application (Figure 7).

Our results indicated that in both examined varieties, the sugarcane pigment contents, nitrogen and total protein was significantly reduced when infested by sugarcane whitefly. Buntin *et al.* (1993) stated that *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) infestation on tomato reduced the amount of photosynthesis. Although tomato plants has shown resistance by limiting stomatal aperture opening and gas exchanges, but the whitefly infestation reduced leaf chlorophyll content and photosynthesis capacity. Palumbo *et al.* (2000) declared that *Bemisia argentifolii* Bellows & Perring feeding reduced the quality of and pure protein of alfalfa. In the other study, the infestations of *B. tabaci* on eggplant caused decrease in leaf area, leaf fresh weight, dry weight, chlorophyll content and photosynthesis rate. So that in infested leaves, the chlorophyll content and photosynthesis rate decreased by 9.7% and 65.9%, respectively (Touhidul Islam & Shunxiang, 2009).

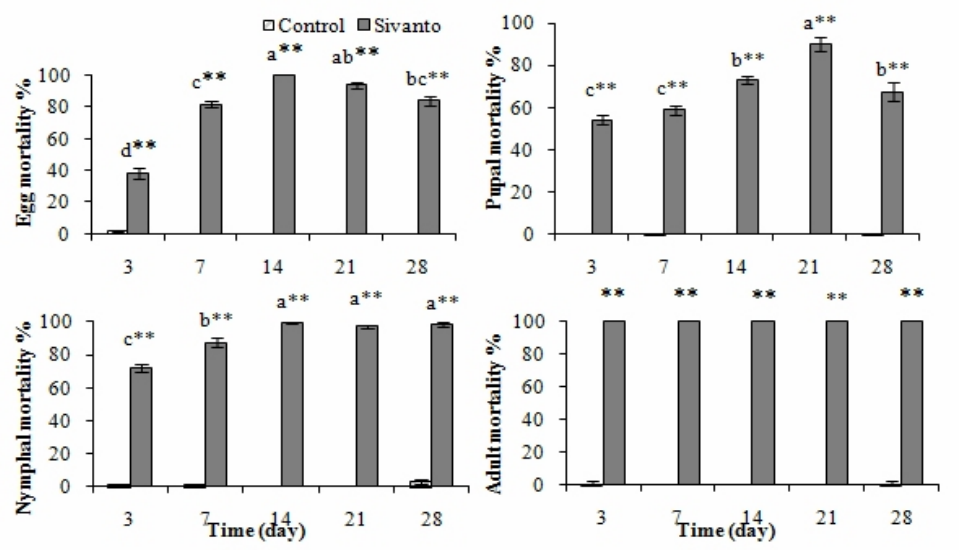

Figure 5: Mortality percentage (± SE) of *Neomaskellia andropogonis* different life stages exposed to flupyradifurone on CP69-1062 variety of sugarcane.

Means followed by the same lower case letter among exposure times for Flupyradifurone are not significantly different using Tukey-Kramer (HSD) test at $P = 0.05$. At each exposure time differences between control and Flupyradifurone denoted with asterisk ($P < 0.01$, t-test, SPSS).

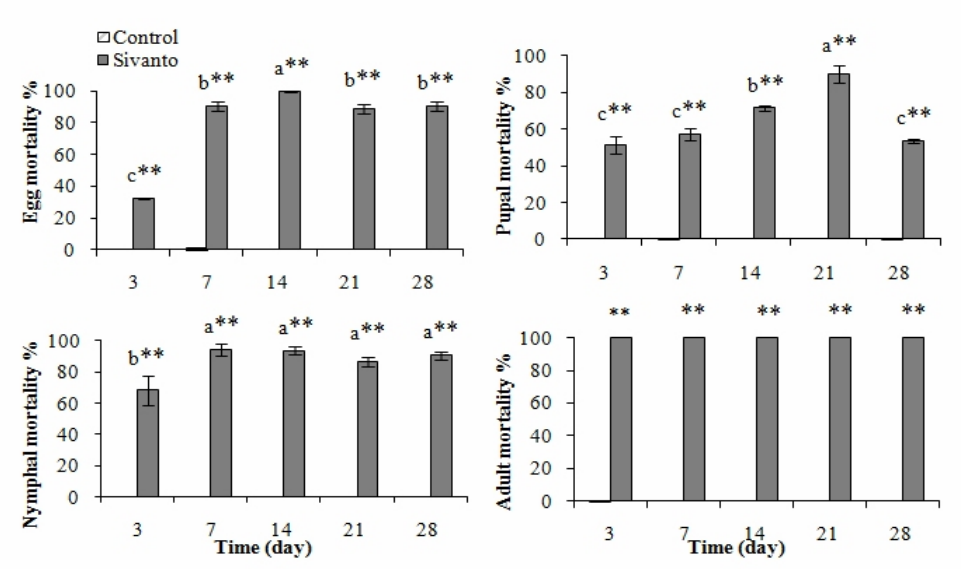

Figure 6: Mortality percentage (± SE) of *Neomaskellia andropogonis* different life stages exposed to flupyradifurone on IRC99-02 variety of sugarcane risk ($P < 0.01$, t-test, SPSS).

Means followed by the same lower case letter among exposure times for Flupyradifurone are not significantly different using Tukey-Kramer (HSD) test at $P = 0.05$. At each exposure time differences between control and Flupyradifurone denoted with asterisk ($P < 0.01$, t-test, SPSS).

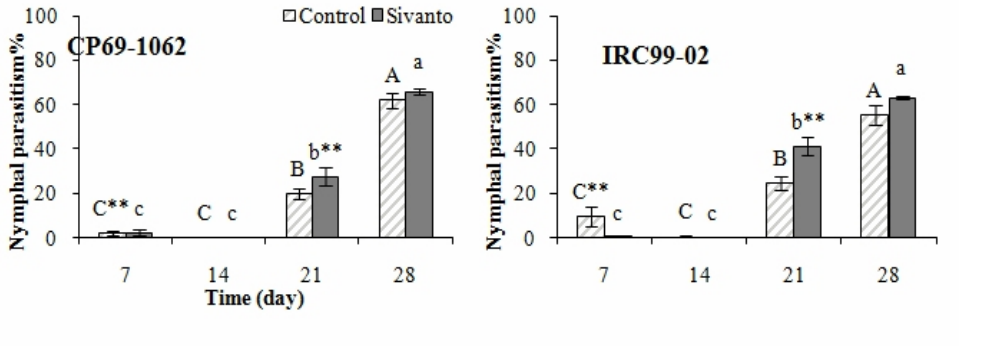

Figure 7: Nymphal parasitism percentage (± SE) of *Neomaskellia andropogonis* exposed to flupyradifurone on CP69-1062 and IRC99-02 varieties of sugarcane.

Means followed by the same upper case letter for control and lower case letter for Flupyradifurone among exposure times are not significantly different using Tukey-Kramer (HSD) test at $P = 0.05$. At each exposure time differences between control and Flupyradifurone denoted with asterisk ($P < 0.01$, t-test, SPSS).

Many studies have been carried out about the effect of *B. tabaci* on reduction of chlorophyll content of different plants such as cucumber, *Cucumis sativus* L. (Shannag & Freihat, 2009), cucumbers, pumpkin, cantaloupe (Al-Shareef, 2011), tobacco, cotton (Li *et al.*, 2013), and blackgram (*Vigna mungo* (L.) Hepper) (Taggar et al., 2015). Our results are in agreement with these reports and the chlorophyll content declined when leaves were attacked by sugarcane whitefly. However, to best of our knowledge there is not any published data about sugarcane whitefly, *N. andropogonis* effects on sugarcane.

Flupyradifurone was effective in reducing whitefly population density on sugarcane. These experiments under sugarcane field conditions demonstrated flupyradifurone can be a good alternative to synthetic insecticides that are currently used for controlling sugarcane whiteflies.

Control of sugarcane whitefly can also be performed by natural enemies such as parasitoids. In Iran, *Encarsia inaron* Walker (Hym.: Aphelinidae) (Malekmohammadi *et al.*, 2012) and *Eretmocerus delhiensis* Mani (Hym.: Aphelinidae) (Khadempour *et al.*, 2014) are reported as two main parasitic wasps of *N. andropogonis* suppressing the nymphal populations. Our results revealed that *E. inaron* and *E. delhiensis* provided significant suppression in sugarcane whitefly nymphs. The present findings agree with the results of Kumar et al. (2017a) who reported that the combination treatment of flupyradifurone with *Eretmocerus eremicus* Howard efficiently controlled *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) Mediterranean (MED) whitefly population. In the other research, Kumar *et al.* (2017b) emphasize the compatibility of flupyradifurone with swirskii mite, *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae) for the control of *B. tabaci* (MED).

CONCLUSIONS

Flupyradifurone was effective in reducing all life stages of sugarcane whitefly population. The application of flupyradifurone with a delayed release of parasitic wasps is recommended to be applied as integrated pest management program (IPM) of *N. andropogonis* under field condition.

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Nenad PEROŠEVIĆ [1]

AGRICULTURE DEVELOPMENT IN THE MUNICIPALITY OF NIKŠIĆ (1945-1991)

SUMMARY

The economic development of Montenegro in the Kingdom of Serbs, Croats and Slovenes/Yugoslavia (1918–1941) failed to bring about substantial progress, and as a result, Montenegro was the most underdeveloped part of the Kingdom of Yugoslavia, along with Bosnia and Herzegovina, Macedonia and Southern Serbia. The lack of a developed industry meant that most of the population of Montenegro was oriented towards agriculture. According to the 1931 census, the agricultural population accounted for 79.6% of all Montenegrins. Economic backwardness and material poverty were further deepened by the wartime devastation from 1941–1945 which hit Montenegro and what was then called the District of Nikšić. In socialist Yugoslavia (1945–1991), Montenegro and the Municipality of Nikšić underwent major socio-economic changes that would eventually have a profound impact on the position and status of agriculture in the economies of Montenegro and the Municipality of Nikšić. One period saw strong economic development, but this was not evenly distributed across all branches of industry. From 1945–1991, agriculture could not keep pace with industry. This can be attributed to several factors. The Municipality of Nikšić is a quintessential example of socio-economic transformation from a typically agrarian environment into an industrial environment under the socialist socio-economic model.

Keywords: Municipality of Nikšić, agriculture, Yugoslavia, Montenegro

INTRODUCTION

The Municipality of Nikšić (Figure 1), covering an area of 2,065 km², is the largest municipality in Montenegro and accounts for 15.0% of the territory of Montenegro (13,812 km²). The complex relief basis, climatic conditions, favorable geographical position and transport links with the rest of Yugoslavia all made impact on the socio-economic development of the Municipality of Nikšić. Until 1948, there was a high percentage of the agricultural population in Montenegro and the Municipality of Nikšić, but the ensuing years saw a fall in this percentage. According to the 1948 Census, the agricultural population accounted for 75.4% in Montenegro and 73.7% in the Municipality of Nikšić

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.
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(Radojičić, 2010; Radojičić, 2015). Having brought the economic potentials of Yugoslavia and Montenegro at their pre-war level in the period of reconstruction of the country in 1945 and 1946, the state attended to the issue of planning the economic development (Andrijašević, 2018).

The First Five Year Economic Development Plan aimed at economic development of Yugoslavia, and thus, the development of Montenegro as well, was adopted in 1947, with an emphasis on the development of industry as a fundamental branch of economy. A village and a farmer were to contribute to the process of industrialization and urbanization, bringing in raw materials, agricultural products and workforce engaged in numerous infrastructure projects. Such orientation had a substantial effect on the agriculture development in the country, and the Municipality of Nikšić is an example that demonstrates all the positive and negative sides of accelerated industrialization, which led to changes in the economic structure of the population, instigated population migration, developed transport links and towns, and diminished villages and affected the lifestyle habits of the agricultural population which, up to that moment, made up the majority population (Perošević and Barović, 2015; Lalatović, 1955; Mićunović, 1955).

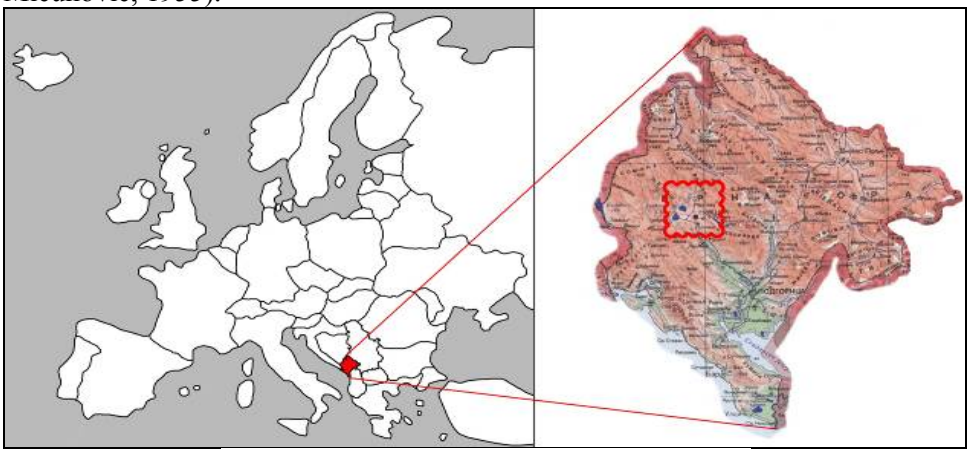

Figure 1. Geographic location of Nikšić field
Source: Vujačić, Duško, Geografske specifičnosti Nikšićkog polja [Geographic Particularities of Nikšić field], Diploma Paper, July 2012.

MATERIAL AND METHODS

The paper is based on historiographical facts and data that have not been published so far and can be found in the historical funds of the State Archives of Montenegro (DACG), Department for Systematization and Processing of Archival Documents Podgorica, (OSOAGP), the funds of the Communist League of the Communist Party of Yugoslavia Nikšić (f. SK KPJ Nikšić), box VII; the State Archives of Montenegro, Department for Systematization and Processing of Archival Documents Podgorica, (OSOAGP), the funds of the District People's Committee of Nikšić (f. SNO Nikšić) box XXVII. The paper also rests upon

statistical data published in the Statistical Yearbooks of Yugoslavia and Montenegro and in the literature. In order to deliver a quality report of the most significant data and fact through text and tables, we employed the methods (historiographic methods, analysis and synthesis methods) which point to the complexity and interdependence of the process of industrialization and de-agrarization of the Yugoslav and Montenegrin economic system in which the Municipality of Nikšić was a prototypical example of the positive and negative effects brought about in the 1945-1991time span.

RESULTS AND DISCUSSION

Agriculture 1945-1991

In the mid-twentieth century, economic underdevelopment in Central and Eastern Europe was essentially equated with the absence of industrialization process and holding on to the agricultural economy. The countries of this region, including Yugoslavia, launched a vigorous industrialization campaign in the 1950s and 1960s, and due to this, by the end of the 1960s, all countries in the region, with the exception of Albania, became industrialized countries. About half of the Gross National Product (GNP) was generated through industry: Czechoslovakia - 61%, Hungary - 57%, Poland - 54%, Romania - 52%, Yugoslavia - 49%, Bulgaria - 46%, and only 20% was generated through agriculture - Hungary, Poland and Yugoslavia. The UN statistical data (1988) show the momentous structural changes that have taken place in the socialist countries of Eastern and Central Europe indicating that industry is a leading branch of economy in the GNP. That same year, the share of industry in GNP in Romania was 62%, in Czechoslovakia - 61%, Bulgaria - 58%, and its share was slightly less in Hungary - 41%, Yugoslavia - 45% and Poland - 48%. Agriculture accounted for 10-15% of GNP in these countries. With the fall of the Berlin Wall in 1989 and the abandonment of the socialist socio-economic model in the countries of Central and Eastern Europe, industry has gone into a sharp decline since 1990. In the 1991-1992 period, industrial production in Poland fell by 40%, in Czechoslovakia by more than 36%, in Hungary by almost 40%, in Yugoslavia by 30%, etc. (Berend, 2001).

It is important to point out that the socialist model of government in Yugoslavia differed from that of other socialist states. This difference was reflected in the position of workers and their role in society. Self-governance, as a form of social structure in Yugoslavia, went through several stages laid down by the Law on the Management of State Economic Enterprises and Higher Economic Associations by the Workers' Collectives (1950), the Constitutional Law (1953), and the Constitution (1963).

However, the normative conceptual and theoretical structure was not implemented in practice, exhibiting both its advantages and disadvantages until the final breakup of the state and the fall of the self-government system in 1991 (Fočo, 2019).

Table 1: Number of people employed in agriculture (in thousands) in certain countries of Eastern and Central Europe in 1990-1992 (Statistical Yearbook of Yugoslavia, 1994).[2]

Country	Year	Total number of people employed in the economy	People employed in agriculture
Czechoslovakia	1990.	8.249	943
Yugoslavia[2]	1992.	2.328	130
Hungary	1990.	4.978	907
Poland	1990.	17.552	4.597
Romania	1990.	11.639	3.097

The economic development of socialist Yugoslavia is characterized by diminishing agriculture in favor of industry and reducing the percentage of the population who worked and lived off agriculture. One of the first measures taken by the authorities to better the position of farmers and agriculture in the state was the decision on agrarian reform and colonization which was to be implemented in 1945-1946. This reform envisaged the handover of a large number of agricultural assets to the state: large estates, farmlands and forests whose total area exceeded 45 ha or 25-35 ha of arable land, etc.

The Agrarian Reform in the Municipality of Nikšić pertained to the assets of four monasteries and 20 private estates. The total area this reform pertained to was 2,054 ha of land (under forest, arable land, meadows, pastures, orchards, vineyards, bushes, etc.), which made up about 9% of the total land affected by the reform in Montenegro (Petranović, B., Zečević, M., 1988; Marović, 1983). Subsequently, aiming at applying the socialist mode of production in agriculture, the state resorted to the establishment of Peasant Working Cooperatives (PWC). A way was sought to further the agricultural sector by exerting collective ownership over the means of production with the definitive liquidation of the "capitalist elements" in agriculture. In the time period from 1947 to 1953, the highest number of PWC in Montenegro was in 1949 and it amounted to 482. After that, the number of PWC declined until their abolition and reorganization in 1953. The undertaken reorganization of the PWC meant that instead of the three property sectors (state, cooperative and individual), two types of households were created - state and individual. An experiment with PWC failed to attain the projected agriculture development. PWC were, by and large, economically unsustainable, while animal husbandry went through a marked period of stagnation (Marović, 2002). In the Municipality of Nikšić, the first Peasant Working Cooperative was established in 1948 and as early as 1949, due to a vigorous and enforced campaign by the state, the number of PWC was 63 with 3,282 households (State Archive of Montenegro, VII). However, after an

[2] It refers to the Federal Republic of Yugoslavia (FRY) made up of Serbia and Montenegro.

apparent economic failure, as early as 1952, the number of PWC plummeted to only nine (State Archive of Montenegro, box XXVII).

A steady decrease in the rural population and, hence, a decrease in population engaged in agriculture indubitably contributed to the decline of agriculture in the Municipality of Nikšić and in Montenegro. The agricultural population in Montenegro saw a steady and constant drop from 1948 to 1991, as indicated by the data in Table 2.

Table 2: Population and percentage of the agricultural population in Montenegro (Radojičić, 2010; Radojičić, 2015)

Year	Population in Montenegro	Percentage of agricultural population in Montenegro
1948.	377.189	75,8 %
1961.	471.891	48,0 %
1971.	529.604	42,6 %
1981.	584.310	13,0 %
1991.	616.553	7,1 %

This trend affected the Municipality of Nikšić as well, which had a particularly strong industrialization in the period from 1947 to 1971 when the largest industrial enterprises were built and came into operation. In 1948, the agricultural population accounted for 73.7% of the total population in the Municipality of Nikšić (38,359), in 1953 – out of 46,589 people the agricultural population comprised 48.4%, in 1991 – the agricultural population made up only 4,1% of the then population of 74,706.

Several factors contributed to a dramatic decline in the agricultural population, the most important of which are as follows: with the opening of new factories, workforce moved from villages to Nikšić, leaving the villages demographically empty; fertile arable fields were seized in Nikšić field for the purpose of building new industrial facilities, which further curtailed the room for agricultural development. For the purpose of construction of industrial facilities in Nikšić field and in Gračanica Valley (Nikšićka župa), large agricultural areas were seized from agriculture: for the needs of Hydro Power Plant Perućica, 4,028 ha were taken away, 2,936 ha of which were agricultural reservoirs: Slano Lake - 1,597 ha, 978 ha[3], Vrtac Lake 1,492 ha - 1,416 ha, Krupac Lake 950 ha - 531 ha, channels and retention of Slivlje 11 ha - 11 ha, reservoir Liverovići 100 ha - 100 ha; Bauxite Mines 92 ha - 92 ha; industrial facilities 150 ha - 150 ha[4]. Significant agricultural areas were also seized due to the construction of sports stadiums in Nikšić, Glava Zete etc. (Radojičić, 2010; Radojičić, 2002).

A particular problem in the development of agriculture in the Municipality of Nikšić was the insufficient number of agricultural machinery and implements

[3] The first figure refers to total area, whereas the latter figure refers to arable land.

[4] 68 ha of these were seized for the biggest industrial facility in Montenegro, Nikšić Steel Mill, which commenced operations in 1956 (Steel Mill, 1996)

(tractors, threshers, plows, seed drills, reapers and mowers) and their negligible use in the agricultural production process. This problem was also plainly evident in the whole of Montenegro. How poor Montenegro was when it came to agricultural machinery is best illustrated by the fact that in 1948 it had only 59 tractors. With the rapid increase in the number of PWC in 1949 and the poor procurement of new tractors, only 35% of arable land in the socialist sector in Montenegro could be ploughed at the end of 1950. In Montenegro, one farm wagon was to cover an area of 15 ha of land, as opposed to area of 5.5 ha covered by one wagon in Slovenia, the most developed republic. The situation did not improve much in 1951 either: out of the total number of tractors in Yugoslavia (6,266), Montenegro had the least (69), while Slovenia had 259 tractors (Marović, 1987; Statistical Yearbook of the Federal People's Republic of Yugoslavia, 1954).

Table 3: Number of agricultural machines and implements in 1951 in the Municipality of Nikšić

Types of agricultural machines and implements	Municipality of Nikšić	Montenegro	Slovenia	Yugoslavia
All kinds of ploughs	621	7.250	30.367	1.178.931
Grain seed drills	29	209	2.276	53.527
Reapers and mowers	9	40	3.616	28.115
All kinds of threshers	8	82	6.876	17.616
Chaff cutters	8	91	61.228	125.323
Tractors	69	7	259	6.266

(Source: Statistical Yearbook of the People's Republic of Montenegro, 1955; Statistical Yearbook of the Federal People's Republic of Yugoslavia, 1954).

Even though the number of agricultural machinery and implements increased over the coming decades, it was still not enough to meet the demands of the contemporary agricultural development, neither in the Municipality of Nikšić nor in Montenegro.

Table 4: Number of the most important agricultural implements and machinery in the Socialist Republic of Montenegro in agricultural enterprises and agricultural cooperatives

Year	Pedestrian-controlled (single axle) two wheel tractors and rotavators	Biaxial tractors	Combine harvester	Trucks
1981.	7	184	8	39
1991.	31	228	24	36

(Source: Statistical Yearbook of the Socialist Republic of Montenegro, 1983; Statistical Yearbook of the Republic of Montenegro, 1993).

a)Animal Husbandry

In an attempt to modernize agricultural production in the first post-war years, the state founded the Institute for Animal Husbandry in Nikšić in 1946, as well as the Institute for Scientific Research and Development of Agricultural Production, established in Titograd in 1950. This Institute was dissolved in 1952 only to be founded again the very same year, also in Titograd, now under the name the Institute for Agricultural Research (Perošević and Barović, 2015; Tomašević, 1955).

As soon as the state systematically initiated the process of development of industrialization and urbanization, agriculture as a branch of economy gradually waned. In addition to the aforementioned factors, which contributed to the decline of agriculture as a branch of economy, the decline of animal husbandry and the poor treatment of livestock, especially goats, was caused by certain administrative measures. Namely, in order to prevent the destruction of forests, to protect fruit growing and to enable afforestation of karst areas and barren lands, The Decree on Prohibition of Goat Breeding in the People's Republic of Montenegro was passed in January 1948, The Decree did not pertain to those places determined by the Minister of Agriculture and Forestry (Official Gazette, 3/1948).

This Decree particularly affected the hilly and mountainous region, where the goat was considered to be the main substitute for large cattle. The largest number of livestock in Montenegro was recorded in 1948 - 1,146,827, and in the Municipality of Nikšić in 1950 - 98,687. After these years the number of livestock decreased steadily both in Montenegro and in the Municipality of Nikšić. The total number of livestock in Montenegro decreased by 58.6% between 1948 and 1991.

Animal husbandry, once a leading industry in the Municipality of Nikšić, lost its former significance and saw a sustained decline in both quantitative and qualitative terms. Sheep and large cattle husbandry in the municipality of Nikšić depended mainly upon the use of mountain pastures, on the plains northeast of Nikšić (Vučje, Konjsko, Bare Bojovića, Krnovo, Lukavica) but also upon the possibility of livestock grazing in the area of Donja Zeta, Nikšić field, and partly in Rudine, Banjani and Grahovo in winter months.

Animal husbandry in *katuns* was characteristic of the Municipality of Nikšić in the observed period, and is still carried out in a similar way even today. The only difference nowadays is that there are far fewer livestock farmers, a much smaller number of livestock and *katuns*, as well as huts and cots in them. Animal husbandry in the Municipality of Nikšić, and in Montenegro, has always had a semi-nomadic character.

It has retained the same feature in the 21st century as well (Radojičić, 2002; Radojičić, 2010; Statistical Yearbook of the Republic of Montenegro, 1993).

Table 5. Livestock numbers in the Municipality of Nikšić 1939-1991

Year	Cattle	Horses	Sheep and goats	Pigs	Total
1939.	19.743	3.441	119.835	2.354	145.373
1945.	6.386	1.370	20.043	520	28.319
1950.	21.424	2.673	70.363	4.227	98.687
1960.	15.041	3.720	66.222	1.550	86.553
1981.	17.786	2.250	54.897	1.397	76.330
1991.[5]	19.649	2.114	54.803	-	76.566

(Source: Statistical Yearbook of Yugoslavia, 1992)

b)Crop and Fruit Growing

The industrialization of the Municipality of Nikšić, set in motion by the 1947 Five-Year Economic Development Plan, had a particularly adverse effect on the development of this branch of agriculture, just like it did on animal husbandry. The construction of reservoirs in Nikšić field and spacious halls of Nikšić Steel Mill, Bauxite Mine, companies Javorak, Metalac, Trebjesa Brewery and other factories, as well as the expansion of the town of Nikšić in the process of accelerated urbanization, deprived agriculture of the highest quality plots of land, which led to a decrease in agricultural land, as indicated by the data in Table 6.

Table 6: Agricultural areas in the Municipality of Nikšić in 1954

Total area/ha	Agricultural area	Total	Plougs and gardens	Orchards	Vineyards	Meadows
214.720	40.835	10.946	4.769	191	14	5.972

(Source: Radojičić, 2010; Statistical Yearbook of the People's Republic of Montenegro, 1955).

In addition, in 1954, there were 29,864 ha of pasture lands and 25 ha of land under reed and moorlands in the Municipality of Nikšić. The following crops were sown and cultivated on the agricultural areas: on 4,769 ha of arable land and gardens - grain was sown on 2,903 ha, industrial plants were sown on 14 ha, vegetables on 874 ha, fodder on 175 ha. Nurseries were not sown and there was 803 ha of uncultivated arable land and fallows. Most common fruit tree was plum with 67,554 trees, then apple with 12,931 trees, pear - 3,778, cherry - 3,150, fig - 2,810, quince - 628 and apricot - 59 (Statistical Yearbook of the Republic of Montenegro, 1955).

The agricultural arable land continued to wane in the following years, and an analysis from 1976 showed that only 2.4% of the total area of the Municipality

[5] There are no data on the number of pigs and goats in 1991 in the Statistical Yearbook of Yugoslavia from 1992. The column “Sheep and goats” refers solely to the number of goats.

of Nikšić (206,500 ha) was covered in arable land and orchards, 0.2% was covered by orchards, vineyards covered 0,1%, meadows 4.6%, pastures 20.1%, forests 44.5% and barren land covered 28.1%. Thus, the total arable land made up only 7.3% (Radojičić, 2010). The decline of arable land inevitably influenced the quantities of crops sown as well as the number of fruit trees in the years to come. For example, in 1982, out of a total of 5,071 ha of arable land and gardens, only an area of 4,650 ha was sown with the following crops: cereals - 1,520 ha, vegetable plants - 1,693 ha and livestock fodder - 1,437 ha. Industrial plants, flowers and nurseries were not sown at all. Fallows and uncultivated arable land accounted for the remaining 421 ha. Of the total number of fruit trees in Montenegro (1,555,300), the most abundant were plum trees - 1,143,050, then apple trees - 275,094 and pear trees - 137,156. Of the total number of fruit trees in the Municipality of Nikšić (99,400), in 1982, there were 74,245 plum trees, 17,445 apple trees, and 7,710 pear trees (Statistical Yearbook, 1983). Towards the end of the observed period, in 1990, the number of agricultural areas in the Municipality of Nikšić dwindled further. The total agricultural area of the Municipality of Nikšić was 54,579 ha; and arable land and gardens covered 4,830 ha, orchards - 380 ha, vineyards - 26 ha, adding up to a total of 5,236 ha. Meadows and pastures covered an area of 49,343 ha. An analysis carried out in 1989 showed that the Municipality of Nikšić did not have agricultural land of satisfactory quality. Most of the quality land was to be found in the following towns: in Podgorica - 17.0%, Pljevlja - 14.5%, Berane - 9.5%, Bar - 7.4%, Nikšić - 7.3%, Ulcinj - 5.7% while in other municipalities the percentage ranged from 0.8% to 3.9% (Statistical Yearbook of Yugoslavia, 1991; Radojičić, 2015).

The comparison of arable agricultural land of Montenegro to that of Slovenia in Yugoslavia in 1990 reveals that Montenegro had a smaller agricultural area but it also had a much lower yield per hectare, used significantly less mineral fertilizers and had far less meat production. Of the total arable agricultural area in Yugoslavia in 1990, there were 14,170 ha of arable land; of which 866 ha in Slovenia and 517 ha in Montenegro. However, Slovenia was the leader in crop yields per hectare, expressed in tons: in Yugoslavia - 115, 68 t/ha; in Slovenia - 142, 19 t/ha; in Montenegro - 44.19 t/ha. The consumption of mineral fertilizers in thousands of tons in Yugoslavia in 1990 was 1,866; in Slovenia – 80 tones; in Montenegro – 1 ton. Meat production (in thousands of tons) in 1990 in Yugoslavia was 1,315 tons; in Slovenia 189 tones; in Montenegro - 17 tones (Statistical Yearbook of Yugoslavia, 1991).

The original purpose of this agricultural land was drastically altered by turning these plots of land, mainly arable land and meadows, into reservoirs, or by using them for housing, construction of industrial enterprises and the activation of mining sites. A particular problem is that these changes were sometimes made without extensive scientific analysis of the feasibility of such ventures, hence the consequences on agriculture were all the more severe. The aspiration towards industrialization of the state, which also initiated the process of urbanization, led to the abandonment of even those rural areas that boasted

good conditions for the development of agricultural production. By moving families from villages to towns, such areas become neglected, and thus meadows were eventually converted into pastures and pastures into barren land.

The research B. Radojičić carried out shows that, as per the European Union criteria, ploughlands, orchards and vineyards are considered arable land. In 2010, there were only 0.054 ha of such areas in the Municipality of Nikšić and only 0.099 ha in Montenegro, which is far below the average in most of the developed Europe (Radojičić, 2010).

c)Fisheries

The most favorable fishing areas in Montenegro are situated on the Montenegrin coast, although there has been a steady decline in fish catches in recent years. In the Municipality of Nikšić, before the construction of artificial lakes, the most favorable conditions for catching fish were in the catchment area of the Zeta River. After the construction of artificial lakes on the territory of the Municipality of Nikšić and in Nikšić field (Vrtac Lake, Krupac Lake, Slano Lake, Liverovići Lake, Grahovo Lake and Bileća Lake), certain preconditions for the development of sport or recreational fishing were created. However, even today, this branch of industry in the Municipality of Nikšić has not been developed, and the only place where something more has been done on artificial lakes in Montenegro regarding that matter is Lake Piva.

There are no precise data on catches of fish or fish stocks in artificial lakes in the Municipality of Nikšić for the observed period from 1945 to 1991. The catch of freshwater fish at the level of Montenegro was as follows: in 1988 - 338 tons, in 1989 - 303 tons, in 1990 - 304 tons and in 1991 - 264 tons. In winter months there are flocks of birds, mostly wild ducks, less frequently geese, cormorants, etc., which could also warrant this type of hunting. So far, this has been at a very low level in the Municipality of Nikšić, both in terms of financial investments and economic gain or profit (Radojičić, 2010; Radojičić, 2015; Statistical Yearbook of the Republic of Montenegro, 1993).

CONCLUSIONS

The industrialization process of Montenegro and the Municipality of Nikšić in the socialist socio-economic system was both indispensable and justified, with indisputable positive results. At the same time, agriculture, which until then was a leading economic activity, was gradually waning. Paradoxically, a farmer and a village that made an undeniable contribution to the industrialization of the state, eventually paid the heaviest price for that progress. The processes of violent collectivisation, unsuccessful experiments with the Peasant Working Cooperatives, material investments and public investments, which were mostly aimed at strengthening the industry, significantly weakened villages and farmers.

On the one hand, initiating the industrialization of the state was the right thing to do because it was a means by which Yugoslavia, and thus Montenegro as

part of it, was to be introduced into the order of the middle-developed countries of Europe. In many respects, the positive effects did arise out of it. Towns went through economic development, which enabled the population to reach better living and working conditions through the process of urbanization. Towns became centers of economic development, and a network of educational, cultural and health institutions expanded and thus appealed to the rural population who wanted to settle in them. The municipality of Nikšić is a quintessential example of such processes that took place in Yugoslavia and Montenegro. Once a municipality and a town, in which agriculture was a leading industry the majority of the population lived off, was gradually transformed into a highly industrial environment. Not only did the industrialization and urbanization of the municipality and the town alter the economic and social structure of the population, but it also changed the geographical features of the municipality. The construction of industrial facilities, the creation of artificial reservoirs and the expansion of the urban area have permanently taken away quality agricultural land, thus exerting a far-reaching effect on villages and agricultural production.

The process of industrialization was vital and indispensable in itself, but the speed it was carried out at, often with no quality expert analysis and assessment of its ultimate effects, brought about more adverse consequences for agriculture development. The full adverse effects of such an economic policy have become especially significant in Montenegro over the last three decades, when the industry has suffered a major decline and consequently led to the dismissal of more workers in this field. A village, which has been steadily declining in the socialist period, cannot appeal to the urban population to live in the countryside and develop agricultural production under present conditions. On the contrary, despite the slowdown of industrial production in towns, the process of turning villages moribund continues, and in the future, it is going to bring about even more severe effects on the economic development of Montenegro and the Municipality of Nikšić.

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THE DISTRIBUTION OF ZINC IN THE WATER, AQUATIC MACROPHYTES AND SEDIMENT OF LAKE SKADAR

SUMMARY

The use of aquatic plants as indicators of water ecosystems is based on their ability to absorb pollutants (heavy metals, chemical substances, etc.). The aim of this study is to determine the zinc content in sediment, water and plants in Lake Skadar, Montenegro, and monitor the distribution and adoption in the roots, stems and leaves of plants. The aquatic macrophytes *Phragmites australis*, *Ceratophyllum demersum* and *Lemna minor* were used as bioindicator plant species to define the contamination level of Zn. The Zn content of sediment, water and plants was tested at six locations around Lake Skadar in each season. The Zn content in the examined sediment was 47.6–135 mg kg^{-1} dry weight. The largest proportion of Zn based on the total amount of the sediment is incorporated into the crystal lattice of minerals (the residual fraction). In descending order, the highest amounts of Zn concentration in the studied macrophytes were as follows: *L. minor* > *C. demersum* > *P. australis*. The highest average content of Zn was detected in the root of *L. minor* (97.8 mg kg^{-1}) in October.

Keywords: Lake Skadar, zinc, *Phragmites australis, Ceratophyllum demersum, Lemna minor*

INTRODUCTION

Aquatic macrophytes are taxonomically close to earth/based plants, but they live in a completely different environment. Their entire cycle ends within a certain aquatic ecosystem, with little potential to avoid pollution, especially if they live in standing water where there is no significant dilution effect. Under such conditions, macrophytes participate in the process of the transfer of toxins by interacting with the atmosphere, water and sediment, and often in real conditions, also with suspended matter, detrius, and the like. Aquatic macrophytes are often in contact with potential pollutants across their whole body. Being similar to all primary producers, macrophytes react to changes in the quality of the environment in which they live (water/sediment) and are good

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bioindicators of surface water condition. The chemical analysis of aquatic plants leads not only to an understanding of the current situation but also to the evaluation of the trends in environmental changes in time and space (Kastratović et. al., 2015).

Zinc is an essential and useful element for plants, mainly as a part of various metalloenzymes. In most aquatic ecosystems, Zn^{2+} can be toxic for organisms. The phytotoxicity of Zn is manifested by morphological deformations: low levels of growth, small leaves and underdeveloped root systems (Jablanović et. al. 2003). The plant requirement for Zn is in the range of 10-50 mg kg^{-1} (Larcher, 2003). The normal range is 1-400 mg kg^{-1}, while the critical range is 100-400 mg kg^{-1} of Zn (Kabata-Pendias and Pendias, 1992).

Most of the zinc in the sediments is in a soluble form, bound to oxides of iron and manganese, carbonates and organic substances, while about 30% is insoluble. The proportion of zinc in the sediment depends mainly on the pH and the redox potential. If the ambience of the environment is anoxic, zinc is in an insoluble, bio-unavailable form, $Zn(OH)_2$. In an acidic environment and in oxidizing conditions, the content of the soluble zinc forms increases, Zn^{+2} and $Zn(OH)^{+}$. Zinc can be released into the water body due to the disturbance of the sediment, which leads to its oxidation and mobilization (Zoumis et. al., 2001).

Our research aims to find the Zn content per fractions of sediments, and estimate its bioavailability, including by analysing content ratios in the sediment, in the water and in the macrophytes, as well as plant tissue distibution, which can indicate the possible absorption or distribution mechanisms and the ability of the plants to bioaccumulate Zn.

All metal concentrations in natural vegetation vary from species to species (Popoviciu et. al., 2017). The aquatic macrophytes used as indicator species in this study Phragmites australis (Cav.) Trin. ex Steud., *Ceratophyllum demersum* L., and *Lemna minor* L. were taken from six locations around Lake Skadar, Montenegro. The plants, water and sediment samples were examined for their zinc content over four different periods of the year.

MATERIAL AND METHODS

Study area

Lake Skadar (19°03'-19°30’E, 42°03-42°21’N) is the largest lake in the Balkan Peninsula. It is located at the border between Montenegro and Albania. Two-thirds of the lake is in Montenegro. During the summer, Lake Skadar has a surface of 370 km^2, while in the winter the area is 540 km^2. The water level also varies seasonally from 4. 7 to 9. 8 m above sea level (Milošević et al., 2017). The lake is 44 km long and 13 km wide.

Sediment samples from Lake Skadar were collected from 6 locations around the lake: 1-Raduš (42° 13' 26, 85" N; 19° 09' 54, 44" E), 2- left estuary of Morača (42° 15' 55, 80" N; 19° 08' 31, 49" E), 3- right esturay of Morača (42° 16' 50, 18" N; 19° 07' 38, 92" E), 4- Plavnica (42° 16' 17, 48" N; 19° 12' 1, 01" E), 5-

Crni Žar (42°17' 49,30" N; 19°22' 23,75"E) i 6- River Crnojevića (42°21' 6,03"N; 19°02' 23,05"E).

Sampling collection

The samples of *P. australis* and *C. demersum* were collected four times during the season, from the beginning of April to the end of October, from six locations. Samples of sediment and water were taken at the same time and from the same places, where the plant material was collected.

Sediment sampling was conducted using an Eckman dredge to a depth of 0–20 cm. The sediment samples were placed in plastic boxes, carefully labeled and transferred to the laboratory for further analysis. Water samples were collected from the depth of 0. 5–1 m using 1. 5 L PET bottles. The samples were stored in a refrigerator (at 5 ± 2 °C).

Preparation of the samples for chemical analysis

Macrophytes

The sampled plant material in the laboratory was first washed with tap water, and then twice with deionized water. The plant parts were cut with stainless scissors, into the roots, stems and leaves of the macrophytes to determine the bioaccumulation diversity of the plant organs. The plant material was then dried at 75°C for 48 hours, and subsequently ground into a fine powder and homogenized. In order to avoid the influence of the matrix, the samples were mineralized. An amount of approximately 0. 5 g (± 0. 0001 g) of the prepared samples was measured and mineralized in a Milestone Microwave Ethos 1, with the mixture of HNO_3 and H_2O_2 (in a ratio of 5 ml : 2 ml). After digestion the solutions were diluted using deionized water to their final volume of 25.0 mL.

Sediment

The sediment samples were dried in air, and in a dryer at a temperature of 75°C for 48 hours. The dried sediment samples were ground in an agate mortar and sieved through a sieve <1. 5 mm. Approximately 0. 5 g (± 0. 0001 g) of the sample under pressure and high temperature was then mineralized with the mixture of HCl:HNO_3:HF (in ratio of 6 ml : 2 ml : 1 ml). After mineralization, the solutions were diluted with deionized water to their final volume of 25. 0 mL.

In order to determine the distribution of the Zn in the sediment we applied a modified BCR (the Community Bureau of Reference of the European Union) sequential extraction procedure to the sample sediment (Pueyo et. al., 2003).

Water

The water samples were filtered through a Millipore filter of 0. 45 μm and stored in plastic bottles of 1L by adding 2 mL of HNO_3.

All the samples of the plants parts, sediments and water were prepared three times and their average value was analyzed. In each batch of ten samples, a blank solution was measured. The concentration of Zn was determined using the ICP-OES technique on a Spectro Arcos instrument.

RESULTS AND DISCUSSION

Table 1. The seasonal minimum and maximum concentrations of zinc in the water (mg dm^{-3}) and the sediment (mg kg^{-1}) and the mean concentration ± standard deviation

Metal		April	June	August	October
Zn	Water	0. 002-0. 008 0. 005±0. 002	0. 002-0. 007 0. 005±0. 002	0. 003-0. 008 0. 005±0. 002	0. 003-0. 008 0. 005±0. 002
	Sediment	47. 6-117 75. 4±24. 0	56. 1-135 79. 1±29. 7	59. 1-128 76. 2±26. 1	53. 2-108 73. 8±19. 7

Table 2. The distribution of Zn (mg kg^{-1}) in fractions of sediments of Lake Skadar:
I- Removable and easy mobile; II- Reductabile; III- Oxidabile; IV- Residuale

Metal	Fraction	I	II	III	IV
Zn	Min. -max. Mean	0.06-0.54 0.22	11.6-37.4 22.7	19.9-32.6 25.2	12.6-59.7 28.0

Table 3. Seasonal changes in Zn content (mg kg^{-1} dry matter) in some parts of *Phragmites australis;* Min. and max. concentrations and the mean concentration ± standard deviation

Part of plant	Minimum - maximum concentrations Mean concentration ± standard deviation			
	April	June	August	October
root	18.4-40.2 28.4±8.33 a(c)	25. 3-49. 1 37.5±9.15 a(b)	36.3-72. 9 52.1±15.8 a(a)	21. 1-79. 4 45. 8±21. 6 a(ab)
stem	5.34-22.5 14.8±5.68 b(b)	17.1-29.1 24.4±4.21 b(a)	13.0-31.7 22.0±7. 67 b(a)	8.28-33.4 16.3±9.68 b(b)
leaf	12.8-31.7 22.4±6.86 ab(b)	13.1-44.8 29.0±11.1 ab(a)	17.4-48.4 30.7±11.1 b(a)	9.42-48.3 25.2±12.9 ab(a)

* The values of individual metals with the same first letter(s) are not significantly different at p = 0. 05 in the column (i. e., between the different parts of the plant);
* * The values in individual parts of the plant with the same letter(s) in parentheses are not significantly different at p = 0. 05 in the row (i. e., between seasons)

The significant translocation of Zn from the root to the above-ground organs was observed. The average seasonal root/stem ratio is 2.39 and root/leaf 1. 61. Schierup and Larsen (1981) find a higher concentration of Zn in the above-ground parts as opposed to other investigated metals, which mainly remain behind in the root and rhizomes. Babović et al. (2010) find the folowing relationships: rhizome/stem = 1.42 and rhizome/leaf = 1.32. Baldantoni et al. (2004) reported the ratio of root/leaves as 4.2 and root/shoots as 1.6. Świerk and Szpakowska (2011) indicate the low mobility of Zn, with a rhizome/leaves ratio of 3.7.

Table 4. Seasonal changes in Zn content (mg kg^{-1} dry matter) in some parts of *Ceratophyllum demersum;* Min. and max. concentrations and the mean concentration ± standard deviation

Part of plant	Minimum - maximum concentrations Mean concentration ± standard deviation			
	April	June	August	October
root	18. 4-40. 2 28. 4±8. 33 a(c)	25. 3-49. 1 37. 5±9. 15 a(b)	36. 3-72. 9 52. 1±15. 8 a(a)	21. 1-79. 4 45. 8±21. 6 a(ab)
stem	5. 34-22. 5 14. 8±5. 68 b(b)	17. 1-29. 1 24. 4±4. 21 b(a)	13. 0-31. 7 22. 0±7. 67 b(a)	8. 28-33. 4 16. 3±9. 68 b(b)
leaf	12. 8-31. 7 22. 4±6. 86 ab(b)	13. 1-44. 8 29. 0±11. 1 ab(a)	17. 4-48. 4 30. 7±11. 1 b(a)	9. 42-48. 3 25. 2±12. 9ab(a)

* The values of individual metals with the same letter(s) do not differ significantly at $p \leq 0.05$

Table 5. Seasonal changes in Zn content (mg kg^{-1} dry matter) in some parts of *Lemna minor;* Min. and max. concentrations and the mean concentration ± standard deviation

Metal		Part of plant	August	October
Zn	Min. -max. Average ± S. D.	root	59. 9-115 78. 6±25. 2	72. 1-115 97. 8±18. 6
	Min. -max. Average ± S. D.	leaf	41. 3-82. 5 58. 6±18. 2	48. 9-109 83. 2±27. 7

The distribution of Zn in the sediment

Zinc is present in the examined sediments of Lake Skadar in the range of 47. 6 to 135 mg kg^{-1} of dry sediment with an average value of 76. 1 mg kg^{-1}. The highest concentrations of Zn were observed in the sediment from River Crnojevica, where there is a canned fish factory with a small production capacity. The total content of Zn at the other sites is relatively uniform. In the short terms, the movement of heavy metals in soil is very slow (Dumitrel *et. al.,* 2017). It can be said that there is a slight amount of Zn in the exchangeable and acid soluble fraction of the sediment, with a maximum of 0. 54 mg kg^{-1}, or 0. 92 % expressed in percentage. Zinc is almost evenly deployed, with a few exceptions, to the II, III and IV fraction sediments of Lake Skadar. There is no higher variation between the sampling sites with regard to the share of Zn in individual phases of the sediment.

In their study Gao et al. (2010) detected zinc in all four phases of the sediment, with the highest concentration in the residual fraction, which is similar to the results of this study. Zinc is released from the sediment as a result of the microbiological oxidation of organic substrates and remobilized by co-precipitation and/or adsorption on the hydrous ferric oxides (Jones and Turki, 1997). The mobilization of Zn from the sediment to the water occurred under

anoxic conditions and soon stopped when the water phase became oxic (Petersen *et. al.*, 1995).

The bioaccumulation of zinc in macrophytes

Phragmites australis

According to the present study, the roots of *P. australis* actively adsorbed Zn, and contained the most Zn during the whole sampling period.

Schierup and Larsen (1981) report maximum increase in Zn in the leaves and stem during the growing season, and that after that, the zinc concentration is reduced. Bragato *et al.* (2009) show almost similar concentrations of Zn in the root, leaves and stem of *P. australis*, from July to October. The zinc concentration in the leaves is 2-3 times higher than in the stem and the rhizome in December.

The distribution of the metal in some parts of the plant is the result of differences in the amount and rate of metal input, primarily by the root pressure and its release into the environment mainly through the transpiration of the leaves (Lasat, 2000; Ravera, 2001). Some metals are accumulated in roots, probably because of some physiological barriers for the transport of toxic elements in traces. The metals essential for metabolic needs are easily transported to the above-ground parts of the plant.

Ceratophyllum demersum

The seasonal concentrations of Zn in the stems ranged from 16. 7 to 75. 6 mg kg^{-1} (the mean value was 38. 7 mg kg^{-1}), and in the leaves from 25. 6 to 114 mg kg^{-1} (the mean value was 69. 2 mg kg^{-1}). Borišev *et al.* (2006) found 20. 6 mg kg^{-1} Zn in the tissues of *C. demersum* and Babović *et al.* (2010) found 106 mg kg^{-1}. Pourkhabbaz *et al.* (2011) observed a higher content of Zn in the stem of *C. demersum*, in the range of 19. 89 to 40. 01 mg kg^{-1}. Fawzy *et al.* (2012) noticed no significant seasonal differences in the concentration of Zn in *C. demersum*. They found a higher content of Zn in the leaves of the plants, which was also supported by El-Sarraf (1995) who found that the Zn content of the leaves was much higher.

The concentration of Zn in the stem of *C. demersum* decreased from April, when it was the highest, and thereafter to the end of the growing season, it remained almost constant. The content of Zn in the leaves from increased from April until the beginning of the growing season, subsequently fell until the end of the growing season and then grew once more to the end of the vegetative phase.

Osmolovskaya and Kurylenko (2005) reported a 5. 3 times higher Zn concentration in the tissues of *C. demersum* from contaminated compared to uncontaminated areas. The content of Zn was slightly higher than *P. australis*, which is also the conclusion of our study.

Lemna minor

The mean seasonal concentration of Zn in the root is 88. 2 mg kg^{-1}, and 70. 9 mg kg^{-1} in the leaf. As was the case with Pb, Jamnická *et al.* (2006) found

a lower value of Zn in the tissues of *L. minor*, 14. 68 mg kg^{-1}, and Iram *et al.* (2012) a higher value, of between 35 and 213 mg kg^{-1} of Zn (and a mean of 106 mg kg^{-1}), compared to the concentration present in the *L. minor* from Lake Skadar. The concentration of Zn in the roots and in the leaf at all locations was higher in October than in August. Significantly higher spatial dispersion results than seasonal results are clear in terms of Zn content. The highest differences were observed in the zinc content of the leaves between the two periods of research.

Khellaf and Zerdaoui (2009) report that *L. minor* can survive in a medium containing of 15 mg dm^{-3} Zn. Although biomass and plant growth rates were reduced at these concentrations, the authors point out that *L. minor* can be a good candidate for the treatment of wastewater contaminated with Zn. Radić *et al.* (2010) determined that, due to the high potential of the bioaccumulation of Zn, *L. minor* has the potential for the phytoremediation of water bodies contaminated with low levels of Zn.

Comparative analysis of Zn accumulation in the examined macrophytes

Table 6. Temporal and spatial mean content of Zn (mg kg^{-1}) for the research season in the organs of *Phragmites australis, Ceratophyllum demersum* and *Lemna minor*

Phragmites australis			*Ceratophyllum demersum*		*Lemna minor*	
Root	stem	leaf	stem	leaf	root	leaf
41.0	19.4	26.8	38.7	69.2	88.2	70.9

Table 7.Mean annual values of BCF for Zn in the organs of *Phragmites australis, Ceratophyllum demersum* and *Phragmites australis, Ceratophyllum demersum* and *Lemna minor* (whole plants) in relation to the sediment

	Phragmites australis	*Ceratophyllum demersum*	*Lemna minor*
Zn	1.19	1.49	2.13

The concentration of Zn in the examined macrophytes decreases in the following order: *L.minor* > *C.demersum* > *P.australis*. The bioaccumulative ability decreases in the same order.The highes value of Zn content was recorded in the root of *L.minor* (97.8 mg kg^{-1}) in October and the lowest in the stem of *P.australis* in April and October (14.8 and 16.3 mg kg^{-1} respectively) (Figure 1). They are different seasonal variations in the concentration of Zn in the tissues of the macrophytes.In *P .australis*, the concentrations in all parts of the plant rise from April to August and then fall until to October.The parts of plant the *L.minor* follow the same trend, that is, they show an increase in concentration from August to October, both in the root and in the leaf. The content of Zn in both plants was higher in the root, and the smaller part is transferred to other organs.In the case of *C. demersum*, most of the metal content is transferred to the leaf,

where the zinc concentration increases from April to June, decreases until August and then rises again until the end of the growth season.

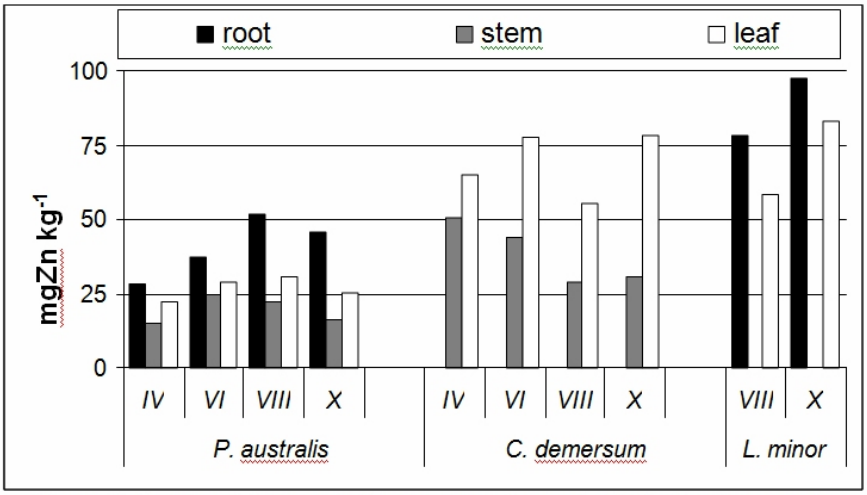

Figure 1. Seasonal changes in Zn concentration in the individual organs of *Phragmites australis*, *Ceratophyllum demersum* and *Lemna minor*

CONCLUSIONS

Zinc is present in the examined sediments of Lake Skadar in the range of 47.6 to 135 mg kg^{-1} of dry sediment with an average value of 76.1 mg kg^{-1}. The highest concentrations of Zn were observed in the sediment from River Crnojevica.

There is a slight amount of Zn in the exchangeable and acid soluble fraction of the sediment, with a maximum 0.54 mg kg^{-1}, or 0.92 % expressed in percentage.Zinc is, with a few exceptions, almost evenly deployed to the II, III and IV fraction sediments of Lake Skadar. There is no significant variation between the sampling sites with regard to the share of Zn in the individual phases of the sediment.

The concentration of Zn in the examined macrophytes decreases in the following order: *L. minor* > *C. demersum* > *P. australis.*The highest value of Zn content was recorded in the root of L.minor (97.8 mg kg^{-1}) in October and the lowest in the stem of *P.australis* in April and October (14.8 and 16.3 mg kg^{-1} respectively).

According to the present study, the roots of *P. australis* actively adsorbed Zn, and contained the most Zn during the whole sampling period.A significant translocation of Zn from the root to the above-ground organs was observed.The concentration of Zn in the stem of *C. demersum* decreased from April, when it was highest, and thereafter to the end of the growing season, it remained almost constant.The content of Zn in the leaves from increased from April until the beginning of the growing season, subsequently fell until the end of the growing season and then grew to the end of the vegetative phase.The concentration of Zn

in the roots and in the leaf of *L. minor* at all locations is higher in October than in August. Significantly higher spatial dispersion results than seasonal results are clear for Zn content in all parts of *L. minor*.

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ANALYSIS OF THE ORGANIZATION OF PROTECTED SPECIES MONITORING IN SERBIA

SUMMARY

Monitoring of protected species within protected areas (PA) is an important component of PA management. In addition to the framework that defines the basic preconditions for PA management and the financing mechanisms that ensure sustainable financing for PA, a structural component of PA management is monitoring of protected species and changes in PA over a long period of time. In Serbia, key monitoring is carried out by the Institute for Nature Conservation as well as by PA managers. This research aimed to identify the management practices utilized for monitoring PA by different groups of managers. To do so, the organization of monitoring of protected species was observed. The survey indicated that monitoring in most PA is conducted by a ranger service, and experts are only present when public enterprises manage national parks. A lack of databases was identified for most PA managers, except the public enterprise Vojvodinašume, and the problems related to reintroduction of species are mostly financial. Therefore, in the next period, it is necessary to improve this aspect of PA management by providing additional financial resources and better organizing the monitoring of protected species in Serbia.

Keywords: protected areas, protected species, monitoring, protected area managers, Serbia

INTRODUCTION

Organization of protected area (PA) management in Serbia includes complex interaction of different actors, rules and responsibilities which are usually interacting with sector of environment and forestry. Sustainable management of PA represents interaction of these two sectors, as well as involvement of different institutions which are coming from state or other management level. Rules and responsibilities are regulated by law and bylaw regulations, and can be on national, regional or international level. Organization

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of PA management includes management framework, structural characteristics of PA management and mechanisms of financing PA, while one of structural characteristics of PA management includes monitoring of protected species (Djordjevic, 2018). According to Law on nature protection (2009) this activity is defined as „…planned, systematic and continues monitoring of nature condition i.e. its biological, geological and landscape diversity“. Monitoring is carried out because the need of data collection of PA characteristics in different periods, assessment of current state and drawing conclusions about changes during that time (Yoccoz et al., 2001). Subject of the monitoring represents biological diversity (Curovic et al., 2011; Milosevic et al., 2019), changes in landscape, historical and cultural changes, land use and human activities, external factors and the impact of management policies and programs (Martinic, 2010). Protected areas ensure conservation of natural resources and contribute to maintaining the ecological balance in the region (Liogchii et al., 2017).

Reasons for monitoring can be scientific and managerial, scientific focus is solely on "… learning, developing and understanding of the behavior and dynamics of the monitoring system…", while managerial character is based on "… providing information related to management decisions" (Yoccoz et al., 2001). This is an important characteristic because "… monitoring that does not provide relevant information to decision makers is not useful for management and unnecessary because it uses human and fiscal resources, which may be directed elsewhere" (Rao et al., 2009). Also, climate change impact on flora has been receiving increasing attention throughout the world (Šimunić et al., 2019; Chivulescu et al., 2019). As one of the most important strategic, developmental and planning aspects of nature protection, Puzovic (2016) cites biodiversity monitoring as "… a way of looking at conditions, and above all, changes in the field of nature protection and biodiversity". Implementation of this monitoring should be done through the nature protection manager, who coordinates the protection and monitoring activities in the PA, while the supervisor monitors the situation and changes (Puzovic, 2008).

Today, the management of PA involves the presence of managers coming from the public and private sectors, and this has conditioned the process of decentralization, which has shifted the focus of PA management to managers who do not traditionally come directly from the public sector (Djordjevic et al., 2014; Djordjevic et al., 2019/a; Nonic et al., 2014). In this research, the PA refers to the areas that have been separated from traditional use and given to the management of individual legal entities. These legal entities comes from the private and public sectors and can be divided on the basis of "… who makes decisions and can be held responsible" (Borrini-Feyerabend et al., 2013). PA managers have a significant role to play, because the use of PA depends directly on their presence and the activities they carry out on the field.

In Serbia management of PA can be given both to public and private sector, while the biggest managers are coming from public sector. Within this sector, public enterprise (PE) "Srbijašume" and PE that manages national parks

(PENP) are the biggest managers and traditional they are coming from forest sector (Djordjevic et al., 2014). Monitoring of protected species is carried out on the level of these PE, other PA managers, as well as by the different institutes (ex. Institute for nature conservation, Institute for biological research etc.) and different environmental NGO organizations.

The goal of this research includes determination of monitoring practices within different groups of PA managers, while subject of this research are structural characteristic of PA management i.e. monitoring practices. The purpose is to establish preconditions for improving of current monitoring in Serbia and develop proposal for its improvement.

MATERIAL AND METHODS

As a research method door-to-door survey was used to collect primary data. Collection of this data was carried out in the period December 2014 - Jun 2015. The survey consisted of three sets of questions (basic characteristics, structural characteristics and financing mechanisms). For this paper, only questions related to monitoring of protected species were used (questions on structural characteristics).

The population was determined on the basis of the PA Register (2012) from which 63 PA were selected. The criteria for the sample selection were: the existence of a PA manager[2], PA size (areas smaller than 10 ha were excluded) and PA categories.

In order to test differences between different managers of PA, previously defined typology of PA managers was used (Djordjevic *et al.*, 2014). Based on this typology, and collected answers from PA managers, the following groups of PA managers were formed: a) PE "Srbijašume" (PES); b) PE "Vojvodinašume" (PEV); c) PENPs[3]; d) other PE (OPE); e) other managers from the public sector (OPS) and f) the private sector (PrS)[4] (Djordjevic et al., 2019/a; Djordjevic et al., 2019/b). Questionnaire was codded using SPSS software (*ver. 21*) (Pallant, 2011) and data were processed by each question using the descriptive statistics and the frequency analysis (Neumann, 2014). Furthermore, χ^2 test of independence was used to determine statistically significant relation (Pallant, 2011). This research also included review and comparative analysis (Wunder et al., 2008; Keča, Marković, 2019) of the elements in the field of nature protection.

RESULTS

In order to test monitoring practices in Serbia, this research studied monitoring method, existence of databases of monitoring species, need for

[2] In Serbia, certain PA do not have a manager (Djordjevic et al., 2014)

[3] PE „NP Đerdap", PE „NP Kopaonik", PE „NP Fruska gora" and PE „NP Tara"

[4] The PrS is not subdivided into smaller groups, but seen as one group, in order to be able to compare the characteristics of managers between the groups of mangers.

reintroduction of different species and its problems, as well as the process of NATURA 2000.

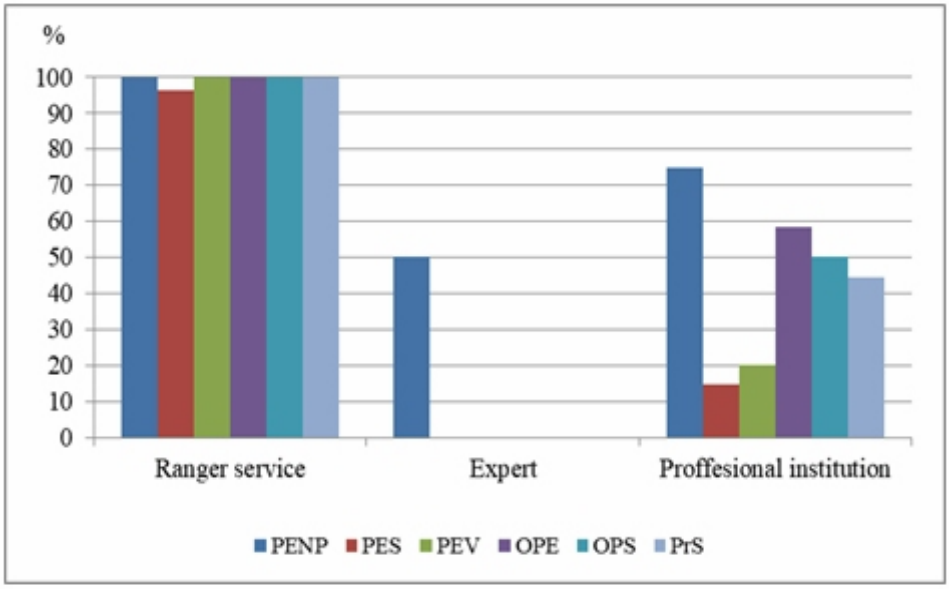

Figure 1. Monitoring method within different groups of managers

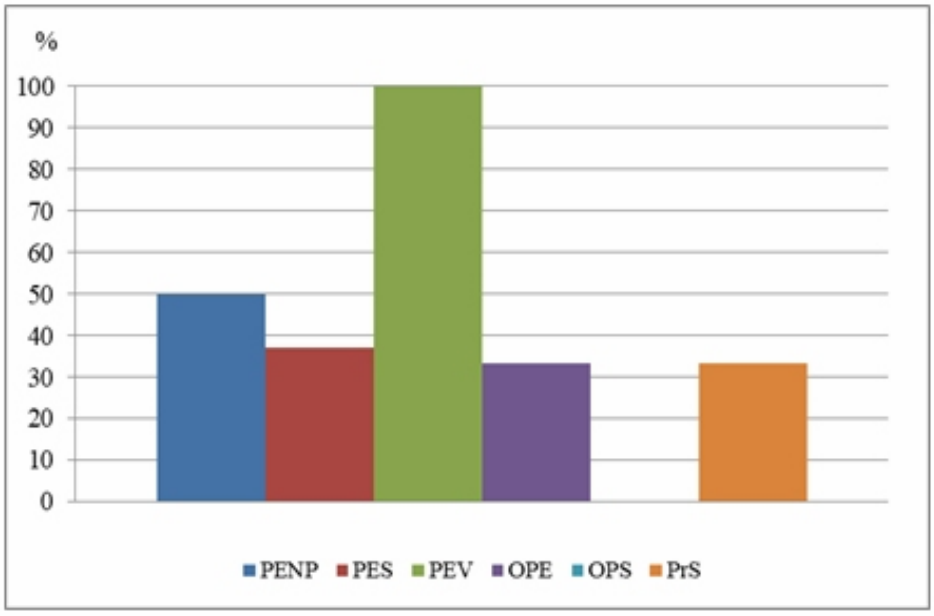

Figure 2. Databases on protected species within different groups of managers

Within this research, three types of monitoring methods were detected. Monitoring is carried by rangers, expert within manager and professional institution. As it can be seen in Figure 1 monitoring by an expert exists only in

the PENP in half of the cases, while the professional institutions involved in monitoring are the highest in the PENP (75.0%) and the least in the PE "Srbijašume"(14.8%). Monitoring by ranger is mostly present as monitoring practice within all groups of managers. Using the $\chi 2$ independence test, a statistically significant relationship was found between the groups of managers and the monitoring method by an expert ($\chi 2 = 12.19$, df = 5, p = 0.03) and professional institutions ($\chi 2 = 12.38$, df = 5. p = 0.03), while no statistically significant relationship was found for monitoring by rangers ($\chi 2 = 1.72$, df = 5, p = 0.89). Effect size value (V = 0.00), for R-1 = 1 was assessed as small, when monitored by an expert, and when monitored by expert institutions, effect size value (V = 0.04), for R -1 = 1 is rated, also small.

Presence of databases are crucial for monitoring of species in long time period and Figure 2 shows these differences. It can be seen that in the PE "Vojvodinašume", in all cases the database exists, unlike other groups of managers. Also, by applying the $\chi 2$ independence test, a statistically significant relationship was found between the groups of managers and the presence of the database ($\chi 2 = 15.86$ df = 5, p = 0.00). Effect size value (V = 0.03), for R-1 = 1, was considered small.

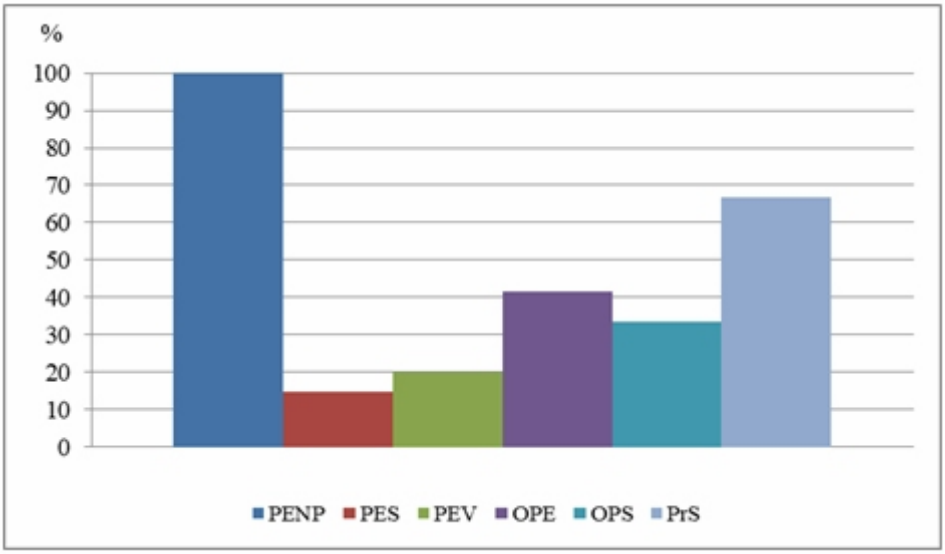

Figure 3. Need for species reintroduction within different groups of managers

Considering the need for reintroduction of certain species, it can be seen that it is completely present at PENP, unlike PE "Srbijašume" (Figure 3). Using the $\chi 2$ independence test, a statistically significant relationship was found between groups of managers and the need for reintroduction ($\chi 2 = 18.46$, df = 5. p = 0.00), and the effect size value (V = 0.01) for R-1 = 1 was estimated to be small.

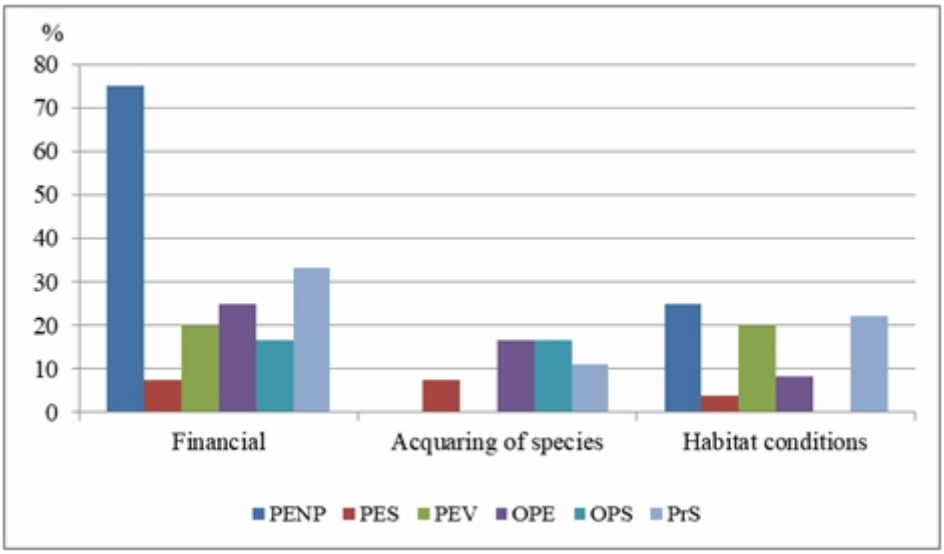

Figure 4. Problems in reintroduction within different groups of managers

In terms of type of problems (Figure 4), financial problems are the least present with PE "Srbijašume" (7.4%), as opposed to PENP (75.0%). Using the same test, no statistically significant relationship was found between groups of managers and financing problems ($\chi 2 = 10.02$, df = 5, p = 0.08), which is the case for acquiring of species ($\chi 2 = 2.87$, df = 5, p = 0.72) and habitat conditions ($\chi 2 = 5.15$, df = 5, p = 0.40).

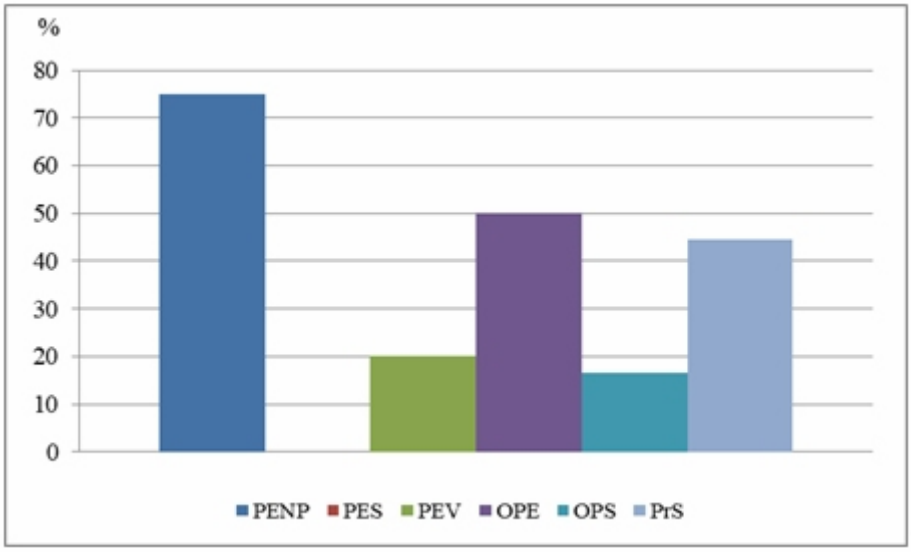

Figure 5. „Natura 2000” in PA within different groups of managers

Identification of Natura 2000 started in in two thirds of cases within PENP, and none of the PA within the PE "Srbijašume" started this process (Figure 5). Using the $\chi 2$ independence test, a statistically significant relationship was found between the groups of managers and Natura 2000 ($\chi 2 = 25.25$, df = 5, p = 0.00), and the effect size value (V = 0.00), for R- 1 = 1 was rated small.

DISCUSSION AND CONCLUSIONS

Regarding to monitoring of protected species in Serbia, it was found that monitoring by an expert only exists in the PENP in half of the cases, while professional institutions are involved in monitoring, mostly within PENP and least within PE “Srbijašume”. Protected species databases are completely present in the PE “Vojvodinašume”, unlike other groups of managers. All PENP have needs for reintroduction of plant and animal species, while the smallest need is within PE “Srbijašume”, while the main problem of reintroduction is financial nature and is very pronounced in PENP. The Natura 2000 process has been started in most of PENP, unlike other groups of managers.

Research on the monitoring of protected species has been conducted within the methodology developed within the international organization IUCN. Thus, research in Slovenia indicates that almost all PA have monitored PA and that the current level of environmental research is appropriate and lacks socio-ecological research (Veenviet, Sovinc, 2008), as is the case with Croatia (Porej and Rajkovic., 2009), while in Serbia, the situation is completely opposite in terms of data collection and research on socio-ecological issues and processes in the PA environment (Piscevic, 2009).

In order to adequately perceive all changes occurring over a long period of time in the PA, it is necessary to introduce appropriate monitoring, however what has been done so far “… is far from meeting the minimum needs and EU standards” (Puzovic, 2016). This author states that professional and scientific institutions do not have the appropriate financial resources to deal with this comprehensively and systematically, whereas the managers of the PA, to whom it is a legal obligation, are generally disinterested or without the professional and material capacity to implement it appropriately, except in rare case of NP and in the area of autonomous province of Vojvodina (Puzovic, 2016). The monitoring of protected species is carried out in all PA in Serbia, mostly through the ranger service of the PA, with the exception of the PENP, who in the half of the cases have an expert involved in these activities. Slovenia also monitors conditions in PA and the current level of environmental research is satisfactory (Veenviet and Sovinc, 2008). On the other hand, in Serbia there is a problem of continuous monitoring, of certain species of plants and animals, which is only carried out in individual cases by PENP. Also, there is a problem of lack of digital databases on protected species of plants and animals in all studied PA managers (exception is PE “Vojvodinašume”).

Proposals for improving of existing monitoring include the establishment of an additional organizational unit for monitoring of protected species at the

level of the PA manager and external involvement of organizations/institution specialized in monitoring. One of the problems in realization of this activity could be limited financial and human capacities, so sustainable model of financing PA needs to be formed. Financing of PA by state should be separated in two directions, one dealing with running costs and other with the costs of improving and monitoring of PA. As a proposal for future research studies, it is suggested identification and analysis of the needs for monitoring of protected species, as well as the necessary organizational, human and financial capacities.

ACKNOWLEDGEMENTS

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THE FIRST REPORT OF THE INVASIVE ALIEN WEED JERUSALEM ARTICHOKE (*Helianthus tuberosus* L.) IN THE REPUBLIC OF NORTH MACEDONIA

SUMMARY

A population of Jerusalem artichoke (*Helianthus tuberosus* L.), an invasive plant A population of Jerusalem artichoke (*Helianthus tuberosus* L.), an invasive plant species native to North America, was recorded in 2016 near Gradište and along the regional road R 1204 (Gradište, Skačkovce, Dobrošane and Kumanovo) in the northern mountainous part of the Republic of North Macedonia. *H. tuberosus* is a new species that is alien to Macedonian flora. Surveys revealed intensive growth and low- to medium-density populations of *H. tuberosus*. The population density was not quantified, but several stands of different sizes were found. An ecological risk assessment based mainly on knowledge about historical invasions in north-western and central European countries showed that this species is a serious threat to Macedonia's biodiversity. Biological invasion of *H. tuberosus* affects global biodiversity, and the invaded ecosystems may suffer from significant loss of economic and cultural value. Specifically, is a threat to biodiversity in wet habitats, natural and extensively managed habitats, riparian areas and swamps. It grows best in habitats that are repeatedly disturbed by floods (i.e. riparian areas), but it may also occur in ruderal and agricultural environments. Although many herbicides can be used to control *H. tuberosus*, their use is limited as the plants are often near waterways, where use of herbicides is not recommended. Other control methods are time-consuming and can be quite costly.

Keywords: invasive weed, environmental impact, control, forecast, Republic of North Macedonia

INTRODUCTION

The Jerusalem artichoke (*Helianthus tuberosus L.*) (syn. *Helianthus tomentosus* Michx.), which is also called the topinambur (Alex et al., 1980), sunchoke, sunroot, girasole, Canada potato, fusichoke, sunroot, or earth apple

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(Wyse and Wilfahrt, 1982), is an angiosperm, C3 warm-season plant species of *Asteraceae* (*Compositae*) family (Monti et al., 2005; Tassoni et al., 2010). Kosaric et al., 1984) reported that there are about 102 common names associated with *H. tuberosus*.

The genus *Helianthus* contains some 70 species, which are annuals, herbaceous perennials or shrubs in North, Central and South America (Rehorek, 1997). H. *tuberosus* is a perennial that is grown as an annual (Anonymous, 2011). According Verburg et al., (1996) and Crawley (1997) *H. tuberosus* belongs to "pseudoannual" group: the death of the whole plant occurs by the end of vegetation, except stem tubers in the soil, from which new shoots – showing total genetic identity with the mother plant – will emerge on the next spring. They are considered as clonal plants without continuous inter-clonal relations in time.

H. tuberosus is a native of North America (Cosgrove et al., 1991) and is thought to have originated in the Great Lakes area (Simmonds, 1976) or possibly in the Ohio and Mississippi River valleys (Wyse et al., 1986). Some authors referred to *H. tuberosus* as being native also in Canada (Swanton et al., 1992). Munro and Small (1997) stated that plants found at wild or ruderal sites in Canada would be escapes from cultivation and distributed to many countries of the world (Swanton et al., 1992). It is unclear whether *H. tuberosus* was used only from wild plants or whether it had already been domesticated when encountered in the region of Massachusetts by Samuel de Champlain in 1605 (Heiser, 1978). Once established, *H. tuberosus* is able to outgrow its competitors as it reaches great heights in a short period of time and thereby shades other plants that are living in the close vicinity (Swanton and Cavers, 1989).

The cultivated forms may have developed in southern Canada, from where they were dispersed to Western Europe early in the 17th century and subsequently to other temperate parts of the Northern Hemisphere (Scoggan, 1979; Kompała-Baba et al., 2005). The first escaped plants were found in the mid 19th century in some countries, the invasive spread began mostly around 1900 and became more rapid in central Europe in the 1930s (Hartmann et al., 1995; Fehér and Končeková , 2001). In the second half of the 20th century it became a serious invasive alien species (Ludwig et al., 2000; Balogh, 2006), and also a common weed problem (Balogh, 2001; Konvalinkova, 2003; Rehorek, 1997) in all parts of Europe. It is on the EPPO list containing the names of the most 34 dangerous invasive species.

In the past it was considered as a typical weed of natural and semi-natural conservation areas. It can locally occur on alluvial weed communities and, due to its intensive vegetative reproduction capacity and shading effect, it can create homogenous stands also (Balogh, 2006). Once established, *H. tuberosus* plants exhibit a rapid increase in plant height, number of leaves and tubers through one life cycle (Swanton and Cavers, 1989). This robust growth habit enables *H. tuberosus* to outcompete most other plant species in arable land. Allelopathy as a type of interference among higher plants is also believed to play an important role

in its intensive spreading (Tessio et al., 2010). This is the first document about the presence of invasive *Helianthus tuberosus* L. in Republic of North Macedonia.

General Description

(*Helianthus tuberosus* L.) (syn. *Helianthus tomentosus* Michx.)

The Jerusalem artichoke (*Helianthus tuberosus* L.) is an erect, rhizomatous perennial herb, up to 3-4 m high, scarcely to moderately branched in upper half of stem, hirsute in most above-ground parts. The root system is adventitious (in plants not grown from seed), fibrous and develops cord-like rhizomes that can reach more than 1 m in length. The apical part of the rhizome is swollen and forms a fleshy tuber. Tubers formed by thickening of short and stout or long and slender underground stolons, ellipsoid to globose, 2-8(-15) x 3-6 cm, whitish, yellow, red or purple, with small scale leaves and axillary buds. Leaves opposite or in whorls of three in lower plant part, in upper part alternate, simple; petiole 2-4 cm long, winged above; blade ovate to ovate-lanceoliate, 10-20 cm long, base tapering into petiole, margin irregularly serrate, apex acute, veins prominent with three main veins.

The inflorescence is a pseudanthium borne alone or in groups at the end of the stem or on terminal axillary branches. The flower head is 5-11 cm in diameter (much smaller than that of the sunflower) and bears many small yellow tubular fertile flowers surrounded by yellow ray sterile flowers, the ligules of which are thought of as petals. Fruit an achene, oblongoid, containing a mottled black or brown seed, 5-7 mm long, flattened at the sides, brownish with dark stripes, thinly hairy (Kays et al., 2008; Fnaec, 2006).

Phenology

In temperate regions, *H. tuberosus* requires at least 6.7ºC of soil temperature for sprout development (Kays and Nottingham, 2007), and cold temperatures are needed to break dormancy (5°C or less) (Denoroy, 1996). Kays and Nottingham (2007) noted that *H. tuberosus* is a photoperiod-sensitive short-day plant that requires long light periods followed by shorter light periods to trigger the shift to reproductive stage of development. In addition, temperature is also important factor affecting floral buds and inflorescence formation (Fenner, 1998; Hassan et al., 2005; Dasumiati et al., 2015). Several reports indicated that low temperature delayed floral bud formation in many plant (Konvalinková, 2003; Kaleem et al., 2010). Therefore, floral bud formation needs sufficiently high temperatures. A cooler temperature can result in a return to vegetative growth. Thus, short day reduced the number of days to flowering.

H. tuberosus is diploid ($2n = 102$) (Duke, 1978), and seed production varies with clone (Konvalinková, 2003) and usually wild clones produce 5 seeds per flower (Kays and Nottingham, 2007). *H. tuberosus* grown in temperate regions had maturity of 125-150 days (Kays and Nottingham, 2007).

MATERIAL AND METHODS

First record in Republic of North Macedonia

On 13th of August 2016, a visit to maize field trials with herbicides located in the Northern mountainous part of the Republic of North Macedonia (Fig. 3) near the village Gradište (Latitude: 42° 1' 23.82" N, Longitude: 21° 53' 9.48" E) and alongside the regional road R 1204 (Gradište, Skačkovce, Dobrošane and Kumanovo) (Fig. 4), revealed an intensive growth of Helianthus tuberosus L. (Jerusalem artichoke), a new alien species to the Macedonian flora.

Figure 1. *Helianthus tuberosus* L. (Jerusalem artichoke) (Photo by Z. Pacanoski)

Figure 2. High dense population of *H. tuberosus* growing in the man-made habitats (Photo by Z. Pacanoski)

Figure 3. Maping of *Helianthus tuberosus* L. (Jerusalem artichoke)

Figure 4. Satellite map of the regional road R 1204

Figure 5. Population of invasive *H. tuberosus* growing in the man-made habitats alongside the regional road R 1204 (Photo by Z. Pacanoski).

Figure 6. Population of invasive *H. tuberosus* growing alongside the regional road R 1204 (Photo by Z. Pacanoski).

On the 30th of September 2016, the site was surveyed to estimate the extent of the invasion. These surveys revealed an intensive growth (intensive flowering stage) and a low to medium dense population of *H. tuberosus*. The population's density was not quantified, but several stands of different sizes were found. The largest stands were approximately 10-15 metres at their widest point. During the second survey, extended the area of observation, and the plant was found mainly in human influenced and man-made habitats such as roadsides, ruderal areas, wastelands near the regional road R 1204, house yards as ornamental plant and river-bed of the Kumanovska Reka (Kumanovo river, Fig. 5. 6. 7. and 8).

Figure 7. Population of invasive *H. tuberosus* growing alongside the River-bed (Photo by Z. Pacanoski).

Figure 8. Population of invasive *H. tuberosus* in the man-made habitats alongside the regional road R 1204, (Photo by Z. Pacanoski)

The green area in the map is the area of the country where the villages Gradište Skačkovce, Dobrošane and city Kumanovo are situated and where the plants were observed and the blue areas are lakes.

RESULTS AND DISCUSSION

Similar to our findings, *H. tuberosus*, according to Alex and Switzer (1976), Gleason and Cronquist (l99l), is frequently found in moist habitats such as river and stream banks, meadows and waste areas, as well as in cultivated fields and orchards (Wyse et al., 1986; Wall and Friesen 1989).

H. tuberosus phytocoenoses occupy refuse dumps, edges of allotments and roadsides, urban wastelands or sites where the fresh soil layer was deposited. They do not cover large areas and can be found in the mosaics with ruderal or nitrophilous plant communities (Kompała-Baba et al., 2005).

Kopecký (1985), Hejný et al., (1979), Oberdorfer (1983) (cit. by Kompała-Baba et al., 2005) placed *H. tuberosus* stands from the ruderal sites into the *Eu-Arction*, the *Dauco-Melilotion* or the *Aegopodion podagrariae* alliances. Species commonly associated with *H. tuberosus* in two grassland populations, mown once per year, in London Ontario are: *Saponaria officinalis* L., *Daucus carota* L. *Elytrigia repens* (L.) Nevski., *Asclepias syriaca* L., *Dactylis glomerata* L., *Achillea millefolium* L., *Trifulium pratense* L., *Plantago lanceolata* L., *Glechoma hederacea* L., *Taraxacum officinale* Weber, *Poa* spp., *Vicia* spp. and *Galium* spp. Weed populations of *H. tuberosus* in southern Ontario have been found in corn, soybean and small grain fields. They are associated with other common weeds such as *Chenopodium album* L., *Amaranthus retroflexus* L., *Amaranthus powellii* Wats., *Abutilon theophrasti* Medic., *Setaria viridis* (L.) Beauv., *Ambrosia artemisiifolia* L. and *Sonchus* spp.

Nearly all documented research concerning *H. tuberosus* applies to areas between latitudes 30 and 50°N. It grows in places where annual precipitation ranges from 310 mm to 2820 mm (mean of 40 cases = 1001 mm) and where annual temperatures are between 6.3°C and 26.6°C (mean of 40 cases = 13.3°C) (Duke, 1979). Favourable climatic conditions might seem to be a key predictor of *H. tuberosus* distribution in Republic of Macedonia. In that context, the place where it was found belongs to region with medium annual precipitation (564 mm) and mean year temperature of 12.6°C (Kostov, 2003). Also, it does well in most soils with pH ranging from 4.5 to 8 (mean of 37 cases = 6.4) and may tolerate salinity. However, it prefers loose, loamy and well-drained soils and is completely naturalized on moist, nutrient-rich, sandy or loamy soils, especially along rivers (Hartmann et al., 1995). Though the plant is tolerant of winds, saline ones have deleterious effects. However, it tends to deplete soils (Kays et al., 2008; FNA, 2006).

Taking into consideration that, *H. tuberosus* was found in human influenced and man-made habitats in border region, probably the introduced pathway may have been human activity. The place of starting point of the invasion was at the Northern part of the Republic of Macedonia much closed to

the Serbia, where *H. tuberosus* is consider as one of the most widespread alien plant species (Stanković-Kalezić et al., 2007; Vrbničanin et al., 2009; Vrbničanin, 2013).

H. tuberosus was brought to Europe for the first time in 1607. Early in the 17th century it was distributed to several European countries: the first plants were mentioned in France, in 1614 it was brought to the Netherlands, 1614 to Italy, 1617 to England and 1627 to Germany. Whereas the motive for the first introduction may have been botanical curiosity, it was soon grown for the edible tubers on a large scale. In the mid-18th century it was widely replaced by the potato as a staple food in central Europe.

The first escaped plants were found in the mid 19th century in some countries, the invasive spread began mostly around 1900 and became more rapid in central Europe in the 1930s (Hartmann et al., 1995). Today, it is cultivated and escaping, often invasive, in many temperate areas in Europe, Asia, New Zealand, and tropical South America (Weber, 2003).

Environmental impact

The discovery of a well-established population of *H. tuberosus*, as a highly invasive alien species in the Macedonian flora, is a significant concern, particularly in the Northern part, where dense stands of *H. tuberosus* monoculture were recorded (Fig. 1 and 2). Biological invasions of *H. tuberosus* affect biodiversity worldwide (Kosaric et al., 1984), and, consequently, the invaded ecosystems may suffer from significant losses in economic and cultural values.

As the species with potentially high negative influence on biodiversity, *H. tuberosus* is the threat to biodiversity in wet habitats, natural and extensively managed habitats (Hartmann et al., 1995; Kowarik, 2003), riparian areas and swamps, as the plant which is able to successfully compete directly with native species for space, light and nutrients (FNA, 2006; Duke, 1983). It grows best in habitats repeatedly disturbed by floods (riparian areas), but may also occur in ruderal and agricultural environments (Zganciková et al., 2012).

In Western European climatic conditions (Belgian, for example), the plant does not produce viable seeds and propagates vegetative. Tubers and pieces of rhizomes are transported with rodents and flowing water, especially winter floods. It is in strong expansion in neighbouring countries, especially in France, Germany and Switzerland. *H. tuberosus* is abundant in natural settings, such as riverbanks of European countries (Schnitzler et al., 2007), especially in Austria (Walter et al., 2005), Croatia (Vendula, 2008), Slovakia (Fehér, 2007), and Ukraine (Protopopova et al., 2006). The plant can produce dense and persistent monospecific populations along rivers, river banks and floodplains where it outcompetes native species, slows down natural colonisation by trees and favours river bank erosion (Krippel and Colling, 2006; Pfeiffenschneider et al., 2014). Invasive populations on river banks can result in damage to flood protection constructions, which can impact on the environment. It produces phytotoxic compounds and can be as competitive as *Fallopia japonica* in alluvial habitats.

H. tuberosus can be a weed of agricultural fields either by invading fields such as forage crops (Park et al., 2001) or when it is used as a crop in crop rotation systems. As not all tubers are removed in harvesting, *H. tuberosus* infests the consecutive crop as a volunteer weed, which can reduce the yield of maize, sugar beet and soybean by 25, 81 or 91%, whereas the yield reductions in wheat, oat, rape and ryegrass were insignificant (Swanton, 1994; Schittenhelm, 1996). Wyse and Young (1979) found that densities of 4 tubers/m of row of *H. tuberosus* reduced corn seed yields by 16-25%. Wyse et al., (1986) found that *H. tuberosus* densities of 1, 2 and 4 tubers per metre of row reduced soybean seed yield by 31, 59 and 77%, respectively.

Soybean height, branches per plant, pods per plant and total seed weight were all reduced by the presence of *H. tuberosus* (Wyse et al., 1986). Soybean leaf area and relative growth rate were reduced by densities of 2 and 4 artichoke tubers per metre of crop row and net assimilation rate was reduced by 4 tubers per metre of crop row (Wyse et al., 1986). Wall and Friesen (1989) found that 4-6 surviving *H. tuberosus* shoots per square metre could reduce seed yield in barley by 20%. *H. tuberosus* may also occur in pastures, but its high nutritional quality may render its presence desirable (Seiler 1988).

The success of *H. tuberosus* as an invasive annual species could be due to the biological factors within diverse habitats which include: (i) a high expenditure of energy on initial growth of stem, branches and leaves; (ii) a large amount of energy allocated to the production of rhizomes and tubers; (iii) a phalanx-like growth morphology, facilitating capture of both above- and below-ground resources; (iv) mobility of nutrients within the plant; (v) seed production; (vi) the ability to regenerate even if severely defoliated; and (vii) the constancy of nutrient allocation to clonal structures (Swanton and Cavers 1989). These factors are complemented by resistance to most diseases and pests, and tolerance of poor soils (Kosaric et al., 1984).

Mechanical Control

Invasive populations in Germany were successfully controlled by various mechanical methods: mowing twice a year in late June and in August gave good control after 2 consecutive years (Wagner, 1988). Large areas can be mowed with agricultural machinery where the soil permits, small infestations or those on soft soils were treated with hand-held trimmers or brush cutters.

Removing the mowed plants did not result in better control. Faster success may be reached by cutting in June and light cultivation. Close monitoring for the right timing is essential: it must be done when the tubers formed in the preceding year are consumed, and new ones have not formed (Hartmann et al., 1995). In light soils, plants can be hand pulled in October or in early spring; if this is done in late spring, too many tubers remain in the soil. In Hungary, efficacy of mowing for *H. tuberosus* control was investigated in the latest years (Fehér and Konĉeková 2012). Balogh (2006) suggested mowing more times within a year when plant shoots reach 50 cm height. Physiological background of this is that

the food reserves of the tubers will deplete until June. Stem tubers of the previous year will destroy from the end of April and entirely die by the end of June (Swanton et al., 1992). Plants use food reserves mainly for the development of the new shoots. The mowing cut the shoots, therefore the possibility or the plants to develop new propagula will considerably decrease.

Tilling in the early summer can help to weaken the weed potential of *Helianthus tuberosus* (Swanton, 1994). Swanton and Cavers propose disking or rot tilling during periods of minimum regeneration.

Chemical Control

Glyphosate and dicamba were both found to give good control of *H. tuberosus* in forage crops in Korea (Park et al., 2001). As a weed, it must be controlled early. Wyse et al., (1986) recommended that in soybeans, *H. tuberosus* growth be controlled within 6 week of planting because of its strong competitive ability. Both rhizomes and tubers can overwinter in the soil and produce shoots the following year (Vanstone and Chubey, 1978).

Application of glyphosate treatments two times in the growing season ensured a 100% weed control effect on *H. tuberosus* in glyphosate resistant soybean fields. Because of the presence of the non-shot forth, dormant tubers *H. tuberosus* control is suggested even after two years (Kays and Nottingham, 2008). Very good (96%) *H. tuberosus* control efficacy was obtained when combined herbicide treatments with glyphosate isopropylamin salt + 2,4 D were applied in autumn after mowing (Labant-Hofman and Kazinczi, 2014).

Also, Swanton (1982) suggested that greater than 90% control of both top growth and re growth of new shoots could be achieved in corn using split applications of dicamba at 0.28 kg a.i./ha or dicamba plus 2,4-D plus mecoprop at 0.55 kg a.i./ha, provided that the split application was separated by a period of 10-14 days. Wall et al., (1986) found that *H. tuberosus* was controlled in barley by a post emergence application of clopyralid at 1.0 kg a.i./ha, or clopyralid at 0.5 $kgha^{-1}$, if combined with 0.5 kg a.i./ha of 2,4-D, or dicamba at 0.2 kg a.i./ha plus 2,4-D at 0.4 $kgha^{-1}$. The combination of clopyralid and 2,4-D was the most effective (Wall and Friesen 1989). Chemical control is most effective at the pre bloom stage of growth. Both top growth and tuber re growth are controlled and further infestation is reduced (Swanton 1982).

CONCLUSIONS

Helianthus tuberosus L. just has been found in Republic of North Macedonia to some initial degree of invasion. However, in some areas, particularly in north-western and Central Europe, it has spread fairly widely and is well established and has become the target of large scale removal campaigns. The climate and topography of the many European regions are favourable for it's grow and expansion. In other Macedonian locations with similar climates to the northern part where *H. tuberosus* was found, it could potentially detected new plants of this species.

Both, vegetative and generative propagation contribute to its invasive potential. Spread is also facilitated by waterways and human transportation. Although many herbicides can be used to control *H. tuberosus*, their use is limited as the plants are often near water ways where herbicide uses is not recommended. The other control methods, however, are time consuming, and could be quite costly.

The prognosis for curbing the spread of *H. tuberosus* in Republic of North Macedonia seems impossible. In very close future, it will rapidly establish itself along rivers, river banks and floodplains, as well as ruderal and agricultural environments in many other Macedonian regions, following the pattern seen over the past three centuries in north-western and Central Europe.

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IMPROVEMENT OF YIELD AND PHYTOCHEMICAL COMPOUNDS OF *Thymus vulgaris* THROUGH FOLIAR APPLICATION OF SALICYLIC ACID UNDER WATER STRESS

SUMMARY

Thymus vulgaris is an important medicinal plant across the world that has been grown in Iran since ancient times. To study the effect of foliar application of salicylic acid (SA) on the yield and phytochemical compositions of *T. vulgaris*, an experiment was conducted in Qazvin in 2017. A factorial experiment based on a randomised complete block design was conducted with three replications. Treatments of SA (0, 100, 150 and 200 mg/L) were applied from the period before flowering until the early stages of flowering under well-watered and withholding irrigation conditions. The results showed that SA had a significant effect on the yield and phytochemical compositions of *T. vulgaris* under both irrigation regimes. SA foliar application led to an increase in total dry matter. The highest total dry matter (1958.83 kg ha^{-1}) was obtained by applying 200 mg/LSA under normal irrigation. The effect of foliar application of SA on the essential oil content of *T. vulgaris* was influenced by the irrigation regime. The highest essential oil content (2.66%) was obtained by applying a foliar spray of 100 mg/LSA under withholding irrigation conditions. Thymol, carvacrol, p-cymene, linalool and γ-terpinene were identified as the main essential oil compounds. The highest thymol content was obtained by foliar application of 200 mg/LSA under withholding irrigation conditions. Our results demonstrate that foliar application of SA reduced the negative effect of water deficit on *T. vulgaris* and increased yield and thymol content. SA foliar application is a simple, eco-friendly and relatively commercially viable method of increasing yield and the synthesis of phytochemicals; therefore, it may be used to improve the dry matter and quality of the essential oils of thyme plants.

Keywords: Dry matter, Essential oil, Stress, Thymus, Thymol.

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INTRODUCTION

Thyme is one of the 8 most important members of the *Lamiaceae* family. More than 360 species of thyme grow in the world, and one of the most important species that commonly cultivated in the world is *Thymus vulgaris* (Lawrence and Tucker., 2002; Zarzuelo and Crespo., 2002). Annual global production for dry matter and essential oils from thyme are estimated around 1500 and 30 tons, respectively. Mediterranean with a value of US$ 1.5 million is the main region of production thyme products. Thyme cultivation area in Iran has been reported to be 50 ha with the estimated annual production of 270 tons per year (MAJ., 2015; McGimpsey., 1993)

T. vulgaris is one of the medicinal plants, native to southern Europe, and has a worldwide distribution (Hosseinzadeh et al., 2015), and in addition to higher quantity and quality of essential oils (higher percentage of essential oil and thymol and carvacrol compared to other species), it also has a relatively widespread distribution in the world, which shows the adaptation and tolerance of the plant to different environmental conditions. This plant's valuable essential oils are of great importance in the pharmaceutical, cosmetic and perfumery industries, and also in preparation of spices (Nickavar et al., 2005).

T. vulgaris, genetically, morphologically and phytochemically has a very high diversity (Nielsen et al., 2017). Phytochemical constituents of thyme mainly consist of monoterpene, sesquiterpene and phenolic compounds (Hosseini et al., 2015; Ebadollahi., 2018). Previous research shows that essential oil compositions in thyme mainly consist of thymol, carvacrol, p-cymene and c-terpinene, and their quantity changes when affected by environmental stresses (Khalil et al., 2018; Mohammadi et al., 2019).

Therefore, achieving the best culture environment by enhancing the performance and active components is one of the most important purposes in the cultivation of medicinal plants (Jaafar et al., 2012). Drought as one of the most important abiotic stresses, and it is a very serious threat to the agricultural industry worldwide, which usually reduces growth, yield and also causes physiological and phytochemical changes (Karim et al., 2015). Improving the drought tolerance is one of the proposed methods to cope with the drought problem that it complies with economic demands, so that plants have the minimum need for water and maximum production in a drought condition. Due to some secondary metabolites and morphological characteristics, medicinal plants have the potential to withstand more stress compared to other plants and they are a suitable choice for cultivation under stressful conditions (Akula and Ravishankar., 2011; Isah., 2019).

One of the most important techniques to increase the rate of accumulation of phytochemicals compounds such as percentage and constituents of essential oils is the use of growth regulators under drought stress (Yucel Candan and Heybet Elif., 2016; Khalil et al., 2018).

External usage of growth regulators has been studied as a method to reduce the negative effects of drought stress and increase the plant compatibility (Khan

et al., 2015; Abbaspour and Babaee., 2017). Salicylic acid or orthohydroxy benzoic acid is one group of the plant growth regulators which is available throughout the plant dynasty. This growth regulator belong to a diverse group of plant phenols which has an aromatic ring with a hydroxyl group and is effective on many physiological processes of plants (Tripathi et al., 2019). The effects of SA and its key roles in the regulation of different physiological processes of the plant have been examined in various studies (Belt et al., 2017).

Given that SA plays an important role in plant growth and increasing performance, recently the use of SA has been increased in raising the resistance of the plant against biotic and abiotic stresses (Sawada et al., 2006; Rivas-San Vicente and Plasencia., 2011; Liu et al., 2015; Shahmoradi and Naderi., 2018). Many reports have shown that SA at low concentrations usually increases photosynthesis (Stevens et al., 2006), uptake and transfer of nutrients, photosynthetic pigments (Mohammadi et al., 2019), activity of antioxidant enzymes (Ananieva et al., 2004; Alam et al., 2013; Habibi., 2017), phytochemicals compounds especially phenolic compounds (Khan et al. 2015; Khalil et al., 2018), essential oil content (Kovácik et al., 2009; Khalil et al., 2018), tolerance to oxidative stress and decreases osmotic stress (Nazar et al., 2011), results of these studies suggest that exogenously applied SA can reduce drought stress impact on plants.

The effects of foliar application of SA, to a great extent, depends on genetics and the environmental factors (Idrees et al., 2011), and also on the applied method and concentration level (Horváth et al., 2007). Some studies show that foliar application of SA has an impact on effective compounds especially the percentage and composition of essential oils of oleoresins in Pinus elliottii (Rodrigues and Fett-Neto., 2009), triterpenes in Nigella sativa (Elyasi et al., 2016), Ocimum basilicum L. (Mirzajani et al., 2015), and Rosemary (El-Esawi et al., 2017).

The purpose of the present study was to evaluate the effect of foliar application of SA on the improvement of yield and the phytochemical constituents of T. vulgaris under water stress.

MATERIAL AND METHODS

The experiment was conducted at the research field of Qazvin, Iran, during 2017. The factorial experiment was conducted in the frame of randomized complete block design with three replications. The first factor was the irrigation regimes (well-watered and withholding irrigation) and the second factor was the foliar application of salicylic acid (0, 100, 150 and 200 mg/L).

The application of the treatments was before flowering until early stages of flowering at 70 days after transplanting seedlings into the field. The soil moisture content of all plants was maintained at the field capacity (FC) level before starting the withholding irrigation treatment. From pre-flowering phase until early stage of flowering (when 50% of the plants entered this stage), withholding irrigation was applied, while well-watered plants were irrigated to 80% of field capacity. The

moisture content of the soil was measured in each plot using TRIME-TDR (Bahreininejad et al., 2013; Mohammadi et al., 2019). From this point on, water stressed plants were no longer irrigated, while well-watered plants were further irrigated until the early stages of flowering. A total of three sprays of SA were carried out. SA was applied using a hand sprayer until the solution began to drip off leaves at sunset. The plots not receiving SA was treated similarly with equivalent amount of distilled water.

The seeds of the *T. vulgaris* German cultivar 'Deutscher Winter was purchased from a Pakan Seed Company, Isfahan, Iran. The seeds were planted into plastic pots (20cm in length and 25 cm in diameter), which were filled with sandy loam soil, pH: 7.2, and kept under light/dark cycle conditions of 16/8 h maintained at 23 °C and 73% relative humidity placed in a greenhouse. Then, when seedlings reach a height of 12–15 cm, they are transplanted to a field. Each experimental plot had three rows with 3 m length and 50 cm distance between rows and 30 cm the distance of seedlings on the planting rows. Results of the physical and chemical analysis of the field soil (0–30 cm depth) described in Table 1. The plant shoots were removed from each treatment at 50% flowering stage, Harvested tissues were weighed and dried at 40 ◦C in a forced air oven for 48h.Plant dry weight, and total phenol and essential oil were extracted and measured.

Table 1. Physical and Chemical analysis of the field soil (0–30 cm depth).

EC (dSm^{-1})	pH	Organic matter (%)	Nitrogen (%)	K (mg/kg)	P (mg/kg)	Texture	Sand (%)	Loam (%)	Clay (%)
2.12	7.2	0.61	0.07	139	12.2	Loam	32	38	30

Total phenols content

Folin-Ciocalteu reagent is used to estimate the total phenols. First, exactly 0.5 g of the sample is grinded in 10-time volume of 80% ethanol by a pestle and mortar. Then, the homogenate is centrifuged at 10,000 rpm for 20 min and the supernatant is collected. The residue is then re-extracted with five times the volume of 80% ethanol. In the next step, the supernatant is centrifuged and dried by evaporating. The residue is dissolved in a certain volume of distilled water (5 ml). Then, different volumes (0.2 to 2 ml) of residue are combined with water in the test tubes. The volume of the resultant mixture in each tube should be 3ml. Next, 0.5 ml of Folin-Ciocalteu reagent is added to the mixture. After 3 min, 2ml of 20% Na2CO3 solution is added to each tube and the mixture is mixed rapidly. Then the tubes are placed in the boiling water for exactly one min. The absorbance is measured at 725 nm against a reagent blank after cooling the mixture. Finally, different concentrations of Gallic acid are used to prepare a standard curve (McDonald et al., 2001).

Measuring the yield of essential oil

The aerial parts of each treatment were dried immediately at room temperature in shade, and then the yields (w/w %) of oils were determined by using of Clevenger Apparatus. The essential oil extraction time was the same for all samples as 2-3 h. The essential oil was dried by anhydrous sodium sulfate, then weight of the pure essential oil was determined, and its value was calculated according to its weight to the dry weight of the plant sample (w/w %). Finally, the obtained essential oils kept in dark sealed vials at 4 ºC until analysis.

Identifying essential oil compounds

GC-FID analyses of the available compounds in essential oil were carried out using a Thermoquest-Finnigan instrument. The analyses were performed on DB-5 fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was nitrogen at a constant flow rate of 1.1 mL per min. The oven temperature program was set 60 (the initial temperature of the oven)-250 ºC at the rate of 5 ºC/min and held isothermally for 10 min at the final. The injector and detector (FID) temperature were set at 250 ºC and 280 ºC. The split ratio was set at 100:1. GC-MS analyses were performed on Thermoquest-Finnigan Trace GC-MS instrument equipped with the same gas chromatography condition as mentioned for GC. The carrier gas was helium at the flow rate of 1.1 mL per min with the split ratio at 100:1. The transfer line and ion source temperature were set at 200 ºC and 250 ºC, respectively. There was a mass spectrometer with 70 eV ionization supply voltage.

Statistical analysis

The collected data were analyzed using SAS 9.1 software. The mean interactions were also compared through the Student-Newman-Keuls test at 5 % level using MSTATC software.

RESULTS AND DISCUSSION

Total dry matter

Based on the results of analysis of variance (ANOVA) showed that the interaction effect of irrigation regimes and foliar applications of salicylic acid has significant effect on a dry matter of the foliage (Table 2). Results of mean comparisons are presented in Figure 1. The results showed that the highest amount of dry matter was obtained, in the normal irrigation conditions and foliar application of 200 mg/L SA, that increased by 25 % compared with, under withholding irrigation conditions and without foliar application of SA (Figure 1). Exogenous SA application significantly increased dry matter in both irrigation regimes.

Increment in dry matter is probably due to increased stabilization of CO2 by exogenous SA application, which in different physiological processes, such as the increase in photosynthetic pigments, promote Rubisco activity, it is ultimately providing more ATP and NADPH and stabilizing carbon and producing more assimilate (Athar and Ashraf, 2005; Lawlor and Cornic, 2002).

Table 2. Analysis of variance (ANOVA) of the effect of salicylic acid and irrigations regimes on *Thymus vulgaris*

Traits	Mean squares for source of variation				
	Block	Factor a (irrigation regimes)	Factor b (application of salicylic acid)	Interaction a×b	Error
d.f	2	1	3	3	10
Total DM	2097.55^{ns}	254018.95^{**}	74772.84^{**}	4987.10^{*}	1029.31
Essential oil yield	0.0017^{ns}	1.05^{**}	0.07^{**}	0.03^{**}	0.001
Total Phenol	0.08^{ns}	287.22^{**}	18.27^{**}	7.23^{**}	0.28

Note: *: Significant at the 0.05 probability level; **: Significant at the 0.01 probability level; ^{ns}: Not significant.

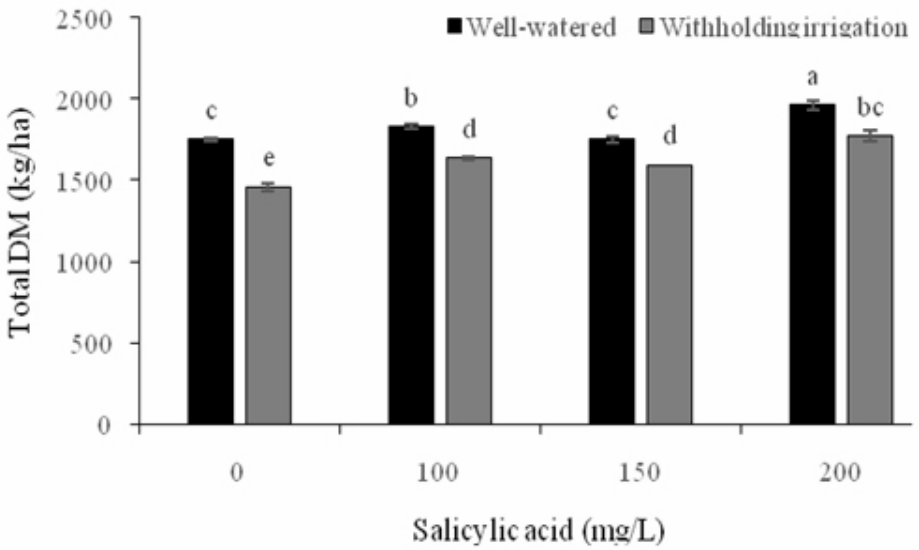

Figure 1: The effects of exogenous application of salicylic acid on total DM of *Thymus vulgaris* under water stress conditions.

Given that SA facilitates absorption of nutrients, it has a positive role in photosynthesis and photosynthetic enzymes, therefore it was expected that plants treated with salicylic acid lead to higher dry matter whether under stress condition or non-stress condition. The increase of dry matter with foliar application of SA under abiotic stress was reported on barley (Habibi., 2011), fennel (Askari and Ehsanzadeh., 2015), Thymus kotschyanus and Thymus vulgaris (Khalil et al., 2018; Mohammadi et al., 2019). The increase in dry mass of water-stressed plants in response to SA may be associated to the induction of antioxidant responses that protect the plant from stress damage (Singh and Usha.,

2003). It is also reported that salicylic acid increases the amount of lignin in the cell wall structure, which can be a factor in increasing plant biomass weight (Hayat et al., 2010). The ability of SA to increase plant dry mass, negating the adverse effects of withholding irrigation, may have significant contributions in improving plant growth and overcoming the yield barrier arising from conditions of limited water availability.

Essential oil content

The results of analysis of ANOVA showed that the interaction effect of irrigation regime and foliar application of SA has a significant effect on content of the essential oil of the leaves (Table 2). According to the results, the highest content of essential oil (2.66%) was obtained in withholding irrigation and foliar application of 100 mg/LSA, and the lowest content (2.003%) was obtained in non-water stress condition and foliar application of SA, but no differences were observed between SA concentrations (Figure 2). The content of essential oil was higher under withholding irrigation condition compared to non-stress conditions.

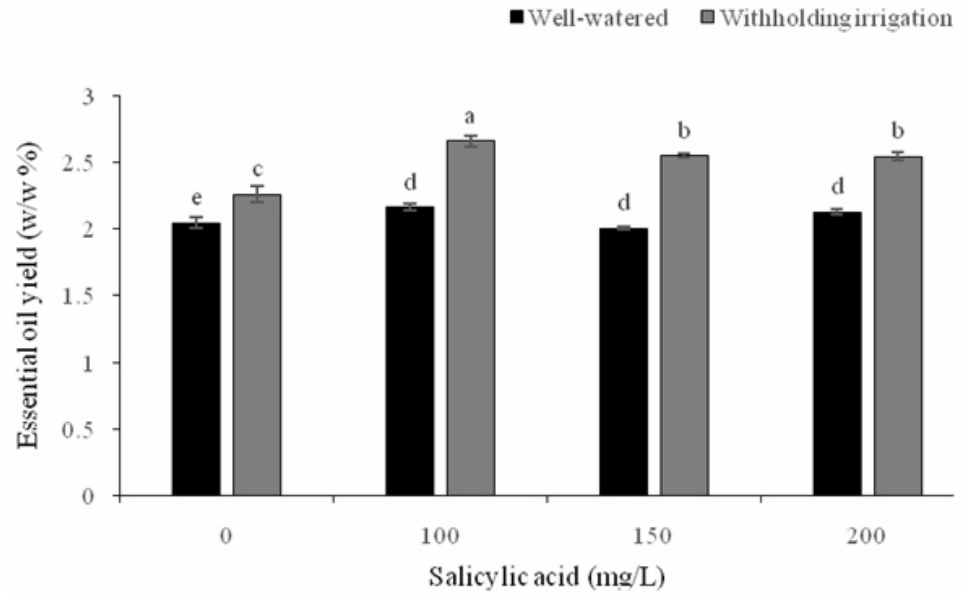

Figure 2: The effects of exogenous application of salicylic acid on essential oil yield of *Thymus vulgaris* under water stress conditions

The increase in essential oil content of the thyme leaves under the withholding irrigation was reported by Simon et al. (1992). The essential oil accumulation by withholding irrigation act as a defense mechanism and it is also because of the increase in the number of glands in the leaves (Simon et al., 1992). Our results indicate, essential oil content increased with increasing SA concentration and water deficit severity. Increase in content of essential oil by foliar application of SA can be due to increased nutrient absorption, increased number of glands of essential oil accumulation, increased synthesis of

monoterpenes, that was conducted in previous studies on *T. vulgaris* (Khalilet al., 2018; Mohammadi et al., 2019) basil and marjoram (Gharib, 2007) and *Mentha piperita* (Saharkhiz and Goudarzi, 2014). In general, in the present experiment, the highest percentage of essential oil was obtained under withholding irrigation and foliar application of 100 mg/L SA. This achievement might be important from economically point of view for thyme essential oil production.

Content of total phenol

The results of ANOVA indicated that interaction effect of irrigation regimes and foliar application with SA had a significant impact on total phenol content (Table 2). The highest total phenol content of 18.431 mg GAE/g DW was observed under withholding irrigation and 200 mg/LSA foliar application which was 58% higher than the control treatment (Figure 3).

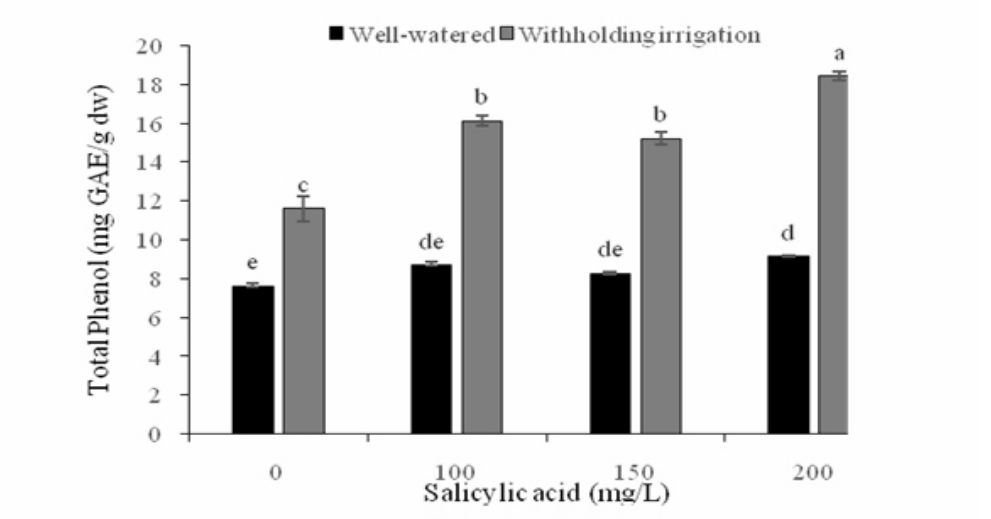

Figure 3: The effects of exogenous application of salicylic acid on total phenol of *Thymus vulgaris* under water stress conditions.

Phenolic compounds are an important group of secondary metabolites in medicinal plants that act as an antioxidant under environmental stress and its quantity usually increases in most plants (Agati and Tattini, 2010).The results of this experiment showed that withholding irrigation increased the amount of phenolic content. However, this increase was greater with SA foliar application. Similar results were reported by Khalil et al. (2018) on *T. vulgaris*.

In order to cope against withholding irrigation, plants increase the synthesis of secondary metabolites like phenol, which have an antioxidant role. SA is known to potentially generate a wide array of metabolic responses in plants, and affect a range of plant functions, including plant phenol content (Hayat et al., 2010). An increased synthesis of this compound in SA was also reported by other researchers (Dihazi et al., 2003; Mendoza et al., 2018; Mohammadi et al., 2019). Increase in total phenol occurs by inducing

Phenylalanine ammonia-lyase enzyme and Tyrosine ammonia-lyase which are key enzymes in the synthesis of this compound in salicylic acid (Beaudoin-Eagan and Thorpe, 1985).

Essential oil constituents

According to the results of GC-MS analysis, 14 compounds were identified. The main constituents included thymol, γ-terpinene, carvacrol, geranial, ρ-cymene (Table 3). Foliar application of SA and withholding irrigation conditions resulted in a significant increase in the amount of thymol compound at both irrigation levels. The highest amount of thymol was observed in withholding irrigation condition with foliar application of 200mg/LSA, and the lowest amount was observed in the normal irrigation and without SA application (Table 3).

Foliar application of SA and withholding irrigation decreased the amount of γ-terpinene compound. The highest amount of this compound was obtained as 16.11% in non-water stress without foliar application of SA. In non-stress condition, foliar application of SA increased the ρ-cymene compound, but under withholding irrigation condition, foliar application of SA decreased content. The highest amount of α-terpineol was obtained by the foliar application of 150 mg/LSA and under non-stress conditions, but under withholding irrigation it was obtained by 200 mg/L foliar application. Also, SA foliar application significantly increased this compound under normal conditions. The highest value was obtained as 5.79% in the foliar application of 150 mg/LSA.

The result indicated that main constituents included thymol, γ-terpinene, carvacrol, geranial, ρ-cymene (Table 3). There have already been numerous reports that the main constituents in the Thyme plants are thymol, p-cymene, carvacrol, and β-caryophyllene (Dehghani Mashkani et al., 2018; Mohammadi et al., 2019).

The results indicated that there is a variation in essential oil compounds in the studied treatments. Thymol was the most abundant compound detected in essential oil. Thymol content increased with increasing SA concentration and water deficit stress. In previous studies increase in thymol amount has been reported under water deficit stress (Bahreininejad et al., 2014; Askary et al., 2018; Mohammadi et al., 2019).

A decrease of γ-terpinene was reported with withholding irrigation and foliar application of SA on *T. vulgaris* (Khalil et al., 2018).

The carvacrol significantly decreased under withholding irrigation condition, which other researchers have found similar results on *T. vulgaris* (Letchamo et al., 1995) and *T. hyemalis* (Aziz et al., 2008). The essential oil compounds had different reactions to withholding irrigation and foliar application of SA. So that foliar application of SA with a high concentration of 200 mg/L led to a significant increase in the compound of oxygenated monoterpenes. Almost all the compounds obtained from this treatment that was at value of 99.92 in this group.

Table 3. Composition of essential oils in *Thymus vulgaris* sprayed with SA (0, 100, 150 and 200 mg/L) under water stress condition.

Compounds	RI[1]	RT[2]	Well-watered				Withholding irrigation			
			0	100	150	200	0	100	150	200
α-terpinene	1012	10.36	1.87 ± 0.0.2	1.63 ± 0.02	-	-	1.26 ± 0.06	1.82 ± 0.05	1.49 ± 0.03	-
ρ-cymene	1028	10.77	7.29 ± 0.2	8.63 ± 0.2	5.51 ± 0.16	18.16 ± 0.4	8.91 ± 0.2	6.5 ± 0.12	7.32 ± 0.11	-
γ-terpinene	1062	11.98	16.11± 0.3	15.6 ± 0.25	-	9.67 ± 0.3	8.13 ± 0.12	7.12 ± 0.2	13.17 ± 0.34	-
Sabinene hydrate	1071	12.1	0.68± 0.02	0.5 ± 0.03	-	0.2 ± 0.03	0.17 ± 0.02	-	-	-
Linalool	1098	12.95	0.58 ± 0.03	1.24 ± 0.05	4.27 ± 0.15	0.23 ± 0.02	0.58 ± 0.01	2.21 ± 0.06	-	4.77 ± 0.1
α-terpineol	1196	15.71	2.35 ± 0.11	1.71 ± 0.04	8.13 ± 0.24	-	-	-	-	10.5 ± 0.21
Thymol methyl ether	1235	16.72	1. 3 ± 0.03	1.23 ± 0.03	-	-	-	-	-	-
Geraniol	1249	17.32	0.18 ± 0.05	0.37 ± 0.02	0.45 ± 0.03	-	0.22 ± 0.02	-	-	2.01 ± 0.05
Geranial	1266	17.96	0.28 ± 0.01	0.42 ± 0.02	6.37 ± 0.3	-	6.2 ± 0.1	5.3 ± 0.14	-	6.47 ± 0.11
Thymol	1291	19.2	64.43 ± 1.98	65.24 ± 2.11	66.32 ± 2.23	67.05 ± 2.54	70.69 ± 1.68	73.12 ± 1.78	74.01 ± 1.43	75.33 ± 1.54
Carvacrol	1302	19.45	3.14 ± 0.12	3.37 ± 0.2	5.79 ± 0.1	3.5 ± 0.13	1.21 ± 0.05	1.13 ± 0.04	1.03 ± 0.02	-
Thymol acetate	1343	20.08	-	-	-	-	-	-	-	-
Carvacrol acetate	1365	20.91	-	-	1.62 ± 0.03	-	-	-	-	0.84 ± 0.04
trans-caryophyllene	1401	21.87	0.42 ± 0.02	-	-	0.25 ± 0.02	0.34 ± 0.03	1.02 ± 0.02	-	-
Total identified (%)			98.63	99.94	98.46	99.06	97.71	98.22	97.02	99.92
Number of constituents										
Monoterpene hydrocarbons			25.95	26.36	5.51	28.03	18.47	15.44	21.98	-
Oxygenated monoterpenes			72.26	73.58	92.95	70.78	78.9	81.76	75.04	99.92
Sesquiterpene hydrocarbons			0.42	-	-	0.25	0.34	1.02	-	-

[1]RI (retention index), [2]RT (retention time, Minute). (t: trace value (< 0.01).

CONCLUSIONS

Foliar application of SA at moderate concentrations increased dry matter yield, essential oil content and some essential oil compounds of Thymus vulgaris the foliage under withholding irrigation conditions. Foliar application of SA (100 mg/L) increased dry matter yield and essential oil content under withholding irrigation, application 200mg/L SA, increased the most important essential oil compounds involving thymol. Overall, the present study revealed that exogenous SA application could overcome the adverse effects of withholding irrigation by further accumulation of phytochemical composition, acting as osmotic and metabolic regulators or substrates and in a part as cell component stabilizers. In general, it can be mentioned that the application of low and highest concentrations of SA improves yield and its high concentration increases some active compounds in *T. vulgaris*.

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EFFECTS OF DIFFERENT PLANT EXTRACTS ON THE MINERAL CONTENT OF BLACKBERRY LEAF (*R. fruticosus*) IN ORGANIC PRODUCTION

SUMMARY

The aims of this study were to investigate the chemical content of plant extracts of nettle (*Urtica dioica*), alfalfa (*Medicago sativa*) and dandelion (*Taraxacum officinale*) and to determine their effect on the chemical content of blackberry leaves grown in organic production. Field and laboratory analysis were conducted over a two-year period. The plant extracts were fermented for 14 days before analysis. The field trial adopted a randomised block design with four repetitions. Each repetition included five blackberry bushes. The following properties were analysed: the chemical composition of plant extracts, macroelement concentration (%), microelement concentration (mg kg^{-1}) and the fresh and dry weights of blackberry leaves (g). Analysis of the macronutrient content revealed the highest N content in the alfalfa plant extract, the highest P concentration in nettle plant extract, while the highest concentration of K was found in dandelion plant extract. The N and P concentrations were higher in all treatments than in the controls. The Fe concentration in blackberry leaves ranged from 70.63 mg/kg^{-1} (treatment with alfalfa plant extract) to 76.68 mg kg^{-1} (treatment with nettle plant extract). The difference in blackberry leaf Mn concentration between the dandelion treatment and the control were highly significant. The nettle and dandelion plant extracts influenced the Cu content of blackberry leaves. The Zn concentration in blackberry leaves treated with plant extracts was significantly higher than that in the controls. The highest dry mass content was found in blackberry leaves treated with nettle plant extract.

Keywords: plant extract, nettle, dandelion, alfalfa, blackberry leaves, micro and macroelements

INTRODUCTION

Historically, humankind has known the effects of plant extracts for hundreds of years and has successfully used them in traditional agriculture (Lawless, 1995). A large number of plants grow freely in nature can serve to

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make quality foliar fertilizer. The prepared extracts from these plants are a source of readily available nutrients for cultivated plants.

In addition to providing the plant with sufficient plant nutrients, plant extracts may in part have insecticidal and fungicidal effects due to the bioactive chemicals present in the solution (Kim et al., 2005; Daoubi et al., 2005). Plant extracts used as foliar fertilizers could be a significant source of readily available elements; however, this may depend on the type and the quality of the soil on which these plants were grown (Popescu et al., 2010; Markoski et al., 2018). Nettle used to make plant extracts is rich in Mg, Ca, and K and also contains significant amounts of the trace elements Zn, Cu, Fe, Cr, Mn, Mo, Si, and V (Gohari at al., 2018).

The use of plant extracts from different plant species gives fruit producers the opportunity to count on quality yield with better marketing possibilities. The application of cultivated plants can be done every 10 to 15 days without the fear of contamination or overyielding. These plant extracts are similar in purpose to those obtained from herbal teas and have been used for a long time on small farms in developing countries (Roy et al., 2005).

The increase in yield of cultivated plants, which have been subjected to foliar treatments, is the result of the nutrient uptake from solution, which results in the intensification of photosynthesis and the accumulation of minerals in the edible part of the plant (Guievence and Badem, 2000; Batra et al., 2002).

The mineral substances from the plant extracts are absorbed by the leaf cuticle, epidermis cells, stoma closure cells and through the hairs on the leaf. The diffusion of nutritients from the leaf surface to the free space within the cell wall is dependent on the difference in the nutrient concentration between the external environment and the concentration within the apoplasts. Nutrient uptake is higher with older leaves, as it has a larger total uptake area compered to younger leaves (Mengel, 2002). Aging of the leaves can affect the permeability of the leaf surface in such a way as to reduce the stoma opening process due to potassium retranslocation during senescence (Jordan and Brodribb, 2007). Young, partially-developed leaves are considered to have easier and faster nutrient uptake than older, fully-developed leaves. However, the influence of leaf age on foliar nutrient uptake has not been fully explained (Fernandez and Eichert, 2009).

The aim of the study was to investigate the chemical composition of plant extracts of nettle, alfalfa and dandelion and to determine their effect on the concentration of minerals in the blackberry leaves grown in organic production.

MATERIAL AND METHODS

Field trials were conducted in the village of Brekinja near Kozarska Dubica in organic blackberry orchard. Laboratory tests of plant extracts were carried out in the Agricultural Institute of Republika Srpska in Banja Luka. Their health status was examined at the Veterinary Institute "Dr. Vaso Butozan" in Banja Luka. Laboratory analyzes of the mineral content of blackberry leaves as well as soil analysis were done in the Laboratory for agrochemistry of the Faculty of

Agriculture in Novi Sad. Field trials and the laboratory tests were conducted in the period of two years.

Plant extracts of nettle, alfalfa and dandelion were fermented for 14 days before the application. The plants used to make these extracts were young, finely chopped and mixed with rainwater. The fermentation took place in the shade with occasional stirring of the solution. One kilo of freshly chopped plant material was used for the nettle extract, while two kilos of chopped plant material was used for alfalfa and dandelion plant extract. After the fermentation, the mixtures were filtered and thereafter diluted with distilled water in 1:10 ratio.

The trial was set up as random block system with four repetitions. Each repetition consisted of five blackberry bushes (Čačanska bestrna variety) planted 3 m x 1.5 m apart.

Treatments were performed with a Villager DM-25 motor sprayer with a capacity of 15 l and operating pressure of 15-30 bars that was being regulated by the throttle. During the application the sprayer operated at almost full throttle. The first treatment started in the middle of May and the following were performed every 10 to 15 days until the first ripe blackberry fruits appeared.

During this study the following properties were analyzed: the chemical composition of plant extracts, macroelement concentration (%), microelement concentration (mg kg-1) and the fresh and dry weight of blackberry leaves (g). Examination of the health status of the plant extracts included testing for the presance of: Salmonella, Staphylococcus, Clostridia, Preoteus species and Escherichia coli.

Mineral content of plant extracts were performed in the in the laboratories of the Department of Agroecology at the Agricultural Institute of Republika Srpska in Banja Luka.

The following macronutrients were tested in the plant extracts:

a) Kjeldahl nitrogen - wet combustion (conc. H_2SO_4 + H_2O_2 + 450°C - Kjeltec system I, Tecator), distillation.

b) Phosphorus - wet burning with a mixture of acids (HNO_3 + $HClO_4$ + H_2SO_4); from solution, determination of phosphorus by staining method (dyeing in yellow) with optical density reading on a spectrophotometer.

c) Potassium - wet burning with a mixture of acids (HNO_3 + $HClO_4$ + H_2SO_4); from solution, potassium readings by flame photometry.

Leaf sampling started after the last treatment of blackberry bushes. Sampling was performed in a matter that 30 leaves was collected from each repetition, resulting in 120 leaves in each treatment. The youngest, fully developed leaves were sampled in mid-July. The samples were weighed, than washed in distilled water, air-dried, and then dried in the oven at 70°C for 48 hours. Afterwards, dried leaves were finely grounded and weighed.

The concentration of macro and microelements in the leaf dry matter was determined in the Laboratory for Agrochemistry of the Faculty of Agriculture in Novi Sad.

The obtained results were analysed by the analysis of variance, and the least significance difference (LSD) test.

Agro-ecological conditions

Soil samples were analyzed at the Laboratory for Pedology at the Faculty of Agriculture in Novi Sad prior to field trial set up. During the course of the trial, meteorological parameterswere gathered at the Hydrometeorological Station located in the Mlječanica Spa near Kozarska Dubica. Latitude and longitude of the field trial are 45°08'N and 16°43'E, respectively, and the climate is moderately continental. The elevation is 157 m.

Soil conditions

The trial was performed on peat soil suitable for growing fruit crops. The soil structure is crumbly, with a favorable water-air regime. The results of chemical analyzes of the soil samples taken before the start of the study are shown in Table 1.

Table 1. Results of chemical analyzes of the soil samples before the treatments

Debth (cm)	pH in KCl	pH in H_2O	$CaCO_3$ (%)	Humus (%)	Total N (%)	Al-$P_2 0_5$ mg/100g	Al-$K_2 0$ mg/100g
0 – 40	6,95	7,67	20,37	3,21	0,21	9,72	20,68

Based on the results of chemical analyzes of soil samples taken at a depth of 0-40 cm, the soil is poorly alkaline (pH 7.67 in H_2O), and it is medium humic in terms of humus content (3.21%). The Ca content is high and thus the soil is classified ascalcareous. The availability of readily available phosphorus is low (9.72 mg $100g^{-1}$), while the potassium content in soil is favorable for fruit production and amounts to 20.38 mg $100g^{-1}$ K_2O. The levels of Pb and Cd in the samples indicate that the soil is suitable for organic production (Tab. 2)

Table 2. Total cadmium (Cd) and lead (Pb) content in the soil

Debth (cm)	Pb (mg kg^{-1})	Cd (mg kg^{-1})
0 – 40	15,5	1,78

The concentration of Pb (15.5 mg kg^{-1}) and Cd (1.78 mg kg^{-1}) at 0-40 cm soil depth is lower than the amounts allowed in the soil intended for organic production.

Weather conditions. The average rainfall during the study was 600 mm, which washigher than the perennial average (442.7 mm). During the vegetation period (IV-VIII), the average air temperaturesvaried compared to perennial average (18.9°C).

RESULTS AND DISCUSSION

Chemical content of plant extracts

Chemical analysis was performed to determine the content of macronutrients in plant extracts of nettle, dandelion and alfalfa (Table 3). Chemical analysis of plant extracts showed that the dandelion and alfalfa extracts were acidic, while the nettle extract had slightly acidic reaction.The pH values measured ranged from 5.2 (dandelion extract) to 6.4 (nettle extract).The highest N concentration was found in alfalfa extract (105.6 mg l-1).Alfalfa extract had twice as much N as the dandelion extract (41.0 mg l-1).The highest P content was measured with nettle extract (56.3 mg l-1).Potassiumconcentrationin dandelion extract was1205,3 mg l-1, whichwas over 40% higher than in nettle and alfalfa extracts.

Table 3. Chemical composition of plant extracts with a fermentation period of 14 days

No.	Extract	pH	N (mg l^{-1})	P (mg l^{-1})	K (mg l^{-1})
1.	Nettle	6,4	56,8	56,3	700,2
2.	Dandelion	5,2	41,0	42,5	1205,3
3.	Alfalfa	5,4	105,6	40,2	670,4

Mineral content of the blackberry leaves

Mineral concentration of blackberry leaves is conditioned by the root activity and the ability of leaves to absorb ions from the applied plant extracts. The intensity of ion uptake depend on the leaf age, position as well as weather conditions during the study.

Concentration of macro and microelements in the blackberry leaves after the treatment with plant extracts are shown in Table 4.

Table 4. Concentration of macroand microelements (mg kg-1) in blackberry leaves before and after treatment with nettle, dandelion and alfalfa plant extracts

Treatment		Macroelements (%)			Microelements (mg kg^{-1})			
		N	P	K	Fe	Mn	Cu	Zn
Control		1,95	0,20	0,65	75,36	7,49	4,12	15,14
Plant extract	Nettle	1,99	0,23	0,63	76,68	8,17	4,57*	16,50*
	Dandelion	1,98	0,26	0,66	72,00	12,06**	4,08*	16,28*
	Alfalfa	1,98	0,26	0,65	70,63	10,86**	3,63	16,27*
LSD	0,05	0,24	0,11	0,14	7,25	1,35	0,42	1,06
	0,01	0,33	0,15	0,19	10,17	1,89	0,59	1,48

Blackberry leaves treated with plant extracts of nettle, dandelion and alfalfa had a higher concentration of nitrogen than the control, but the differences

found were not statistically significant.The highest concentration of N was in blackberry leaves treated with nettle extract (1.99%).

The highest average P concentration (0.26%) was found in blackberry leaves treated with dandelion and alfalfa extracts, and the lowest in the control (0.2%).All samples treated with nettle, dandelion and alfalfa extracts had higher P contentcompared to the control, however, the differences were not statistically significant. Blackberry leaves treated with nettle extract were found to have lower average P concentration of 0.03% compared to leaves treated with alfalfa and dandelion extracts.

The highest concentration of K was found in leaves treatedwith dandelion extract (0.66%). No statistically significant differences in K concentration were found between the control and the treatments with plant extracts. However, blackberry leaves treated with nettle extract had a 0.03% lower K concentration than the control. Potassium concentration was the same in leaves treted with alfalfa extract and the controle (0.65%).

Different localities and soil types significantly affect the mineral composition of blackberry leaves (Licina and Oparnica, 2000a). The unfavorable agrochemical and physical properties of the soil significantly condition the mineral composition of the fruit leaves, and this is especially true for the N, K, Mn and Fe content (Licina and Oparnica, 2000 b).

Iron content in blackberry leaves after the treatments varied from 70,63 mg kg^{-1}(alfalfa extract) to 76,68 mg kg^{-1} (nettle extract). Blackberry leaves in the control treatment had a higher Fe content than the leaves treated with dandelion and alfalfa extracts.The differences in Fe content between the control and treatment with alfalfa extract were 4.73 mg kg^{-1} and were not statistically significant.

The optimal concentration of Fe in the leaves of strawberries and berry fruits is within the narrow range of 70 - 80 μg/g (70-80 mg kg^{-1}) of dry matter (Bergmann, 1983).The Fe concentration in healthy blackberry leaves was 142 μg/g (142 mg kg^{-1}) in dry matter, while in diseased leaves it reached significantly higher values of 351 μg/g (351 mg kg^{-1}) in dry matter (Stevanovic et al., 1999). In this study Fe concentration found in blackberry leaves was within the optimal range.

The concentration of Mn in the leaves treated with nettle, dandelion and alfalfa extracts was higher than the control. The highest average concentration of Mn was found in blackberry leaves treated with dandelion extract (11.88 mg kg^{-1}). A high concentration of Mn was also found in blackberry leaves treated with alfalfa extract.The differences in Mn concentration between treatments with dandelion and alfalfa extracts compared to controls were statistically significant. Treatment with nettle extract increased the Mn content of the blackberry leaves, but the differences compared to control were not statistically significant.

Optimal concentration of Mn in the blackberry leaf ranges from 40-150 μg/g (40-150 mg kg^{-1}) of dry matter. Symptoms of the Mn deficiency occur at values lower than 10 μg/g (10 mg kg^{-1}) of dry matter (Bergmann, 1983).

Manganese concentration in the leaves in the control treatment and the treatment with nettle extract was below the limit of 10 mg kg^{-1}.

During this study, nettle and dandelion extracts were found to statistically significant increase the concentration of copper in the blackberry leaves. At the same time, the concentration of copper in the blackberry leaves treated with alfalfa extract was lower than the control.

Copper is considered to be an essential microelements for higher plants, and its role in the transport of matter is predominantly catalytic. Plant deficiency symptoms are observed at concentrations less than 4 μg/g (4 mg kg^{-1}) of dry matter, and signs of excess at concentrations greater than 30 μg/g (30 mg kg^{-1}) of dry matter (Ubavić et al., 2001).During this study lower concentration of 4 mg kg^{-1} of dry matter was found in blackberry leaves treated with alfalfa extract (3.63 mg kg^{-1}), indicating its deficiency.

All treatments with plant extracts resulted in increased Zn concentration in the blackberry leaves relative to the control. The differences in Zn concentration between the control and the treatments were statistically significant. The highest concentration of Zn was found in blackberry leaves treated with blackberry extract (16.50 mg kg^{-1}).

Concentrations of Zn in blackberry leaves during the study were within the optimal range between 20 and 70 μg/g (20-70 mg kg^{-1}) of dry matter (Bergmann, 1983). The concentration of Zn in the blackberry leaves in all treatments was below 20 mg kg^{-1} of dry matter. During the two-year trials, the average concentration of Zn in the blackberry leaves was 34 mg kg^{-1} of dry matter (Glišić et al., 2006), which is higher than the results obtained during this study.

Fresh and dry blackberry leaf weight

The effect of plant extracts on the weight of fresh and dried blackberry leves was observed as well as as the proportion of dry weight per fresh weight-dry matter content (Tab. 5).

Table 5. Fresh and dry blackberry leaf weight

Treatment	Fresh leaf weight (g)	Dry leaf weight(g)	Dry matter content (%)
Control	43,80	18,35	42,06
Nettle	49,23	20,74	42,25
Dandelion	44,25	17,93	40,27
Alfalfa	48,57	20,42	42,14
Average	46,46	19,15	41,68
LSD 0,05 0,01	18,84 26,41	8,86 12,42	2,10 2,94

The mass of fresh blackberry leaves treated with nettle, dandelion and alfalfa extracts was higher than the control, but the differences found were not

statistically significant. The average fresh leaf weight ranged from 43.80 g (control) to 49.23 g (nettle extract).

The highest leaf dry weight was obtained by treating fresh leaves with nettle extract (20.74 g). At the same time, the leaves that were treated with the dandelion extract had the lowest dry weight (17.93 g). Comparing treatments with the control indicates no statistically significant differences in the weight of dried blackberry leaves treated with different plant extracts.

The average dry matter content was 41.68%. The highest proportion of dry matter was found on the samples treated with nettle extract (42.25%) and the lowest in the treatment with dandelion extract (40.27%). The differences in the dry matter content between the control and the treatments were not statistically significant. Fresh blackberry leaves harvested in the spring can be used for chewing, brewing and teas. Freshlysqueezedblackberry leaf juice is used in folk medicine for compressused with ulcers and insect bites. After harvesting, and dryingblackberry leafscan be used to make teas and tea blends that are used in alternative medicine (Gursky, 1978).

CONCLUSIONS

The dandelion and alfalfa plant extracts were acidic and the nettle slightly acidic reaction. Chemical analysis of the macroelement showed that alfalfa plant extract had the highest nitrogen content (105.6 mg l^{-1}), nettle had highest phosphorus content (56.3 mg l^{-1}), while the dandelion extract had the highest potassium content (1205.3 mg l^{-1}).

In blackberry leaves treated with nettle, dandelion and alfalfa plant extracts, nitrogen and phosphorus concentrations were higher than in control treatment, but the differences found were not statistically significant. Potassium concentration was the same or below the levels in the control treatment (nettle extract treatment).

The differences in manganese concentration between blackberry leaves in the control treatment and dandelion and alfalfa plant extracttreatments were statistically highly significant.

A lower copper concentration than 4 mg kg^{-1} of dry matter was found in blackberry leaves treated with alfalfa extract (3.63 mg kg^{-1}), indicating its deficiency. The concentration of manganese in blackberry leaves in control and nettle extract treatments was below the 10 mg kg^{-1} limit when symptoms of its deficiency appear.

A significantly higher concentration of copper was found in blackberry leaves treated with nettle and dandelion plant extract compared to the control.

The concentration of zinc in the leaves treated with the plant extracts was significantly higher compared to the control. The highest dry matter content was found in blackberry leaves treated with nettle extract.

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OVERCOMING SEED DORMANCY OF *SENEGALIA GALPINII* AND *VACHELLIA ROBUSTA* THROUGH SCARIFICATION PRE-SOWING TREATMENTS

SUMMARY

The seeds of *Senegalia galpinii* (Burtt Davy) Seigler and Ebinger and *Vachellia robusta* (Burch.) Kyalangalilwa and Boatwright have hard coat-imposed dormancy that prevents water and air from entering the seeds, which is essential for the germination process. The specific objectives of this study were to determine the number of seeds per pod for the study species; determine the size and weight of seeds; and test the effects of scarification pre-sowing treatments on the speed, uniformity and total percent germination of seeds. Seeds of both species were subjected to 10 different pre-sowing seed treatments: the control, mechanical scarification, soaking in concentrated sulphuric acid (for 15, 30, 45 and 60 minutes), immersion in boiling water (for 1, 3 and 5 minutes), and soaking in boiling water (and cooling down for 24 hours). The germination data were examined using an analysis of variance and Tukey's honestly significant difference test to separate significantly different treatment means. The results showed that the mean number of seeds per pod was 7 ± 0.2 and 10 ± 0.03 for *S. galpinii* and *V. robusta*, respectively. For *S. galpinii*, the mean length, width and breadth of seeds were 12 ± 0.2, 10.4 ± 0.1 and 2.7 ± 0.03 mm, respectively. For *V. robusta*, the mean length, width and breadth of the seeds were 10 ± 0.1, 6.1 ± 0.1 and 4.2 ± 0.1, respectively. The mean thousand-seed weights were 275 ± 3 and 183.6 ± 6 g for *S. galpinii* and *V. robusta*, respectively. The results indicated that seeds treated with mechanical, sulphuric acid and boiling water scarification had significantly higher mean germination percentages than the controls for *S. galpinii*, whereas for *V. robusta*, mechanical scarification, exposure to sulphuric acid and immersion in boiling water and then cooling down for 24 hours yielded a better total percent germination than the controls. The highest mean germination percentages for *S. galpinii* (92–100%) were observed for seeds

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treated with sulphuric acid (15, 30, 45 and 60 minutes). For *V. robusta*, mechanical scarification resulted in the highest mean germination percentage (96%), and the boiling water treatments, except for the treatment with 24 hours of cooling down, resulted in the lowest mean germination percentage. The two study species possess seed-coat-imposed dormancies that require pre-sowing seed treatments. Based on the results, the best treatments to release dormancy in both *S. galpinii* and *V. robusta* are sulphuric acid and mechanical scarification, as these yielded the highest, fastest and most uniform germination of seeds. Appropriate recommendations are proposed.

Keywords: dormancy, germination percentage, pre-sowing treatments, seed size and seed weight.

INTRODUCTION

The socio-economic and ecological importance of forest resources and trees outside of forests is reflected in their contribution to livelihood diversification of rural and urban communities, wood and food security, animal feed, health care and environmental conservation (Teketay, 2004-2005). Despite the recognized socio-economic and ecological importance, forest resources and trees outside forests have been declining both in size (deforestation) and quality (degradation), especially in Africa, but also elsewhere in the world, over the past many years.

The major drivers of deforestation and forest degradation are clearing of forests and woodlands for cultivating crops and cutting of trees and shrubs for various purposes, notably for fuelwood, charcoal and construction materials. The fact that planted forests have been very far from meeting the demand for wood for various purposes indicates the inevitability of deforestation. The underlying causes of deforestation are, however, closely linked with the mutually reinforcing factors, i.e. poverty, population growth, poor economic growth and the state of the environment (Teketay, 2001, 2004-2005). Deforestation had caused and continues to cause environmental degradation in the form of land and water resources degradation as well as loss of biodiversity.

The rapid decline of the extent of natural forests has been a great concern in many African countries. Hence, there has been a continuous call by professionals and governments alike to plant trees or establish planted forests in order to address the huge demand of goods and services by both rural and urban communities. As a result, the goal of sustainable forest management has received considerable attention recently in international negotiations. The Rio Declaration (UNCED) and several of the United Nations conventions, as well as the United Nations Forum on Forests (UNFF) and other international processes, meetings and key publications have recognized the critical role of forest resources, including planted forests, in achieving sustainable development and mitigating the effects of climate change (Evans, 2009). The significance of planted forests and recognition of their contributions to a range of development goals are anticipated to increase in the coming decades. Planted forests provide not only

wood, fibre and fuel, but also other non-wood forest products. Moreover, they sequester carbon, rehabilitate degraded lands, help in restoring landscapes, protect watersheds and agricultural soils, and provide recreational areas and amenities.

The study species, namely *Senegalia galpinii* (Burtt Davy) Seigler and Ebinger (synonym: *Acacia galpinii* Burtt Davy) and *Vachellia robusta* (Burch.) Kyalangalilwa and Boatwright (synonym: *Acacia robusta* Burch.), are among the excellent candidates for establishing planted forests because of their multi-purpose uses and very fast growth. However, similar to many other members of the Fabaceae, their seeds possess hard seed coats that are impermeable to water, hence, prevent germination. Seed coat impermeability has been reviewed by several authors (Ballard, 1973; Tran and Cavanagh, 1984; Cavanagh, 1987; Egley, 1989) while its ecological significance has been discussed by Fenner (1985), Baskin and Baskin (1989), Gutterman (1993) and Bewley and Black (1994). Seed coat imposed dormancy is a delaying mechanism, which prevents germination under conditions, which might prove to be unsuitable for establishment, thereby, distributing germination both in time and space (Teketay, 1996a, b, 2005). This, in turn, increases the chances that some seeds will successfully germinate to complete the life cycle. The ability to remain dormant for a long period is associated with seeds of species from unpredictable environments and climate, with very variable rainfall trends, such as those found in Botswana, where most of the *Senegalia* and *Vachellia* species grow.

A high level of seed dormancy is a characteristic feature of many plants of dry regions, and it either completely prevents germination or allows very few seeds to germinate over a long period of time. Therefore, to obtain rapid, uniform and high germination, the dormancy imposed by the hard seed coat should be removed. Lack of knowledge of the germination requirements of leguminous species is an obstacle for their successful artificial regeneration (Teketay, 1996a, b; Mojeremane et al., 2017, 2018; Odirile et al., 2019; Setlhabetsi et al., 2019). Different scarification techniques, such as mechanical, acid and boiling/hot water scarification have been widely used (Teketay, 1996a, b, 1998, 2005; Aref et al., 2011; Tadros et al., 2011; Missanjo et al., 2014; Rasebeka et al., 2014; Fredrick et al., 2016) because they can improve germination by overcoming seed dormancy within a relatively short period of time (Tadros et al., 2011; Mojeremane et al., 2017; 2018; Odirile et al., 2019; Setlhabetsi et al., 2019). But, no single pre-treatment method has been reported to be effective across plant species (Amusa, 2011).

Apart from seed dormancy, seed size, including weight, is an important component in plant fitness, and it is thought commonly to be an important focus of selection on the life histories of plants (Janzen, 1977; Saeed and Shaukat, 2000) since the likelihood of dispersal, germination and survival can all depend on seed size (Howe and Kerckhove, 1980; Schaal, 1980; Saeed and Shaukat, 2000). Studies have shown significant effects of seed size within a species on percent germination (Milberg and Andersson, 1994), rate of germination

(Marshall, 1986), seedling size (Zhang and Maun, 1990) and seedling competitive ability (Gross, 1984). Seed size can show considerable variation within population, and this variability is often associated with variability in seedling size (Schaal, 1980). The individual seed size (mass) in a species varies from nearly constant (Fenner, 1985) to as high as 16-fold (Thompson and Pellmyr, 1989). In general, large seeds have a higher seedling survival rate, higher growth and better field performance than small seeds (Ambika *et al*. 2014; Steiner *et al*., 2019).
Despite the practical importance, there is no information on the numbers of seeds per pod, seed sizes (length, width and breadth), single seed masses and thousand seed weights of both *S. galpinii* and *V. robusta*.

Therefore, the general objective of this study was to determine characteristics of seeds and identify the best scarification seed treatments that result in the fastest and highest as well as uniform germination of the two study species. The specific objectives of the study were to: (a) determine the number of seeds (intact, eaten/dead and aborted seeds) of the study species per pod; (b) determine the size (length, width and breadth) and weight (mass of single seeds and thousand seed weight) of seeds; and (c) test the effects of scarification seed treatment methods (pricking, sulphuric acid and boiling/hot water) on the speed, uniformity and total percentage germination of seeds of the study species.

MATERIAL AND METHODS

Study site

The seeds used in the study were collected in Gaborone, Botswana, located at 24° 39' 11.7252" S and 25° 54' 24.4512" E. It is located at about 15 km away from the South African border. The city lies at an elevation of 994 m (Odirile et al., 2019) and has a hot semi-arid climate, usually with hot summers and sunny days all year round.

Study species

Senegalia galpinii (Burtt Davy) Seigler and Ebinger

The species is also commonly known as the monkey-thorn (English) and mokala, mophoka and mpungwane (Setswana) (Setshogo and Venter, 2003). Monkeys like taking cover in its wide branches and may also eat the pods and seeds, hence, the common name. The tree is fast-growing, reaching 25-30 m, and deciduous, losing its leaves during the southern African winter from April to July. It has a yellowish-brown fissured bark, which is flaking in thin papery pieces as well as short, broad and hooked prickles. Its creamy to light yellow flowers appear during the growing season from September to October and the reddish to purplish brown pods ripen during February to March (Mutshinyalo, 2003).

The species grows naturally in open, wooded grassland, open woodland and often near streams. The trees can survive hot and dry conditions (Mutshinyalo, 2003). The species is indigenous to northern and eastern Botswana, Malawi, South Africa, Zambia, and Zimbabwe (Mutshinyalo, 2003; van Wyk and van Wyk, 2011).

Many insects, such as bees, and wasps visit the flowers. The ripe pods burst open, releasing the seeds, and the seeds are also dispersed by animals eating the pods. In the wild, the plant is grazed and used for shade during the hot summer by different animals including giraffes, kudus and elephants. Many birds often prefer nesting in this tree as it provides protection. The tree makes a stunning tree along roads where there is enough space. It also provides shade on hot summer days, making it an ideal tree for planting on a lawn where some sun can penetrate (Mutshinyalo, 2003). The strong black hooked thorns are ideal for making security hedge (Timberlake, 1980; Venter and Venter, 2013). The roots are burnt and used to cure headaches while dried and crushed pods are used for ear infections, heated pods are used for swellings, wood ash is used to sooth wounds, and the root infusions are used as cough medicine (van Wyk and van Wyk, 2011).

Vachellia robusta (Burch.) Kyalangalilwa and Boatwright

Vachellia robusta is known by the common names: broadpod robust thorn, false umbrella thorn and splendid acacia (English) as well as moga, moku and moshaoka (Setshogo and Venter, 2003). It is represented by fast-growing small to medium-sized trees, reaching 10 m (usually around 5 - 8 m) high, with a slightly flattened crown. The main stem is grey to blackish with rough hairy branches. The white thorns are straight and paired. The leaves are twice-compound with 2 - 5 pairs of pinnae and 10 - 15 pairs of dark green and glossy leaflets. The flowers are creamy white, which are produced from July to October, followed by the dehiscent greyish brown, straight and broad pods, adorning the tree from November to August (Mokobori and Hankey, 2015).

The species occurs in a diverse range of habitats and is component of many plant communities. The species is commonly found in open forests and woodlands, often near streams, where one can find large specimens. In southern Africa, it is very common in the warm dry savannas, up to 1,800 m altitude. It is resistant to drought and frost. It is distributed from tropical Africa southwards to Namibia, eSwazini, Ethiopia, Mozambique, Somalia and South Africa. It has also been introduced elsewhere, e.g. in South Asia (Mokobori and Hankey, 2015).

Leaves are browsed by kudu and other mammals. The strongly scented flowers attract bees and butterflies, and many other insects. The pods and leaves are eaten by herbivores, which distribute the seed in their dung. The seeds are parasitized by Bruchid beetle larvae, which eat the developing seed. Many birds will rifle through the dry seed pods looking for these beetle larvae. Birds, such as Sparrows and Finches, like to build their nests in the densely thorny branches of the trees since the thorns offer excellent protection from predators (Mokobori and Hankey, 2015). The species is modulated by nitrogen-fixing Rhizobium soil bacteria, which colonize inside the roots, where they fix atmospheric nitrogen, which is unavailable to plants and convert it to ammonia (Mokobori and Hankey, 2015).

The wood is considered of rather poor quality, but is sometimes used for making furniture and shelving. It is also used as firewood. The pulping properties

of the wood have been rated as good. In traditional medicine, the powdered root is applied to swellings, and a decoction of the roots is used to treat dysmenorrhea (pain during menstruation), infertility in women and bilharzia (schistosomiasis), whereas a decoction of the stem bark is used to treat gonorrhoea, abdominal pain and skin conditions. The leaves are used to treat snake bites. The species is commonly grown as an attractive garden tree and occasionally grown as a bonsai. The trees can provide a spectacular display when flowering and are very good garden trees, even in frost-prone regions (Timberlake, 1980; Lemmens, 2006; van Wyk and van Wyk, 2011; Mokobori and Hankey, 2015).

Methods

Number of seeds in a pod

The number of seeds of the two study species in a pod were determined from five replications of 10 pods each. The seeds were, then, categorized as intact, aborted or dead/eaten. Once extracted from pods, seeds were immersed in cold water, and only those that sank and settled at the bottom of the container were used for the experiment. The floated seeds, which represented non-viable seeds, were discarded.

Size and weight of seeds

The sizes, i.e. length, width and breadth of seeds, of the two study species were determined by measuring five replications of 10 seeds each using a digital calliper.

The weight of single seeds (seed mass) was determined by weighing five replicates of 10 seeds using a digital sensitive scale. Similarly, five replications of 100 seeds from each study species were weighed to determine the weight of thousand seeds.

Experiments and treatments

In this study, three experiments containing 10 treatments, including the control, were carried out. The three experiments were mechanical scarification, exposure to sulphuric acid and exposure to boiling water. The treatments in the experiments were completely randomized in four replications.

Experiment 1 - Mechanical scarification

In this experiment, 100 seeds of each study species, with four replications of 25 seeds, were used. In all these seeds, 1-2 mm of the seed coat was removed using scissors so that the seeds could imbibe water, which is required to initiate germination.

Experiment 2 - Exposure to sulphuric acid

In this experiment, four periods of exposure of seeds of the study species, i.e. 15, 30, 45 and 60 minutes, to concentrated sulphuric acid (98%) were used by employing the method described by Teketay (1996a). For each period of exposure, the four replications of 25 seeds were put into four 100 ml heat-resistant non-corrosive glass beakers containing sulphuric acid by making sure that all the seeds were covered by the acid. The seeds were continuously stirred to ensure their uniform exposure to the acid. After the specified periods of exposure,

the seeds were sieved out of the acid using an acid-resistant sieve while the acid was drained off simultaneously into another beaker. The seeds were, then, thoroughly washed and rinsed to remove all the acid in a running tap water and distilled water, respectively.

Experiment 3 - Exposure to boiling water

In this experiment, three periods of exposure of seeds of the study species, i.e. 1, 3 and 5 minutes, to boiling water were used. For each period of exposure, four replications of 25 seeds were put into four separate coffee filter papers and immersed into a cooking pot with boiling water for the specified period, after which they were removed and immersed in a small bucket containing cold distilled water to cool them down for a few minutes.

Four replications of 25 untreated seeds were used as control for all the experiments. In all the experiments and the control, each replication containing the 25 seeds were enclosed in 8 mm petri dishes lined with cotton wool. The cotton wool was continuously kept moist by adding distilled water whenever necessary until the end of the experiments. Seeds were considered to have germinated when the radicle penetrated the seed coat and reached 1 - 2 mm. The germinated seeds were counted and recorded on daily basis. The germinated seeds were removed from petri-dishes after counting and recording. The experiments were terminated after 30 days. Seeds that had not geminate after 30 days were tested for their viability by a cutting test.

Data analyses

The data collected was subjected to both descriptive statistics and One-Way ANOVA using Statistix Software, Version 10 (Statistix 10, 1984-2003). Before the ANOVA, the germination percentage data were arcsine transformed to meet the requirement of normality (Zar, 1996). Significant differences of means were tested using Tukey's Honestly Significant Difference (HSD) at the significance level of $P < 0.05$.

RESULTS

Status of number of seeds in a pod

The mean numbers of seeds per pod were 7 ± 0.2 and 10 ± 0.3 in *S. galpinii* and *V. robusta*, respectively. Of these, the mean numbers of intact, eaten and aborted seeds in S. galpinii were 6 ± 0.3, 4 ± 0.1 and 1.2 ± 0.1, respectively. In the case of V. robusta, the mean numbers of intact, eaten and aborted seeds were 7 ± 0.4, 1 ± 0.2 and 2 ± 0.2, respectively (Table 1).

Single and thousand seed weights

The mean mass of single seeds of *S. galpinii* and *V. robusta* was 0.269 ± 0.007 and 0.197 ± 0.0008 grams, respectively. Similarly, the mean thousand seed weights were 275 ± 3 and 183.6 ± 6 grams for *S. galpinii* and *V. robusta*, respectively.

The highest mean germination (100 and 98%) for *S. galpinii* were those from seeds treated with sulphuric acid (30, 45, 60 and 15 minutes), followed by those treated with mechanical (88%) and boiling water (85%, allowed to cool in

24 hours) and boiling water (15 minutes, 74%) while the control and boiling water (3 and 5 minutes) were not significantly different from each other (Table 3).

Table 1. Status (intact, eaten and aborted) and number of seeds pod^{-1} of the study species (mean values ± standard error of means).

Species	Status and Number of Seeds							
	Intact		Eaten		Aborted		Total	
	Number	Range	Number	Range	Number	Range	Number	Range
Senegalia galpinii	6 ± 0.3	1 – 10	0.4 ± 0.1	0 – 4	1.2 ± 0.1	0 - 4	7.4 ± 0.2	4 – 11
Vachellia robusta	7 ± 0.4	0 – 12	1 ± 0.2	0 - 4	2 ± 0.2	0 - 9	10 ± 0.3	3 – 14

Size of seeds

The mean length, width and breadth of *S. galpinii* seeds were 12 ± 0.2, 10.4 ± 0.1 and 2.7 ± 0.03 mm, respectively. For *V. robusta*, the mean length, width and breadth of the seeds were 10 ± 0.1, 6.1 ± 0.1 and 4.2 ± 0.1, respectively (Table 2). Seeds of *S. galpinii* were longer and wider than those of *V. robusta* while *V. robusta* exhibited more breadth than *S. galpinii*.

Table 2. Seed size (mm) of the study species (mean values ± standard error of means).

Species	Size of Seeds (mm)					
	Length	Range	Width	Range	Breadth	Range
Senegalia. galpinii	12 ± 0.2	12 – 13	10.4 ± 0.1	10.2 – 10.7	2.7 ± 0.03	2.7 - 2.9
Vachellia robusta	10 ± 0.1	9.6 - 10	6.1 ± 0.1	6 – 6.4	4.2 ± 0.1	4 – 4.4

Germination of seeds

The results indicated that the seeds treated with mechanical, sulphuric acid and boiling water scarification had significantly higher mean germination percentages than the controls in the two study species *[S. galpinii* - One Way ANOVA: (F (9, 39) = 34, P = 0.00001) and *V. robusta* - One Way ANOVA: (F (9, 39) = 33, P = 0.00001)] (Table 3).

For *V. robusta*, the mechanical scarification had the highest mean germination (96%), followed by those treated with sulphuric acid for 60 and 45 minutes (86 and 72%, respectively) and boiling water for 24 hours (70%). Untreated seeds (34%) and those treated with boiling water for 1 (24%), 3 (12%) and 5 (6%) minutes exhibited the lowest mean germination percentages (Table 3).

Table 3. Means and ranges of the cumulative germination of seeds of the study species subjected to different pre-sowing seed treatments (± standard error of the means).

Treatment	*S. galpinii*		*V. robusta*	
	Germination (%)	Range (%)	Germination (%)	Range (%)
Control	9 ± 10^{d}	4 - 24	34 ± 6^{de}	24 - 52
Mechanical Scarification	88 ± 11^{abc}	72 - 96	96 ± 2^{a}	92 - 100
Sulphuric Acid (15 minutes)	92 ± 6^{abc}	88 - 100	32 ± 6^{de}	16 - 44
Sulphuric Acid (30 minutes)	100 ± 0^{a}	100 - 100	52 ± 6^{cd}	40 - 68
Sulphuric Acid (45 minutes)	98 ± 4^{ab}	92 - 100	72 ± 8^{bc}	52 - 88
Sulphuric Acid (60 minutes)	98 ± 4^{ab}	92 - 100	86 ± 4^{ab}	76 - 96
Boiling Water (1 minute)	74 ± 16^{c}	60 - 88	24 ± 3^{def}	16 - 28
Boiling Water (3 minutes)	39 ± 13^{d}	24 - 52	12 ± 5^{ef}	0 - 20
Boiling Water (5 minutes)	23 ± 12^{d}	16 - 40	6 ± 3^{f}	0 - 12
Hot Water (boiling water allowed to cool in 24 hours)	85 ± 4^{bc}	80 - 88	70 ± 8^{bc}	56 - 84

Means separated using Tukey's Honestly Significant Difference (HSD) Test at $P \leq 0.05$. Means within columns followed by the same letters for each species are not significantly different.

Rate of Seed Germination

The results revealed that seeds of *S. galpinii* that were exposed to sulphuric acid and mechanical scarification exhibited the fastest and uniform germination, reaching > 91% and > 85% cumulative germination, respectively, within six days after sowing followed by those treated with hot water and boiling water for 1 minute, reaching > 71% and > 66% within six days (Figure 1), respectively.

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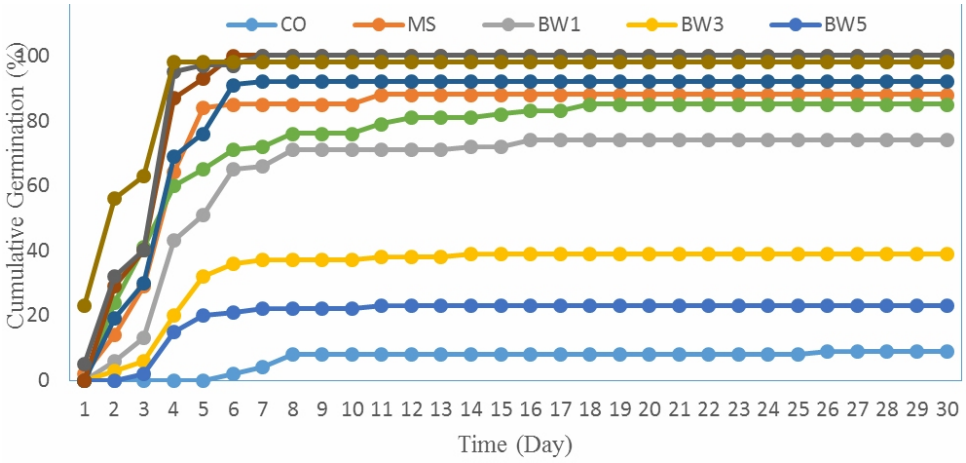

Figure 1. Cumulative germination percentage of *Senegalia galpinii* recorded for 30 days (CO = Control, MS = Manual scarification, BW1 = Boiling water for 1 minute; BW3 = Boiling water for 3 minutes, BW5 = Boiling water for 5 minutes, HW24 = Boiling water allowed to cool in 24 hours, SA15 = Sulphuric acid for 15 minutes, SA30 = Sulphuric acid for 30 minutes, SA45 = Sulphuric acid for 45 minutes and SA60 = Sulphuric acid 60 minutes).

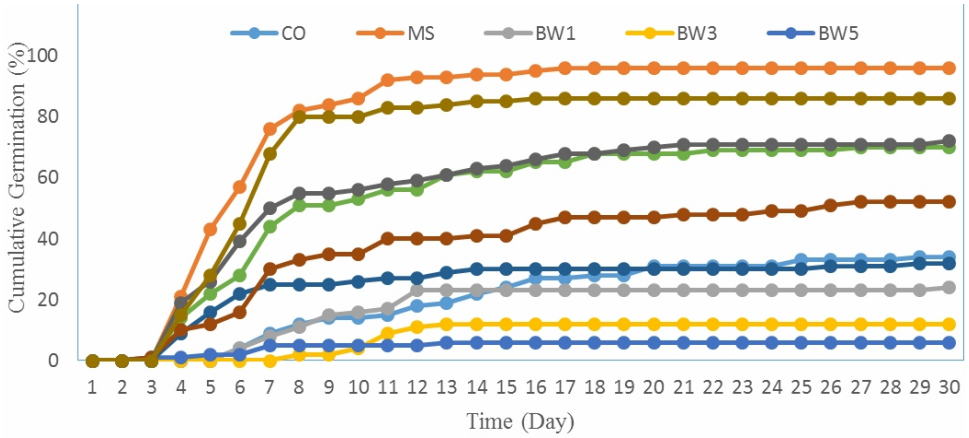

Figure 2. Cumulative germination percentage of *Vachellia robusta* recorded for 30 days (CO = Control, MS = Manual scarification, BW1 = Boiling water for 1 minute; BW3 = Boiling water for 3 minutes, BW5 = Boiling water for 5 minutes, HW24 = Boiling water allowed to cool in 24 hours, SA15 = Sulphuric acid for 15 minutes, SA30 = Sulphuric acid for 30 minutes, SA45 = Sulphuric acid for 45 minutes and SA60 = Sulphuric acid 60 minutes).

On the other hand, untreated seeds (control) and seeds treated with boiling water for 3 and 5 minutes exhibited not only the lowest, but also the slowest germination.

The results also showed that in the case of *V. robusta*, seeds treated with mechanical scarification and sulphuric acid for 60 minutes exhibited the fastest and uniform seed germination, reaching >82% and >80% cumulative germination, respectively, within eight days (Figure 2). On the other hand, seeds treated with boiling water for 1, 3 and 5 minutes exhibited, not only the lowest, but also the slowest germination

DISCUSSION

It has been demonstrated that seed size, represented by length, width, breadth and weight, is a life history trait that may affect the fitness of parent plants and the population regeneration process (Harper *et al*., 1970; Harper, 1977; Fenner, 1985; Gross and Kramer, 1986; Silvertown, 1989; Chacon *et al*., 1998; Finch-Savage and Bassel 2016; Steiner *et al*., 2019). This implies that seed size affects the germination, emergence, plant growth and performance of plants in the field. It has been shown that plants from large seeds had larger and more leaves, greater plant weight and more vigour than plants from small seeds (Elliott *et al*., 2007). Large seeds have been shown to exhibit higher germination and emergence than small seeds and produce larger and more vigorous seedlings, which may enhance survivorship (Hendrix, 1984; Sikder *et al*. 2009; Steiner *et al*., 2019).

Seeds of *S. galpinii* exhibited longer and wider, but less broad, seeds as well as heavier single seed masses and thousand seed weights than those reported

for *V. robusta* (Table 2) and *V. rehmanniana* Schinz. Similarly, *V. robusta* had longer and broader, but comparable width of seeds with *V. rehmanniana* (Mojeremane et al., 2017). Seeds of *S. galpinii* were longer and wider than those of *Vachellia erioloba* (E. Mey.) P.J.H. Hurter, but *V. erioloba* exhibited longer and wider seeds than those of *V. robusta* as well as broader and comparably broader seeds than *S. galpinii* and *V. robusta*, respectively (Odirile et al., 2019). Both *S. galpinii* and *V. robusta* exhibited longer, wider and broader seeds as well as much heavier single seed masses and thousand seed weights than several other similar leguminous species, e.g. *V. tortilis* (Forssk.) Galasso and Banfi (Odirile, 2018), *Dichrostachys cinerea* (L.) Wight and Arn., *Senegalia erubescens* (Welw. ex Oliver) Kyal. and Boatwr. and *Vachellia nilotica* (L.) Delile (Kahaka et al., 2018), *Peltophorum africanum* Sond. (Mojeremane *et al*., 2018), *Sesbania bispinosa* (Jacq.) W. Wight (Mafote, 2018), and other species, e.g. *Ziziphus mucronata* Willd. (Malatsi, 2019). The large seeds possessed by both *S. galpinii* and *V. robusta* would confer advantages of having higher germination, as evidenced in this study, and emergence as well as developing larger and more vigorous seedlings, which ensure higher survival in the field.

Most evidences (Tran and Cavanagh, 1984; Cavanagh, 1987; Egley, 1989) implicate that the continuous layer of tightly packed palisade cells in the seed coat as containing the major barrier to water entry into seeds. Therefore, rupture of the seed coat is necessary to trigger germination in many hard-seeded species with impermeable seed coats (Ballard 1973; Baskin and Baskin 1989; Bewley and Black 1994; Rolston 1978; Teketay, 2005). Hence, seeds have to be pre-treated before sowing to overcome the hard seed-coat imposed dormancy that will stimulate water imbibition and their germination. Among the different pre-sowing seed treatments, scarification using mechanical removal of the seed coats (about 2 mm) and exposure of seeds to different durations in sulphuric acid are known to be consistently effective, resulting in rapid, uniform and high germination in different species (Cavanagh, 1987). For some species exposure of seeds to different durations in boiling water has also been shown to increase germination (Teketay, 1996a, b, 1998, 2005).

In the present study, scarification of seeds of *S. galpinii* with mechanical means, sulphuric acid and boiling water resulted in better germination percentages (23 - 100%) than the untreated seeds (control = 9%). Similarly, seeds of *V. robusta* seeds treated with mechanical scarification, sulphuric acid and boiling water (allowed to cool for 24 hours = 70%) (duration = 30, 45 and 60 minutes) exhibited better germination percentages (52 - 96%) than those in the control (34%). However, seeds of *V. robusta* treated with sulphuric acid (for 15 minutes = 32%) and boiling water (for 1, 3 and 5 minutes) showed lower germination percentage (6 - 24%) than the control, suggesting that these treatments are not effective to improve germination of seeds significantly since the seeds were sensitive to high temperatures.

The present study confirms results from earlier seed germination studies on other leguminous species, e.g. species of *Acacia* (Larsen, 1964; Clemens et al.,

1977; Bebawi and Mohamed, 1985; Sniezko and Gwaze, 1987; Danthu et al., 1992; Masamba, 1994; Teketay, 1996a; Tadros et al., 2011), *Afzelia* and *Baikiaea* (Botsheleng, *et al*., 2014), *Albizia* (Babeley *et al*., 1986; Msanga and Maghembe, 1986; Khan and Tripathi, 1987; Teketay *et al*., 1996a, 2018), *Cadaba* (Teketay, 1996a), *Caesalpinia* (Ngulube, 1989; Teketay, 1996a), *Erythrina* (Teketay, 1994) and *Leucaena* (Oakes, 1984; Babeley and Kandaya, 1985; Duguma et al., 1988; Teketay, 1996a; Tadros et al., 2011), *Calliandra* and *Sesbania* (Albrecht, 1993), *Entada*, *Delonix* and *Prosopis* (Teketay, 1996a), *Faidherbia* (Fredrick *et al.*, 2016), *Vachellia* and *Peltophorum* (Mojeremane et al., 2017; Mojeremane et al., 2018; Kahaka et al., 2018; Odirile et al., 2019), *Dicrostachys* and *Senegalia* (Kahaka et al., 2018), and *Philenoptera* (Setlhabetsi et al., 2019), which demonstrated that scarification of seeds through mechanical, sulphuric acid and boiling water pre-sowing treatments improved germination.

In the natural habitat, fire (Dell, 1980; Sabiiti and Wein, 1987), animal ingestion (Lamprey et al., 1974; Russi et al., 1992; Gardener et al., 1993), abrasion (Gutterman, 1993), soil acids and soil organisms (Bewley and Black, 1994) as well as fluctuating temperatures (Probers, 1992) have been implicated to be responsible for breaking seed coat-imposed dormancy. Moist heat, mimicked by boiling water in the present study, has also been reported to improve germination (Martin et al., 1975; Jeffery et al., 1988). Moist heat is thought to simulate more nearly conditions in a forest fire since moisture is available from thermal degradation and combustion of woody fuels and from moisture present in the fuels (Teketay, 2005). Fire is an annual recurrent phenomenon across many habitats in Botswana, including forests and woodlands. Therefore, heat generated from fire may act as one of the factors stimulating germination.

Pods of *S. galpinii* (Mutshinyalo, 2003) and *V. robusta* (Mokobori and Hankey, 2015) are browsed by different animals. Hence, passage of the hard seeds through the digestive tracts of animals may be another means by which their scarification is effected making them ready for germination when they are dispersed in the dung of animals. The diurnal fluctuation of temperature between day and night may also be another factor which stimulates germination in the natural habitats.

CONCLUSIONS

Results from the present study show that the barrier to germination of seeds of *S. galpinii* and *V. robusta* is the hard seed coat, which prevents water uptake. Hence, before the seeds can germinate, they require mechanisms, which overcome this barrier. The results also indicated that the barrier can be removed through different scarification pre-sowing treatments. For *S. galpinii*, mechanical scarification as well as exposure to sulphuric acid and boiling water significantly improved percent germination of seeds while for *V. robusta* only mechanical scarification, exposure to sulphuric acid and immersing the seeds in boiling water, which was allowed to cool down for 24 hours, gave better percent germination than the untreated seeds. The results also confirmed that sulphuric

acid and mechanical scarification treatments resulted in the highest, fastest and uniform germination percentages relative to the control and boiling water treatments.

Therefore, extension agents and researchers that have plans to raise seedlings of *S. galpinii* and *V. robusta* should undertake scarification treatments using mechanical scarification, sulphuric acid and boiling water (only for *S. galpinii*), before sowing the seeds in order to render the seed coats permeable to water and trigger germination. Mechanical scarification and boiling water treatments are recommended for nurseries since they are safer and require less skill to administer while sulphuric acid treatments can be used in research laboratories.

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WEED CONTROL DURING THE FIRST VEGETATION OF BLACK POPLAR (*POPULUS NIGRA* L.) PLANTATION

SUMMARY

Black poplar (*Populus nigra* L.) is fast-growing species, demanding in terms of light and soil and very sensitive to weeds, especially in the first vegetation. Therefore, developing effective, environmentally friendly weed control measures is an urgent task for herbologists. There is an inverse relationship between the amount of weed mass and the productivity of black poplar; the lowest height, biomass yield and energy yield (87.1 cm, 0.41 t/ha and 8.06 GJ/ha, respectively) were found in the control treatment with the highest weed mass accumulation (3062 g/m^2). The practice of mechanical weed control influenced the accumulation of weed mass in the black poplar plantations. Three consecutive cultivations of interrow space and three consecutive harrowing sessions at an interval of 14 days reduced weed mass 2.7 times. Soil mulching with sawdust almost completely destroyed the weeds in the experimental plots.

Keywords: poplar, average productivity, weed infestation, leaf area.

INTRODUCTION

Poplar (*Populus spp.*) is undoubtedly one of the most promising bioenergy crops due to very fast growth and ability to produce more than 15 m^3 of wood in a short time (Berguson et al., 2010). There are up to 4.0 million hectares of unproductive agricultural land suitable for energy trees cultivation in Ukraine. The cultivation of energy wood on plantations, in particular, the representatives of *Populus* L., has been studied relatively recently In Ukraine (Vysotska, 2016; Kharytonov et al., 2017). Energy plantations will facilitate the rational use of the country's land resources. Poplar biomass has been used in EU countries as a feedstock for the production of bioethanol and pellets for house heating at least last decade (Branco et al, 2019; González - García and Bacenetti, 2019). Poplar trees are distinguished from other tree species by many valuable biological features. Firstly, they grow very fast. Poplar trunks are suitable for cellulose

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production already in 20 years (Klasnja et al., 2012; Karacic et al., 2006). Secondly, the possibility of easy vegetative multiplication using winter stem cuttings, root cuttings, and root shoots as well as easy seed reproduction. Thirdly, easy natural and artificial, intro - and interspecific hybridization that facilitates the production of new fast - growing clones (Kutsokon et al., 2014). Poplar is common in forests throughout the Northern Hemisphere in both temperate and subtropical zones (Kutsokon et al., 2014). Mostly it occurs in North America and Eurasia (Panacci et al., 2009; Labrecque and Teodorescu, 2005; Karacic and Weih, 2006). Today, Canada is one of the leading countries in the world for the production and use of poplar wood biomass (Labrecque and Teodorescu, 2005). Its use as the secondary energy source in the country increased from 3.5% in 1970 to 6.5 % (Dickmann, 2006). Plant survival and intensive growth of new poplar plantations are affected by weed competition (Kauter et al., 2003). It was shown the importance of weed control in the first year of energy trees cultivation regardless of the genotype and growing conditions (Broeckx et al., 2012; Albertsson et al., 2014).

Weeds in poplar planting are strong competitors for water, nutrients, and light. Aboveground competition affects the morphological and physiological features of plants, such as leaf area, plant height, and biomass, photosynthetic activity, which directly or indirectly affects the ability of plants to consume light (Balandier et al., 2006). Underground competition, above all, for nutrients and water is more important and has a greater impact on the growth and development of both cultivated plants and weeds. In this case, there is a more complex mechanism of root system activity that involves such soil properties as density, structure and microorganisms community (Phillips et al., 2014; Caldwell et al., 1986; Casper and Jackson, 1997). As a result of competition with weeds, a decrease in growth of more than 50 % and an increase in the die-off rate of trees in plantations are observed (Thompson and Pit, 2003). Therefore, it is urgent to substantiate a competitive power of black poplar plantings and to develop an effective weed control system for the Right-Bank Forest Steppe of Ukraine.

The main objective of this study was to evaluate the efficiency of available environment-friendly mechanical and environmental weed control practices for black poplar.

MATERIAL AND METHODS

This case study was carried out from 2017 to 2018 in field experiment at the Salyvinky Experimental Farm of the Institute of Bioenergy Crops and Sugar Beet NAAS (IBCSB) in Ksaverivka village, Vasylkiv District, Kyiv Region located in the zone of unstable soil water supply of the Central Forest Steppe of Ukraine, with the moderately continental climate. The soil for the experiment was meadow chernozem or molisols (Kravchenko et al., 2012). Complete cultivation was carried out before planting. One-year black poplar cuttings of 25 cm in length were planted in the middle of April. Planting design was 150 cm x 75 cm x 75 cm. The distance between the plants in a row was 0.59 cm. Plant density was 15,000 per hectare. Sowing area was 50 m^2 and accounting area 25 m^2. The

experiment was carried with three replicates. Weeds observation was carried out using fixed frames measured 1.25 m x 0.20 m = 0.25 m ^{2} that were permanently set in four places diagonally in each treatment (Tsyliuryk et al, 2017). The first and second registration of weeds was made in early May and second decade of August accordingly.

The yield of the above-ground part of plants was determined by the method of cutting the above-ground parts at the experimental sites and expressed in either g/m^2 or t/ha. The experiment was established in energy plantations of Salix viminalis in its first growing season according to the following design: (a) without weed treatment; (b) three consequent cultivations between rows at an interval of 14 days; (c) three consequent harrowing between rows using mounted chain harrow at an interval of 15 days; (d) three consequent manual weed cutting (cut height 1.5−3.0 cm at an interval of 14 days); (e) topsoil mulching with 15-cm sawdust layer; (f) six consequent hand weedings (to total destruction of weeds).

RESULTS AND DISCUSSION

At the time of the first records of the number of weeds observation, the dominating weeds were *Elymus repens* (L.) Gould (9.9), *Setaria glauca* (L.) P. Beauv (3.7), *Chenopodium album* L. (4.1), *Echinochloa crus-galli* (L.) P. Beauv (3.4) and other species. The total number of weeds averaged 33 units (Table 1).

Table 1: Weed infestation of black poplar stands, 2017−2018 (plant/ m^2)

Weed species	Date of counting				
	13.05	13.06	13.07	13.08	13.09
Echinochlea cruss-galli (L.) P. Beauv	3.4	21.3	22.4	22.6	22.5
Setaria glauca (L.) P. Beauv	3.7	22.8	29.3	29.8	29.8
Chenopodium album L.	4.1	5.7	7.9	8.5	8.5
Sinapis avrensis	2.2	6.2	7.7	8.0	8.0
Thlaspi avrense L.	2.1	8.4	9.8	10.0	10.1
Polygonum lapathifolium	1.3	1.3	2.8	3.3	3.3
Solanum nigrum	1.8	2.7	3.2	5.9	6.2
Elytrigia repens (L.) Gould	9.9	15.9	16.1	17.2	17.2
Other species	4.5	5.6	8.2	8.3	8.3
Total weeds	33.0	89.9	107.4	113.6	113.9

Weed mass accumulation in black poplar stands was significantly affected by an applied weed control practice (Table 2). Carrying out three cultivations of inter-row space at an interval of 14 days (treatment B), as well as three harrowing (treatment C), reduced the weed mass 2.7 times. Hand weeding (treatment D) reduced it 2.9 times. The application of a 15-cm layer of wood sawdust (treatment E) almost completely destroyed the weeds. Calculations of projective leaf area in one-year black poplar stands revealed very small values of this area, in the first half of the growing season especially. Average values of projective leaf area at

the time of the first counting (13 May) showed that all weed species formed 28.3 % of the projective leaf area in total.

Table 2: The efficiency of mechanical weed control systems in black poplar stands on the value of weed mass accumulation, the average for 2017−2018 (g/m^2)

Weed species	Treatment					
	A	B	C	D	E	F
Echinochlea cruss-galli (L.) P. Beauv	322	114	121	111	-	-
Setaria glauca (L.) P. Beauv	218	101	103	97	-	-
Chenopodium album L.	611	232	217	230	-	-
Sinapis avrensis	237	81	79	85	-	-
Thlaspi avrense L.	211	46	41	43	-	-
Polygonum lapathifolium	187	65	66	61	-	-
Solanum nigrum	829	312	321	274	-	-
Elytrigia repens (L.) Gould	134	97	101	72	7	-
Other species	313	79	82	71	3	-
Total weeds	3062	1127	1131	1044	10	-
$LSD_{0.05}$	1.8					-

Poplar seedlings had not yet formed a significant projective coverage yet. It was less than 1 % (Table 3).

Table 3: Projective leaf area of weeds in black poplar stands, 2018 (%)

Weed species	Date of counting				
	13.05	13.06	13.07	13.08	13.09
Echinochlea cruss-galli (L.) P. Beauv	1.1	14.2	13.7	12.4	9.1
Setaria glauca (L.) P. Beauv	1.1	14.4	13.2	11.6	8.9
Chenopodium album L.	1.1	15.7	16.1	17.9	13.3
Sinapis avrensis	9.7	18.3	21.7	20.6	18.6
Thlaspi avrense L.	9.5	16.1	18.3	18.8	15.7
Polygonum lapathifolium	1.5	7.2	10.1	11.9	9.9
Solanum nigrum	1.1	2.9	3.1	4.1	3.7
Elytrigia repens (L.) Gould	1.3	6.3	2.6	1.7	0,8
Other species	1.9	4.9	1.2	1.0	0,9
Total weeds	28.3	100	100	100	80.9

As a result of the active processes of plant growth and development after 30 days, i.e. on 13 June, the situation in the field changed. Culture plants formed leaves and started forming new shoots. Their projective leaf area increased to 5 %. Wild plants filled all available ecological niches and completed projective cover to 100 %. The largest share in the formation of the projective leaf area was fixed among the next several weed species: *Sinapis avrensis* (18.3 %), *Thlaspi avrense* L. (16.1 %), *Chenopodium album* (L.) (15.7 %), *Setaria glauca* (L.) (14.4 %), *Echinochlea cruss-galli* (L.) (14.2 %), *Elytrigia repens* (L.) (6.3 %). Different intensity of weed infestation in the experimental plots significantly affected the growth rate of black poplar seedlings, biomass accumulation and then, in a consequence, energy yield (Table 4).

Table 4: The average productivity of black poplar under different weed control systems, 2016−2018

Parameter	Treatment						
	A	B	C	D	E	F	$LSD_{0.05}$
Shoot height (cm)	87.1	130.5	130.1	133.2	166.3	171.0	5.9
Yield (t/ha)	0.41	0.77	0.76	0.81	1.10	1.16	0.09
Energy yield (GJ/ha)	8.06	15.22	15.04	15.93	21.66	22.91	1.4

There is an inverse relationship between weed number and poplar productivity. Thus, the lowest values of height, biomass yield, and energy yield (87.1 cm, 0.41 t/ha and 8.06 GJ/ha, respectively) were found in the control treatment, where the highest amount of weed mass was found as 3062 g/m^2 (Table 2).

The plants of black poplar had approximately the same height (130.1−133.2 cm), dry biomass yield (0.76−0.81 t/ha) and energy yield (15.04−15.93 GJ/ha) in treatments B, C and D, where approximately the same weed mass amount (from 1044 to 1131 g/m^2) was measured. Similar results were obtained in our field experiments to see best weed control method in willow plantation (Fuchylo et al., 2019).

Treatment E, with the application of sawdust as mulch, provided almost complete destruction of the weeds (only 10 g/m^2 remained). This treatment result can be compared with treatment F after six consecutive weedings.

CONCLUSIONS

Black poplar, as a fast-growing, and demanding to light and soil crop, is very sensitive to weeds, especially in the first vegetation.

There is an inverse relationship between the amount of weed mass and the productivity of black poplar. Thus, the lowest values of height, biomass yield, and energy yield (87.1 cm, 0.41 t/ha and 8.06 GJ/ha, respectively) were found in the control treatment with the highest weed mass accumulation (3062 g/m^2).

The practice of mechanical weed control influenced on the accumulation of weed mass in the black poplar plantations. Three consecutive cultivations of

interrow space and three consecutive harrowing at an interval of 14 days reduced weed mass 2.7 times. Soil mulching with sawdust almost completely destroyed the weeds in plots with poplar.

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EFFECT OF SUCROSE ON THE PHYSIOLOGY AND TERRESTRIC ACID PRODUCTION OF *PENICILLIUM AURANTIOGRISEUM*

SUMMARY

Penicillium aurantiogriseum (*P. aurantiogriseum*) is a post-harvest pathogen that causes significant losses in agricultural production during storage. It plays an important role in food and feed spoilage, and it contaminates agricultural products with mycotoxins that are potentially harmful to human and animal health. *P. aurantiogriseum* is one of the most toxic species in the genus *Penicillium*, and it is often isolated from foods, vegetables, fruits and permafrost sediments from the Arctic and Antarctic. It has also been isolated from the marine environment. Thus, it is resistant to several types of stress related to nutrients and growing conditions. This study aimed to determine the effect of sucrose on the physiology of *P. aurantiogriseum* in order to control its growth and toxigenesis. Mycotoxin production was determined by TLC technique. Our results show a close relationship between the physiological state of *P. aurantiogriseum* and the secretion of mycotoxins under carbon stress conditions. The physiological state of the pathogen reveals a correlation between increased sucrose concentration and the intensity of aging signs. Aging signs begin to disappear at a sucrose concentration of 400 g/l, which allows the normal characteristics of P. aurantiogriseum to reappear. It is suggested that this transformation is meant to avoid the action of sucrose. Terrestric acid production was recorded at the time of appearance of aging signs. Terrestric acid is always maintained, even after returning to a normal physiological state, but its production was diminished. The growth of *P. aurantiogriseum* can be controlled by modifying the sucrose concentration in growth medium. This allowed us to determine the critical concentration at which the pathogen suffered and thus reached the phase of decline earlier while mycotoxin production was minimal.

Keywords: *P. aurantiogriseum*, sucrose, physiology, aging signs, terrestric acid.

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INTRODUCTION

Mycotoxins, as toxic secondary metabolites of molds, have been detected in several human or livestock foods (Khaddor et al., 2006). The ingestion of mycotoxins represents a real menace to human and animal health (Faid and Tantaoui-Elaraki, 1989). *P. aurantiogriseum* is a particular species of *Penicillium* genus. It is ubiquitous in the terrestrial and marine environment (Yu et al., 2010; Sonjak et al., 2005). It is a post-harvest pathogen causes significant losses of agricultural production during storage (Khaddor et al., 2006). This species is recognized as a prolific source of biologically active secondary metabolites. Mycotoxins of *P. aurantiogriseum* are of great importance given their largely variable effects between harmful and beneficial to human and animal health (Khaddor et al., 2007). By this double effect, mycotoxins of *P. aurantiogriseum* could be used in pharmaceutical industry because of their therapeutic potential and also in agri-food industry which can minimize their unsafe effect by controlling growth factors. Previous studies had identified some mycotoxins such as penicillic acid, aurantiamine, and terrestric acid on *P. aurantiogriseum* (Khaddor et al., 2007).

Terrestric acid is the least common toxin in *Penicillium* species (Peberdy, 1987). There are few studies of natural contamination with terrestric acid and its toxicity, and little is known about the factors influencing its production. The production of terrestric acid by *P. aurantiogriseum* was demonstrated in 1971 by Turner et al. (1971). The offending substance was found to be phytotoxic (Gausman, 1991) and cardiotoxic (Frisvad and Samson, 2004.).

Sucrose is the most used carbon source in growth media for development and production of *Penicillium* mycotoxins. Except for sucrose, the substitution between carbon sources in a growth medium does not present a large difference in colony production (Bode et al., 2002). This production is also influenced by the addition of nitrate. The addition of complex accessory factors such as yeast extract to the medium increases the rate of growth while having little effect on the colony (Smith et al., 1981; Pitt, 1973). Glycerol facilitates the consistent development of *Penicillium* colonies (Pitt, 1973) and it is an excellent carbon source for mycotoxin production (Mulè et al., 2004). Hocking and Pitt (1979) recommend the use of glycerol to adjust water activity with the least harmful effects on fungal growth. Several studies have used G25N (25% glycerol nitrate) as an identification and purification medium, but not for the production of mycotoxins (Park et al., 2014; Zhao et al., 2014). Khaddor et al. (2007) showed that penicillic acid and aurantiamine are produced by *P. aurantiogriseum* in CYA (Samson and Gams, 1984) and terrestric acid in YES liquid (Samson and Gams, 1984). Therefore thus, we suggest that the basic medium that will be used for the growth and toxigenesis of *P. aurantiogriseum* is the G25N (Pitt, 1973) medium added at different sucrose concentrations (to get the G25N* medium).

The present work is devoted to study the effects of sucrose on the physiology and terrestric acid production of *P. aurantiogriseum*. Thus, it may help to control the growth conditions of *P. aurantiogriseum* in order to improve

the production of its mycotoxins with therapeutic interest or restrict its growth to minimize the harmful effects of this species.

MATERIAL AND METHODS

Fungal Strains

The strain of *P. aurantiogriseum* is part of the collection of the Environmental and Food Biotechnology Research Team (EFBRT) used in previous studies (Bouhoudan et al., 2018; Khaddor et al., 2007; Maouni et al., 2002). The stored strain is placed in the MEA (malt extract agar) and incubated at 25°C for 7 days. After incubation, the spores were suspended in 0. 1 % of tween 80. The density of suspension was adjusted to 107 spores /ml.

Growth medium

P. aurantiogriseum was inoculated on G25N* medium (at different concentrations of sucrose from 0 g/l to 700 g/l). The dishes are incubated for 10 days at 25°C.

Determination of mycelial dry weight and colony diameter

Physiological studies are based on morphology, texture, color, growth rings, growth status, mycelial weight, and colony diameter of *P. aurantiogriseum* colonies, as well as the aspect of its hyphae.

Mycelia were harvested by filtration using Buchner funnel. Then they were washed thoroughly with distilled water and the excess of water was removed by plotting with filter papers. The mycelia were dried at 80°C until constant weight obtained which is a dry weight (Zain et al., 2009). Radial growth was estimated by measuring the diameter of each colony with a ruler (Zain et al., 2009). All the experiments were performed in triplicate.

Mycotoxins extraction

The toxigenesis study was made according to the method reported by Bouhoudan et al. (2018). The colonies grown in G25N* medium were added with 25 ml of chloroform and agitated during 2 minutes. The chloroform phase recovered was filtered on anhydrous sodium sulfate. The filtrate was concentrated to the rotavapor and then evaporated to dryness in nitrogen current. Thin Layer Chromatography (TLC) highlights mycotoxins in concentrated filtrates thus obtained. The thin layer chromatography technique (TLC) adopted in this study is described by Mills et al. (1995). The TLC plates used are 60 Kieselguhr F254. Mycotoxins standards used by the reference of migration forehead (Rf) were patulin (P), citrinin (C), ochratoxin A (OTA), penicillic acid (PA), and griseofulvin (Gi). Ten ml each of ethanol extract and of standard solutions (1 mg/ml) were spotted on TLC plates. Elution systems used are toluene-ethyl acetate - formic acid (5/4/1, v/v/v) and chloroform - acetone - 2-propanol (85/15/20, v/v/v). The plates were examined in daylight and by ultraviolet 365 and 254 nm after spraying the spots by ANIS (p-anisaldehyde solution) and 8 min heating to 120°C. The ratio (Rf), color and fluorescence intensity of the extracts were compared with different reference concentrations of P, C, Gi, PA and OTA

(Cunniff, 1995). Fluorescence intensity was expressed by a variable number of "+" signs (Hameed et al., 2012).

Statistical study

Statistical analysis of the obtained results was performed by the test "Duncan's multiple range" at the threshold of 5% [Stat Soft]. For each medium, nine tests were performed. The averages obtained in the nine trials (n = 9) were compared by analysis of variance (ANOVA) with the Ducan's Multiple Range test at the 5% threshold. This test is then used to define more precisely if the factor (carbon source) has seen a really significant effect on the response (mycotoxin production and lipase and fungal growth).

RESULTS

Macromorphological characteristics of P. aurantiogriseum

Our results revealed that the mycelia weight and the diameter of *P. aurantiogriseum* colonies were increased with increasing concentration of sucrose (Figure 1 and Table 1).

Table 1: Average colony diameters, dry weight and AT intensity of P. aurantiogriseum as a function of sucrose concentration of G25N medium.

Sucrose concentration (g/l)	Mycelial Dry weight (g/100 ml) (95% IC)	Colony Diameter (cm) (95% IC)	Intensity of Terrestric Acid
0	0.68 d	1.98 d	–
30	4.00 ab	40.00 ab	+
50	4.48 a	41.00 ab	+
100	4.88 a	45.00 b	+
200	6.05 c	50.00 e	++
300	6.96 c	55.00 c	+++
400	8.72 e	36.37 a	++++
500	4.03 ab	57.70 c	++
700	3.19 b	62.77 f	++
(-) No (+) low, (++) medium, (++) strong (+++) very strong			

On the same column, 2 results followed by the same letter do not differ significantly at the 5% threshold. For each concentration of YES, nine tests were performed. The averages obtained in the nine trials (n = 9) were compared by analysis of variance (ANOVA) with the Ducan's Multiple Range test at the 5% threshold.

At different sucrose concentrations ranging from 0 to 700 g/l, signs of aging are observed at the macroscopic level. *P. aurantiogriseum* colonies change color in a centrifugal direction. They are whiter from the center to the periphery. The colony diameter increases with the concentration of sucrose. The rough shape extends centrifugally over the entire colony. We also noted a change of relief that results in the elevation of the colony central area. The aging degree is proportional to the sucrose concentration used (Figure 1).

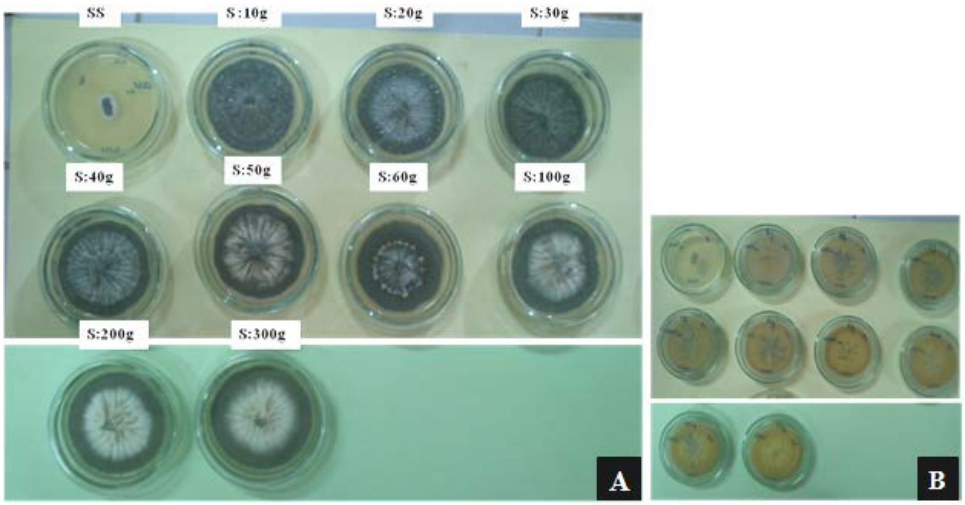

Figure 1: Physiological modification of *P. aurantiogriseum* in the medium containing different sucrose concentrations (g/l); A: front view of the colony, B:reverse view of the colony; S: sucrose; SS: no sucrose

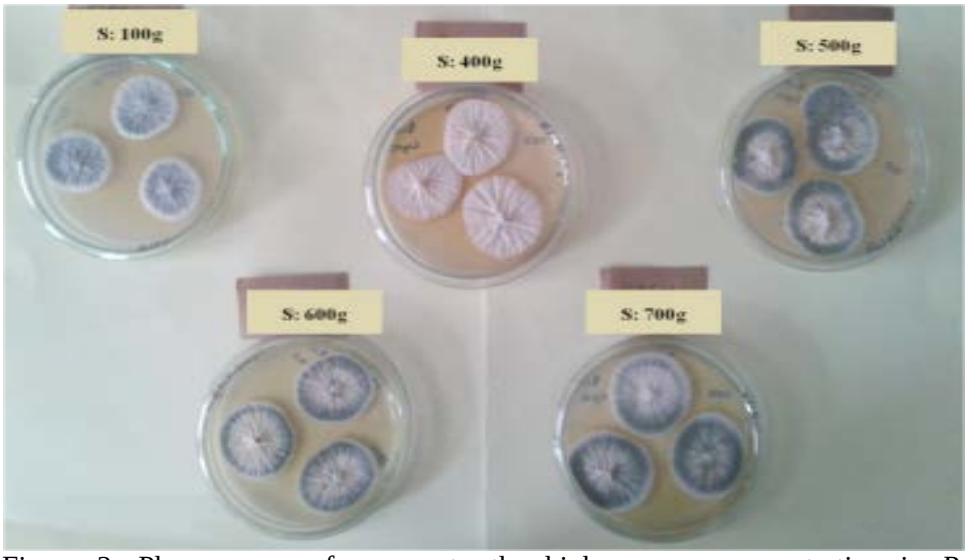

Figure 2: Phenomenon of escape to the high sucrose concentration in *P. aurantiogriseum.* At the concentration of 400 g/l, the strain begins to resume its normal characters and the aging signs disappear. S: sucrose

The use of increasing sucrose concentrations allowed us to observe signs of suffering and aging reflected by the physiological activity of the strain. This results in morphological changes on the colonies (Table 1).

There was a critical concentration (400g/l) for which the strain responded aggressively to the concentration and this appeared at the macroscopic level

where the colony has a whitish and very rigorous appearance. The aging signs begin to disappear from the sucrose concentration of 400 g/l, revealing the normal characters of *P. aurantiogriseum.* We have considered this transformation as an escape phenomenon to the action of sucrose (Figure 2).

Micromorphological characteristics of P. aurantiogriseum

At the microscopic level, *P. aurantiogriseum* showed an identical appearance on all sucrose concentrations added to G25N* medium. We distinguished two areas: a central zone, which contains older cells and a peripheral zone, which represents the young cells.

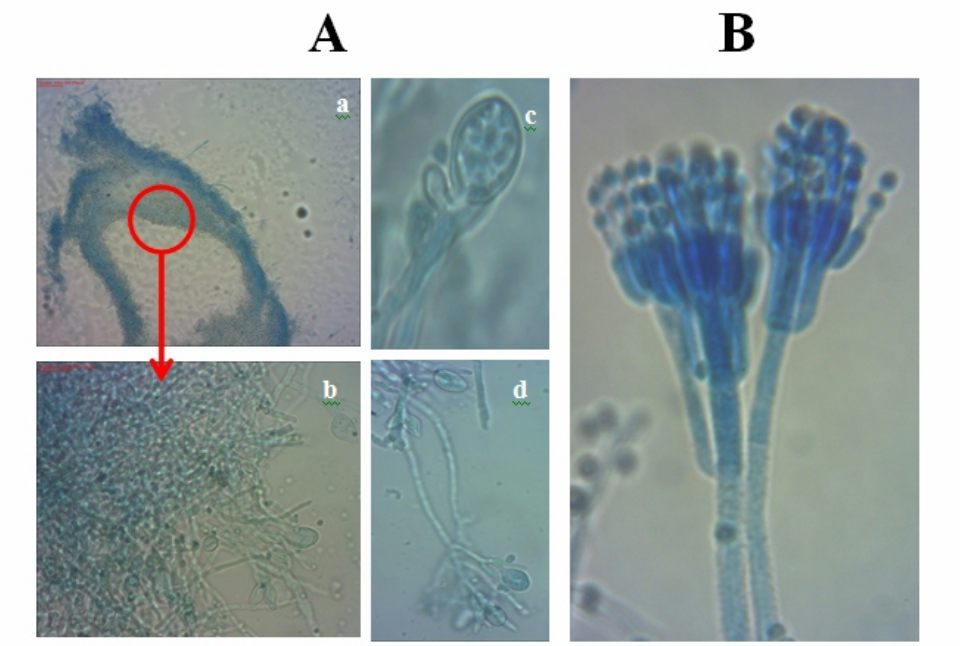

Figure 3: Thallus appearance in the same colony of *P. aurantiogriseum* at the concentration of 100 g/l of sucrose:
A: center of the colony; the cells lose branched hyphae and show an abnormal appearance: (a) with a resolution x10 (b) with a resolution x 40. The thallus shows conidiophores without phialides and metulae clearly differentiable: (c) with a resolution x100, (d) with a resolution x40.
B: periphery of the colony; the strain retains its asexual reproduction type with the presence of conidiophores as well as spores.

During carbon stress, the colonies of *P. aurantiogriseum* showed a dispersed morphology. In the colony center, where sucrose began to decline, we observed empty hyphae compartments emerged and the diameter of growing hyphae decreased significantly (Figure 3A-b). Throughout the prolonged decrease, the fraction of the empty hyphae compartments increased, but the exoskeleton of the cell wall appears to have remained intact (Figure 3A-a). We also observed asexual reproductive structures morphologically paralyzed which

resembled to low-density conidiophores without clearly distinguishable phialides and metulae (Figure 3A-c and 3A-d). At the peripheral zone, the mycelia appear normal with a penicil containing phialides and metules (Figure 3B). All hyphae have the same extension ratio and the same diameter; extension zones have the same shape and size. In differentiated mycelia, there is a hierarchy such that parental hyphae extend faster, have larger extension areas, and are wider than the branches they support.

Production of terrestric acid

Our results revealed that the mycotoxins profile of P. aurantiogriseum was greatly affected by the sucrose concentrations of G25N* growth medium (Table 1). Toxigenesis study allowed us to detect a significant production of terestric acid at the time of the appearance of aging signs. The aging signs are more important when the concentration of terrestric acid produced is high.

DISCUSSION

Morphological response

Sugars act not only as nutrients, but also as important regulators of gene expression. The influence of sucrose concentrations on *Penicillium* growth has been extensively studied (Cunniff, 1995). Thus, the identified phenotypic responses are likely caused by changes in fungal growth rate (Hameed et al., 2012;Zain et al., 2011;Gasch et al., 2000). Our results revealed that the mycelium weight, the colony diameter, and the terrestric acid production of *P. aurantiogriseum* were significantly affected by the increase in sucrose concentrations added to the G25N*medium. We have observed that the high sucrose concentration in the medium induces a kind of trauma in the strain. This was reflected first by a change at the morphological level and then at the behavioral level. The changes in *P. aurantiogriseum* growth parameters, possibly induced by sucrose, could be related to the significant decrease in the total sugar content of the cell walls observed after the stationary phase. However, the oldest cells in the center did not find oxygen for survival and so began to increase. The shape of the colony became rougher because of the intense growth of cells and the high concentration of sucrose gave an aged appearance. According to Sinclair (2002), the accumulation of sugar resulted in over-expression of free radicals in mitochondria, leaded to a mitochondrial dysfunction and consequently accelerated cell aging.

Physiological response

In the presence of high sucrose concentration in the medium, all the metabolism of *P. aurantiogriseum* developed as rapidly as possible by extension of the hyphal end. During exponential growth, all mycelial hyphae of biomass contribute to growth. However, as the hypha spreads, the nutrients must diffuse through the hyphae and the mycelia in the center become progressively limited in nutrients so that exponential growth is limited to the periphery. According to Zain et al. (2009), filamentous fungi respond to carbon deficiency with very specific

responses, including fungal cell wall degradation (autolysis) and the onset of asexual spore formation. Similar results reported morphological data from *Aspergillus oryzae*. Pollack et al. (2008) indicate a clearj transition between thick and thin compartments in response to carbon starvation. This was also observed in this study suggesting, as well, that hyphal diameters can be used to distinguish aged and young cells formed during growth. On the other hand, the microscopic analysis of the thallus showed us a change in the reproduction type at the same colony (unpublished results). The area showing aging signs (center of the colony) shows sexual reproduction with presence of ascospores while moving away from the center to the periphery. The reproduction remains asexual; this can be explained by the degree of sucrose resistance in function of age. The modification of reproduction type in older cells is probably due to the production of mycotoxins secreted during the stationary phase. Li et al. (2008) report that some mycotoxins have easily observable effects on morphological differentiation and can induce sexual sporulation.

Metabolic response

In this work, the study of toxigenesis has shown that the production of terrestric acid is proportional to the intensity of the aging signs. Indeed, the high sucrose concentration in the medium caused a kind of cellular stress and therefore led to overproduction of terrestric acid in the cell during the stationary phase. This means that the terrestric acid probably caused the early cells aging. These results are in agreement with Chander (1981) who reported that the high concentration of the carbon source causes the production of mycotoxins in molds. In addition, Rouvier (2002) and Meisner (1974) showed that terrestric acid is produced in the Krebs cycle, a process that occurs in mitochondria, which enhance its involvement in the respiratory cell metabolism. Consequently, the intensity of cell growth in the central zone of *P. aurantiogriseum* colony caused respiratory problems related to mitochondrial dysfunction (Coppola and Ghibelli, 2000;Sámi et al., 2001). This dysfunction, reported by Moore and Truelove (1970) and Meisner and Chan (1974) leads to early cell aging

Escape phenomenon at high concentration of sucrose

This phenomenon appeared in the concentrations from 500 to 600 g/l of sucrose. Our strain was normal in appearance with good biomass and mycelium production and moderate production of terrestric acid. We consider this finding as escape phenomenon at the high sucrose concentration. According to other studies (Nilsson and Bjurman, 1998; Robin et al., 2001; Park and Gander, 1998; El-Kady et al., 1995) molds are well known for their ability to adapt to high osmolarity environments through the polyols intracellular accumulation. *Penicillium* species accumulate glycerol as major osmoregulation substance (Blomberg and Adler, 1992; Harris, 1981; Hocking, 1986). Our analysis suggests that the presence of glycerol in the medium (G25N*) creates some cell permeability. Thus allows the oxydo-reductase genes to hinder protein activity and consequently maintaining the cells during the growth phase.

On the other hand, we suggest that between 10g /l and 400g /l of sucrose, *P. aurantiogriseum* was forced to adapt to the sucrose stress. It used its panoply of intracellular proteins to maintain growth and survival, which was remarkable in colony diameter and terrestric acid production. At certain levels, the difficulty of nutrients absorption by the colony center cells and mycotoxins production have led to a premature senescence which has been reflected in the relief degree and rigorously in the colony morphology.

However, at a concentration of 600 g/l of sucrose, *P. aurantiogriseum* behaved as being in osmotic shock. This leads it to call, in addition to its protein heritage, its genetic heritage by a signaling cascade in order to respond to the stress. This reaction allows the strain to maintain its growth in a normal state. This explains its normal morphology and the minimal production of terrestric acid compared to other concentrations.

CONCLUSIONS

This study is a continuation of a previous study (Bouhoudan et al., 2018) showing the effect of different carbon sources on the production of secondary metabolites in *P. aurantiogriseum*. Other stress-related studies are running on 3 different strains of *Penicillium* by analyzing their metabolic profile with the HPLC-MS method.

In conclusion, this study demonstrated that cell autolysis, morphological changes, and terrestric acid production by *P. aurantiogriseum* are influenced by the sucrose concentration in the medium. Indeed, terrestric acid production was faster and more important by increasing the sucrose concentration in the medium. Therefore, the carbon stress can prove to be an effective procedure to reduce the life duration of *P. aurantiogriseum* and control the terrestric acid production or to accelerate the aging process that occurs during sucrose stress. The possibility of recovering large quantities of terrestric acid is a key advantage that could later allow studying this mycotoxin well.

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SOIL ATTRIBUTES, SOIL ORGANIC CARBON AND RELATIONS WITH RUBBER-TREE MORPHOLOGY IN A THREE-DECADE-OLD ORCHARD

SUMMARY

Successful establishment of a rubber tree plantation may be affected by the initial soil management at the time of planting. However, research on the long-term development of rubber tree plantations and initial soil treatment is scarce. Thus, this study evaluated agronomic characteristics, organic carbon and its stock and their relation to soil attributes in a 32-year-old rubber tree plantation. The experiment adopted a randomized block design with two rubber tree clones (RRIM 600 and FX 2261) and five planting systems (P1: 0.4 x 0.6 m planting hole; P2: 0.35 x 0.4 m planting hole; P3: 0.45 x 0.45 m furrow; P4: 0.35 x 0.8 m planting hole; P5: 0.25 x 0.5 m planting hole) with four replications. The trunk circumference at breast height, height of first bifurcation, bark thickness, total organic carbon in litterfall and carbon stocks in soil were evaluated. The planting systems used for the rubber trees and clones did not influence the trunk circumference at breast height or height of first bifurcation. The bark thickness of FX 2261 was greater than that of RRIM 600. The soil's physical attributes were not affected by different planting systems or clones. The soil of the rubber tree plantation includes significant amounts of carbon absorbed from the atmosphere as CO_2.

Keywords: *Hevea brasiliensis*, tree biometrics, bark thickness, litterfall, soil carbon stock.

INTRODUCTION

Rubber-tree (*Hevea brasiliensis* Muell Arg.) is native of the Amazon region and belongs to the *Euphorbiaceae* family. Plantations can be cultivated in several Brazilian regions since there are rubber-tree clones well adapted to the

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diverse climatic variations found in the country. In addition to latex production, rubber-tree plantations can be used to recover deforested and degraded areas, because the cover provided by the canopy and litter deposition protect the soil against erosion, maintain moisture and cycle nutrients (Naime et al. 2009), and also, can store significant amounts of carbon in tree biomass, in soil and in the latex produced, which is a raw material for many industrial products (Diniz et al. 2015).

The cultivation of rubber-trees has promoted the recovery and improvement of soil attributes such as aeration, physical structure, water retention capacity, nutrient cycling and the stock of carbon in soil (CStk) (Chaer & Tótola 2007). Adequate soil management can enhance soil physical and chemical properties recovering natural fertility and increasing the sustainability of the agroecosystem (Cardoso et al. 2010). The quantification of these changes is very important to improve the management of tree plantations (Neves et al. 2007).

The cultivation of long cycle forest species, whose economic uses are not dedicated exclusively to wood production, has comparative advantages in relation to the short-cycle tree species because carbon remains stored in the vegetation for longer periods (Cotta et al. 2008). Carbon fixation in soil is a natural process related to the transference of atmospheric carbon to the litter decomposition process in soil. Lal (2006) emphasizes that 5 to 15% of the total soil biomass is transformed into humic and fulvic acids. This phenomenon depends on physical, chemical and biological characteristics of soil, climate, nature of the deposited material and on crop management.

The increase of CStk occurs slowly in forest ecosystems through biomass (mostly plant residues) annually deposited in the form of fallen leaves and dead roots. Pandey et al. (2007) further highlighted that in forest ecosystems, the main contribution of organic matter occurs via production, accumulation and decomposition of litter, which is a fundamental process of carbon cycle in the ecosystems. This litter deposition is directly related to silvicultural management, especially in regions of high temperatures and rainfall throughout the year (Lal 2005). Epron et al. (2004) also emphasized that CStk in soils of rubber-tree plantations is a function of plantation age, soil textural class, climate, and management practices.

Moreover, the relation between organic carbon and carbon stock with soil physical attributes in different production systems became a critical practice and is frequently present in discussions in the scientific community. In this context, this study evaluated the tree characteristics, organic carbon and its stock and related them with the soil attributes of a rubber-tree plantation.

MATERIAL AND METHODS

The experimental area

This study was conducted in an experimental area of the Federal Institute of Triângulo Mineiro (Campus Uberaba), located in Uberaba, State of Minas Gerais, located at 19º39'19" S and 47º57'27" W, 795 m above sea level. The

rubber-trees were planted in January 1986 in an area that was a degraded natural pasture covered by species of the genus Brachiaria (Trin.) Griseb grass.

The region's climate

The climate of the region, according to Köppen, is classified as Cwa, i.e. tropical hot, with hot rainy summer and cold, dry winter, with average annual precipitation and temperature of 1600 mm and 21ºC, respectively (Alvares et al. 2013).

Soil type

The soil in the area was classified as a dystrophic Red Latosol, medium texture (Santos et al. 2013). After 32 years, the analysis of the soil superficial layer, down to 0.2 m depth showed: 210, 710, 80 g kg^{-1} of clay, sand and silt, respectively; pH (H_2O): 5.5; P (Mehlich^{-1}): 3.3 mg dm^{-3}; K^+: 2.9, Ca^{2+}: 22, Mg^{2+}: 10, and H^+Al: 20 mmol dm^{-3}; organic matter (OM): 16 g dm^{-3} and 68% of base saturation.

Experimental design

The experimental design was a randomized blocks, as a 2x5 factorial, with two clones (RRIM 600 and FX 2261) and five different planting systems (P1- 0.4 m by 0.6 m depth planting hole, made with post-hole digger (control); P2 - 0.3 m by 0.4 m depth planting hole, made with post-hole digger (these treatments had the upper soil layer returned to the bottom of the hole); P3- 0.45 m wide by 0.45 m depth furrow (soil was returned to the furrow with hoe); P4- planting hole made with a drill attached to a tractor, with 0.35 m in diameter x 0.8 m depth, without lateral scarification (sandy soil); P5- planting hole made with a drill attached to a tractor, with 0.25 m in diameter x 0.5 m of depth), with four replications.

Limestone (29.5 and 19.5% of CaO and MgO, respectively) was broadcast applied at 2 t ha^{-1}. Pre planting fertilization was done in the planting hole or in the furrow with 200 g of 6-30-6 + 6 (NPK + Mg), 10 g of FTE Br-12 and 0.06 g Borax 0.3%. This same fertilization was repeated in the subsequent year plus 0.15 kg KCl, as side dressing. The soil surface around the saplings (0.8 m diameter) was weeded during the first two years after planting. Subsequently, weed management was done with a rotary brush cutter between planting rows as needed. No other cultural practices or mineral fertilization was done in the area for 32 years.

The useful experimental plot was 160 m2 and consisted of 8 plants spaced 8 x 2.5 m, equivalent to a planting density of 500 plants ha^{-1}.

The following rubber-tree morphological parameters were evaluated: circumference at breast height (CBH); height of the first bifurcation (HFB) from soil level, and bark thickness (BT).

Physical Attributes

Undisturbed soil samples were collected by the method of volumetric rings, in 48 x 53 mm (diameter x height) rings coupled to a Uhland auger, at 0-0.05 and 0.05-0.10 m to determine soil density (SD). These samples were

saturated, weighed, and dried in an oven at 105º C for 24 hours. Pore size distribution was determined with the same undisturbed sample, which was saturated with water for 24 hours, and then subjected to suction at 0.60 m of water column height for the estimation of macro-porosity (Ma), total porosity (TP) and micro-porosity (Mi) according to Embrapa (2017).

Soil water content

The second set of soil samples was collected at the same day and depths to evaluate the soil water content, which was homogenized to obtain the moist and dry weights of the soil. These samples were packed in aluminum containers, weighed and dried in forced circulation oven at 105º C for 24 hours when the volumetric soil water was estimated (Embrapa 2017).

Determination of total organic carbon

In each treatment, 0.5 x 0.5 m and 0.5 m depth trenches were opened. Two undisturbed samples were collected in each trench with the aid of a volumetric ring (Teixeira et al. 2017), one sample to determine soil density and the other one to determine the content of total organic carbon (TOC), at the soil depths of 0-0.05 and 0.05-0.10 m. TOC content was determined according to the methodology of Walkley & Black (1934) adapted by Yeomans & Bremner (1988).

Organic carbon stock of the soil

Carbon stocks (CStk) in the soil were calculated according to the method of Fernandes and Fernandes (2013), using the following equation:

$CStk\ (t\ ha^{-1}) = (C \times SD \times e) /10;$

where CStk (t ha^{-1}) is the carbon stocked in soil, C is the total organic carbon (TOC) in the considered layer (g kg^{-1}); SD is soil density (Mg m^{-3}) and e is the thickness of the soil layer considered (cm).

Quantification of litterfall

The amount of litterfall was estimated on a single occasion. The samples were collected between plants using a 0.5 x 0.5 (0.25 m^2) metal frame, where all the rubber-tree residues were collected. In each experimental plot, four simple litterfall samples were collected to make one composite sample (n = 40).

The samples were packed in paper bags, dried in a forced circulation oven at 65º C for 72 hours, when dry mass was estimated. The stock of litter for each clone was quantified through the expression: Stock of litter (t ha^{-1}) = mass (t) x area of the metal frame (ha).

Statistical analysis

The results were submitted to analysis of the normality of the distribution of errors (Lilliefors' test) and homogeneity of variances (Cochran's test). Given the assumptions of normality and homogeneity were accepted, the values were submitted to analysis of variance (ANOVA) and the averages compared by Tukey's test at 5% probability ($p<0.05$). The software R Core Team was used for the statistical analysis.

RESULTS AND DISCUSSION

Morphological parameters

There were no significant differences ($p<0.05$) among treatments (planting systems) or clones for circumference at breast height (CBH) and height of the first bifurcation (HFB), which ranged from 0.64 to 0.8 m and from 3.18 to 4.87 m for the clone RRIM 600, and from 0.68 to 0.88 m and from 3.49 to 4.56 m for the clone FX 2261, respectively (Table 1). Such dimensions of CBH and HFB the rubber-tree plants evaluated in this study indicate that they are suitable for latex production.

Table 1. Circumference at breast height (CBH), bark thickness (BT) and height of first bifurcation (HFB) of RRIM 600 (RR) and FX 2261 (FX) rubber-tree clones subjected to different planting systems after 32 years.

Planting System	CBH (m)			BT (cm)			FBH (m)		
	RR	FX	Average	RR	FX	Average	RR	FX	Average
	m			cm			m		
P1	0.80	0.86	0.83a*	1.09	1.17	1.13a	3.18	3.81	3.49 a
P2	0.71	0.76	0.73a	1.09	1.15	1.12a	4.34	4.45	4.39 a
P3	0.69	0.79	0.74a	1.06	1.19	1.12a	4.16	3.79	3.97 a
P4	0.64	0.88	0.76a	1.05	1.17	1.11a	4.87	4.26	4.56 a
P5	0.69	0.68	0.68a	1.10	1.12	1.11a	3.91	4.97	4.44 a
Average	0.71a	0.79a		1.08b	1.16a		4.1 a	4.2a	
CV (%)	17.03			6.99			14.85		

* = averages followed by different letters differ by the Tukey test ($p<0.05$). P1 = In pits of 0.4 m by 0.6 m depth, made with post-hole digger (control); P2 = In the pits with 0.3 m by 0.4 m depth, made with post-hole digger; P3 = Furrow with 0.45 m wide by 0.45 m depth; P4 = In holes made with a drill attached to a tractor, with 0.35 m in diameter x 0.8 m depth; P_5 = In holes made with a drill attached to a tractor, with 0.25 m in diameter x 0.5 m of depth. CV: coefficient of variation.

These variables are directly related to latex productivity since rubber-trees with greater HFB and CBH have greater production of dry rubber (Costa et al. 2010). Pereira and Pereira (2001) emphasized that rubber-tree plants should reach the minimum CBH at the earliest age possible because this is the first parameter considered to start tapping activity, which must range from 0.45 to 0.5 m.

The BT values found differed among clones ($p<0.05$) with the BT of clone FX 2261 approximately 7% thicker than the bark of clone RRIM 600. The CBH

and the BT correlate phenotypically, indicating that plants with great CBH tend to have great BT and, therefore, greater productivity (Campbell et al. 2010; Mosque & Oliveira, 2010). According to the results found in the present study, the clone FX 2261 has the potential for superior latex production due to thicker bark when compared with the clone RRIM 600. However, this similar response between FX 2261 and RRIM 600 was not observed by Melo et al. (2004), who found that RRIM 600 presented the greatest latex production among the genotypes evaluated.

Soil physical attributes

The table of analysis of variance (F test) of the interactions among clones, planting systems and soil depths, shows that there were no significant interactions ($p>0.05$) for the attributes evaluated, nor for the factors individually (Table 2).

Table 2. Probability of the analysis of variance (F test) of the triple interaction (clones x planting systems x soil depths) for soil density (SD), macroporosity (Ma), microporosity (Mi), total porosity (TP), and macropore density (Ma / TP).

Source of Variation	*df*	SD	Ma	Mi^{ns}	TP	Ma/TP
		p value				
Clone	1	0.7617	0.2337	0.6932	0.3448	0.2015
Planting syst.	4	0.3665	0.2171	0.1948	0.5602	0.1545
Soil depth	1	0.0001	0.0009	0.7945	0.0007	0.0056
Clone*Planting syst.	4	0.7279	0.1390	0.7680	0.2446	0.1832
Clone*Soil depth	1	0.0606	0.8591	0.3252	0.2722	0.7730
Planting syst.*Soil depth	4	0.9618	0.9902	0.1390	0.3943	0.8591
Clones* Planting syst.*Soil depth	4	0.8674	0.8593	0.6291	0.9898	0.6249
CV (%)		6.93	26.27	15.04	9.72	22.74

These results corroborate with other studies in rubber-tree plantations, which, after its establishment, regardless of the planting technique, the system stabilizes in time. The annual deposition of litter on soil surface, with the renewal of the rubber-trees root system and invasive plants that colonize the nearby areas, provide a high input of organic matter causing positive changes in soil structure (Eucalyptus - Chaer & Tótola, 2007; Eucalyptus, soybean, rice, Brachiaria - Neves et al. 2007; native Brazilian forests - Cardoso et al. 2010).

The homogeneous responses from the different planting systems are a result of soil stability since the planting process - more than three decades earlier. Also, the regular input of organic matter on the soil surface resulted in significant improvements in the soil microporosity in both soil layers (Table 3).

Table 3. Soil density (SD), macroporosity (Ma), microporosity (Mi), total porosity (TP) and macropore density (Ma / TP) at different soil depths in a 32-year-old rubber-tree plantation. Uberaba, MG, 2016.

Soil depth	SD	Ma	Mi	TP	Ma/PT
(m)	kg dm^{-3}	%			
0 - 0.05	1.34 a*	23.90 a	31.22 a	55.13 a	43.49 a
0.05 - 0.1	1.45 b	18.73 b	31.54 a	50.27 b	36.59 b
CV (%)	6.93	26.27	15.04	9.72	22.74

*= averages followed by different letters in each column differ by the Tukey test ($p<0.05$).

Soil micropores are one of the most stable soil characteristics, especially in stabilized systems, such as rubber-tree plantations. In planting systems with great soil movement and short cultural cycles, as in soybean, maize and wheat, the micropores are effectively changed after each soil mobilization (Albuquerque et al. 1995; Bertol et al. 2004; Sales et al. 2016).

According to Torres et al. (2015), the deposition of plant residues on the soil protects it from erosion caused by rainfall and reduces soil moisture fluctuation. Besides, plant residues provide energy as organic matter to the soil microbiata, which produces substances responsible for the formation and stabilization of soil aggregates. These effects, added to the aggregation promoted by rubber-tree roots, reduce the variations in soil density and microporosity of soil top layers.

The density of soil macropores (Ma/TP) is an indication of favorable conditions for root development. Taylor and Stewart (1972) highlighted that approximately one-third of the total soil porosity are macropores, and that lower values of macropores indicate soil compaction - compaction that can reduce space for satisfactory root development. The critical threshold of Ma considered prejudicial for root growth is 10% of the soil space, and an ideal proportion for annual crop production is about 1/3 of Ma (33%) and 2/3 of Mi (67%) (Kiehl 1979).

In this study, the density of macropores in the soil layers evaluated was greater than the optimal density (Ma/TP > 0.33); therefore, with enough space for unrestrained root growth (Table 3). Macropore density in the top 0.10 m of the soil surface indicates an influence of the degradation and incorporation of organic matter in the soil physical attributes. This superficial layer is in direct contact

with plant debris fallen from the rubber-trees and from the Brachiaria grass, which was the most common weed in the area.

Quantity of litter and carbon stock

No interaction was observed between clones and planting systems, nor differences between the levels in each factor ($p>0.05$), for the amount of litter on the ground or the total organic carbon (TOC) found in the layer of 0-0.10 m. The average amount of litter and TOC were 26.38 t ha^{-1} (CV = 29.80%) and 207.62 g kg^{-1} of soil (CV = 15.35%), respectively.

For Pimentel Gomes and Garcia (2002), the variability of an attribute can be classified according to the magnitude of its coefficient of variation (CV), which can be low when it is less than 10%, moderate when between 10 and 20%, high between 21 to 30% and very high when above 30%. The CV (%) values for the amount of litter and TOC were classified as high and moderate, indicating that these variables can considerably change from one sampling place to another. However, for all treatments, there were high inputs of organic matter in the soil under rubber-tree plantation.

The stock of carbon (CStk) in the soil did not show any significant interaction with the planting systems or the rubber-tree clones ($p>0.05$), but differed between soil layers ($p = 0.0010$). The top soil layer (0 - 0.05 m) presented 7.12 t ha^{-1}, while the second layer (0.05 - 0.10 m) presented 5.57 t ha^{-1} of carbon. These values of carbon stored in the soil indicate that the rubber-tree is a forest species capable of fixing significant amounts of atmospheric carbon into the soil (Cheng et al. 2007; Nizami et al. 2014; Diniz et al. 2015).

Litter decomposition is done by soil microbiota, consuming approximately 80% of the organic matter present, while transforming the remaining in humic substances (Anderson & Domsch 1990). The contents and characteristics of soil organic matter are the result of its production and decomposition rates, chemical composition of the plant residues, soil texture, aeration and pH, climate prevalent conditions, soil microbiota diversity and the interaction among all of them (Silva et al. 2009; Nascimento et al. 2010).

In this sense, crop management and soil interactions with the vegetation that fixes carbon are important for increasing organic matter in the soil. Studies are still required to better understand the quality of the plant residues produced by different forest species and their impact on the physical, chemical and biological properties of the soil (Caldeira et al. 2008), as well as the time in which these changes occur. Such studies may assist in the selection of the most appropriate clones for the formation of rubber-tree plantation, promoting a better balance of the ecosystem and contributing to reduce the environmental impact of the activity.

CONCLUSIONS

The systems of planting *Hevea brasiliensis* seedlings did not affect the circumference at breast height nor the height of the first bifurcation, three decades after the establishment of the rubber-tree plantation.

The bark of the clone FX 2261 was significantly thicker than that of RRIM 600, indicating that FX 2261 has greater potential for latex production.

The soil physical attributes were not affected by the different systems of rubber-tree planting, but differences were observed between the soil layers, indicating that the system has reached environmental stability;

The rubber-tree plantation, regardless of the clone or planting system, incorporates significant amounts of carbon to the soil, in quantities similar or superior to many forest species.

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ASSESSMENT OF SPATIAL AND SEASONAL NITRATE VARIATION OF GROUNDWATER IN THE IRRIGATED PERIMETER (TADLA PLAIN- MOROCCO)

SUMMARY

The Nitric pollution in the groundwater of the Tadla plain increases the risk of degrading the quality of water resources and creates a risk to human health and the environment. This study aims to establish the state of pollution of the groundwater by nitrates in Béni Aamir and Béni Moussa aquifers, to follow its temporal evolution and to map its spatial distribution using Surfer software. To assess the contamination of well water by nitric pollution, 200 samples were collected in four seasonal campaigns between March 2017 and May 2018, with a rate of 43 samples taken per season. The spatial distribution of nitrates in the groundwater shows that the majority of the catchment points reached by this pollution are located in agricultural areas. Additionally, there was a range of 160.15 mg/L (4.65–164.5 mg/L). The temporal distribution of nitrate in the groundwater of Béni Aamir and Béni Moussa shows an increase in response to the agricultural intensification in this region, and a variation depending on the period of withdrawal. Including the increase in the quality degradation in all wells, from 1% in campaign N°01 to 7.06% in campaign N°02 and to 5.7% in campaign N°03, and to 0% as very bad quality in campaign N°04. The fact that the campaign N°02 has been carried out during the agricultural season, proves the link between the Nitrate pollution and chemical products uses during this period. Therefore, the impact of chemical products uses on the groundwater quality in this region.

Keywords: environnement, groundwater, nitrate, pollution, Tadla Plain.

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INTRODUCTION

Groundwaters are the second largest freshwater reservoir on Earth after that of the polar glaciers. Aquifers are therefore exploited for irrigation but above all for the supply of drinking water to the population (Matzeu et al, 2017). This essential resource for life is too often exposed to problems of domestic, industrial or agricultural pollution (Zeddouri et al, 2013). In Morocco, water availability has so far been very limited and is likely to decrease significantly in the long term. As well as, the World Bank's 2018 forecast estimations triggered the alarm bell. Indeed, renewable water resources per capita are likely to be reduced by half, from 750 m3/hab/year in 2000 to 500 m3/hab/year in 2030, thus classifying Morocco as a country in a situation of chronic water stress (El Menouar, 2012). The deterioration of the quality of water resources through the proliferation of different sources of pollution (fertilizers and pesticides, untreated wastewater discharges, uncontrolled solid waste discharges, urbanization, etc.) is as significant a threat as that linked to quantitative imbalance (Nouayti et al, 2015).

Since the 1980s, the Tadla plain has undergone considerable progress in terms of hydro-agricultural development, which is still ongoing in other extension areas. It actively contributes to the increase of national agricultural production (Rico et al, 2000). Nevertheless, the emergence of problems of natural resource degradation, including nitric pollution of groundwater, has incited the managers of the perimeter to implement promising projects aimed mainly at improving the competitiveness of irrigated agriculture in Tadla through rational management and preservation of the quality of its resources (Hafiane et al., 2019; El Hamidi et al, 2018). In this context, an intensification of research on the whole plain is becoming necessary in order to determine the sensitivity of the resource to any form of pollutant introduced from the soil surface, and hence the importance of establishing the state of groundwater pollution by nitrates and of monitoring its temporal evolution. Nitric nitrogen, which is largely derived from human activities, is a major health and environmental problem when it is found in the natural environment, particularly when the resource is destined for the production of drinking water (Zhai et al, 2017).

The diagnosis of the nitric quality of groundwater, conducted on groundwater in the region's agricultural zone, showed that nitrate levels are higher than the water potability standard set at 50 mg/L. This spatial variability of water nitrate levels also indicates diversity in their origin, and given the data on the annual increase in nitrate level in groundwater show that the water situation is likely to become worrisome if appropriate measures are not taken immediately. Thus, our study consists in deepening the current state of knowledge through the identification and analysis of nitric pollution.

MATERIAL AND METHODS

Study zone

The Tadla plain (Fig. 1) is located approximately 200 km southeast of Casablanca in the Beni-Mellal province. It extends over a large portion, estimated

at 3,600 km², of the middle basin of Oum-er-Rbia between the High Atlas in the south and the Phosphate plateau in the north (Hafiane et al, 2019).

The plain is intersected for approximately 160 km from east to west by the Oum-er-Rbia wadi, which splits it into two vast hydraulically independent irrigated areas: the Beni Amir and Beni Moussa perimeters on the right and left banks of the Oum-er-Rbia, respectively. The Beni Amir are irrigated from the waters of Oum-er-Rbia (Kasba-Zibania diversion dam), and the Beni Moussa from the waters of the Oued el Abid (Bin el Ouidane dam). The total irrigated area is about 124,000 ha (Taibi et al, 2015).

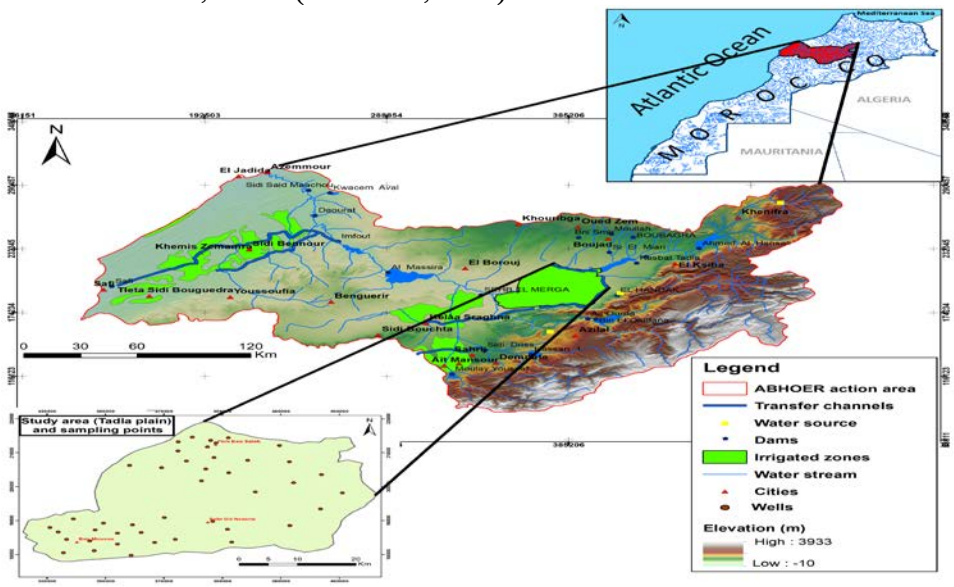

Fig. 1. Geographical location of the study zone in Central Morocco

Sampling and Analysis

Sampling is carried out on both groundwaters (Fig. 2). The sampling points are then determined and located on maps developed using the Geographical Information System (GIS). These points are selected according to the information collected through the survey, the study covers forty-three (43) wells located in areas where the sources of pollution are major, these are intensive pastoral or agricultural fields. The 43 wells chosen are strictly among those where chlorination is not practiced. They have easy access to agricultural fields. Water samples are placed in sterile polyethylene vials rinsed with distilled water and sample water beforehand. These water samples are then transported in a cooler at 4°C and processed in the laboratory within 24h of collection.

On the basis of groundwater analysis campaigns covering both dry and wet periods, water samples were collected in collaboration with the Tadla Regional Office for Agricultural Development (ORMVAT) and the Oum Er Rbia Hydraulic Basin Agency (ABHOER) during the period March 2017 - May 2018,

for a total of 43 wells per campaign. The analyses were carried out according to the NM ISO 7890-3 (2012) standard at the "ABHOER" laboratory. The physical parameters were measured in situ, focusing primarily on temperature, electrical conductivity and hydrogen potential. The coordinates of the water points are verified using a GPS.

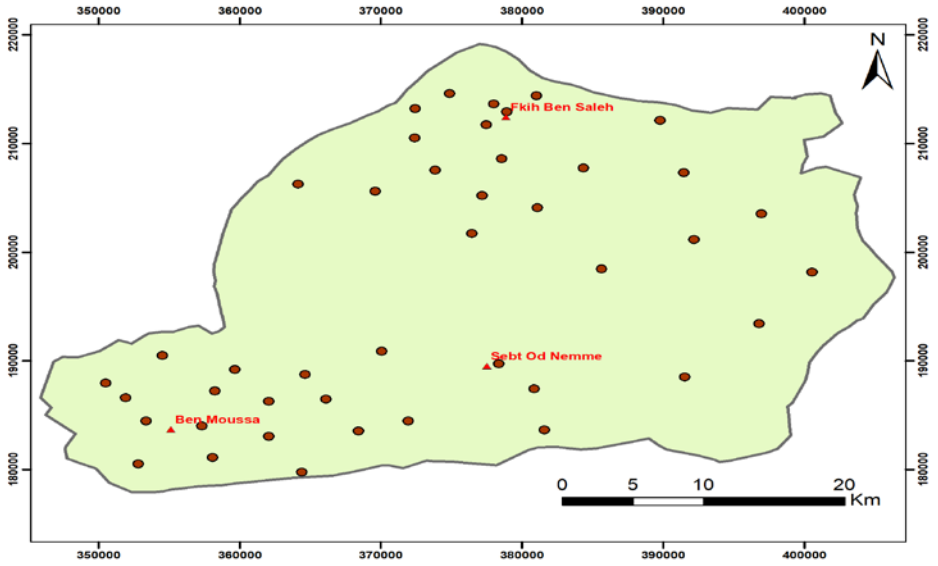

Fig. 2. Wells water in the perimeter of (Beni Moussa & Beni Aamir)

Samples of (1L) one liter are taken in polystyrene vials and deposited in a refrigerated place, then sent to the ABHOER water chemistry laboratory in Beni Mellal within 24h of the sample being collected. A spatio-temporal portrait of each campaign according to the season (dry or wet) was well drawn up to determine the geographical nitric pollution in the area studied by the Surfer software.

RESULTS AND DISCUSSION

The determination of water quality requires the positioning of each element measured on an evaluation grid containing the threshold limits of each parameter, thus permitting the degree of water pollution to be determined. During this study, the evaluation system adopted is the one that subdivides water quality into four levels: Very Good (blue color), Acceptable (green color), Mediocre (yellow color), Poor (red color), Very Poor (purple color). This evaluation grid is applied on the basis of the results of the analyses obtained.

The results obtained concerning the sampling points are part of a study that covers the period between March 2017 and May 2018, which revealed the following observation (Fig. 3).

The groundwaters Beni Aamir and Beni Moussa are more affected by nitric pollution in campaign 02, i.e. all the points showed 41.6% Poor to very Poor quality followed by both campaigns 01 and 03 such that 39.7% Poor to very Poor quality. While the campaign 04 showed excellent to average quality in all points 96.16% with low contamination 5.7% to poor quality in all wells. This period, the groundwater was the most favorable period in terms of quality status compared to others.

There was also an increase in the degradation of groundwater quality in all wells from 1% very poor quality in campaign 01 to 7.06% in campaign 02 and 5.7% in campaign 03 followed by 0% very poor quality in campaign 04 in all wells, this is explained by the decrease in fertilizer use and groundwaters regeneration through the succession of dry and wet periods.

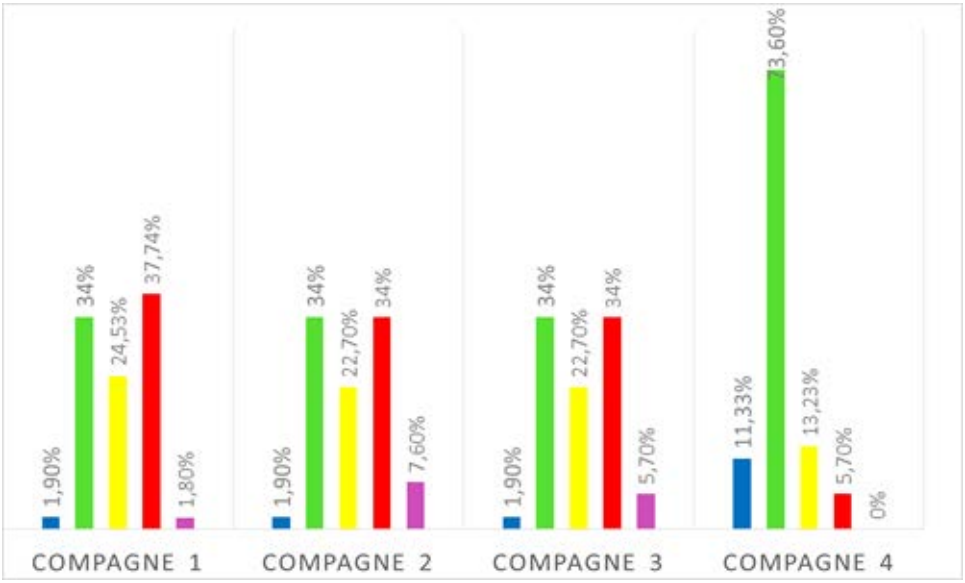

Fig. 3. Statistical analysis of nitrate results for the four campaigns

The interpretation of the map (Fig. 4) through spatial analysis showed that the wells studied have different spatial variations from one well to another, these values vary between 4.65 mg/L to 150 mg/L, upstream an excellent quality was recorded in well 28 (4.65 mg/L), while well 24 showed a very poor quality (146.98 mg/L).The wells (5, 6, 7, 8, 12, 32, 34, 35, 36, 37, 38, 39 and 43) showed values ranging from 5 to 25 mg/L with good quality. The wells (3, 4, 14, 16, 17, 20, 22, 23, 29 and 42) showed values ranging from 25 to 50 mg/L with average quality.

As for the wells (1, 2 ,9 ,10,11,13,15,18,19,21,25,26,27,30,31,33,40 and 41), they showed values ranging from 50 to 100 mg/L with poor qualities. The spatial analysis of the map (Fig. 5) showed that the wells studied have different spatial variations across wells, values range from 4.65 mg/L to 154

mg/L. The upstream part is more affected by contamination (very poor to poor quality) while the downstream part has an excellent to average quality except for two wells (32 and 43) with very poor quality. Whereas the well (4) has an excellent quality. The wells (1, 2, 3, 5, 6, 7, 8, 9, 15, 16, 17, 18, 19 and 37) have shown values ranging from 5 to 25 mg/L with good quality. The wells (11,12 ,14, 22,23, 27,35,36, 41and 42) showed values ranging from 25 to 50 mg/L with average quality.

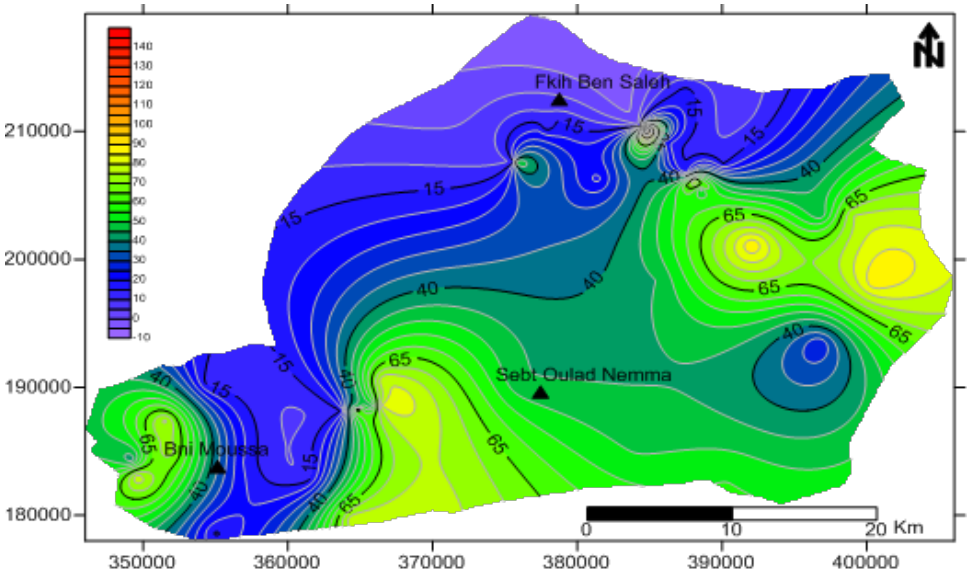

Fig. 4. Map of spatio-temporal variation of nitrates during the campaign 01.

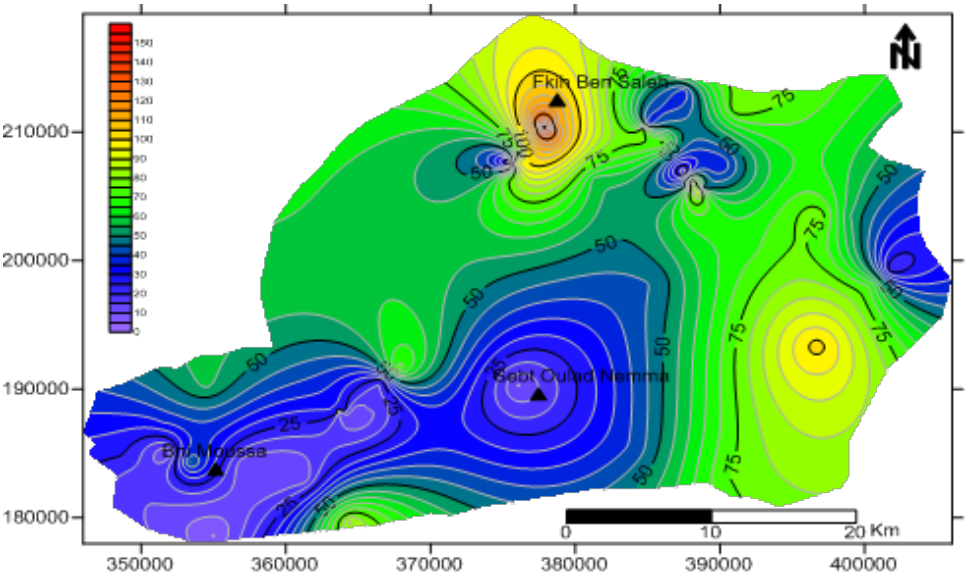

Fig. 5. Map of spatio-temporal variation of nitrates during the campaign 02.

The wells (21, 23, 24, 25, 28, 29, 33, 34, 39, 40 and 43) have reported values ranging from 50 to 100 mg/L with poor quality. Also, the wells (31, 32 and 30) showed values > 100 mg/L with very poor quality. The interpretation of the map (Fig. 6) by spatial analysis has also shown that the wells have different spatial variations from one well to another, the values vary between 5.7 mg/L and 164.5 mg/L, the upstream part is more affected by contamination (very poor to poor quality) with the exception of the four wells (30, 31, 40 and 42) with good quality.

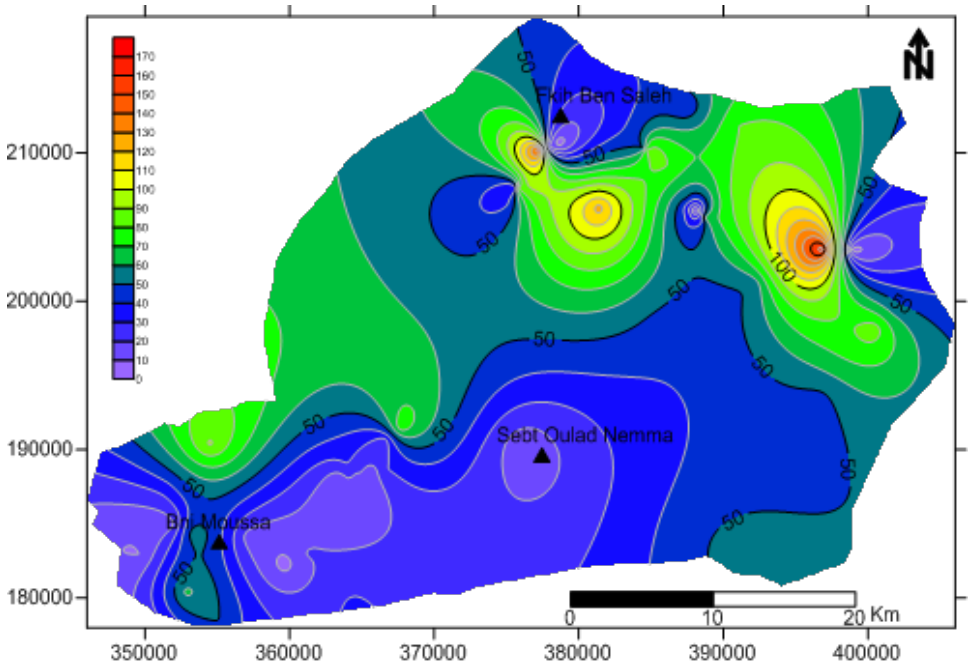

Fig. 6. Map of spatio-temporal variation of nitrates during the campaign 03.

The wells (2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 15, 16, 17, 19 and 20) showed values ranging from 25 to 50 mg/L with average quality especially in the downstream Beni Moussa zone. The wells (21, 23, 24, 25, 26, 28, 29, 33, 34, 35, 38, 39, 41, and 43) have reported values ranging from 50 to 100 mg/L with poor quality. As for the wells (1, 5, 7, 14, 18, 22 and 32), showed values > 100 mg/L with very poor quality.

Interpretations of the map (Fig.7) by spatial analysis showed generally average to good quality with the exception of the three wells (23, 31 and 43) with poor quality. The wells (30 and 35) showed an average quality, and wells (1. 9, 10 and 15) have reported values with very poor quality. Generally, season 04 remains the good period compared to contributions to other periods.

It can be concluded that the four nitrate maps showed a spatio-temporal variation in quality according to the sampling periods, these variations are due to intensive and irrational agricultural activities.

According to all the maps (Fig.4.5.6 & 7) it can be concluded that nitrate levels are very high throughout the southern part of the groundwater, more precisely the southwestern part of Beni Moussa and the northwestern part of Beni Aamir and this is due to the flow direction from the NE to the SW, as demonstrated with (Arauzo et al., 2017; Chidya et al., 2016; Fetouani et al., 2008; Ouedraogo et al.,2016). So according to this spatial diffusion of pollution, it can also be deduced that nitric pollution is more concrete in the agricultural areas that are most affected.

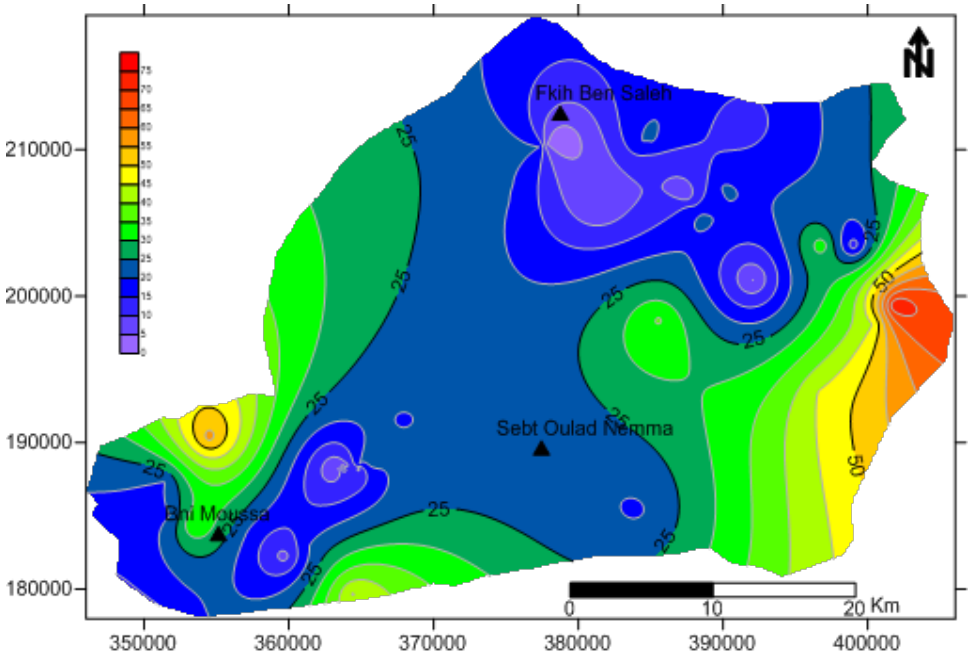

Fig. 7. Map of spatio-temporal variation of nitrates during the campaign 04.

The main sources of industrial pollution are located in the groundwater of Beni Amir and Beni Moussa (sugar factories, canneries, oil mills and dairy plants); have a major impact on the increase in nitrate levels and organic matter. Each map shows a North-South progression of the nitrate pollution front throughout the groundwater. The variation in the spatial and temporal profile of the observed high nitrate levels would be related to the degree of urbanization. Indeed, the South-East and South-West parts are cities, villages (Béni Mellal city and Oulad Moussa Commune) highly urbanized with an industrial session. Agriculture contributes to groundwater pollution because of the often-irrational use of fertilizers and pesticides that farmers add to improve the productivity of the plot.

The quantity of nitrogen leached to the groundwater or runoff reaches the Tadla groundwater by leaching. According to (Barakat et al., 2016; Bentekhici et al., 2018; Lalami et al., 2014; Lagnika et al., 2014; Nouayti et al., 2015), they have come to similar conclusions. The origin of nitrates in groundwater can be

multiple. Nitrates are very often the result of agricultural activities (fertilizers, industrial breeding) as demonstrated with (Hamutoko et al., 2016; Moussa et al., 2018). This degradation seems to be mainly due to human activity; this conclusion is similar to (Zhai et al., 2017).

Nitrogen fertilization of the surrounding agricultural areas at the points studied, waste water, lost wells and livestock waste, the results achieved are similar to the conclusions reached by (Barakat et al.,2016; Fetouani et al.,2008; Lagnika et al., 2014; Nouayti et al., 2015; Matzeu et al., 2017; Vrzel et al., 2016), including animal excreta. The high nitrate levels sometimes correspond to the reduction of nitrates to nitrites by reducing aerobic bacteria, the same conclusions of which can be found in a study by (Heriarivony et al., 2016; Lamribah et al., 2013). In addition, in natural water, whether groundwater or surface water, nitrate mineralization (N mineralization) can have several origins. Indeed, in the Tadla plain, work that reports excess nitrate in groundwater is increasingly frequent (Barakat et al., 2016; Chidya et al., 2016; El Bouqdaoui et al., 2009; Stuart et al., 2011).

This spatial variability in nitrate levels can also be due to the nitrogen provided by meteoric waters as demonstrated by (Shen et al.,2019). The high levels of nitrates found in the waters of the Quaternary aquifer are of urban origin due to the infiltration of domestic wastewater into the ground, sewage, septic tanks, latrines and the decomposition of organic matter, according to (Chidya et al., 2016; El Bouqdaoui et al., 2009; Martinelli et al., 2018). Furthermore, nitrate levels above the WHO and NM guidelines have been reported in Moroccan groundwater by (Barakat et al., 2016; Bricha et al., 2007; El Bouqdaoui et al., 2009; Fetouani et al.,2008; Nouayti et al., 2015). It can also be deduced that the spatial variation in nitrate quantity is probably related to the depth and design techniques of the wells. In fact, the contamination of the groundwater is due to the piezometric level, it is noted that these results are similar to the those of (Ahoussi et al., 2013; Barakat et al., 2016; Fetouani et al.,2008; Martinelli et al., 2018) which showed that the groundwaters are the richest in nitrates. These, given their shallow depth, are the most vulnerable.

Finally, let us not underestimate the health risk associated with the consumption of this groundwater by the sedentary population, and precisely by infants. According to (Ameur et al., 2016; Shen et al.,2019; Zhai et al., 2017; Degbey et al., 2011), the same result was also found that young children were the most vulnerable group to exposure to nitric contamination.

CONCLUSIONS

The spatial distribution of nitrates in the waters of the Quaternary aquifer in the irrigated area shows that the majority of the points that capture this aquifer are subject to nitrate pollution. This variation in nitrate levels is related to population growth and rapid urbanization in the area, and also with a view to ensuring agricultural development, agricultural supply in high productivity sectors has significantly improved. This positive momentum has been further

boosted by the Green Morocco Plan (GMP), which has led to the expansion of these products and the increased use of fertilizers and pesticides. This highlights the great pressure exerted by human activities on the groundwater of the said aquifer, which contributes to the degradation of its quality. This spatialized approach to nitric contamination allows interested organizations to ensure sustainable management of groundwater resources. It is a decision-making support tool for spatial planning and development by identifying the areas most threatened by nitric pollution.

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HYDROSEDIMENTOLOGICAL DYNAMICS IN THE GUARANI AQUIFER SYSTEM, RIBEIRÃO PRETO, STATE OF SÃO PAULO, BRAZIL

SUMMARY

This research evaluated the effects of agriculture on hydrosedimentological dynamics in the city of Ribeirão Preto in the State of São Paulo, Brazil. Physical and chemical analyses of the soils of the Ribeirão Preto hydrographic sub-basin were carried out. A water erosion susceptibility map was generated using the Revised Universal Soil Loss Universal Equation as well as water level and potentiometric depth maps of the studied region. Using geophysical data, a local three-dimensional geological model was prepared that clearly differentiates the outcrop regions of the Guarani Aquifer System. The research results indicate that the key reasons for hydrosedimentological changes under intensified erosion processes are conventional management and forest fragmentation, which cause soil losses above the region's average tolerance limit of 8.5 Mg ha^{-1} $year^{-1}$. Apart from soil damage, the calculated soil loss of about 1 million tons per year leads to a high risk of contamination in the Guarani Aquifer System. In regions located north of Ribeirão Preto, where the Guarani Aquifer System is shallowest, the risk of contaminants diffusing through agriculture is very high. In this context, modelling hydrosedimentological dynamics is of great importance as it enables accurate evaluation of the natural susceptibility of the aquifer to diffuse contamination. It also helps to identify sites that exceed the tolerance limit for soil loss, which are critical for conservation. However, there are no safe levels of

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soil loss, and attention should be paid to all areas that have soil loss values above what is considered natural. Soil losses for areas in forests were calculated as 0.03 Mg ha^{-1} $year^{-1,}$ which is well below the average for land under agricultural use (2.03 Mg ha^{-1} $year^{-1}$). The results of this research reveal critical hydrosedimentological dynamics in the studied area that affect both the quantity and quality of the water of the Guarani Aquifer System..

Keywords: Hydropedology; Soil Management; Aquifer; RUSLE.

INTRODUCTION

The surface and groundwater interaction takes place in different forms and degrees of intensity, with the surface water being the main cause for the groundwater contamination (USGS, 1998). In general, surface water is hydraulically connected to groundwater. However, how these occur and the magnitudes of their interactions are not easy to estimate. Land use changes representing the natural balance between morphogenetic and pedogenetic rates and are influencing erosion processes in the studied basin. This can result in sediment layers being deposited, which based on their composition, can contaminate the hydrogeological resources. In fact, over time, they can become a secondary source of contamination, principally by getting deposited on the floodplains.

Quantifying the susceptibility to soil loss or sediment production by water erosion helps to evaluate and quantify the sediments that can be carried by water in an area (Sakuno et al., 2020; Chalise et al., 2019; Tavares et al., 2019; Khaledi Darvishan et al., 2019; El Mouatassime et al., 2019; Spalevic et al., 2017). Therefore, crossing these data with the piezometric surface of a specific area can provide an overview of the interactions and the material exchange that occurs.

Taking into consideration the mentioned above, the aim of this study was to evaluate, using systemic analysis, the interaction between the exposed areas of the Guarani Aquifer System (GAS) and the surface dynamics and the uses upstream of this region influencing the production and transport of the sediments by water erosion.

MATERIAL AND METHODS

The study of this research was the area of the Municipality of Ribeirão Preto (Figure 1), located in Northwest of the São Paulo state of the south-eastern Brazil. In 2017, the population of this municipality was estimated at around 682,302 inhabitants (IBGE, 2017). Based on the Köppen classification, the climate in Ribeirão Preto is of the mesothermal tropical, subtype - Cwa (Alvares et al., 2014). The study area belongs to the hydrographic basin of Rio Grande, which limits the municipality to the north and it is drained mainly by the Ribeirão Preto subbasin, whose headwaters are found in the centre-south portion and in the north of the subbasin.

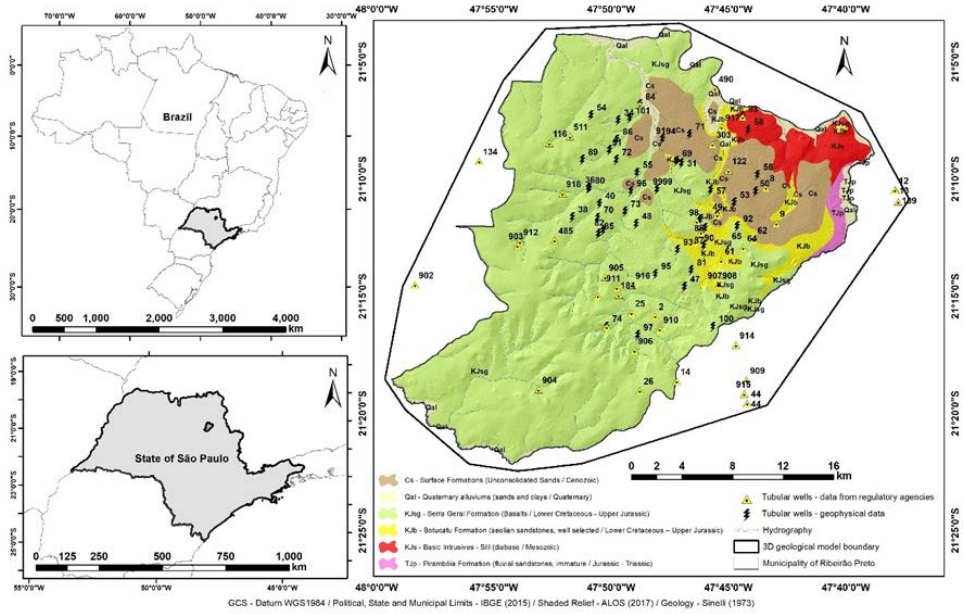

Figure 1. Study area: The locations, geological, geophysical data collection and sites of the tubular wells.

The region constitutes part of the Paraná Sedimentary Basin and is composed of Cretaceous basaltic rocks from the Serra Geral Formation, as well as the Jura-Cretaceous sedimentary rocks from the Botucatu and Piramboia Formations. These formations together constitute the São Bento Group. However, some portions are covered by Cenozoic sandstones (Figure 1). In the study area, the Botucatu Formation is basically composed of sandstones having cross-stratifications, typical of the desert environments, with the characteristic fine to medium grain and well-selected grains, having high hydraulic conductivity and specific storage. The Piramboia Formation, on the other hand, consists of fluvio-lacustrine sandstones with plane-parallel stratifications and fine to medium granulation and, often, with wave and current marks. In these sedimentary rocks, thick successions of flood basalts and diabase intrusions from fissural magmatism were placed (Soares et al., 1973; Sinelli et al., 1980). Finally, thick soil profiles cover almost the whole municipality (Figure 2) (São Paulo, 2017).

The data from 128 tubular wells (Figure 1) drawn from the public agencies DAERP and SIAGAS, were interpolated by kriging in the ArcGIS 10.0 Geographic Information System (GIS) were used to obtain the depth of the static water level and the GAS potentiometric surface in the region.

To identify the outcropping areas of the Botucatu Sandstone, geophysical data from 45 tubular wells from Empresa Perfil Master and construction profile data from 38 tubular wells (Figure 1) from the Department of Water and Sewage of Ribeirão Preto - DAERP were used.

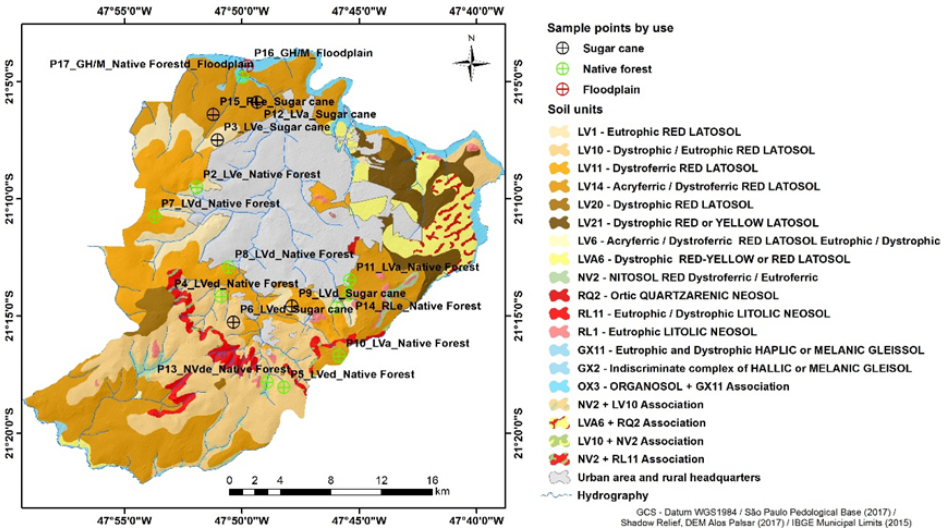

Figure 2. Map showing the pedological units second Brazilian Soil Classification System (EMBRAPA, 2018) in the Ribeirão Preto Municipality (Source: São Paulo, 2017) and sample points of the soils.

These data enabled the interpretation of the geophysical profiles using the software 'Display 6, Century Geophysical'. In the interpretations, four basaltic lava flows from the Serra Geral formation, were identified, just as demonstrated by of Fernandes et al. (2010). As well as the dry and wet phases in the formation of Piramboia (Sinelli, 1973).

From the profiles, the depth and type of lithology of the layers were obtained. Then, a 'dropdown' style table, '.txt' format was created, with the coordinates X (longitude), Y (latitude) and Z (depth) of the layers. The table was then transferred to the GMS 10.1 software by producing a well-shaped file (Figure 3A). Next, a set of blank cross-sections was generated connecting the tubular wells (Figure 3B) using the finite element method (Figure 3C) (GMS, 2015). Finally, a shapefile of the geological boundary of the model (Figure 3E) was created. This was interpolated in three-dimensions in an irregular triangular network (TIN) with 50 m spacing between the triangle edges (Figure 3D). This enabled the creation of a three-dimensional model of the geology of the municipality to accurately identify the outcrop regions of the Botucatu Sandstone.

The hydrosedimentological dynamics was evaluated and the sediment produced was quantified by applying the Revised Soil Loss Universal Equation (RUSLE) (Equation 1), by Renard et al., (1997) with support for SIG ArcGIS 10.0.

$$A = R \times K \times L \times S \times C \times P \quad (1)$$

where: 'A' is the soil loss (Mg ha^{-1} $year^{-1}$); 'R' is the erosivity through rain (MJ mm^{-1} ha^{-1} h^{-1} $year^{-1}$); 'K' is the soil erodibility (Mg ha^{-1} MJ^{-1} mm^{-1}); 'L' and 'S' represent the topographic factors, calculated by the relationship between the

slope and the ramp length (dimensionless) and 'C' and 'P', respectively, soil cover factors and as practices of management and conservation

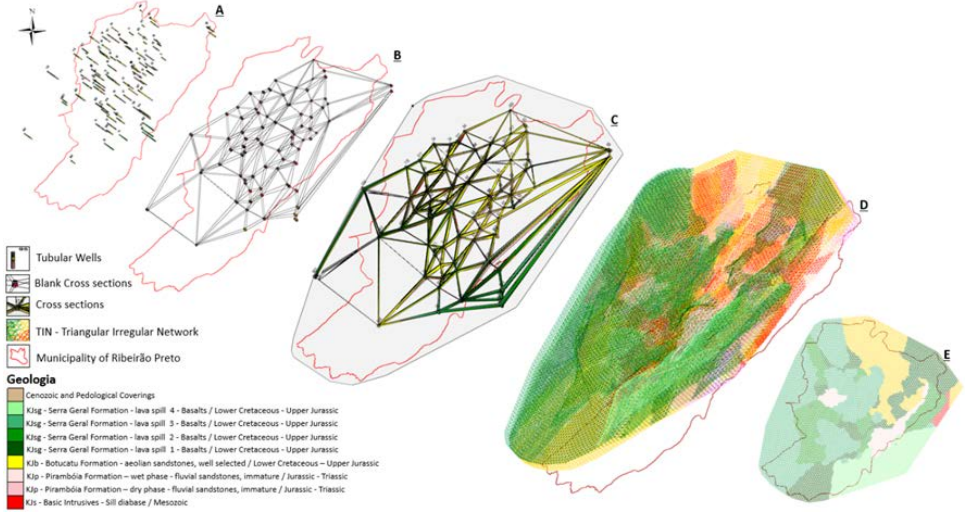

Figure 3. Methodological procedures for the elaboration of the geological model: Stage A - plot of the tubular wells; Stage B - connection between the wells (blank cross-sections); Stage C - attribution of the lithology to the sections created (cross-sections); Stages D and E - creation of the Irregular Triangular Network (TIN) and three-dimensional geological model.

To accomplish this, 17 sample points were chosen, distributed within the main pedological units of the municipality (Figure 3) and inside the Ribeirão Preto hydrographic subbasin. Among these, 11 points were under native forest, which shows the soil pattern without anthropic alterations, 5 were under temporary use of raising sugarcane, a clear indication of the main anthropic dynamics and 1 in the lowland floodplain at the mouth of the Ribeirão Preto river, in a the area of deposition of sediments. Regarding the soil types sampled, two (2) points were under forest and one (1) under sugarcane for the following soil units: Acriferric Red Latosol (LVa); Eutrophic Red Latosol (LVe); Dystrophic Red Latosol (LVd) and Eutrophic / Dystrophic Red Latosol (LVed). In the Dystrophic / Eutrophic Red Nitosol (NVde) and Eutrophic Litolic Neossolo (RLe), occupying restricted areas; one (1) sample was drawn from the native forest and one (1) from agricultural use. To complement the evaluation of the hydrosedimentological dynamics, two (2) points were collected from the floodplain region, one (1) under forest and one (1) under sugarcane, all being taken at the mouth of the Ribeirão Preto River, where the Gleisols and / or Melanic (GH / M) are dominant. The protocols and procedures of Lemos and Santos (2011) were adopted for soil collection. Among the soil classes, the Latosols are the most highly significant economically, as well as the most abundant in the municipality.

In order to calculate the RUSLE K factor, samples were collected in May 2018, from each soil unit described, with and without deformed structure, at 0 to 0.30 and from 0.30 to 0.60 m depths. From the soil samples, several physical, chemical and morphological attributes of the soils were obtained: particle size and clay dispersed in water, and the flocculation index (EMBRAPA, 1998); soil permeability to water using field infiltrometers and with triplicate (Zhang, 1997; Dane and Topp, 2002); aggregate stability from the soil sieving method in water (Kemper and Rosenau, 1986), which enabled the geometric weighted average diameter (DMG) to be calculated (Kemper and Chepil, 1965). An analysis of soil fertility was also done (EMBRAPA, 1998).

The RUSLE was calculated following the procedures listed: K Factor obtained by the indirect method of evaluating soil erodibility of Denardin (1990), from the data sampled in each soil unit; R Factor obtained from Trindade et al. (2016) and the study area shows an erosivity of 6500 MJ mm ha^{-1} h^{-1} $year^{-1}$; LS Factor (Figure 3A) was calculated employing the method of Hickey (2000), with the data derived from the Digital Elevation Model (DEM) SRTM 2, and corrected by the ArcGIS Fill function which eliminates anomalous pixels. In order to apply the Hichey (2000) algorithm, altimetry and slope were used as input data, from which the flow direction was obtained. From this to the area of contribution and accumulation of flow, by D∞ method of Tarboton (1997); C Factor (Figure 4B) was obtained from the Earth Explorer image / multispectral bands repository of the Landsat-8 OLI satellite (Operational Land Imager), from 05/09/2015, with atmospheric and geometric treatment, georeferenced to the World Geodetic System - WGS 1984. Then, the 8 classes of land use and cover were defined: urban area, roads and rural headquarters; floodplain hygrophilous fields; water bodies; temporary crops; permanent crops; pasture; native forest and mining. The image produced by stacking all the multispectral bands, in a false-colour composition (R5G4B6), using a 2% linear contrast, was segmented in the ENVI 5.3 software.

From the segmentation, the digital image classification was performed, by applying the non-parametric statistical algorithm, Support Vector Machines (SVM) (Huang et al., 2002) available in EnviFX. This classifies each segment based on the spectral attributes of the shape and texture of each use and ground cover, based on the training data and multispectral bands. The image was cropped, according to the shapefile of the municipality limits (IBGE, 2013). The values of the factors of land use ‘C’ and conservation practices ‘P’ (Figure 4B) were taken from the specialized literature.

In ArcGIS 10.6, by map algebra, the product of the natural factors' R ',' K 'and' LS 'and the anthropic factors' C' and P 'were obtained, the water erosion susceptibility map was generated and the soil loss rates were quantified for the entire area. To assess areas with unsustainable water erosion rates, the Soil Loss Tolerance limit was also calculated, based on the method of Bertol and Almeida

(2000). Then, the map of the soil units (Figure 2) was related to the erosion susceptibility map, with the TPS broken down for each soil unit.

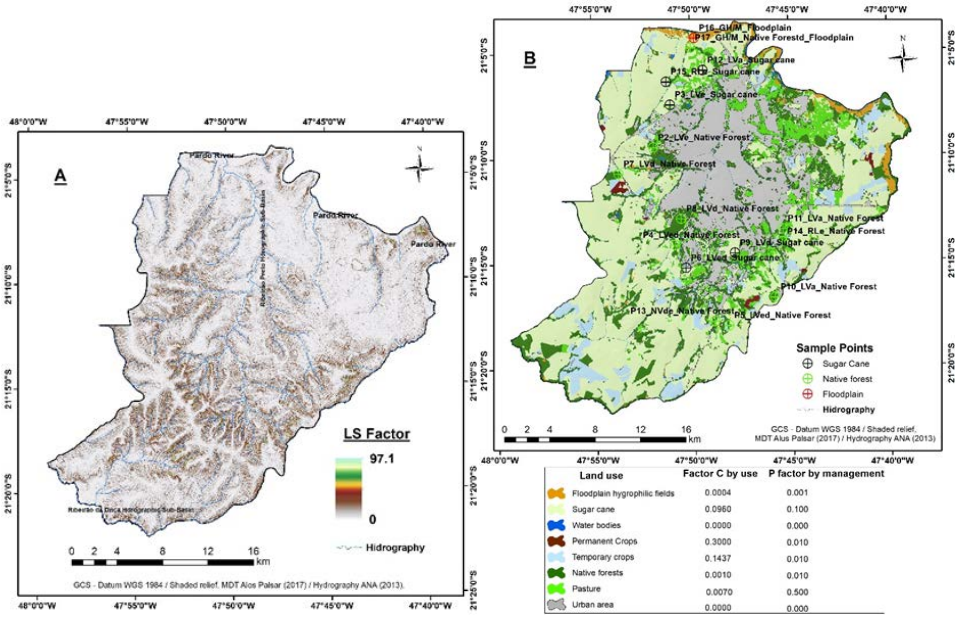

Figure 4. A - Topographic factor 'LS'; B - Use and management map with values of the factor of use, and coverage factor 'C', as well as conservationist practices factor 'P'.Sources: Eucalyptus under no-tillage and native forest (Martins et al., 2010); Soy under no-tillage (Bertol et al., 2001); Sugarcane without burning at harvest and with terracing (Andrade et al., 2011); Conserved pasture and degraded pasture (Roose, 1977) and Flood plain area (Oliveira et al., 2007).

RESULTS AND DISCUSSION

According to the geological model (Figure 5), of Botucatu Sandstones outcrops are located in the north of the municipality, near the Rio Grande floodplain. The yellow portions shown in Figure 5, indicate the Botucatu Sandstone outcrops, and are recharge areas of the GAS, and are therefore, influenced by the runoff of practically of the whole Ribeirão Preto subbasin, (Figure 5A). Even in the areas the Botucatu Sandstones (Figures 1 and 5), are cover by the soils and by the Cenozoic Sandstones, the runoff waters are able to penetrate through these layers and recharge the aquifer.

According to the particle size of the soils (Figure 6A), when these are formed in the areas of the outcrop of the Botucatu Sandstone, the sand content is highest, reaching at the 400 g kg^{-1} at point 16, which is covered with indiscriminate floodplain soils, and 380 g kg^{-1} in the point 12, at the areas of Acriferric Red Latosol.

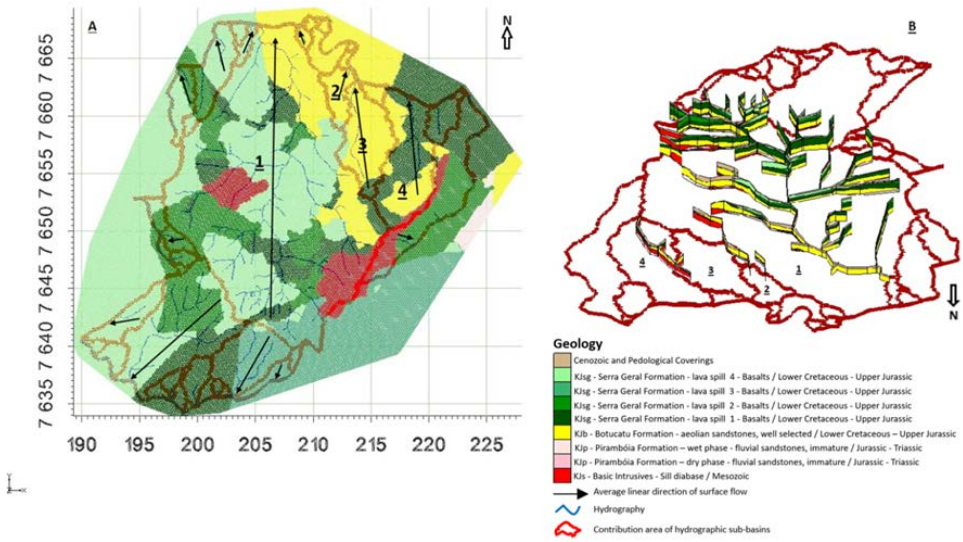

Figure 5. A - São Bento Group's geological model, hydrography and areas of contribution to the hydrographic sub-basins of the Municipality of Ribeirão Preto; B - Geological profiles drawn over the hydrography that flow to the outcrop areas of the Botucatu Sandstone.

In these latter soil type, when formed on the basaltic rocks of the Serra Geral Formation, it reached a maximum of 120 g kg^{-1}, what reflected in the permeability of the Latossols over the region of the outcrop of the GAS, with 99 mm h^{-1} permeability and 24 cm s^{-1} infiltrability at point 12 (Figure 6B). However, in the regions upstream of the Ribeirão Preto Subbasin, above the basalts, the soils show less permeability and thus the runoff increases, transporting the sediment to the lower and flat areas, precisely in the free GAS areas, where the higher infiltration rate is achieved.

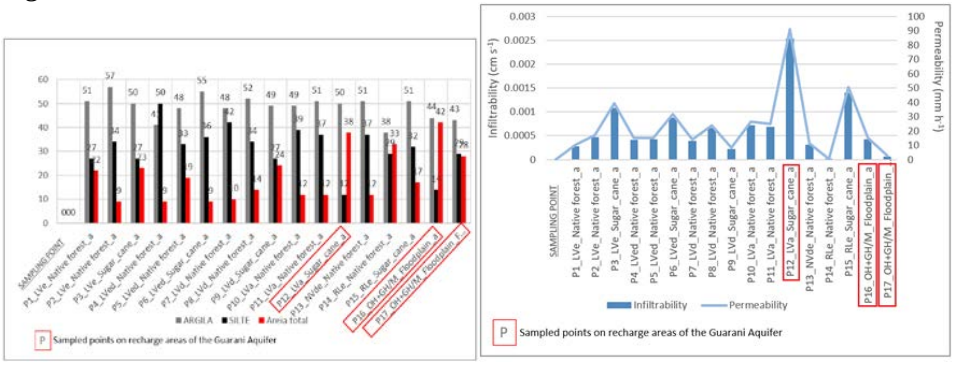

Figure 6. A – Histogram illustrating soil granulometrical composition; B - Histogram illustrating soil infiltration and permeability.

The soils formed over the Free GAS, also show higher levels of acidity and aluminum and lower Cation Exchange Capacity - CTC, indicative of lower

fertility, In the Ribeirão Preto Municipality these regions are located in the areas with lowest altitudes. The soils on the Serra Geral Formation rocks, however, are more clayey with greater agricultural workability because of their higher CTC, moisture retention, lower acidity and better fertility. As the iron content is high, these soils have the characteristic red colour and therefore are generally classified as Latossols and Argisols. They occur i regions higher than 525 m altitude, located in the north region of the municipality.

The Latosols sampled under the native forest (MN) and agricultural use (UA), generally presented, on average, organic matter (OM) content, of 54.4 g kg-1, and 28 g kg-1 respectively. For soils under agricultural use, this value was lower and indicative of the degradation of this soil attribute, caused perhaps by the conventional management methods used. This condition is aggravated by deforestation and burning the sugarcane harvest in some cases, which decreases the deposition of plant debris and reduces the base exchange capacity of these soils. Low levels of OM, along with mechanization management techniques, can also be linked to the lower average values in the DMG of the Latosols surface horizon at the sites with sugarcane, of 4.7 mm as against the 4.85 mm with native forest.

In Latosols, in basaltic and diabase areas (Figures 1 and 2), management can produce structures that are harmful to the growth of seedlings and roots, due to less aeration, infiltration and water movement in the soil profile and therefore favouring erosive processes. Thus, inadequate management practices and the lack of conservation methods favour the disintegration of soil structures and cause latosols to have low permeability, of the order of 26 mm h-1, on average, for soil under agricultural use, and 26.6 mm h-1, for soil under native forest. The resulting low permeability arises mainly from the clayed texture, although the compaction caused by mechanization also has influence. This characteristic of low permeability together with the clayey texture and the average geometric diameter of the soil aggregates under agricultural use, suggest a possible change in the hydrosedimentological dynamics, including the likelihood of an increase in the surface runoff and concomitant rise in the transport of the sediments and potential contaminants employed in the agricultural production and in urban centres, which can contaminate the GAS groundwater.

When comparing samples taken from Latosols at depths of 0 to 30 cm and 30 to 60 cm, the surface horizons contain about 22.2% of the total sand in agricultural use and 13.4% in native forest; however, the 30-60 cm layer contains 20.6% of total sand in agricultural use and 14% in native forest. This implies a probable concentration of the largest grains in the superficial horizons, because fine particles are carried by the superficial flow, confirming the proposition that the anthropic use has altered the characteristics of these soils. At Ribeirão Preto Municipality, the GAS both in free and confined areas near Free GAS are the most utilized for public supply, because of the low levels of mineralization and silica. However, the GAS displays an unconfined behaviour and has acquired its chemical characteristics from the unsaturated aquifer zone (Gallo and Sinelli,

1980). The stable isotopes of the oxygen-18 and deuterium reveal homogeneous values for the aquifer, suggesting that recharge in the urban area occurs only due to local precipitation. The radioactive isotopes tritium and carbon 14 indicate an identical dynamic and reveal that the waters are recent and do not contain artificial tritium, which implies infiltration after 1950 (Gallo and Sinelli, 1980). In this context, the flow of surface water can diffuse agrochemical contaminants in outcrops of GAS. Latosols over GAS Livre have good permeability, due to the high sand content and, in depth; the water has relatively fast circulation and has a recent origin, which favours the exchange of water with the surface. Besides this, the underground water level depth (Figure 7A) that in the northern region of the Ribeirão Preto reaches the surface of terrain in the north area of the municipality in the Pardo River Plain, which increases susceptibility to contamination by diffuse contaminants in the GAS.

The piezometric surface (Figure 7B) flows from northeast to southwest, which implies that part of the flow of sub-basins 1, 2, 3 and 4 (Figure 5) flows over the Free GAS. These flows can be returned to the southwest in depth, due to the GAS piezometric surface (Figure 7B), reaching urban tubular wells with surface contaminants. This increases the vulnerability of GAS in the region where a large part of the local population is concentrated. The piezometric surface reveals drawdown induced by the large concentration of wells and high pumping rates, mainly in the central region of the city (Montenegro et al., 1988; São Paulo, 2008; Conceição et al., 2009). In addition, in the outcrop region of the SAG, the level of groundwater has the lowest depths (Figure 7A), which coincides with the lowest altitudes. Due to this hydrosedimentological configuration, the SAG in the outcrop areas is vulnerable to all contaminants from runoff.

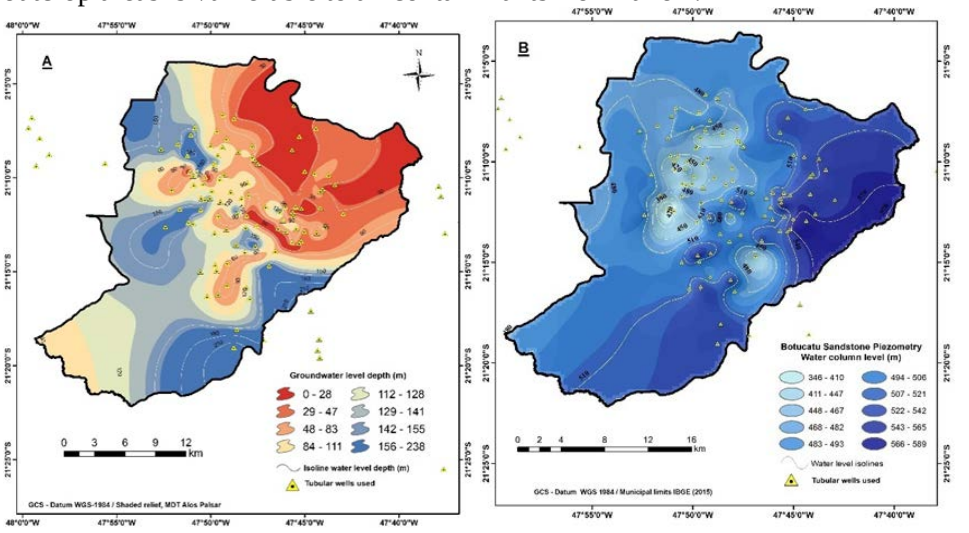

Figure 7. A. Map showing water level depth; B. Map showing the potentiometric surface of the underground water in Ribeirão Preto – SP.

Soil use and management can introduce a series of new alien substances to the natural environment due to the application of agrochemicals, as well as dragging and agitating the soil particles, as shown by London (2011). As a result, free aquifers can be negatively affected by the improper use of agrochemicals and the acceleration of the dynamics and surface hydrosedimentological flows. Thus, the geological units that comprise the GAS would have experienced a series of changes from the time it was formed, which includes the oxidation of grain surfaces and the translocation of groundwater clays. These geological units are also affected by the alteration of clay minerals, such as the change of kaolinite to smectites, and the establishment of secondary porosity, due to the dissolution of the unstable minerals (feldspars and ferromagnesian silicates) and carbonate-induced cementation (Araújo et al., 1999; França et al., 2003). By way of example, the rate of water erosion on average in the area under study is 1.42 Mg ha^{-1} $year^{-1}$ (Figure 8A). Around 5% of the municipality is above the TPS limit; however, when we remove the urban areas from the floodplain and water bodies, it extends to around 7% of the municipal agricultural area, 3,155 ha of the 48,527 ha were demarcated for agriculture and livestock in 2017.It is vital to note that no safe rate of soil loss can be set (Figure 8A). The TPS varied between 8.5 and 9.7 Mg ha^{-1} $year^{-1}$ for the Latosols, 8.5 Mg ha^{-1} $year^{-1}$ for the Nitosols and 6.5 Mg ha^{-1} $year^{-1}$ for the Neosols (Figure 8B).

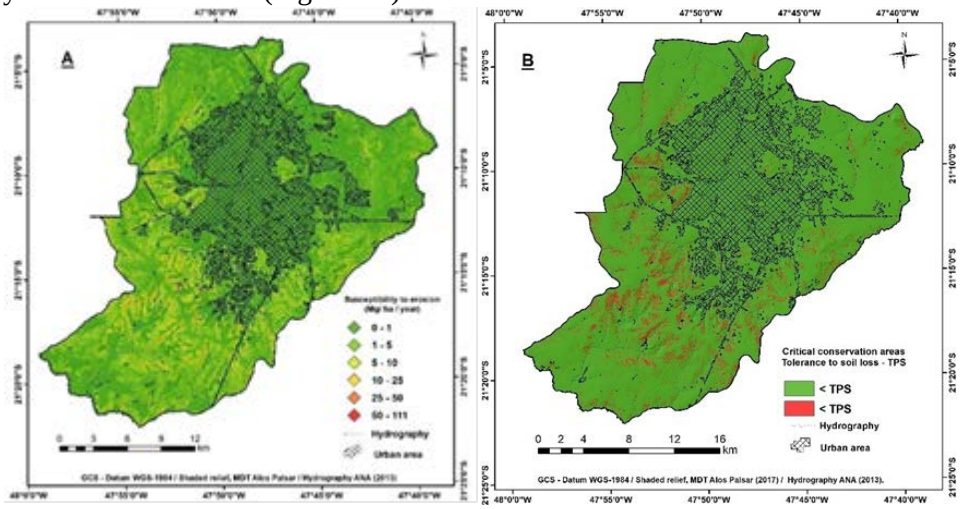

Figure 8A. - Map showing the erosion susceptibility classes; B –soil loss above and below the Tolerance to Loss of Soil limit in Ribeirão Preto Municipality – SP.

However, it is important to recognize that no safe levels of soil loss can be prescribed, mainly because the rates of pedogenesis are not known and also due to the synergistic effects on other environments including rivers and the atmosphere. With TPS values like these, areas with soil losses less than the TPS cannot be assumed to be sustainable. They will necessitate measures to mitigate the soil loss rates to levels closer to those recorded for the native forests.

Considering the average soil losses by land-use class (Table 1), sugarcane, which occupies the largest relative area, has the highest susceptibility to water erosion, with an average loss estimated at 2.83 Mg ha^{-1} $year^{-1}$. Then, the land-use classes with higher erosion susceptibility are silviculture and soybean, with average losses estimated at 1.23 Mg ha^{-1} $year^{-1}$ and 0.35 Mg ha^{-1} $year^{-1}$, respectively. The forests present the lowest erosion rate with 0.03 Mg ha^{-1}, $year^{-1}$ of soil loss. It is noteworthy that the lowland and urban areas have been excluded from the RUSLE calculation, first because it is a sediment receiving area and not a loss area and second because of the waterproofing of the soils.

Table 1. Susceptibility to soil loss for the different classes of uses and management in Ribeirao Preto.

	Area	Average rate of soil loss	Maximum Erosion	Total by class	Contribution to total erosion
Use	ha	Mg ha^{-1} $year^{-1}$	Mgha^{-1} $year^{-1}$	Mgha^{-1} $year^{-1}$	%
Urban area	14446.1	0.00	0.0	0.0	0.0
Hygrophilic fields	1257.9	0.02	4.5	4.5	0.0
Sugarcane	31030.0	110.11	959090.0	959090.0	93.2
Water bodies	223.8	0.00	0.0	0.0	0.0
Temporary culture	4286.7	28.37	15700.3	15700.3	1.5
Permanent culture	254.0	38.99	3419.8	3419.8	0.3
Native forest	8817.8	7.30	2574.1	2574.1	0.3
Pasture	5115.0	27.32	47741.6	47741.6	4.6
Total	65431.2	1.4	110.1	1028530.4	100.0

Losses which exceed the TPS limit (Figure 8B) are mostly between 10 and 25 Mg ha^{-1} $year^{-1}$ and are observed in regions practicing conventional management methods, mainly sugarcane and the permanent and temporary crops. Losses in the range of 25 to 100 Mg ha^{-1} $year^{-1}$ are noted, particularly, in the areas of conventional sugarcane management. These losses are due to the combined effect of the slope, the management employed (Figure 4B) and soil erodibility. Concentrated losses are found in the Eutrophic Latosols unit, one of the most widely utilized in agriculture because of the high fertility and extensive area occupied (Figure 2). Such losses are also found in the Neosols, which is a shallow and sandy soil founded in regions characterized by the highest slopes and erodibility. Neosols present low value of DMG, OM, and CEC, which accentuated the soil particle transport.

The practices of agricultural mining and the accidental burning of sugar cane worsen this situation in all soil classes, as in the State of São Paulo it is prohibited to burn sugar cane for harvest. It must be highlighted that in the

tropical regions because the kaolinite soils are naturally flocculated due to the aggregation between the clay, silt and sand particles, they exhibit a more porous and permeable behaviour, an essential attribute for their formation and maintenance of such soils. If this is neglected, with the water hydric regime being shorter than three dry months on average per year, the rates of water erosion can escalate. However, in agriculture, the conventional management practices used cause the break-down of soil structure thus minimizing the natural porosity and negatively influencing the infiltration rates, thereby enhancing the transport of these particles and the OM via the runoff waters.

In the regions of the permanent crops, namely silviculture and/or coffee, soil losses are mostly linked to the low soil cover due to the early years of plant development. Thus, the soil protection by the dossel is lower, which exposed the soil to the rainfall mechanic impact (Martins et al., 2010).

In the areas of the temporary crops, however, the continuous changes in coverage result in several instances when the soil is exposed, opening it up to the erosive action of rainfall. The use of level cultivation techniques, no-till and terracing, for instance, can remedy this situation. Soil losses in the pasture areas are mostly caused by common burning during dry periods, overgrazing and the absence of management techniques, which can be reduced by implementing rotated grazing, reducing the number of cattle per area and correcting the soil.

In such a scenario, the susceptibility to erosion has been estimated to exceed one million tons per year for the municipality of Ribeirão Preto, which can exert disastrous and long-term consequences for the economy of this municipality, due to the natural resources of soil and water getting deteriorated, which historically have ensured economic support.

Thus, assessing the hydrosedimentological dynamics of region provides an overview of the energy and matter flow patterns between the various environmental systems, like the surface and groundwater, facilitating inferences to be drawn regarding the possible interactions. This enables the adoption of measures to reduce the levels of potential contamination, based on precautionary and preventive concepts. Erosion raises the susceptibility level of the aquifer to diffuse the contamination because it ravages the surface soil layers, thus raising the permeability and lowering the water depth level (water table). These erosions encourage runoff which can transport the soil with the agrochemicals in the water courses. Particularly for the Ribeirão Preto, the GAS outcrop area is close to the Ribeirão Preto subbasin, in the Rio Pardo alluvial plains, causing the danger levels of contamination to escalate because of the proximity of the water level, land use and type of occupation. Besides, it must be recognized that CONAMA Resolution No. 430 (CONAMA, 2011) mainly investigates contaminants present in lethal doses, normally from the point sources, neglecting the chronic contaminations, like those arising from diffuse contamination by agrochemicals, which pose greater challenges to study.

Therefore, in the north-eastern region, where the GAS begins and extends into some areas of the urban area, the aquifer faces higher contamination risks,

caused by the potentially existing activities of contamination. Besides, the water demand has steadily escalated, which induces extensive lowering cones of groundwater level of the aquifer in the urban area, and which locally alter the direction of the flows by attracting and increasing or decreasing the flows from the areas close to these locations in the urban portion. Besides, the presence of highly deteriorated deactivated wells, which need to be, sealed off by the government or via legal requirement, which, through surface runoff, are indicative of still one, more threat to the protection of quality of the groundwater.

With reference to expanding the use of the lowering cone (Conceição et al., 2009) it is suggested the tubular wells be constructed far away from the areas of highest exploitation, using alternate pumping methodologies to enhance the supply; another way is to regularize the water collection in the Rio Pardo subbasin, and set up environmental preservation areas and a vulnerability to pollution zone; one more method would be to improve agricultural management and control the types of fertilizers and pesticides applied and establish maximum permitted limits, in synchrony with intensive environmental education. Therefore, the hydrosedimentological dynamics in the specific area requires assessment to discover the water erosion hot spots, as revealed by the regions that exceed the established TPS limit (Figure 8B). This data can contribute towards defining the natural susceptibility of the aquifer to diffuse contamination.

Unsatisfactory use of land and poor management directly affect the soil loss rates (Figure 8A), by raising the agricultural production expenditure and lowering the productivity. However, while these harmful and indirect impacts must be considered, it is noteworthy, as examples, that the eutrophication of the water bodies, trophic chain contamination with the pesticides and heavy metals applied heightened quantities of greenhouse gas emissions and loss of environmental and ecosystem services are some other effects (Carvalho, 2008). Such environmental degradation of the resources in turn produces negative effects on all the physical and biological resources in the ecosphere (Morgan and Nearing, 2011). These deleterious effects can be reduced in regions characterized by anthropic actions by implementing simple conservation management practices, including no-till, planting along the counter lines, terracing, rotated grazing, as well as the protection of permanent preservation areas, like the riparian forests. These reparative actions are of paramount importance because of the surface flows in the drainage networks and which can negatively affect the GAS.

Although empirical data to validate the model are lacking, it is crucial to note that because of the paucity of environmental data in Brazil, this work offers a panoramic view of the areas that release and receive the hydrosedimentological flows, thus indicating the regions susceptible to erosion in this landscape, the water to be eroded and sediment deposition. Thus, the combination of the anthropic factors, as well as the uses and management of the specific soils enables the critical areas to be demarcated for the preservation and maintenance of the natural resources, which must be the focus of the sustainable management alternatives.

CONCLUSIONS

Based on the investigation conducted in this study, the following conclusions were drawn:

1 - To identify the hot spots of water erosion, the hydrosedimentological dynamics can be used to define the natural susceptibility of the aquifer to diffuse contamination.

2 – Further studies are required to investigate the effect of agricultural practices on groundwater and aquifers. However, according to local characteristics such as soil porosity, types of rocks, and water level depth, these water resources may be contaminated.

3 – The superficial hydrosedimentological dynamics of the Ribeirão Preto hydrographic sub-basin and adjacent areas can carry particles to the Botucatu Sandstone outcrops, which depending on its composition; can contaminate the Guarani Aquifer System in this point and many others with a similar configuration.

4 - No safe levels have been prescribed for soil loss, as all areas deserve equal attention, especially those having soil loss values exceeding values considered natural. In this case, the soil loss in the native forest for the municipality was 0.03 Mg ha-1 year-1, which is well below the average for agricultural use, which is 2.03 Mg ha-1 year-1. This situation can be considered critical and cause serious problems to the hydrosedimentological dynamics of the area, negatively influencing the water quantity and quality of the Guarani Aquifer System.

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PRELIMINARY RESULTS OF THE INFLUENCE OF SOWING DATES ON THE MOST IMPORTANT SUNFLOWER AGRONOMIC TRAITS

SUMMARY

Timely, well-made sowing is one of the essential prerequisites for successful sunflower production. In order to determine the influence of the sowing date on the most important agronomic sunflower traits, an experiment was conducted according to a random block design at the Osijek location. The experimental material included 22 hybrids of the Agricultural Institute Osijek and three introduced foreign hybrids. Sowing was performed on two dates (21st April and 13th May 2015). The following traits were analyzed: plant height, head diameter, grain yield, oil content, and oil yield. Significantly higher values were found for the plant height and head diameter in the second sowing date and the oil content in the first sowing date. Grain and oil yields were higher but not statistically significant at the second sowing date. The Experimental hybrid OS-H-10 had the highest yield of grain (6.970 t ha^{-1}) and oil (3.448 t ha^{-1}), while the experimental hybrid OS-H-4-2 had the highest oil content (55.61 %).

Keywords: sunflower, sowing date, hybrid, grain yield, oil content, oil yield.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is primarily grown because of the oil used in human consumption, but also as a raw material for the processing industry, animal feed, and beekeeping. In the year 2015 there are about 25.5 million hectares in the world, of which around 16.5 % in the European Union. Sunflower is the most important oilseed crop in Croatia. In the period 2000–2014,

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areas under sunflower ranged from 20615 to 49769 ha, with grain yields from 1.57 to 3.20 t ha^{-1} (FAO, 2020).

The priority task in the production of any crop, including sunflower, is to achieve optimal values of the most important agronomic traits. These are mainly quantitative traits, implying that their expression is determined by genetic and environmental factors, as well as their interaction. Environmental factors, which are extremely important in sunflower production, are highlighted by many authors (Miklič et al., 2007; Gadžo et al., 2011; Škorić, 2012). The yield per unit area is the result of the action of factors of variety in interaction with environmental factors (Živanović et al., 2017; Maksimović et al., 2018; Dončić et al., 2019; Rajičić et al., 2019; Rakaščan et al., 2019).

Of many environmental factors, priority should be given to weather, soil and agrotechnical measures applied. The agrotechnical task is to enable the optimal growth and development of cultivated plants, with achieving maximum yield in the quantity and quality of invested labor and resources (Vratarić, 2004). Agrotechnical measures help the plants to adapt by reducing the impact of unfavorable conditions (abiotic and biotic stresses) on the most important agronomic traits.

When applying a semi-intensive technology, the impact of environmental factors (weather and soil) on the quantity of sunflower yield can be reduced substantially (Sárvári, 2010). Considering the occurrence of increasingly dry vegetation seasons, especially in the summer, with less precipitation, higher temperatures, extremely high daily maximums, but also fluctuations in weather parameters over a short period (daily, weekly) (Mijić et al., 2017; Jug et al., 2018), determination of the most favorable sowing date is a very important part of the application of quality agrotechnical measures (Balalić et al., 2007). Therefore, this study aimed to determine the effect of sowing date on the most important agronomic traits of sunflower: plant height, head diameter, grain yield, oil content and oil yield of 25 sunflower hybrids at the Osijek location during 2015 and to identify the best hybrid combinations for further breeding work.

MATERIAL AND METHODS

The study included 22 new sunflower hybrid combinations of the Agricultural Institute Osijek and three introduced hybrids (standard 1-3) that occupied significant harvesting areas under sunflower in the Republic of Croatia. The sowing was done on two dates (21st April and 13th May 2015) in the experimental field of the Agricultural Institute Osijek in a randomized complete block design with three replicates for each sowing date. Other elements of the experiment were: length of the basic plot 5 m, width 2.8 m, number of plants on the basic plot 88, the spacing between rows 70 cm, row spacing 23 cm, the spacing between blocks 1.5 m.

Standard agrotechnique for sunflower production was applied. In the fall, basic fertilization was carried out with 300 kg ha^{-1} of NPK fertilizer (7:20:30) and 50 kg ha^{-1} of UREA (carbamide, 46 % N). Prior to sowing, 200 kg ha^{-1} NPK

(7:20:30) was applied with and furrow closing. The sowing was done by hand. In phase four of the permanent leaf pairs (Schneiter and Miller, 1981), 100 kg ha^{-1} of KAN (calcium ammonium nitrate, 27 % N) was applied. Weed protection was performed after sowing, and before emergence with a combination of herbicides metolachlor + fluchloridone + oxyfluorfen (1 + 1.5 + 0.5 l ha^{-1}). Boscalid + dimoxystrobin (0.5 l ha^{-1}) in the butonization phase was used for disease control. The harvest was done with a plot combine harvester.

The most important agronomic traits of sunflower were analyzed. Plant height and head diameter were measured at the physiological maturity stage. The grain yield from the plot was calculated per hectare according to the standard (9% moisture and 2% impurity). The oil content was determined by a magnetic resonance analyzer (MQA 7005 NMR Analyzer), and the oil yield was calculated based on grain yield and oil content. The obtained values of the analyzed traits were systematized by hybrids and sowing dates for statistical processing by analysis of variance (ANOVA), and mean values were compared by the LSD test (SAS, 2003).

Weather conditions and soil traits

According to the data presented in Table 1, the year 2015 was dry. Specifically, the precipitation deficit in the observed period in 2015 (I-IX month) was by 21% less (110.9 mm) and in vegetation period by 38% less (151.6 mm) respectively, compared to the long-term average (2000–2014). Particularly pronounced lack of rainfall was observed in April, June, July and September, that is, during most of the vegetation period.

Temperatures in each month were higher than the long-term average, except April. The highest temperatures were noted in July and August. The trend of increasingly dry years, with precipitation deficits and elevated temperatures, was evident in 2015.

Table 1. Monthly precipitation (mm), the average monthly temperature (°C), and their long-term average (2000–2014)

Month	Precipitation (mm)		Temperature (°C)	
	2015	2000–2014	2015	2000-2014
I-III	167.9	127.2	-	-
IV	10.6	52.4	12.4	12.6
V	108.6	78.2	17.9	17.4
VI	44.9	85.1	21.3	20.8
VII	7.6	56.9	24.9	22.5
VIII	50.8	65.0	24.1	22.0
IX	29.1	65.6	19.0	16.7
Amount (I-IX)	419.5	530.4	-	-
Amount (IV-IX)	251.6	403.2	-	-
Average (IV-IX)	-	-	19.9	18.7

Source: Croatian Meteorological and Hydrological Service

The type of soil was an eutric cambisol, clayey loam texture with neutral reaction (pH in KCl 6.61). The humus content in the soil was 2.26, which classifies it in low humus soils, but is well supplied with phosphorus (36.90 mg/100 g soil) and potassium (29.43 mg/100 g soil).

RESULTS AND DISCUSSION

The sowing date had a significant effect on plant height, head diameter, and oil content (Table 2). Studied hybrids differed significantly for all traits, and the interaction of sowing date and hybrids was significant only for oil content.

Large variations in plant height within the sowing dates were found between the hybrids, indicating divergence of the material selected for the study (Table 3). It is also clearly evident that the plants in the second sowing date were, on average, statistically significantly higher than the plants in the first sowing date (214 cm versus 202 cm), which is in agreement with the studies of Liović et al. (2015). Different results were obtained by Krizmanić et al. (2001) and Vratarić (2004). Higher plant height values in the second sowing date may be related, among other factors, to above-average rainfall during May (108.6 mm), which greatly influenced the expression of this trait.

Table 2. Analysis of variance

Source of variation	Mean square				
	Plant height	Head diameter	Grain yield	Oil content	Oil yield
Sowing date	5162.7**	29.9**	$0.963^{n.s.}$	15.34**	$0.057^{n.s.}$
Hybrid	2225.3**	23.9**	3.773**	53.69**	0.868**
Sowing date x Hybrid	$68.0^{n.s.}$	$3.02^{n.s.}$	$0.416^{n.s.}$	2.00**	$0.069^{n.s}$

^{ns} F - test not significant; ** F- test significant on level P<0.01

In the first sowing date, the average head diameter was 21.2 cm and was statistically significantly lower than the second sowing date (22.1 cm). Variations were expressed for individual hybrids so that the head diameter values ranged from 18.7 to 27.7 cm in the first sowing date, while in the second date, these variations were even more emphasized (19.3-32.3 cm). The head diameter is of great importance for successful sunflower production and depends on the sowing date (Ahmed et al., 2015). Balalić et al. (2016) point out that head diameter affects the number of flowers and grains per head, which directly affects the grain yield per plant. According to Škorić (2012), the head diameter in sunflower hybrids is generally 20 to 30 cm. Unlike the plant height and head diameter, where the values were higher in the second sowing date, the oil content was statistically significantly higher in the first sowing date (Table 4), with large variations in both sowing dates (first term: 43.71-55.20 %; second term: 41.82-56.38 %). Oil content is a complex trait determined by genetic and environmental factors and is an important component of oil yield (Leon et al., 2003, Balalić et

al., 2012). Krizmanić et al. (2012; 2013) indicate that the oil content is greatly influenced by temperature and air humidity, by environmental factors, as well as soil type, and the application of agrotechnical measures.

Table 3. Variations in traits' values and averages by sowing date for plant height and head diameter

Sowing date	Plant height (cm)		Head diameter (cm)	
	Average	Variation	Average	Variation
I	202	168-237	21.2	18.7-27.7
II	214	180-249	22.1	19.3-32.3
Average	208		21.6	
LSD 5%	2.8		0.6	

Large variations in grain and oil yields between individual hybrids have been identified within the sowing date. Although there were no statistically significant differences between sowing dates for grain and oil yields, it should be noted that they were larger in the second sowing date. Most authors (Krizmanić et al., 2001; Crnobarac et al., 1996; Crnobarac and Dušanić, 2000; Vratarić, 2004; Liović et al., 2015) concluded that delayed sowing in similar agro-ecological conditions leads to a decrease in grain and oil yields.

Table 4. Variations in traits' values and averages by sowing date for grain yield, oil content and oil yield

Sowing date	Grain yield (t ha^{-1})		Oil content (% in D.M.)		Oil yield (t ha^{-1})	
	Average	Variation	Average	Variation	Average	Variation
I	5.689	2.899-6.780	51.37	43.71-55.20	2.655	1.418-3.404
II	5.849	3.089-7.161	50.73	41.82-56.38	2.694	1.475-3.492
Average	5.769		51.05		2.675	
LSD 5%	ns		0.31		ns	

Table 5 shows the average values of the first and second sowing date for the analyzed traits. After the LSD test, the investigated hybrids were ranked. The plant height was ranked in 13 ranks (a-m), head diameter 7 (a-g), grain yield 10 (a-j), oil content 14 (a-n), and oil yield 12 (a-l). This way of presenting data provides insight into the statistical justification of differences between individual hybrids; respectively, there are no statistically significant differences between the hybrids marked with the same letter. By grain and oil yields, as the most important agronomic characteristics, highlighted several experimental hybrids.

Table 5. Average traits value for both sowing dates

Hybrid	Plant height (cm)	Head diameter (cm)	Grain yield (t ha^{-1})	Oil content (% in D.M.)	Oil yield (t ha^{-1})
OS-H-10	239 ^{ab}	22.3 ^{bc}	6.970 ^{a}	54.39 ^{bc}	3.448 ^{a}
OS-H-20	222 ^{cde}	21.2 ^{bcdefg}	6.634 ^{ab}	50.40 ^{j}	3.042 ^{b}
OS-H-120	219 ^{def}	21.8 ^{bcde}	6.259 ^{bcde}	52.73 ^{ef}	3.001 ^{b}
OS-H-163	209 ^{ghijk}	21.8 ^{bcde}	6.104 ^{bcdef}	53.82 ^{cd}	2.988 ^{bc}
OS-H-28	226 ^{cd}	21.3 ^{bcdef}	5.949 ^{bcdefgh}	55.15 ^{ab}	2.986 ^{bc}
OS-H-30	216 ^{defgh}	22.0 ^{bcd}	6.188 ^{bcde}	52.24 ^{efg}	2.942 ^{bcd}
STANDARD 1	185 ^{l}	22.3 ^{bc}	6.268 ^{bcde}	50.43 ^{j}	2.879 ^{bcde}
OS-H-16	201 ^{k}	21.0 ^{cdefg}	6.191 ^{bcde}	50.75 ^{hij}	2.858 ^{bcdef}
OS-H-2	214 ^{efghi}	20.3 ^{cdefg}	6.182 ^{bcde}	50.67 ^{ij}	2.848 ^{bcdef}
OS-H-103	207 ^{hijk}	21.8 ^{bcde}	5.670 ^{efgh}	55.10 ^{ab}	2.844 ^{bcdef}
OS-H-36	230 ^{bc}	22.2 ^{bcd}	6.027 ^{bcdef}	51.76 ^{fgh}	2.834 ^{bcdef}
OS-H-5	218 ^{defg}	21.2 ^{bcdefg}	6.378 ^{abcd}	47.75 ^{lm}	2.766 ^{bcdefg}
OS-H-4-2	205 ^{ijk}	20.3 ^{cdefg}	5.438 ^{fghi}	55.61 ^{a}	2.753 ^{bcdefg}
OS-H-4	203 ^{kj}	20.2 ^{defg}	5.961 ^{bcdefg}	49.22 ^{k}	2.671 ^{cdefgh}
OS-H-29E	226 ^{cd}	20.7 ^{cdefg}	5.655 ^{efgh}	51.46 ^{ghij}	2.650 ^{defghi}
OS-H-42	211 ^{fghij}	19.8 ^{efg}	5.589 ^{efgh}	51.38 ^{ghij}	2.618 ^{efghij}
OS-H-21	243 ^{a}	23.2 ^{b}	5.875 ^{cdefgh}	48.34 ^{kl}	2.577 ^{efghij}
OS-H-22/67	231 ^{bc}	21.2 ^{bcdefg}	6.548 ^{abc}	42.77 ^{n}	2.545 ^{fghij}
OS-H-50	205 ^{ijk}	22.3 ^{bc}	5.827 ^{defgh}	47.28 ^{lm}	2.501 ^{ghijk}
OS-H-22	187 ^{l}	19.7 ^{fg}	5.265 ^{hi}	51.66 ^{fghi}	2.473 ^{ghijk}
STANDARD 2	189 ^{l}	22.0 ^{bcd}	5.491 ^{fghi}	47.16 ^{m}	2.358 ^{hijk}
STANDARD 3	182 ^{lm}	19.2 ^{g}	5.319 ^{ghi}	48.27 ^{kl}	2.335 ^{ijk}
OS-H-44	174 ^{m}	21.0 ^{cdefg}	4.866 ^{ij}	52.29 ^{efg}	2.315 ^{jk}
OS-H-49	184 ^{lm}	21.3 ^{bcdef}	4.571 ^{j}	52.59 ^{ef}	2.188 ^{k}
OS-H-46-7	181 ^{lm}	30.0 ^{a}	2.994 ^{k}	53.14 ^{de}	1.446 ^{l}
Average	207	21.6	5.769	51.05	2.675
LSD 5%	10	2.0	0.686	1.08	0.317
Min	174	19.2	2.994	42.77	1.446
Max	243	30.0	6.970	55.61	3.448

CONCLUSIONS

Based on the analyzed data for plant height, head diameter, grain yield, oil content, and oil yield of sunflower hybrids in two sowing dates, the following conclusions are:

1. The sowing date had a significant impact on plant height, head diameter, and oil content.
2. Statistically significant differences were found between the hybrids for all analyzed traits.
3. The interaction of the sowing date and hybrid was only significant for the oil content.
4. In the second sowing date, higher values were determined for all analyzed traits except for oil content.
5. Several experimental hybrids have been distinguished by grain and oil yield and oil content and can be considered as promising hybrids for further research.

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CONSUMER MOTIVES AND BARRIERS TOWARDS OLIVE OIL

SUMMARY

This paper analyses the motives for and barriers to the consumption of olive oil in Montenegro. The structure of respondents' attitudes was analysed using a survey questionnaire with questions focused on consumers' attitudes towards olive oil. The obtained results were statistically processed using descriptive statistics and SPSS software. The results of the survey revealed a correlation between age and frequency of olive oil consumption. Based on the revealed habits and low olive oil consumption, there is a need for additional education on the importance of consumption and its health benefits and nutritional value. To improve the consumption of domestic olive oil, respondents emphasised the need for more affordable prices and better marketing.

Keywords: Motives, Barriers, Consumer, Olive oil, SPSS

INTRODUCTION

Based on numerous research activities, olive oil and extra-virgin olive oil (EVO) have proven to be an indispensable element in the Mediterranean diet and olives are among the leading crops in this and the broader region. The focus of research of numerous scientists was income and cultivated area (Pupo D'Andrea, 2007; Marchini et al. 2010; Teresa Del Giudice et al, 2015). For the purposes of this work, literature concerning consumer behaviour was analysed depending on their attitudes towards the particular characteristics of olive oil consumption. The period from 2001 to December 2019 was selected. Numerous databases were used, including: Thomson Reuters (ISI) Web of Knowledge; Scopus et al. Methodological approach was used to select the papers predominantly analysing consumer preferences, motives and barriers. Particular emphasis was laid on the effect of the various attributes for the preferred product (Jiménez-Guerrero et al. 2012; Mtimet et al. 2008, 2011, 2013; Di Vita et al. 2013; Sillani et al. 2014).

The attributes analysed for the needs of this paper included analysis of the papers addressing the Geographical origin (Del Giudice and D'Elia 2001, S.Dekhili et al. 2005, 2011; Cicia et al. 2012; Aprile et al. 2012; Piccolo et al. 2013; Panico et al. 2015, Moric et all 2017), Brand (Baourakis and Baltas 2003; Cicia et al. 2005; Bracco et al. 2009; Dekhili and d'Hauteville 2009;

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Gázquez-Abad and Sánchez-Pérez 2009; Joel Espejel et al. 2009; Jiménez-Guerrero et al. 2012) and Price (Lazaridis 2004; Mtimet et al. 2014, Gázquez-Abad and Sánchez-Pérez 2009, Carlucci et al. 2014, Cicia et al.2002; Martínez et al. 2002; Scarpa and Del Giudice 2004, Cicia and Perla 2000).

MATERIAL AND METHODS

For the purpose of this paper, the survey was conducted in the following municipalities: Podgorica, Danilovgrad, Bar, Ulcinj, Budva, Kotor, Herceg Novi, Tivat. The questionnaire was divided into two parts: the first part consists of a set of questions concerning the descriptive statistics for sociodemographic indicators, while the second part includes questions concerning the economic aspect of consumers to olive oil products.

The poll was conducted in late December 2018. It was carried out by trained survey takers. The survey included students - respondents above 18 years of age. On a sample N=129 respondents, metric properties of the scale were assessed. The scale reliability is high. Results obtained were analysed and statistically processed using the descriptive statistics methods and SPSS program.

RESULTS AND DISCUSSION

The obtained results of the survey concerning the sociodemographic indicators, household incomes and so on show as statistically significant a group of respondents of 45.4% who consume olive oil daily and once a month. The dominant participation of the respondents is with high school and three household members, whose monthly income ranged from € 401 to € 600. (table 1).

As the main reason for non-consumption of olive oil, apart from the high price the taste, smell, lack of habit or achieved saturation level were particularly highlighted. Quality and price are the most important factors in the process of making a decision on buying olive oil. Almost 2/3 respondents pay particular attention to the brand, while the most frequently bought packaging was 0.75l. Approximately 40% of respondents consider marketing communication to be important and partly important, while 2/3 prefer extra virgin olive oil and virgin olive oil (table 2).

In the buying process, the most significant sales channels are supermarket (38.4%) and direct purchase from the manufacturer (30.4%). Home consumption applies to 80% of the total number of respondents. In terms of price levels at the domestic market, more than 60% believe that prices are high and play a significant role in the buying process, but also that the domestic supply of olive oil is satisfactory. Special emphasis was laid on domestic olive oil. The most represented of the foreign countries are Greece and Spain. Quality is one of the main reasons for buying foreign oil.

Respondents agree with the assertion in most cases, and also, on average, they provided similar answers. Analysis of the results obtained implies also the examination of the correlation between certain questions.

Table 1. Descriptive statistics for sociodemographic indicators, education level, household income

Sex of olive oil purchaser, n (%)	
Male	66 (51.2)
Female	63 (48.8)
Age of main olive oil purchaser (years), mean (SD), median	46 (1.341) 2.83
Education level of main ollive oil purchaser, n (%)	
Bachelor degree or higher	24 (18.6)
Diploma	31 (24.0)
Vocational	69 (53.5)
No post-school qualification	6 (3.9)
Household places, n (%)	
Rural	84 (65.1)
Urban	22 (17.1)
Peri urban	23 (17.8)
Number of household members	
1	5 (3.9)
2	10 (7.8)
3	43 (33.3)
4	34 (26.4)
5	27 (20.9)
More than 5	10 (7.8)
Household income (EUR €), n (%)	
to 200 eur	14 (11.0)
From 201 to 400 eur	33 (26.0)
From 401 to 600 eur	42 (33.1)
From 601 to 800 eur	25 (19.7)
From 800 to 1000 eur	5 (3.9)
More than 1000 eur	8 (6.3)
How often do you consume ollive oil?	
Daily	34 (26.6)
Once a month	24 (18.8)
2-3 times a week	31 (24.2)
1-3 times a month	21 (16.4)
I dont consume ollive	18 (14.1)

SD – standard deviation.

Table 2. Descriptive statistics for economic aspect of consumers to olive oil products.

Why not consume ollive oil? a) High price 12 (19.4) b) Health reasons9 (14.5) c) Other41 (66.1) Which factor has a dominant influence when buying olive oil? a)Quality 70 (56.0) b)Brand 8 (6.4) c)Price 24 (19.2) d)Packaging 7 (5,6) e)Pack size 1 (0.8) f)Producer 12 (9.5) g)Other factors 3 (2.4) Is Brand Important When Buying Olive Oil? a) extremely important (31) 24.6 b) very important (17) 13.5 c) moderately important (37) 29.4 d) neutral (20) 15.9 e) slightly important (1) 0.8 f) a little important (8) 6.3 g) is not important at all. (12) 9.5 What size of pack of olives do you use most often? a) 0.20 l (31) 24.6 b) 0.50 (17) 13.5 c) 0.75 l (37) 29.4 d) 1.00 l (20) 15.9 e) 2.00 l (1) 0.8 f) other (8) 6.3 Does economic propaganda have an impact? a) Yes (31) 24.6 b) Mostly (17) 13.5 c) No (37) 29.4 What types of olive oil do you prefer? a) Extra virgin (38) 30.0 b) Virgin (46) 37.4 c) Malsin oil lamps (12) 9.8 d) Refined olive oil (23) 18.7 e) Refined mixed with virgin (2) 1.6 f) Olive oil (2) 1.6	Where do you usually buy olive oil? a) hypermarket 31 (24.8) b) supermarket 48 (38.4) c) directly from the manufacturer 38 (30.4) d) other, where? 8 (6.4) Where do you usually consume olive oil? a) Restaurant 15 (12.0) b) the house 109 (87.2) c) other, where? 1 (0.8) Do you think that the price of olive oil in the domestic market is high? a) I totally agree 19 (15.0) b) I agree 58 (45.7) c) I have no opinion 35 (27.6) d) I disagree 11 (8.7) e) I disagree at all 4 (3.1) Does the level of retail prices play a role in buying olive oil? a) has no role 18 (14.3) b) has a role 78 (61.9) c) I don't know 30 (23.8) Is the domestic market supply of olive oil satisfactory? a) Very satisfactory 30 (23.6) b) Satisfactory 48 (37.8) c) I'm not sure 42 (33.1) d) Unsatisfactory 6 (4.7) e) Very unsatisfactory 1 (0.8) Which olive oil do you prefer? a) domestic 76 (61.8) b) foreign 22 (17.9) c) equally 25 (20.3)

Table 3. Correlations between 2 and 8.

		Q2	Q8
Q2	Pearson Correlation	1	-,289**
	Sig. (2-tailed)		0.001
	Sum of Squares and Cross-products	230.248	-68.289
	Covariance	1.799	-0.538
	N	129	128
Q8	Pearson Correlation	-,289**	1
	Sig. (2-tailed)	0.001	
	Sum of Squares and Cross-products	-68.289	243.430
	Covariance	-0.538	1.917
	N	128	128

**. Correlation is significant at the 0.01 level (2-tailed).

Table 4. Correlations between 4 and 8.

		Q4	Q8
Q4	Pearson Correlation	1	-0.093
	Sig. (2-tailed)		0.294
	Sum of Squares and Cross-products	89.550	-13.766
	Covariance	0.700	-0.108
	N	129	128
Q8	Pearson Correlation	-0.093	1
	Sig. (2-tailed)	0.294	
	Sum of Squares and Cross-products	-13.766	243.430
	Covariance	-0.108	1.917
	N	128	128

In difference to the examination of the correlation between a larger number of questions, for example, questions No.4 and 8 or 7 and 8, a significant correlation was not established, while between questions 2 (Age of main olive oil purchaser) and 8 (How often do you consume olive oil?), the correlation was confirmed and was .289** (Table 3-5)

Table 5. Correlations between 7 and 8.

		Q_7	Q_8
Q7	Pearson Correlation	1	-0.027
	Sig. (2-tailed)		0.763
	Sum of Squares and Cross-products	205.969	-6.000
	Covariance	1.635	-0.048
	N	127	126
Q8	Pearson Correlation	-0.027	1
	Sig. (2-tailed)	0.763	
	Sum of Squares and Cross-products	-6.000	243.430
	Covariance	-0.048	1.917
	N	126	128

CONCLUSIONS

The analysis made and assessment of the motives and barriers to olive oil consumption using the SPSS software has not been used previously in the area analysed. The paper aimed to use this analysis to consider the main motives and attitudes of consumers from the socio-economic aspect.

The results obtained, as well as the analyses of organic and other products in Montenegro, showed positive consumer attitudes towards quality (56%) and price (19.2%) (Jovanovic et al., 2004, 2012, 2016, 2017).

When it comes to the brand, the responded stated that it was moderately (24.6%), or extremely important (29.4%) Baourakis and Baltas 2003; Cicia et al. 2005; Bracco et al. 2009; Dekhili and d'Hauteville 2009; Gázquez-Abad and Sánchez-Pérez 2009; Joel Espejel et al. 2009; Jiménez-Guerrero et al. 2012). With regard to packaging, sizes of 0.75 l (29.4%) and 0.2 l (24.6%) prevail. The opinions of the respondents about whether marketing communication has a significant impact on buying olive oil were divided- 35.4% of respondents found that it did not have an impact, while 34.6% considered it to have an impact.

The most favoured olive oils are extra virgin (30.9%) and virgin (37.4%) (Pupo D'Andrea, 2007; Marchini et al. 2010; Teresa Del Giudice et al, 2015). Supermarket (38.4%) and direct purchase from the manufacturer (30.4%) are the dominant sale channels for olive oil.

In most cases, the respondents consumed olive oil at home (87.2%) or in the restaurant (12%).

When asked whether the olive oil price at the domestic market was high, 45.7% agreed with the assertion, while 3.1% completely disagreed. With regard to the supply of olive oil at the domestic market, 37.8% of respondents found it satisfactory, while 33.1% stated they were not sure. Domestic olive oil and quality is preferred by 61.8% of respondents (Lazaridis 2004; Mtimet et al. 2014, Gázquez-Abad and Sánchez-Pérez 2009, Carlucci et al. 2014, Cicia et al.2002;

Martínez et al. 2002; Scarpa and Del Giudice 2004, Cicia and Perla 2000). Spanish and Italian oils (Arbeguino and Monini) prevail in the consumption of imported oils.Also further studies are needed to gather wider and deeper evidence of the effect of different technologies on odour and to calculate the economics of the use these technologies.

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THE EFFECT OF ROTARY TILLER AND OTHER MACHINES FOR TILLAGE ON THE SOIL STRUCTURE AGGREGATES

SUMMARY

The soil tillage is still the most complicated agro-technical measure where more than 30% of the total energy is deployed for the plant production. In the field conditions, the effect of rotary tiller as well as other machines for the supplementary treatment of the soil structure aggregates was examined in this study. The following tools and machines were used for the soil tillage: harrow (HA), rotary tiller (RT) and disc harrow (DH). In particular, it is interesting to determine the effect of rotary tiller and other machines for the soil tillage on the treatment quality as well as on the excessive attrition and making the powder structure and its degradation. By the soil tillage with different tools and machines (harrow, rotary tiller, disc harrow), there were changes in the condition of the soil aggregates, i.e. the decrease of the total content mega structural aggregates for 17.72%. At the soil treatment by rotary tiller (RT), the statistically significant higher values of the structure coefficient ratio were found in regard to the tillage with the harrow as well as the disc harrow for all examined depths 1.94-2.75 and high content micro structure aggregates (diameter <0.25 mm) of 6.37%. The values of volumetric mass before the tillage were in the great range from 1.06 to 1.29 g/cm^3 depending on the depth, while the average values of the volumentric mass (VM) of the examined soil after the treatment were in the range from 0.92 to 1.17 g/cm^3. By testing the ratio of volumetric mass (X_{ZM}), the statistically significant differences were determined for the type of treatment while the measured depth has not shown the statistically significant difference. Contrary to the ratio of volumetric mass (X_{ZM}), the ratio of structure coefficient (X_k) showed a significant difference in the soil treatment by rotary tiller while the measured depth had a statistically significant differences in all three variances.

Keywords: soil tillage, rotary tiller, structure aggregates, structure coefficiant, treatment intensity, soil bulk density.

INTRODUCTION

The soil tillage is still the most complicated agro-technical measure where more than 30% of the total energy is deployed for the plant production (Marković

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et al., 1995, Moteva et al., 2017). The rotary tiller (rotary cultivator) are machines which are used for shattering the lumps as well as for the attrition of soil especially in adverse conditions (hard, dry soil), backfilling and mixing with the compost soil and plant leftovers, destroying of weed (that is reproduced generatively), husking of stubble and the basic treatment, especially in the orchards, vineyards, gardens and so on. The rotary tillers as individual machines are used less because of relatively high energy consumption in relation to the tools with passive working gears (Marković et al., 1996; Bajkin, 2006).

The main reason why the rotary tillers should be used more is a significant working effectiveness, easy handling, flat soil area after the treatment and good soil attrition (Páltik et al., 2003, Salokhe and Ramalingam, 2003). During the treatment and under the effect of a certain mechanic power, the soil is shattered, torn, decomposed into pieces, aggregates, sputtered and it produces the mass made of very different aggregates, lumps and dust by size (Vučić, 1987). The analysis of the soil structure shows a figure of the content quantity of certain structural elements fractions, their reciprocal relation especially the content of micro (diameter <0.25 mm), macro (diameter 0.25-10 mm) and mega aggregates (diameter >10 mm) (Ponjičan et al., 2011a).

The environment significance of the soil structure is large and it is taken for the key of the soil fertility. The structure is a regulator of water-air and thermal relations. A large venting and water-tightness of the soil is a prerequisite for a good anaerobic, biological and biochemical activity (Miljković, 1996).

Upon all types of the soil structure and from the agronomic aspect, the most favorable soil structure is the crumbly soil structure, i.e. the aggregate of 0.25-7 mm and in some cases even to the 10 mm, but in the drier regions the aggregates of 0.25 to 2-3 mm are the most favorable from the aspect of the soil fertility (Vučić, 1987). The soil tillage quality can be effectively assessed by measuirng the physical properties of the soil. The volumic mass (soil bulk density) is especially suitable for determining the treatment effects before and after the soil treatment (Nozdrovický, 2007; Ponjičan et al., 2009).

By decreasing the excessive and unnecessary attrition, the energy used for the soil treatment would be reduced (Matjašin et al., 1988; Mitrovic et al., 2017) and the soil resources would be kept better by lower release of CO_2 (Lal, 2000). The analysis of the soil structure aggregates was done for different machines' constructions of the soil tillage. The main task of examination is to determine the quality of the soil tillage by different types of machines for pre-sowing soil preparation. It is specifically interesting to determine the effect of rotary tiller and machines for the soil treatment on the quality of cultivation, but also on the excessive attiration and making of the powder structure as well as its degradation.

MATERIAL AND METHODS

The examination of the rotary tiller and machines effect for the supplementary soil tillage on the structure (aggregate composition) of the soil was carried out in the field conditions on the locality of Mokro, municipality of Pale,

Entity of Republic of Srpska, Bosnia and Herzegovina (latitude 43° 52′ 33″ and longitude 18° 36′ 26″), during 2019 on the soil type dystric cambisol (brown acid soil). By the texture composition, the dystric cambisol belongs to the type of sandy loam which means that the soil has favorable physical properties because of a suitable relation of water and air in its micro and macro pores. The machines' examination for the soil tillage on the structure was carried out on the previously ploughed soil.

For the soil tillage, the following machines were used:

•harrow (HA)
•rotary tiller (RT)
•disc harrow (DH)

The depth of examination of tools and machines was 10 cm for the harrow and disc harrow and 15 cm for the rotary tiller. After the tillage, taking of the soil samples was carried in different depths (upper layer 0-5 cm, middle layer 5-10 cm and lower layer 10-15 cm). For the needs of determining the structure coefficient, the soil samples were taken in the damaged soil of the average mass 2 kg, by seam thickness of 5 cm, to the depth of 20 cm in three repetitions. For the needs of the soil bulk density, the samples were taken in non-damaged (natural condition) by the Kopecky cylinders of 100 cm^3 (Dugalić and Gajić, 2005).

The physical characteristics of the soil were determined by measuring the soil bulk density and structure coefficient (Nozdrovický, 2007; Petrovic et al., 2010; Sakin et al., 2011). For the needs of examination, the methodology and laboratory equipment ware used by the accredited Laboratory for soil of the Federal Institute for Agro-pedology. The mechanical content of the soil was determined by B-international pipet method and the texture category was determined by the triangle of Fere (Soil survey manual, 1995). The volumetric soil mass (soil bulk density) was determined by the method of Kachinsky and by the Kopecky cylinder of 100 cm^3, drying on 105°C till the constant mass. (Hadžić et al., 2004).

The structure coefficient (k) was determined by the method of the field dry sieving (Radojević and Petrović, 2011) and by the sieves that is used for determining the soil structure content and whose hole size is defined by the Savinov method (Hadžić et al., 2004). The structure coefficient (k) is defined as a mass relation between macro structural aggregates (0.25-10 mm) and sum of mega (>10 mm) and micro (<0.25 mm) structure aggregates (Šein et al., 2001; Ponjičan et al., 2012):

$$k = \frac{m_{macro}}{m_{micro} + m_{mega}}$$

The intensity of the soil tillage was determined via the soil bulk density ratio (X_{ZM}) and the structure coefficient ratio (X_k), (Ponjičan et al., 2011a). Soil bulk density ratio (X_{ZM}) was assessed via the relation of the volumetric mass value after the tillage (ZM_{NO}) and the volumetric mass before the cultivation (ZM_{PO}) by the machine for the soil tillage throughout the equation:

$$X_{ZM} = \frac{ZM_{NO}}{ZM_{PO}}$$

The structure coefficient ratio (X_k) was calculated via the relation of the structure coefficient value after the tillage (k_{NO}) and the structure coefficient before the tillage (k_{PO}) throughout the equation:

$$X_k = \frac{k_{NO}}{k_{PO}}$$

The assessment of the measured data was done in the licensed Excel program 2010 and IBM SPSS Statistics 21. The testing was carried out at the point of significance of 5% by F-test analysis variance and Tukey (HSD) test.

RESULTS AND DISCUSSION

The examined type of soil (brown acid soil – distric combisol) had the following percentage of the mechanical fractions: coarse sand 6.20%, medium sand 52.80%, powder 35.30% and clay 5.70% and it belongs to the category of sandy loam. During the tillage, the soil humidity varied in the intervals from 9.31 to 16.90% at the depth of 0-10 cm, at the average of 12.32% at the depth of 0-5 cm, 12.52% at the depth of 0-10 cm, 13.18% at the depth of 10-15 cm. The examination was carried out by the optimal values of the instantaneous soil humidity where it comes to the formation of a favourable crumbly soil structure and minimal used energy for the soil tillage (Birkás, 2008a).

The reliable indicator of soil dispersion and degradation represents the presence of microstructural aggregates <0.25 mm (Vučić, 1987). The mass part of microstructural aggregates was the largest on the surface soil seam 0-10 cm which was prepared by the machines for the surface preparation with the passive work gears of harrows and disc harrows and a classical rotary tiller.

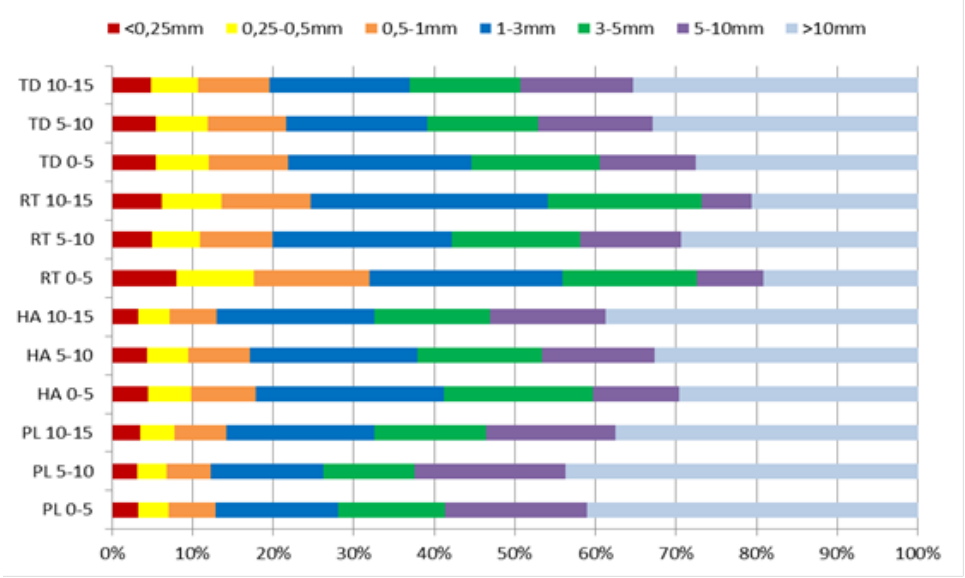

Figure 1. Percentage share of structural soil aggregates in relation to tillage type and measurement depth

The results of structure aggregates examination through the sieving by concretely determined sieves and by the Savinova method are shown in the figure 1. The examined soil was a field. Then, the main tillage was done by a plough at the depth of 20 cm and then a thorough tillage was carried out by different tools and machines. A high share of coarse soil aggregates was found by the main soil tillage (>10 mm), approximately 40.76% at the depth of 0-15 cm, while the average percentage share of macro aggregates (0.25-10 mm) was 55.95% and the share of micro aggregates (<0.25 mm) was 3.28% at the examined soil depth.

By the soil tillage with different tools and machines (harrow, rotary tiller, disc harrow), there were changes in the conditions of soil aggregates, i.e. the total content decrease of mega structural aggregates. Cultivating the soil by a harrow (HA) at the depth of 0-15 cm, the percentage content of mega structural aggregates was determined in the amount of 33.65%, at the depth of 0-15 cm. The content of macro structural aggregates after the soil cultivation by a harrow was 62.34%, while the content of micro structural aggregates had the value of 4.01%. Unlike the harrow tillage, the treatment by a disc harrow (TD) showed the following percentage values of the structural aggregates' content at the depth of 0-15 cm: the content of mega structural aggregates 31.95%, macro structural aggregates 62.80% and micro structural aggregates 5.25%.

Table 1. Structure coefficient (k) and share of micro structural aggregates (<0.25 mm) depending on the tillage type and measurment depth.

Parameter		Structure coefficient - k				< 0.25 mm (%)			
Depth (cm)	Tillage	HA	RT	TD	Average	HA	RT	TD	Average
0-5		1.96	2.69	2.04	2.23*	4.47	7.99	5.45	5.97*
5-10		1.71	1.94	1.61	1.76	4.29	4.98	5.41	4.90
10-15		1.39	2.75	1.50	1.88	3.26	6.15	4.88	4.76
Average		1.69	2.46*	1.72		4.01*	6.37*	5.25*	

*Testing was carried out by Tukey (HSD) $p<0.05$

Assessment scale of aggregate soil content per values of structure coefficient (Šein, 2001)

good structure k>1.50
satisfactory 0.67<k<1.50
unsatisfactory k<0.67

The content of mega structural aggregates at the depth of 0-15 cm was 23.04%, macro structural aggregates 70.58% and micro structural aggregates 6.37% for the soil tillage by rotary tiller (RT) and it is significantly higher from the previous two variances. A few authors state that the optimal relation of macro structural aggregates can be higher than 70%, whereby the perennial examination of the relation of structural aggregates (mega: macro: micro) was increased from 35:55:11% to 13:79:8% to the benefit of macro structural aggregates which are

the most favorable for the growth and development of cultivated cultures (Birkás, 2008b). Upon the previously mentioned statements, it can be concluded that the high quality surface soil preparation was carried out by utilizing the rotary tiller (RT) where the closest relation of structural aggregates was calculated of 23:71:6.

A detailed figure for the comparison of the soil tillage quality between the examined machines is especially determined by the structure coefficient (k) (Ponjičan, 2008). At the tillage with the rotary tiller (RT), the statistically significant higher values of structure coefficient ration were determined in regard to the tillage by a harrow and disc harrow for all examined depths (Table 1.)

According to the assesment scale of the aggregate soil content (Šein, 2001), after the tillage with the above mentioned machines and tools in all measurement depths, an enhanced and satisfactory structure of examined soil was found. Comparing the quality of treated soil by harrow (HA) and disc harrow (TD), the statistically significant derogations of structure coefficient were not found, while the tillage with the rotary tiller (RT) had a statistically significant variances of the structure coefficient from 2.46.

In terms of depth of the examined soil profile 0-15 cm and by all measurement combinations, the statistically significant difference was found in the soil cultivation by the mentioned machines and tools only at the depth of 0-5 cm. At the depth of 10-15 cm for rotary tiller (RT), the highest value of the structure coefficient k = 2.75 was derived and that represents unnecessary attiration of soil which negativly affects on the energetic balance of the soil tillage. At the same time, it unfavorably affects the water and air regime of soil and it comes to the increased mineralization of soil and unnecessary as well as excessive release of CO_2 (Lal, 2000).

The secure indicator of dispersion and degradation of soil represents the existance of micro structural aggregates (<0.25 mm) (Vučić, 1987). By the increase of the soil attrition intensity comes to the growth of the micro structural aggregates content (Birkás, 2008a).

By testing the machines for soil tillage, the statistically significant difference was found for all types of cultivation, the highest content of micro structural aggregates was determined for RT 6.37%, and the lowest for HA 4.01% (Table 1). During the soil tillage, RT on the soil surface layer 0-5 cm, the statistically significant value of 7.99% was found for micro structural aggregates, while treating the soil with HA and TA the value was 4.47% and 5.45%. Regarding the increase of the examined depth, i.e. measurement depth the share of micro structural aggregates decreases for all examined variances. By testing the machines for tillage on the soil surface seam of 0-5 cm, there is a significant difference in an average value of the micro structural aggregates content of 5.97% and that is one of the indicators of the soil dispresion and its degradation.

The volumetric mass (soil bulk density) shows the mass of absolute dry soil in the natural, undamaged state in the volume unit. Its values are used for the assessment of the soil compression level and for calculation of the total soil porosity. Before the tillage, the volumetric mass values had been in the range

from 1.06 to 1.26 g/cm^3 depending on the depth, approximatelly 1.18 g/cm^3. According to the expectations, for the soil conditions before the tillage, higher values of volumetric mass were measured that is highly expressed at the depth of 10-15 cm from 1.26 g/cm^3. The values of volumetric mass above 1.6 g/cm^3 represent the barrier for the growth of root system of cultivated plants and it also comes to the water stagnation as well as to the anaerobic processes in the soil. From the stated reasons, it is necessary to decrease the number of technological processes during the soil tillage (Resulović and Čustović, 2002).

The average values of volumetric mass (VM) or soil bulk density of the examined soil for all types of tillage (Table 2) were statistically significant, while the effect of tillage at the measurment depth showed the statistically significant difference only on the soil surface layer of 0-5 cm.

Table 2. Volumetric mass or soil bulk density (VM) depending on the trillage type and measurement depth.

Parameter		VM (g/cm3)			Average
Depth (cm)	Tillage	HA	RT	TD	
0-5		1.09	0.81	0.96	0.95*
5-10		1.17	0.99	1.09	1.08
10-15		1.25	0.97	1.09	1.10
Average		1.17*	0.92*	1.05*	

*Testing was carried out by Tukey (HSD) $p<0.05$

Similar values are stated (Ponjičan et al., 2009) who had obtained values of the volumetric mass on treated soil at the depth of 10 cm in his examinations, but the value was from 0.990-1.186 g/cm^3 depending on the pre-culture and the previous type of tillage. In the same way, Larney and Bullock (1994) state in their examinations that humidity and type of soil tillage have a significant effect on the value of volumetric mass when it comes to the decrease of the soil volumetric mass at the depth of 0-6 cm in the average of 0.26 g/cm^3.

The intensity of attrition of the soil aggregates relates the state of soil before and after the tillage and it is expressed in the soil bulk density ratio (XVM) as well as the structure coefficient ratio (Xk). The values of the volumetric mass ratio for higher intensity of attrition of soil aggregates are lower, but the values of structure coefficient ratio are higher (Figure 2). During the minimization of the energy used for the soil tillage, it is necessary to know the parameters of the attrition intensity of the soil aggregates as the soil bulk density ratio and the structure coefficient ratio (Matjašin et al., 1998; Asl and Singh, 2009; Ponjičan et al., 2011b).

By testing the soil bulk density ratio (X_{VM}), the statistically significant differences were found for the type of tillage (Figure 2a), while the measurement depth has not shown the statistically significant difference. Contrary to the soil bulk density ratio (X_{VM}), the structure coefficient ratio (X_k) showed the

significant difference in the soil tillage by rotary tiller (Figure 2b), while the measurement depth had a statistically significant difference in all three variances. The proven values of the structure coefficient ratio (X_k) have shown a higher intensity of the soil aggregates attrition during the soil tillage by the rotary tiller where there is unnecessary attrition and degradation of soil at the depth of 10-15 cm which is accordance with the results (Ponjičan et al., 2011b).

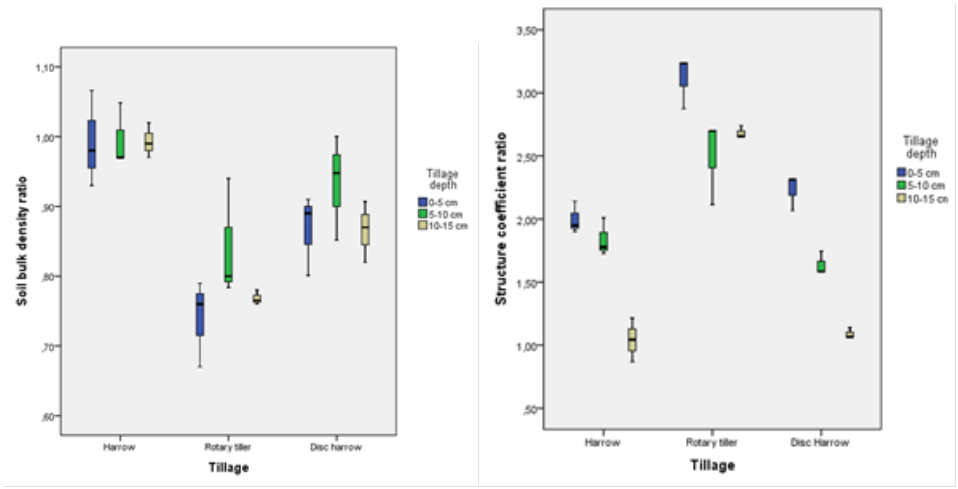

Figure 2. Tillage intensity depending on the tillage type and measurement depth: 1.soil bulk density ratio, 2.structure coefficient ratio

CONCLUSIONS

Based on the examinations carried out in the field conditions for different types of machines for pre-sowing soil preparation as well as measuring the physical properties of soil, it was found that there are clearly expressed differences in the quality of tillage and the intensity of soil attrition depending on the type of tillage and depth of examination (measurement).

The enhanced tillage quality is determined for the soil tillage by a rotary tiller, and the relation of structure aggregates of 23:71:6 with a significantly favorable form per depth. However, at the depth of 10-15 cm for rotary tiller (RT), the highest value of the structure coefficient k=2.75 was determined which represents unnecessary soil attrition and it affects the degradation of the soil. Before the tillage, the values of soil bulk density (VM) were in the range from 1.06 to 1.29 g/cm^3 depending on the depth, approximately 1.18 g/cm^3, and after the soil tillage the average values (VM) had the following values in the range of 0.92 (RT) to 1.17 (HA). Selecting an optimal system of the soil tillage, the energy efficiency of the soil tillage can be increased by the minimal soil degradation for the purpose of the environment protection. The quality of soil tillage and the attrition intensity of the soil aggregates must be arranged to the needs of growing plants.

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ANALYSIS OF POSSIBILITIES OF REDUCING THE QUANTITY OF MINERAL FERTILIZER APPLICATION USING DIFFERENT TYPES OF ORGANIC FERTILIZERS IN CARDINAL GRAPE VARIETY

SUMMARY

This paper presents the results of a three-year study (2010–2012) on the influence of different types and combinations of fertilisers on the agro-biological, economic and technological characteristics of the Cardinal variety of grapes. The study was carried out in the experimental vineyard of the Biotechnical Faculty in Podgorica to explore the possibility of reducing the use of mineral fertilisers by applying various organic fertiliser sources.

Ten variants of fertilisation were applied: control (500 kg ha^{-1}, NPK 8:16:24), cattle manure (25 t ha^{-1}), poultry manure (10 t ha^{-1}), peat (12 t ha^{-1}), combination of 75% mineral fertiliser and 25% cattle manure, combination of 75% mineral fertiliser and 25% poultry manure, combination of 75% mineral fertiliser and 25% peat, combination of 50% mineral fertiliser and 50% cattle fertiliser, combination of 50% mineral fertiliser and 50% poultry manure, and combination of 50% mineral fertiliser and 50% peat.

The results demonstrate the possibility of using organic fertilisers to partially replace mineral fertilisers. The application of 50% mineral fertiliser and 50% organic fertilisers produced the best results in terms of grape yield and the physical and chemical characteristics of bunches and berries of the Cardinal variety..

Keywords: Grapes, Cardinal, mineral fertilizer, organic fertilizer.

INTRODUCTION

Production of table grapes is an important part of the economy of Montenegro. This branch of plant production is mostly present in Podgorica vineyard region. Due to very favourable climate, in this region are ideal conditions for cultivation of table varieties of different epochs of ripening. Cardinal, which is distinguished by large and beautiful bunches and berries, but also with very pleasant aroma, is the most common among the table grapes. Due

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to the above mentioned characteristics, a significant part of produced grapes is exported.

Cardinal variety originates from California, and was developed by crossing varieties Flame Tokay and Ribier. It is widespread in almost all countries of the world, especially in Spain, Italy, Romania and Mexico (Mirošević, 2008). The area under this variety in Montenegro is about 180 ha (Savić, 2007). Particularly good results are achieved in regions where at the time of maturation there are no abundant precipitation and where during winter there is no danger of low temperatures (Cindrić, 2000).

Fertilization is one of the most important agro technical measures in the production of grapes, significantly influencing the yield and quality of grapes (Delgado et al., 2004). In conventional agricultural production, fertilization is based on mineral-rich synthetic fertilizers, since it is the most efficient and quickest compensation of the deficit of certain nutrients. However, it should be kept in mind that their long-term and uncontrolled application can cause certain environmental problems (soil, water and air pollution), but also lead to deterioration of the soil structure and reduction of soil microflora (Korać, 2011). In addition, the excess of minerals, especially nitrogen fertilizers, leads to the accumulation of harmful residual substances (nitrates and nitrites) in grape berries and leaves (Abd El-Wahab, 2011; Montasser et al., 2003). These problems can be partially mitigated by application of organic fertilizers that in the past represented the standard method for vineyard fertilization. Since organic fertilizers contribute significantly to preserving natural resources, but also to reducing ecosystem degradation, their use in modern viticulture becomes increasingly important (Francis and Daniel, 2004). In comparison with mineral fertilizers, organic fertilizers (manure, compost, green manure, worm compost and other organic sources of nutrients) are less important in the fertilization of vineyards due to chemical bound of nutrition elements with organic matter from which they are gradually released during the mineralization process (Džamić, 2000). However, the real significance of organic fertilizers is far greater, as soil structure, air, water and heat regimes are substantially improved with their usage, soil is enriched with microorganisms and pH is reduced (Abbas et al., 2012; Sial et al., 2007). Due to all above mentioned, but also possible negative effects that can be caused by the uncontrolled use of mineral fertilizers, organic fertilizers are a very important source of support for the production of safe raw materials, suitable for export (Ghobrial, 2006).

Considering that most vineyards are built on skeletal, mostly infertile soils of the southern part of Montenegro, they are growing and yielding under conditions of insufficient essential nutrition elements. The application of mineral and organic fertilizers is one of the most important agro technical activities in the production of this important culture. Mineral and organic fertilizers complement each other in maintaining fertility of the soil and can be replaced according to the nutrient content (Popović, 2007).

The possibility of using organic fertilizers in order to increase growth and development, as well as for improving of yield and nutritional status of the vines, was examined by many researchers (Zafar et al., 2012; Popović, 2007; Kassem and Marzouk, 2002 and Omar, 2005). Although this is not a new idea, there is still not enough information about the impact of organic fertilizers on the yields and quality of table varieties in Montenegro. For these reasons, experiments have been set up with the aim of examining the influence of the reduced quantities of mineral fertilizers in the fertilization of Cardinal using various sources of organic fertilizers in the conditions of the Montenegrin vineyard region.

MATERIAL AND METHODS

In the period 2010-2012 the influence of various organic fertilizers, and mineral fertilizer (NPK 8:16:24), as well as influence of these various combinations on the agro-biological and economical and technological characteristics of the Cardinal variety was analysed in the Podgorica vineyard region. The experimental vineyard of the Biotechnical Faculty was used for this research.

The experiment was carried out in the early grape variety Cardinal, grafted on rootstock Kober 5BB. The vineyard was planted in 2005, with 2.50 m between and 1.2 m within row distance. The vine form is double horizontal cordon, about 80 cm high. The research involved 300 vine plants. Short pruning was applied with two long cordons with five buds and two cordons with two buds. The experiment was performed in three repetitions with 10 vine plants each. The fertilization was carried out in the furrows at a depth of 25 cm during the winter grape period in the last week of January and was applied in the following variants: V1 (control variant) - 100% mineral fertilizer (500 kg / ha NPK 8:16:24) ; V2 - cattle manure 25 t ha-1; V3 – poultry manure, 10 t ha-1; V4 - peat, 12 t ha-1; V5 - 75% mineral and 25% cattle manure; V6 - 75% mineral and 25% poultry manure; V7 - 75% mineral fertilizer and 25% peat; V8 - 50% mineral and 50% cattle manure; V9 - 50% mineral and 50% poultry manure and V10 - 50% mineral fertilizer and 50% peat.

The following parameters were followed:

- Grape yield was obtained by weighing harvested grapes on each vine plant, and the bunch weight was determined from the ratio of yield and number of bunches. After harvest, the average weight (g) of berries was measured.

- Sugar content in grape juice is determined aerometrically (Oeschle must balance), and proportion of the total acid in the grape juice by neutralization of acids and their salts with n/10 NaOH solution using bromothymol blue indicator.

During the studied period, the highest temperatures were measured in 2012, both on an annual basis and during the growing period. The average temperature of the vegetation period in this year was 23.4°C and was slightly higher compared to 2011 (23.1°C) and significantly higher than 2010 when it was 21.7°C. The Podgorica vineyard region is characterized by a high Winkler index, which in the years of experiment was > 2300, > 2 600, > 2 600, in 2010, 2011 and

2012, respectively. The amount of rainfall in the vegetation period was 771.7 mm in 2010, 309.8 mm in 2011 and 911.6 mm in 2012 (Monstat, 2013).

Analysis of the results was performed using analysis of variance for completely random block design using LSD test for paired comparisons on two levels of significance: 0.05 and 0.01.

RESULTS AND DISCUSSION

Bunch weight is essentially a biological feature of a variety that depends on the agro ecological conditions of the production area to a large extent (Cindrić, 2000). In the three-year average (Table 1), the lowest weight of bunches had variants with individual application of organic fertilizers V2, V3 and V4 (200 g, 205 g and 209 g, respectively), and the highest weight had the variant V10 in which combination of 50% of mineral fertilizer and 50% peat was applied - 265 g. Statistical data processing has shown that all variants with a combination of mineral and organic fertilizers have a significantly higher weight of bunches than the variants with individual application of organic fertilizers or variant with mineral fertilizers. The positive impact of combined use of organic and mineral fertilizers on the bunch weight is also reported by Abd El Wahab 2011.

Number of bunches - The lowest number of bunches per vine plant had variants with individual application of organic fertilizers in the three-year average - V2 and V3 (17.4, 17.6), while highest bunch number was found in variants V10 and V7 (18.7). All the studied varieties of fertilization had a significantly higher number of bunches than variants V2 and V3. A significantly higher number of bunches was determined in variants V10 and V7 in comparison with variant V1.

Grape yield as an absolute indicator of fertility, and depends on a large number of factors, among which the most important are the biological specificity of the variety, the plant nutrition and ecological conditions that prevail in production area (Popović, 2012). The highest yield of grapes was measured in variants V10, V7 and V9 (5.0 kg/vine, 4.8 kg/ vine and 4.7 kg/vine), while the lowest yield was found for variant V2 - 3.5 kg/vine. Fertilizer variant with mineral fertilizer (V1), yielded 4.2 kg/vine. In the three-year average on all variants with combined application of fertilization, a significantly higher yield was obtained in variants where organic fertilizers were applied individually. The variance analysis showed that the variants V10 and V7 had significantly higher yields of grapes compared with the variant on which only mineral fertilizer was used (V1).

The yield of grapes in the examined years was within the limits indicated by Cindrić, 2000; Božinović, 2010; Dardeniz, 2014 et al.

Average berry weight – All studied fertilizers significantly influenced the average berry weight of the Cardinal varieties. In the three-year average, the lowest weight of berry had variants with the use of 100% organic fertilizers (V2 -

5.7 g, V3 - 5.8 g and V4 - 5.9 g). A significant increase in the weight of the berries was found in vines fertilized with 50% mineral and 50% organic fertilizers (V10 - 6.7 g, V9 - 6.4 g). The positive effect of combined application of organic and mineral fertilizers on this vine property is due to the higher content of organic matter in the soil, as well as to improved structure and physical properties of the soil (Abd El Wahab, 2011 and Gamal, 1992).

Tab. 1. Influence of organic and mineral fertilizers on yield and physical characteristics of bunches and berries.

Variant	Number of bunches				Bunch weight (g)				Yield (kg/vine)				Berry weight (g)			
	2010	2011	2012	2010-12	2010	2011	2012	2010-12	2010	2011	2012	2010-12	2010	2011	2012	2010-12
V1	18.1	18.1	18.3	**18.2**	233	230	235	**233**	4.2	4.2	4.3	**4.2**	6.0	6.1	6.2	**6.1**
V2	17.0	17.4	17.8	**17.4**	184	196	220	**200**	3.1	3.4	3.9	**3.5**	5.5	5.6	6.0	**5.7**
V3	17.3	17.6	18.0	**17.6**	193	200	222	**205**	3.3	3.5	4.0	**3.6**	5.6	5.8	5.9	**5.8**
V4	17.5	17.9	18.2	**17.9**	197	204	225	**209**	3.4	3.7	4.1	**3.7**	5.7	5.9	6.1	**5.9**
V5	18.1	18.0	18.5	**18.2**	238	248	260	**249**	4.3	4.5	4.8	**4.5**	6.4	6.0	6.2	**6.2**
V6	18.4	18.5	19.0	**18.6**	240	253	255	**249**	4.4	4.5	4.9	**4.6**	6.2	6.2	6.4	**6.3**
V7	18.7	18.6	18.9	**18.7**	251	257	262	**257**	4.7	4.7	5.0	**4.8**	6.4	6.3	6.6	**6.4**
V8	17.0	18.3	19.3	**18.2**	228	255	269	**251**	3.9	4.7	5.2	**4.6**	5.7	6.5	6.8	**6.3**
V9	17.3	17.9	19.7	**18.3**	232	260	267	**253**	4.0	4.8	5.3	**4.7**	5.8	6.5	7.0	**6.4**
V10	16.6	19.3	20.1	**18.7**	241	275	280	**265**	4.0	5.3	5.6	**5.0**	6.0	6.9	7.1	**6.7**
ẋ	17.6	18.2	18.8	**18.2**	224	238	250	**237**	3.9	4.3	4.7	**4.3**	5.9	6.2	6.4	**6.2**

Parameter	Number of bunches		Bunch weight		Grape yield		Berry weight	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2010-2012	0.470	0.633	11.165	15.047	0.409	0.671	0.409	0.551

Sugar content in must - Optimal mineral nutrition is essential for achieving higher carbohydrate content in grape. Potassium and phosphorus have highest importance of all macronutrients, and nitrogen and in specific optimal limits (Popović, 2007). Table 2 presents the results demonstrating that the applied fertilizers in all the studied years significantly influenced the chemical composition of the berries, that is, the content of sugar and acids in the must. In the three-year average, the variant with cattle manure had the lowest content of sugar - 14.12%, while the highest content was measured in the V10 variant - 14.94% and V9 - 14.80%. All the examined fertilizer combinations had significantly higher sugar content compared to variant V2, and variants V10 and V9 had higher amount of sugar compared to the variant fertilized with poultry

manure (V3) and peat variant (V4). The results of the study are in line with the results by Omar (2005), demonstrating in seedless variety Thomson seedless a significant increase in sugar content in must in combined use of mineral and organic fertilizer.

Table 2. Influence of organic and mineral fertilizers on chemical characteristics of berries

Variant	Sugar content (%)				Acid content (g/l)			
	2010	2011	2012	2010-12	2010	2011	2012	2010-12
V1	14.35	14.55	14.83	**14.58**	4.18	4.00	3.88	**4.02**
V2	13.87	14.12	14.37	**14.12**	4.65	4.38	4.22	**4.42**
V3	13.97	14.31	14.60	**14.31**	4.55	4.39	4.10	**4.34**
V4	13.96	14.54	14.70	**14.44**	4.40	4.23	4.00	**4.21**
V5	14.41	14.62	14.82	**14.62**	4.25	3.97	3.90	**4.04**
V6	14.53	14.73	14.62	**14.57**	4.32	4.02	3.85	**4.06**
V7	14.64	14.87	14.90	**14.80**	4.13	3.92	3.75	**3.93**
V8	14.00	14.77	15.17	**14.60**	4.30	4.10	4.02	**4.14**
V9	14.13	14.80	15.27	**14.80**	4.38	4.18	4.13	**4.23**
V10	14.23	15.13	15.37	**14.94**	4.22	3.99	3.97	**4.06**
Average	14.20	14.57	14.86	**14.57**	4.33	4.11	3.98	**4.14**

Parameter	Sugar content		Acid content	
	LSD 0.05	LSD 0.01	LSD 0.05	LSD 0.01
2010-2012	0.438	-	0.289	-

Acid content in must is an important indicator of the quality influencing the taste and harmony of the grapes (Popović, 2012). Due to the specific pedological and climate conditions, the grapes produced in the southern parts of Montenegro are characterized by high sugar content and low acid content. In a three-year average, a statistically significant increase in acidity was found in variants with the individual application of organic fertilizers (V2 - 4.42 g/l, V3 -

4.34 g/l, V4 - 4.21 g/l). The smallest content was measured in the V7 variant - 3.93 g/l. El-Shenawy and Fayed (2005) had similar results in the variety Crimson seedless and Abd-El Wahab (2011) in the Red Globe variety.

CONCLUSIONS

Based on a three-year study of the fertilization of Cardinal variety with different types and combinations of organic and mineral fertilizers in the agro ecological conditions of Podgorica vineyard, it can be concluded:

-The highest yields of grapes, bunch and berry weight had fertilization variant in which mineral and organic fertilizers were combined in a ratio of 50% + 50%.
-All variants of combined application of organic and mineral fertilizers have had a positive effect on the content of sugar and acids in grape must.
-Given the very small number of available information related to the fertilization of grape vines with organic fertilizers, these studies should continue in the following period.

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