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CONTENT

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THE EFFECT OF DIRECT USE OF LAKESIDE BIOMASS AS SOIL AMENDMENT ON THE PRODUCTIVITY OF DRY BEAN CROP

SUMMARY

The riparian areas of the lake Mikri Prespa in the northwest of Greece are surrounded by extensive reedbeds (mainly *Phragmites australis* and *Typha angustifolia* reed) that spread around the perimeter of the lake. The removal of this vegetation is often problematic and imposes difficulties in management and costs. The goal of the present study was to evaluate the effect of the direct use of reed biomass on soil fertility and the performance of common bean (*Phaseolus vulgaris* L.) type "Plake", the main crop in the Prespa region. In a two year field experimentation we tested two fertilization methods, manure and chemical fertilization, combined with three reed biomass doses (0 as a control, 10 tn ha^{-1} and 20 tn ha^{-1}) resulting in five different reed addition treatments (0-0, 10-10, 10-0, 20-20 and 20-0 for the two consecutive years and each fertilization method respectively). Total chlorophyll content measured from start of flowering until physiological maturity was not affected by the incorporation of reed biomass in both fertilization methods. The chemical fertilization significantly affected bean yield exceeding the application of manure in all treatments of reed plant material addition. Reed plant addition treatments affected bean yield with all treatments showing higher values compared to the control (0-0) while the addition of 20 tn ha^{-1} for one year (20-0) significantly exceeded the control regardless of the fertilization method. Further research is needed to explore the best agricultural practice on the effect of reed addition on arable land improvement and the bean crop.

Keywords: Common reed biomass, Soil amendment, Chlorophyll concentration, Bean yield, Prespa area.

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INTRODUCTION

The riparian areas of Lake Mikri Prespa are surrounded by extensive reedbeds that spread almost all around the perimeter of the lake. Different methods have been applied worldwide to manage the overgrowth of mainly *Phragmites australis* and *Typha angustifolia* reed either individually or in combination, including removal of aboveground biomass by cutting, grazing, or burning (Marks *et al.,* 1994; Hazelton *et al.,* 2014; Volesky *et al.,* 2016). The management of common reed cuttings can be accompanied by the exploitation of significant quantities of biomass for energy production and in agriculture. The reed is widely used as animal feed although it has lower nutritional value than other forage crops, it is cheap and easily available (Häkkinen, 2007; Thevs *et al.*, 2007; White, 2009). The harvested biomass of *P. australis* has been exploited for the production of biogas and the sludge which is a by-product of the process is used as organic fertilizer (Hansson and Fredriksson, 2004; Wysocka-Czubaszek *et al.,* 2018).

The production of compost from the removed reed can significantly improve soil composition by increasing organic matter and different plant nutrients but it does not produce any other useful energy and has increased production costs (Mamolos *et al.,* 2011; Hansson and Fredriksson, 2004). Common reed has recently been used to produce biochar (Croon, 2014; Yang and Chen, 2017) a solid, carbon-rich product after pyrolysis of biomass which has been used with positive results for the restoration of cultivated soils in Australia and Canada (Barbiero *et al.,* 2017). *P. australis* has a high N and P uptake capacity (Jiang *et al.,* 2007) with the highest content of nutrients towards the end of the growing period transporting most of them to the rhizomes (Gessner, 2001). The addition of reed after summer cutting directly in the field can improve the structure of the soil, increase the percentage of organic matter and in general the fertility of the soil. However, the addition of nutrients to the crop can be significantly low due to the high C:N ratio (Hansson and Fredriksson, 2004). After decomposition of straw with high C: N ratio, immobilization of N was observed, affecting the growth of corn (Cheshire *et al.,* 1999), while when mainly reed was used as a direct soil conditioner, the growth and yield of corn were not affected due to non-availability of N in the plants (Huijser *et al.,* 2004). The presence of seeds and stolons in the reed pieces may increase the presence of weeds in the field (Prew *et al.,* 1995) but this was not the case when mainly *P. australis* was used as green compost in corn cultivation for two years (Huijser *et al.,* 2004). The transport of cut biomass is the most important obstacle to its utilization (Carson *et al.,* 2018) as low density increases transport costs, however, wetlands often border agricultural land, thus providing an opportunity for cut biomass to be used as a soil conditioner, potentially recycling a significant amount of nutrients.

The aim of the present study was to evaluate the effect of the direct use of reed biomass on the productivity of common bean (*Phaseolus vulgaris* L.) type "Plake", the main crop in the Prespa region regarding the agronomic and

physiological behavior of the plants in relation to the soil improvement treatments applied.

MATERIAL AND METHODS

Field experimentation

An experiment was installed for two years (2019 and 2020) in three selected locations in farmer's fields in the area of Prespa in Greece, using a split plot experimental design with fertilizer treatments as the main factor and reed biomass applications as the second factor. We used two fertilization methods, manure at a dose of 50 t ha^{-1} and chemical fertilization based on soil analysis for each experimental field, combined with three reed biomass doses (0 as a control, 10 tn ha^{-1} and 20 tn ha^{-1}) resulting in five different reed addition treatments (0-0, 10-10, 10-0, 20-20 and 20-0 for the two consecutive years and each fertilization method respectively) with three replications. The reed was harvested from the riparian areas of the lake Mikri Prespa during autumn with a special harvester, chopped into 2 mm pieces and spread to the experimental fields with a spreader used to spread manure following the experimental design. Sowing of the experimental fields took place after April 20th until the beginning of May in both years with a local population of climbing indeterminate bean type "Plake". Soil analysis to evaluate the effect of reed addition treatments on soil characteristics was performed in the start of the experimentation and at the end of each cultivation year in all three experimental fields.

Chlorophyll measurements and harvest

Total chlorophyll content was measured with a hand-held dual-wavelength meter (SPAD 502, Chlorophyll meter, Minolta Ltd., Japan) at three developmental stages from start of flowering until physiological maturity (SPAD1 to SPAD3) in twenty-four plants per plot. The measurements were made on developed leaves and the central leaflet, between the central nerve and the leaf blade and were four in total (two on either side), two near the base of the leaf and two in the middle of the leaf in each plant. Harvest was performed with a special harvester after physiological maturity and yield ha^{-1} was estimated. For each sample the 100 seed weight was measured using a seed counter (Contador Pfeuffer, Germany).

Statistical analysis

The statistical analysis of the results was performed with Analysis of Variance (ANOVA) with three factors (location, fertilization and reed addition treatments) while comparison of means was conducted by Least Significant Difference (LSD) at significance level $p < 0.05$.

RESULTS AND DISCUSSION

The analysis of variance of agronomic and physiological parameters measured in all three experimental sites in the final growing season is shown in Table 1. A significant effect of location on yield, 100-seed weight and

chlorophyll SPAD levels in the three developmental stages was observed. These differences can be attributed to the initial soil properties of the experimental fields, with the most fertile and with the best soil characteristics experimental field showing the highest yield characteristics and the highest chlorophyll concentration (data not shown).

Table 1. Analysis of variance of different parameters measured in three locations of the lake Mikri Prespa affected by Location (L), Fertilization (F) and the five reed addition treatments (R) (0-0, 10-0, 10-10, 20-20 and 20-0 tn ha^{-1} for the cultivation periods 2019 and 2020 respectively).

Parameter	Location (L)	Fertilization (F)	Reed addition (R)	Lx F	L x R	F x R	L x F x R
df	**2**	**1**	**4**	**2**	**8**	**4**	**8**
Yield	***	**	**	***	***	**	**
100-seed weight	***	NS	*	***	***	NS	NS
SPAD 1	***	NS	*	NS	***	NS	NS
SPAD 2	**	NS	*	NS	NS	NS	NS
SPAD 3	***	NS	NS	NS	NS	NS	NS

df=degrees of freedom, *, **, *** denotes a difference at the levels of $P<0.05$, $P<0.01$ and $P<0.001$ accordingly and NS=no significant

The fertilization factor significantly affected bean yield with the application of chemical fertilization significantly exceeding the application of manure in all reed addition treatments and all three experimental locations. The 100-seed weight and the level of total chlorophyll in all three growing stages measured were not affected by the different fertilization methods but only by the location.

The reed addition affected bean yield with all the treatments showing higher values than the control (0-0 reed material addition for both consecutive years) regardless of the fertilization treatment, with the treatment of 20 tn ha^{-1} for one year (20-0) to have a statistically significant difference in relation to the control (0-0) but not with the other reed addition treatments (Table 2). This could be attributed to the better utilization of water due to the possible improvement of soil water capacity by the addition of plant reed material which was supported from the final soil analysis showing that with higher doses an increase in organic matter mainly in sandy soils could occur. Similarly, wheat straw soil

incorporation increased crop yields (Yang *et al.*, 2016), organic matter (Zhu *et al.*, 2014) and soil nutrients (Zhang *et al.*, 2018). Also improved the physical properties of the soil such as hydraulic conductivity and water holding capacity (Mandal *et al.*, 2004; Singh *et al.*, 2007).

Table 2. Effect of fertilization treatment (manure or chemical fertilization) and reed addition treatments (0-0, 10-0, 10-10, 20-20 και 20-0 tn ha^{-1} for the cultivation periods 2019 and 2020 respectively) in agronomic and physiological characteristics of bean crop at three different experimental locations.

Fertil.	Reed addition (2019-2020)	Yield (tn ha^{-1})*	100-seed weight (g)	SPAD 1	SPAD 2	SPAD 3
Manure						
	0-0	4,06b	68,7a	39,8a	40,7a	31,6a
	10-0	4,41ab	68,4a	39,1a	39,8a	31,5a
	10-10	4,36ab	68,7a	38,8a	39,7a	31,5a
	20-20	4,27ab	68,9a	39,0a	41,0a	31,8a
	20-0	4,57a	70,3a	39,8a	41,5a	32,4a

*means in the same column followed by the same letter are not significantly different (t-test, P<0,05)

Chemical fertilization						
	0-0	4,20 b	67,1a	39,6a	40,0a	32,1a
	10-0	4,27ab	68,8a	39,6a	41,1a	32,4a
	10-10	4,60ab	68,1a	38,3a	40,1a	32,8a
	20-20	4,45ab	68,9a	39,9a	40,9a	31,8a
	20-0	4,70a	69,2a	39,5a	41,4a	34,2a

*means in the same column followed by the same letter are not significantly different (t-test, P<0,05)

In addition, the growth of microorganisms was positively affected with the addition of plant biomass (Liu *et al.*, 2010; Zhang *et al.*, 2016). The 100-seed

weight did not differ statistically between the reed addition treatments but in the 20-0 treatment there was a slight increase compared to the control. The addition of reed plant material didn't seem to affect the availability of the main nutrients' nitrogen, phosphorus and potassium in both fertilization methods (data not shown). This could explain that the levels of total chlorophyll in the three developmental stages for all the reed addition treatments did not differ from the control in both manure and chemical fertilization treatments (Table 2). After decomposition of straw with very high C: N ratio, immobilization of N was observed, affecting the growth of corn (Cheshire *et al.*, 1999) but when mainly reed plant material was used as a direct soil conditioner, the growth and yield of corn were not affected compared to the control (Huijser *et al.*, 2004).

CONCLUSIONS

The repeated large addition dose of reed in bean cultivated soils of the lake Mikri Prespa slightly increased soil organic matter especially in sandy soils having a possible positive effect on soil water capacity and thus affecting bean yield. This observation could lead to the adoption of practices for continuous and systematic addition of reed. Adding higher doses than those tested in the present study may have had a more significant effect, however this carries the risk of incomplete degradation of the reed and the development of nutritional problems or other dysfunctions in the rhizosphere. Further research would be useful to explore best agricultural practice on the effect of reed addition on arable land improvement in the Prespa area.

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INFLUENCE OF CITRATE NANOPARTICLES ON PHOTOCHEMICAL ACTIVITY, RESISTANCE TO PATHOGENS AND PRODUCTIVITY OF WHEAT

SUMMARY

In field experiments, increase the quantum efficiency of PSII (Fv/Fp), the Rfd-values (the chlorophyll fluorescence vitality indices) and the content of photosynthetic pigments in wheat leaves at pretreatment of wheat seeds with solutions of citrate nanoparticles has been shown. The green weight of plants at bacterial and phytoplasmas infecting at pretreatment with solutions of citrate nanoparticles, was increase, than without pre-treatment, in order: GeNPs>I-Se>SeNPs>VNPs as it has been shown. The Fv/Fp-values in leaves infected with phytoplasmas of plants at pretreatment of VNPs or I-Se solutions were more, than without pretreatment. The Fv/Fp-value in leafs with bacterial infecting at pretreatment of GeNPs with compared to infected plants without pretreatment was increased has been shown. The Rfd-value in leaves at pre-sowing treatment of GeNPs or I-Se and bacterial infecting compared to infected plants without the pr-treatment was more has been shown. This value at infected plants with phytoplasmas at pretreatment of GeNPs or VNPs, compared to infected plants without pretreatment, was increased. In greenhouse experiments, Fv/Fp-values of plants leaves infected of phytoplasmas, bacteria and wheat streak mosaic virus (WSMV) at pre-treatment of solutions of citrate nanoparticles compared to infected plants without pretreatment has been increased. The pretreatment with nanoparticles GeNPs caused grain production by 15.6% increased has been shown. The weight grains/10 in plants on variant with pretreatment with Ge

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nanoparticles (with consortium of soil microorganism) and bacterial infecting were more, than without pretreatment has been shown. The weight grains/10 in plants on variant at pre-treatment of plants of nanoparticles solution and infected with phytoplasmas compared to infected plants without the pre-treatment increased in order: GeNPs>I-Se>VNPs>SeNPs.

Keywords: Wheat, bacteriosis, phytoplasmas, WSMV, nanoparticles.

INTRODUCTION

Nanoparticles, their compounding and nanomaterial's, that created with help nanotechnologies have been actively studied in recent decades with the aim of their application in various fields of science, including crop production (Sanzari, 2019; Mittal D et al, 2020). Due to the instability of metal nanoparticles in solutions, they are stabilized, particularly, with citrates, which provides both an increase in the stability of their solutions and their high reactivity. Specialty of the reactivity of citrates of nanoparticles is that molecules with thiols or amines easily displace citrate from the surface and firmly bind to metal surfaces. Negatively charged metal nanoparticles can easily penetrate into plant cells, affecting metabolism (Ranoszek-Soliwoda et al., 2017). Shinohara S. et al (2018) fined, that nanoparticles stabilized by citric acid remain stable at high ionic strengths and therefore exist in solution as individual particles.

Various nanoparticles can be used as fertilizers, growth stimulants, antimicrobial and anti-stress agents. The protection effect of nanoparticles for plant diseases is due to both direct antimicrobial action and the activation of plant defense mechanisms (Elmer et al., 2018; Mittal D et al, 2020).

The effect of pre-seed treatment with nanoparticles and their compounds has been demonstrate by many researchers (Siddiqui, S.A., et al, 2021; Acharya P et al, 2020; Honchar L. et al, 2021; Prażak R. et al, 2020; Davydova, N.V. et al, 2020; Pereira E.S. et al, 2021; Khalaki A.M. et al, 2021).

The results investigation Siddiqui, S.A. et al (2021) showed demonstrated, that selenium in the form of nanoparticles has an order of magnitude that is less toxic than in the form of selenous acid. In studies of Acharya P et al (2020) demonstrated that seed priming with AgNPs can enhance seed germination, growth, and yield while maintaining fruit quality of watermelons. It was shown, that pre-sowing treatment of chufa tubers with nano-particles solutions of manganese, zinc, copper and iron with a concentration of 60 ppm and re-application of these solutions after seedling emergence significantly increased the weight of the plant (excluding the weight of seeds), and the most effective were treatments with copper and iron (Honchar L. et al, 2021). It was shown, that application of AgNPs influenced not only seed germination in the laboratory but also the dynamics of emergence of bean seedlings and their growth and development in field conditions were shown. The biological significance of the effects induced by AgNPs varied depending on the concentration of nanoparticles and on growth conditions (Prażak R. et al, 2020).

It was found, that pre-sowing seed of spring wheat treatment by a composition of FeNPs at a concentration of 10–5% and Zn NPs at a concentration of 10–4% contributed to a 27% increase in the seed germination energy index and root weight compared to the control (Davydova, N.V. et al, 2020).

Seed nano-priming can change seed metabolism and signaling pathways, affecting not only germination and seedling establishment but also the entire plant lifecycle, that the promotion of stress and diseases resistance out coming in the reduction of pesticides and fertilizers. Thus, use of nano-based technology for seed treatment has big potential move to a more sustainable agriculture (Pereira E.S. et al, 2021). It was shown, that nano-priming increased seed germination, seedling growth and development, vigor, rate of seedling emergence and subsequent performance in most of the medicinal and forage plants. While, also negative effects of nano-priming on seed germination, seedling and plant growth traits were observed (Khalaki A.M. et al, 2021).

The effect of seed treatment with nanoparticles of Au, Ag, Cu, Zi, Fe, Mn, Mg, Ce, Ti, Si and their oxides on plants in more was studied (Ruttkay-Nedecky et al.,2017; Mittal D et al, 2020). At the same time, the effect of selenium and esp germanium, vanadium and nanoparticles on the physiological and biochemical properties of plants has been little studied. Although these elements are not essential for plants, they have different beneficial properties.

For example, Ge NPs have the advantages of low toxicity and excellent biocompatibility. it was found that, was discovered that Ge NPs have the intrinsic peroxidase-like catalytic activity. Compared with natural enzyme (horseradish peroxidase, HRP), Ge NPs maintain higher catalytic stability after treatment at different pH, temperature and ionic strength (Hu J. et al, 2019).

In plants, low levels of V have beneficial effects on plant growth and development. Nevertheless, excessive V provokes numerous deleterious effects (Chen L. et al, 2021).

Physiological function of selenium in plants is not fully-known and the physiological reactions of various plants to selenium vary very much. At the same time, fortification of crops with selenium can be one of the ways enabling to increase the content of selenium in human and animal food chain (Ježek P., 2012). Iodine, silicon, and selenium are considered elements not essential for the metabolism of plants, but these elements (as traces) are vital for humans. It has been found that by using I, Se, and Si in crop plants, applied in seeds, plants, or fruits, favorable responses are obtained such as increased growth and tolerance to stress (due to with a higher concentration of antioxidants). In this connection, the use of these elements is a useful technique for the nutritional improvement of crop plants, both in antioxidant level and biofortification (Medrano-Macías J., 2018). Iodine (I) and selenium (Se) are beneficial elements, as both play important roles in humans, that is why often using plant biofortification by agrotechnical or biotechnological approaches in order to improve, among other things, the accumulation this nutrients in food and fodder (Smoleń S. et al, 2016).

Therefore, the aim of our work was investigation of influence of citrate-stabilized of nanoparticles of germanium, vanadium and selenium on photochemical activity, chlorophyll content, resistance to pathogens and productivity of plants wheat.

MATERIAL AND METHODS

For experiments in field conditions and in the greenhouse were used: 0.75% solutions of citrate nanoparticles Ge – GeNPs (Ge content: 500 mg/l) and citric acid; 0.75% V NPs (V content 300 mg/l) and citric acid; 1% Se NPs (Se content: 100 mg/l) and citric acid; («Nanomaterials and nanotechnology» Ltd, Ukraine); 0.5% BP I-Se (a preparation of biologically active iodine in a complex with solution Se) (preparation "Jodis-concentrate plus Se") consists of purified water enriched with polyatomic ions I, 80 mg/dm³, Se citrate, intended as a biologically active food additive (SPC «Iodis», Ukraine).

In the field's conditions, wheat plants (*Triticum aestivum* L.) variety`s Pecheryanka were grown on experimental plot of the D.K. Zabolotny Institute of Microbiology and Virology of NASU in 2019–2020. The area of the experimental plot is 50 m^2, the soil is sod-podzolic. Repetition in the experiment four times.

In the soil before sowing was added consortium of soil-forming microorganisms (the biological product (BP) "Extrakon" (Ukraine)). BP Extrakon consists of a consortium of soil cellulosolytic and heterotrophic microorganisms inoculated into a peat-like substrate, which are in a functionally active state and are closely connected by trophic bonds: (*Sporocytophaga mixococcoides*, *Sorangium cellulosum*, *Cellvibrio mixtus*, *Trichoderma viridae*, *Pseudomonas fluorescens*, *P. putida*, *Bacillus subtilis*, *B. sphaericus*, *B. megaterium*, *B. pumilus*).

The general scheme field`s and greenhouse`s experiments with using pre-sowing treatment of citrate nanoparticles: 1 – control (intact plants); 2 – 1 mg/lSeNPs+ addition in soil of BP Extrakon; 3 – 0,8 mg/l I-Se solution+ addition in soil of BP Extrakon; 4 – 2,25 mg/l VNPs solution addition in soil of BP Extrakon; 5 – 3,75 mg/l GeNPs solution addition in soil of BP Extrakon.

Bacterial culture *Pseudomonas 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., 2017).

In the greenhouse`s and field`s experiments, artificial inoculation of Pecheryanka`s variety spring wheat plants in the boot stage was carried out with bacterial suspension of the strain of *P. syringae* pv. *atrofaciens* D13 (the causal agent of basal glume rot of cereals). Density of the suspension was 1×10^9 CFU/ml. Artificial inoculation of plants was done by injecting a bacterial suspension into a stem in 10-fold repeatability on each of the variant.

In the greenhouse`s and field`s experiments phytopathogenic phytoplasma

of *Acoleplasma laidlawii* var. *granulum* 118 UKM BM-34 was cultured on a liquid nutrient medium CM IMB-72 (pH = 7.8) in a thermostat at 32 °C for 72 hours. Artificial inoculation with phytoplasma of *A. laidlawii* var. *granulum* 118 UKM VM-34 in the boot stage was performed with the method of subepidermal injection in the steam.

In the greenhouse, inoculation of wheat plants with freshly prepared virus-containing material was carried out in the phase of the two true leaves, by the method of mechanical inoculation of leaves with preliminary dusting with the carborundum. Isolation of viral material was performed by homogenization of freshly cut leaves of diseased plants with clear symptoms of the wheat streak mosaic virus (WSMV) with the addition of 0.1 M phosphate buffer pH 7.0. The plant homogenate was filtered through a nylon sieve and used for mechanically infect of plants. Infection of plants was carried out of a glass spatula or fingers in disposable gloves soaked in inoculum. Excess inoculum was washed off with water (Diagnose von Krankheiten und Beschädigungen an Kulturpflanzen. Diagnosemethoden, 1984).

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

The device is equipped with a liquid crystal display (128 × 64 pixels) and a remote optoelectronic sensor with a wavelength of 470 ± 15 nm, the area of the irradiation spot not less than 15 mm² and illumination within it not less than 2.4 W/m². The spectral range of fluorescence intensity measurements is in the range from 670 to 800 nm. The data measured by the device was transferred to a PC via the USB port of the computer using the software "Floratest", which comes with the device and allows you to display this data in tabular or graphical form. Measurement of fluorescence induction was carried out on flag leaves of wheat in the heading-flowering phase. Dark adaptation of leaves before measurements was in range: 6-20 minutes. Replications measurements on each variant – threefold. The evaluation of the influence of experimental factors was performed by changes in the value of the quantum efficiency parameter PSII: Fv/Fp(m) (Fv = Fm(p) – F_0), where F_0 – the minimum fluorescence; F m (p) – maximum (peak) fluorescence, Fv – variable fluorescence; Fs – is the steady-state Chl fluorescence; Rfd-values – the chlorophyll fluorescence decrease ratio (chlorophyll fluorescence vitality indices): Rfd = (Fm(p)-Fs)/Fs (Sharma et al., 2015; Lichtenthaler et al., 2007; Huliaieva, et al. 2018).

The pigments contents in flag leaf of wheat in field`s experiments were measured with the extraction method in DMSO with followed by spectrometry (Hisox, Israelstam., 1979).

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

RESULTS AND DISCUSSION

It is known that the method of induction of chlorophyll fluorescence is quite sensitive and allow quickly and non-invasively assess photochemical changes under the influence of various factors (Maxwell, Johnson, 2000). It is known that the action of stressors leads to changes of Fv/Fm(p) (quantum efficiency of PSII), which reflects the damage and inactivation of PSII (often called photoinhibition). Changes in this indicator are often used to assess the impact of stress factors (Murchie, Lawson, 2013; Sharma, et al., 2015). The results investigation of Peña-Olmos et al. (2013) showed that toxicity caused by an excess of iron induced an increased level of stress in the broccoli plants, causing decreased of the value of photosystem II's (PSII) maximum quantum yield (Fv/Fm). The maximum quantum efficiency of photosystem II was inhibited in citrus plants under saline stress (Sousa J.R.M., et al, 2016). In investigations Jia M. et al (2019) were shown, that the Fv/Fm of wheat leaves was tended to increase with increasing N application. In our field`s experiments, at the portable field chlorophyll fluorometer using, was shown increase quantum efficiency of the PSII (Fv/Fm) at pre-treatment of citrates of nanoparticles solution in order: Ge (17.5%)>I-Se (8.8%)>Se (7.0%) have been shown (Fig.1 a). This is indicates rise amount of photochemically reactive centers and an improvement in the absorption of light quanta by PS II of the photosynthetic apparatus of wheat plants.

The Rfd-value for effect of application investigating of nanoparticles give important information, as is indicator of photosynthetic activity of leaves (Lichtenthalera, Babania, 2000). The Rfd-value also has been called vitality index of green leaves (Haitz, Lichtenthaler, 1988). Many researchers often use the Rfd parameter to assess the effect of stress factors on the photosynthetic apparatus. In investigations of Perera-Castro et al (2018) the Rfd-value has been demonstrated as a more sensitive parameter for thermic damage assessment. According to Rfd-values, the photosynthetic apparatus, independent of symbiotic status, becomes increasingly damaged under progression of drought, which was accompanied with a sharp drop in stomatal gas exchange parameters (Polcyn W. et al, 2019). In our results was shown, that the Rfd-values increased at pre-treatment of citrates of nanoparticles solution of GeNPs by 11.4%. This is value have been equal to control at pre-treatment of wheat of I-Se and reduced at pre-treatment of VNPs (23.9%) (Fig 1 b).

In results investigations Wang Z. B. et al (2018) was suggested that low concentrations of GeO_2 could alleviate photoinhibition and 5.0 mg (GeO_2) L^{-1} was the most effective, due to its function of scavenging free radicals and lowering accumulation of reactive oxygen species, which was proven with higher antioxidant enzyme activities. The application of 5 μM V increased plant growth, induced floral bud development, and accelerated flowering of pepper plants was shown (García-Jiménez A., 2018). A chlorophyll assay which was conducted of the chlorophyll *a+b* in leaves increased on variants pre-treatment in order: I-Se>GeNPs>VNPs>SeNPs has been shown (Table 1).

Table 1. Photosynthetic pigments content in flag leaves of wheat plants at the pre-sowing treatment of solutions of citrates nanoparticles and application of the BP Extrakon

Variants	Chl. *a*+*b*	Car.
Control (without treatment)	1,06±0,05	0,17±0,009
Pre-treatment of 1mg/l SeNPs+Extr.	1,68±0,08	0,52±0,015
Pre-treatment of 0,8 mg/l I-Se+Extr.	2,49±0,16	0,30±0,010
Pre-treatment of 2,25 mg/l VNPs+Extr.	2,30±0,14	0,58±0,03
Pre-treatment of 3,75 mg/GeNPs+Extr.	2,42±0,12	0,45±0,02

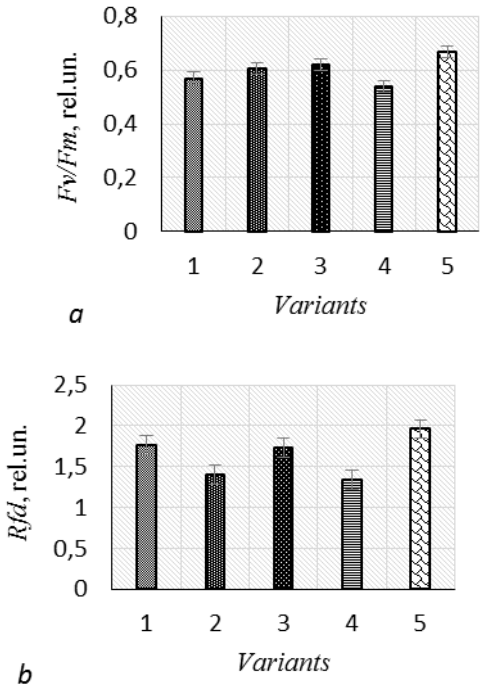

Figure 1. The quantum efficiency of PSII (Fv/Fm) (a) and Rfd-values (chlorophyll fluorescence vitality indices) in wheat leaves at the pre-treatment of wheat seeds with solutions of citrate nanoparticles and application BP Extrakon (Extr.) (heading stage): 1 – Control; 2 – SeNPs+Extr.; 3 – I-Se+Extr.; 4 – VNPs+Extr.; 5 – GeNPs+Extr.

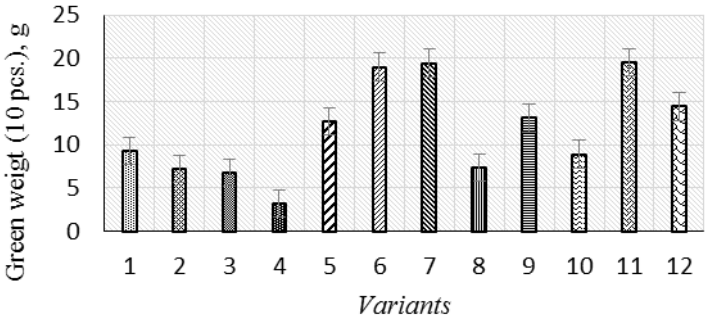

Figure 2. Influence pre-treatment of NPs on the green weight of wheat plants (10 pcs., booting stage): 1 – Control; 2 – D13; 3 – 118; 4 – VNPs, Extr.+D13; 5 – VNPs, Extr.+118; 6 – GeNPs, Extr.+D13; 7 – GeNPs, Extr.+118; 8 – SeNPs, Extr.+D13; 9 – SeNPs, Extr.+118; 10 – I-Se, Extr.+D13; 11 – I-Se, Extr.+118; 12 – I-Se, Extr.+(D13+118).

The content carotenoids, which protect of chlorophyll molecules from photooxidation, increased in order: VNPs>SeNPs>GeNPs>I-Se (see table 1). In investigations Mohamed T (2021) was shown, that the BioSeNPs increased of content of photosynthetic pigments (chlorophyll and carotenoids), parameters of gas exchange, i.e., transpiration (Tr), net photosynthesis (Pn) and has been strong antifungal activity. At application of V the chlorophyll concentration in pepper plants has been varied according to the type of plant part analyzed was shown (García-Jiménez A., 2018). In results investigations Abedini, M., Mohammadian, F. (2018) was indicated that V at all applied concentrations (0, 3.25, 7.5 and 15 mg L^{-1}) significantly decreased the plant's growth, and total protein and carotenoid contents, but increased the total chlorophyll and soluble sugars contents. Also, the obvious induction in the phenylpropanoid pathway was seen in response to V application. In comparison to the control, only at the simultaneous application of I + Se significantly increased of root biomass of lettuce was shown (Smoleń S et al, 2016).

On the other hand, in our previous studies, the antimicrobial effect of V, Ge, I-Se nanoparticles was shown (Huliaieva H.B. et al, 2020). In this regard, further studies were aimed investigation the photochemical activity and growth of wheat plants at pre-treatmant by these nanoparticles with infecting with pathogenic phytoplasmas and bacteria. At infected of wheat plants with infectious agent of bacteriosis (*Pseudomonas syringae* pv. *atrofaciens*) and phytoplasmas (*Acholepasma laidlawii* var. *granulum*) were reduced of green weight of plants (10 pcs.) by 10.8 and 26.9% (Fig. 2).

On variants with at pre-sowing treatment of plants of citrates nanoparticles solution and bacterial and phytoplasmas infected plants versus control increased of green weight of plants in order: GeNPs>I-Se>SeNPs>VNPs have been shown. The most significant growth of wheat biomass was in variants of the pre-

treatment: GeNPs (with Extrakon) at infected plants with bacteria and phytoplasmas, I-Se (Extrakon) both at infected with *A.laidlawii* 118 and at mix infection (D13+118) has been observed (See fig. 2). The pre-treatment VNPs and SeNPs (and Extrakon) was more effectiveness at infected plants of phytoplasmas has been shown (See fig. 2). This effect from using of Ge, Se, V nanoparticles at bacterial and phytoplasmas infected plants can be explained by the induction of systemic resistance, which developed to varying degrees. It is possible due to: fruition the intrinsic peroxidase-like catalytic activity of the Ge (Hu J. et al, 2019), induction of the phenylpropanoid pathway was inducted in response to V application (Abedini, M.; Mohammadian F. 2018) and increased of concentration of antioxidants in plants at application I and Se (Medrano-Macías J., 2018). Introduction of I, Se and SA into the nutrient solution significantly affected proline accumulation in lettuce leaves and roots was shown (Smoleń S et al, 2016).

In field and greenhouse experiments, two weeks after inoculation, we observed the appearance of characteristic symptoms: yellow mosaic with intermittent strokes on the leaves (on variants of virus-infected plant) (Fig. 3), dark brown spots on caulis (at bacterial infecting) (See fig. 3). The early yellowing and drying of leaves as well as ear of wheat with splay out on plants infected with phytoplasmas to milk-ripe stage have been observed (Fig. 3).

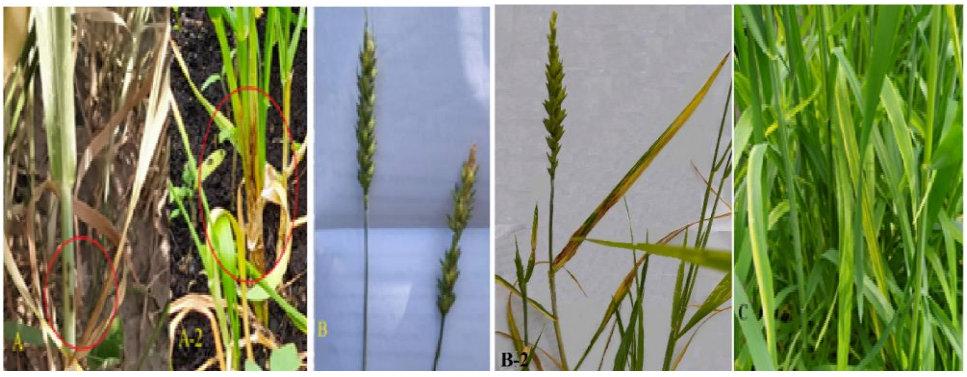

Figure 3. The characteristic symptoms of infesting: (A, A-2) – the plant wheat with bacterial infecting (*P.syringae* pv. *atrofaciens*); (B, B-2) – the wheat plant infected with phytoplasmas (*A.laidlawii*) (milk-ripe stage); C – yellow mosaic with intermittent strokes on the leaves (on variants of virus-infected plant (WSMV)).

The quantum efficiency of PSII (Fv/Fm) in leaves of plants at infected with phytoplasmas reduced by 6.3% in field's experiments. The Fv/Fm–value increased by 13.6% at phytoplasmas infected plants with pre-treatment of VNPs and with application of I-Se it value was tended to increase in compared to infected plants without treatment. In leaves of plants at pre-treatment of GeNPs and bacterial-infected the Fv/Fm-value increased compared to control on 14%

have been detected (Fig. 4 a). Thus, using nanoparticles of V, Ge and I-Se at inoculation plants with phytoplasmas promoted an increase in the quantum efficiency of PS II in comparison with infected plants without treatment.
The Rfd-value – vitality indices of green leaves – increased in leaves of wheat at bacterial infected and decreased at phytoplasmas infected (Fig. 4 b).

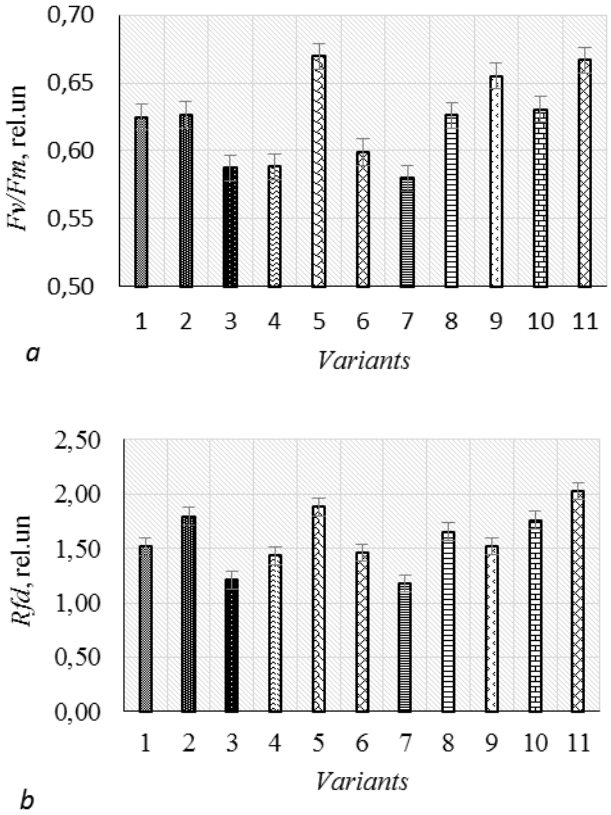

Figure 4. Influence of infected of wheat plants with infectious agent of bacteriosis (*Pseudomonas syringae* pv. *atrofaciens*) and phytoplasmas (*Acholepasma laidlawii* var. *granulum*) after pre-treatment of NPs with application of Extrakon on Fv/Fm and Rfd-values (fields experiments, heading stage): 1 – Control; 2 – *Pseudomonas syringae* pv. *atrofaciens* D13; 3 – *Acholepasma laidlawii* var. *granulum* 118; 4 – VNPs, Extrakon (Extr.) + D13; 5 – VNPs, Extr. +118; 6 – SeNPs, Extr. + D13; 7 – SeNPs, Extr. + 118; 8 – I-Se, Extr. +D13; 9 – I-SeNPs, Extr.+ 118; 10 – GeNPs, Extr.+ 118; 11 – GeNPs, Extr.+D13.

In investigation Berger S. et al (2007) decline of the F_V/Fm and the Rfd parameter was detected from 24 h after the inoculation with bacteria *P. syringae*.

Previous studies (Gulyaeva A.B. et al, 2016) was founded that when infected wheat plants with four pathogenic strains of *P. syringae* pv. *atrofaciens* (different in aggressiveness), from 13 to 16 days gradually decreased the

maximum quantum efficiency of photochemistry PS II (Fv /Fm). Whereas 19 days after the inoculation, the value of this parameter was almost no different from the control. In the same time an increase in the Rfd-value in plants leaves bacterial-infected, together with a decrease in plant biomassmay be is associated with redistribution of assimilates and the inclusion of protective mechanisms against the development of infection. In particular, it may be associated with an increase in the process of photorespiration (antagonistic to photosynthesis), which is involved in protection photosynthetic apparatus from stress (Wingler et al., 2000; Timm, 2016).

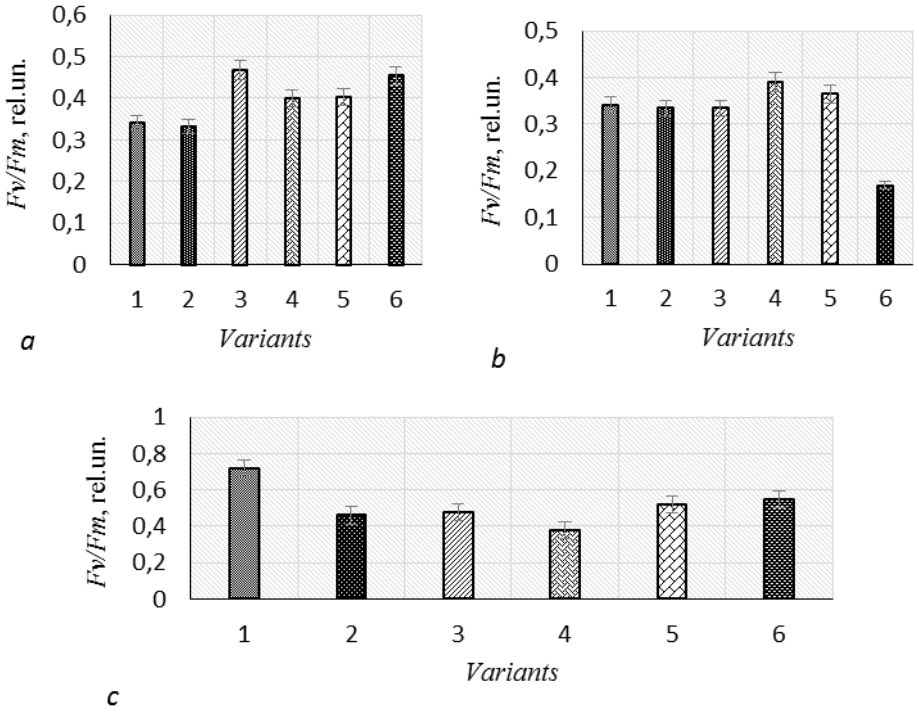

Figure 5. Influence of infected of wheat plants with infectious agent of bacteriosis (*Pseudomonas syringae* pv. *atrofaciens*), phytoplasmas (*Acholepasma laidlawii* var. *granulum*) and WSMV after pre-treatment of NPs with application of Extrakon on Fv/Fm-values (greenhouse experiments, heading stage): (a) 1 – Control; 2 – D13; 3 – GeNPs, Extr.+D13; 4 – SeNPs, Extr.+D13; 5 – VNPs, Extr.+D13; 6 – I-Se, Extr.+D13. (b) 1 – Control; 2 – Phytoplasma (phytopl.); 3 – GeNPs, Extr.+Phytopl.; 4 – I-Se, Extr.+Phytopl.; 5 – VNPs, Extr.+Pytopl.; 6 – SeNPs, Extr.+Phytopl. (c) 1 – Control; 2 – WSMV; 3 – Extrakon+SeNPs+WSMV; 4 – Extrakon+I-Se+WSMV; 5 – VNPs, Extr.+WSMV; 6 – GeNPs, Extr.+WSMV.

The Rfd-values in the leaves of infected plants with phytoplasmas an increased at pre-sowing treatment with VNPs (55.6%), GeNPs (45.5%) and I-Se (25.6%) were observed. This value increased in leaves at bacterial-infected plants at pre-sowing treatment of GeNPs (12.8%) (See fig. 4 b).

In greenhouse experiments increase of the Fv/Fm-value on variants of pre-treatment with: I-Se (14.7%), VNPs (5.9%) (at phytoplasmas infected plants), GeNPs (42.4%)>I-Se (36.4%)>V, Se (21.2%) (at bacterial infected plants) and GeNPs (19.6%)>VNPs (13.0%)>SeNPs (4.3%) (at WSMV infected plants) has been shown (Fig. 5 a-c). This indicates an increase in stress resistance at pre-sowing treatment with these nanoparticles.

In investigation (Liu Y et al, 2016) was shown, that addition of exogenous Ge to the salt solution, as well as soaking the seeds in Ge, attenuated the salt stress effects in a manner dependent on the dose of Ge, as indicated by the increased percentage of seeds that germinated and improved seedling growth. In investigations Rojek J et al (2019) was founded, that higher rate of plant survival in the presence of $VOSO_4$ and the relatively high photosynthetic parameters and anthocyanin contents in the cells vanadium (IV) compound can have positive effects on plants that are grown under stress conditions. The stressed plant's growth rate was the highest in Se nanoparticle concentrations of 5 and 10 μg kg. The plant leaf plate surface area after Se nanoparticle application was almost 2 times larger compared to stressed plants grown without Se nanoparticle addition to the soil (Gudkov S.V. et al, 2020).

The grain weight /10 plants increased on variants at bacterial-infected plants and pre-treatment of GeNPs (with Extrakon) (36.2%) (Fig. 6).

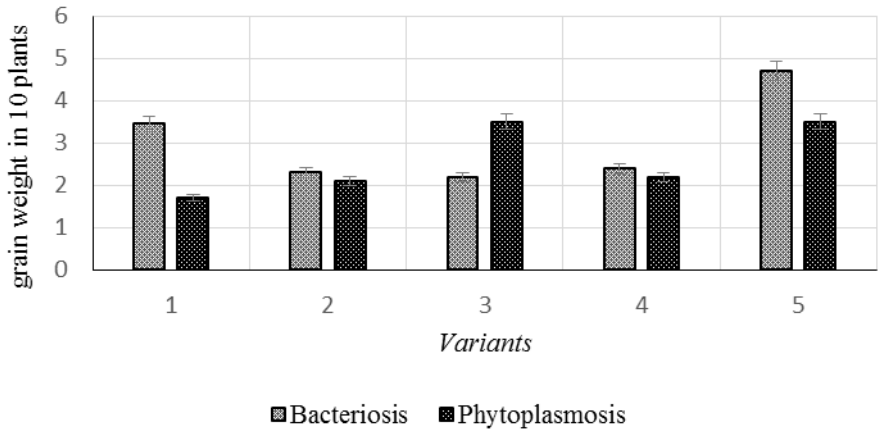

Figure 6. The grain mass / 10 plants during pre-sowing treatment of nanoparticles citrate solutions (with the Extracon) and bacterial and phytoplasmas infected plants: 1-bacteriosis/phytplasmosis; 2 – SeNPs+infected plants; 3 – I-Se+infected plants; 4 – VNPs+infected plants; 5 – GeNPs+infected plants.

In field condition, at bacterial and phytoplasmas infected plants, heads wheat was small and unformed, with flat grain has been shown (Fig. 7 A (2-5)).

The grain production was increased by 15.6% in variants with pre-treatment with GeNPs have been shown. Whereas in other variants it decreased (Fig. 7 a). The

weight of 1000 grains increased by 16,4% at pre-treatment of GeNPs. At pre-treatment of SNPs and VNps this value was equal to control, but pre-treatment of I-Se it decreased by 10,9% (Fig. 7 b). Thus, during the pre-sowing treatment with 2,25mg/l VNPs, 1mg/l SeNPs and 0,8 mg/l I-Se nanoparticles a phytotoxic effect was observed, which manifested itself in a decrease in wheat productivity was shown.

The condition of ears and grains at bacterial and phytoplasmas infected plants wheat at pre-sowing treatment of with citrate nanoparticles of SeNPs, I-Se, VNPs and GeNPs improved has been observed (Fig. 8 B-E).

The grain weight /10 plants on variants increased at pre-treatment of citrates nanoparticles solution and infected with phytoplasmas in compared to infected plants without pre-treatment, in order: GeNPs (by two)>I-Se (by two)>VNPs (29.4%) >SeNPs (23.5%) were shown.

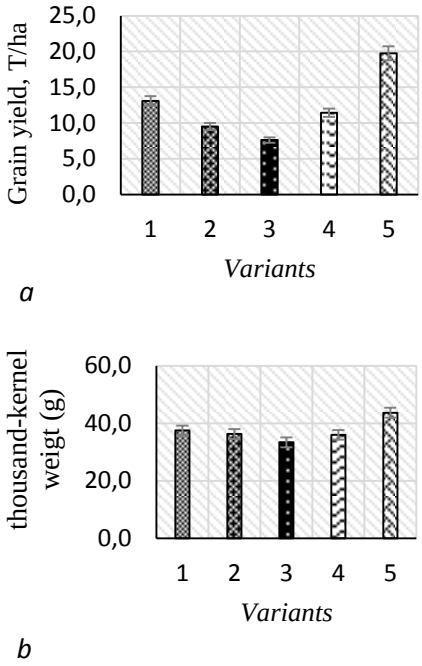

Figure 7. Influence of pre-treatment of wheat with citrate nanoparticles on (a) grain yield (t/ha) and thousand-kernel weight (b): 1 – Control; 2 – SeNPs; 3 – I-Se; 4 – VNPs; 5 – GeNPs.

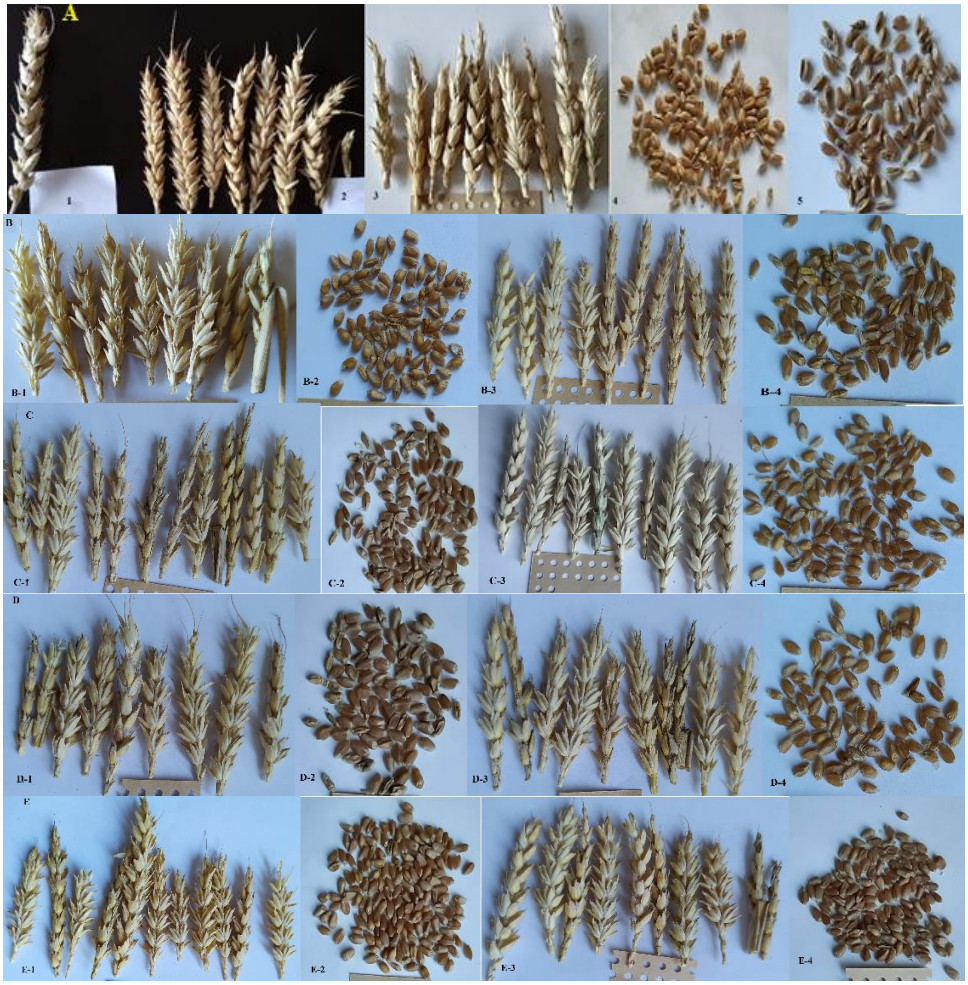

Figure 8. Ears and grains at pre-sowing treatment with citrates of nanoparticles SeNPs, I-Se, VNPs; GeNPs at bacterial and phytoplasmas infected plants: (A) 1 – Control; 2,3 – bacteriosis; 4,5 – phytoplasmosis; (B) 1,2 – SeNPs+bacteriosis; 3,4 – SeNPs+phytoplasmosis; (C) 1,2 – I-Se+bacteriosis; 3,4 – I-Se+phytoplasmosis; (D) 1,2 – VNPs+bacteriosis; 3,4 – VNPs+phytoplasmosis; (E) 1,2 – GeNPs+bacteriosis; 3,4 – GeNPs + phytoplasmosis.

Therefore, pre-sowing treatment of wheat seeds of citrate nanoparticles (2,25 mg/lVNPs, 1mg/lSeNPs, 0,8mg/lI-Se, 3,75mg/lGeNPs) with application BP Extrakon stimulated increasing the chlorophyll content and photochemical activity of leaves. An increase in the quantum efficiency of PSII was observed: at pre-sowing treatment VNPs, GeNPs, I-SeNPs at phytoplasmas infected plants, GeNPs, I-Se, VNPs at bacterial-infected plants and GeNPs,VNPs, SeNPs at WSMP-infected.

The pre-sowing treatment nanoparticles of VNPs (2,25mg/l), SeNPs (1mg/l) and I-Se (0,8 mg/l) a phytotoxic effect was observed, which manifested itself in a decrease in wheat grain productivity was shown. At the same time, pretreatment with 3.75 mg / L GNPs, 2.25 mg / L VNPs, 1 mg / L SeNPs, and 0.8 mg / L I-Se increased plant resistance at bacterial and phytoplasmas infected plants, which was manifested in increasing the mass of grains and improving their quality.

This indicates an increase in stress resistance. However, an increase in grain yield was observed only in the variant of pre-sowing treatment with 3,75 mg/l GeNPs.

CONCLUSIONS

The pre-sowing treatment of wheat seeds with citrate nanoparticles of VNPs, SeNPs, GeNPs and I-Se with application BP Extrakon have been favor increase the chlorophyll content in leaves has been shown.

In the field experiments, at the portable field chlorophyll fluorometer using was shown increased the quantum efficiency of PSII (Fv/Fm) at the pre-treatment of citrates of nanoparticles solution in order: GeNPs (17.5%)>I-Se (8.8%)>SeNPs (7.0%). The Rfd-values increased at the pre-treatment of citrates of nanoparticles solution of GeNPs by 11.4%.

At infected of wheat plants with *Pseudomonas syringae* pv. *atrofaciens* and *Acholepasma laidlawii* var. *granulum* was reduced of green weight of plants (10 pcs.) by 10,8 and 26,9%. At the same time, plants infected at treatment with citrates GeNPs, I-Se, SeNPs, VNPs of nanoparticles had a higher biomass in comparison with the control. The most significant growth of wheat biomass on variants of the pre-treatment: GeNPs (with Extrakon) in plants with bacterial and phytoplasmas infected plants, I-Se (Extrakon) both at infected with 118 and at the mix infection (D13+118) has been observed. The pre-treatment both VNPs and SeNPs (and Extrakon) was more effectiveness at infected plants of phytoplasmas has been shown.

The Fv/Fm was more at infected plants with phytoplasmas and the pre-treatment of VNPs by 13.6%. In leaves of plants at application of GeNPs and bacterial infected, the Fv/Fp-value was the more compared to control on have been shown. The Rfd-values in the leaves of plants at inoculation with phytoplasmas increased at the pre-sowing treatment of VNPs (55.6%), GeNPs (45.5%) and I-Se (25.6%) were observed. This value increased in leaves at bacterial infected plants and the pre-sowing treatment GeNPs (12.8%).

In greenhouse experiments was shown increased of the Fv/Fm-value on variants with the pre-treatment with: I-Se (14.7%), VNPs (5.9%) (at phytoplasmas infected plants), GeNPs (42.4%)>I-Se (36.4%)>V, Se (21.2%) (at bacterial infected plants) and GeNPs (19.6%)>VNPs (13.0%)>SeNPs (4.3%) (at WSMV infected plants).

The weight grains/10 in plants on the variant at the pre-treatment with Ge nanoparticles (with consortium of soil microorganism) with bacterial infected plants were more, than without pre-treatment has been shown.

Wheat plants on variants with pre-treatment citrates nanoparticles solution with phytoplasmas infecting was shown increased of the grain weight /10 plants, in compared to infected plants without pre-treatment, in order: GeNPs (twice)>I-Se (twice)>VNPs (29.4%) >SeNPs (23.5%).

The pre-treatment with nanoparticles GeNPs caused grain production by 15.6% increased has been shown. The weight of 1000 grains increased by 16.4%.

The pre-sowing treatment nanoparticles of with 2,25mg/l VNPs, 1mg/l SeNPs and 0,8 mg/l I-Se the phytotoxic effect was observed, which manifested itself in a decrease in wheat grain productivity was shown. However, we observed an improvement in grain with pre-sowing treatment with these nanoparticles and infection with pathogenic.

Thus, increase in the quantum efficiency of PSII: at pre-sowing treatment VNPs, GeNPs, I-Se (at phytoplasmas infected plants), GeNPs, I-Se, VNPs (at bacterial infected plants) and GeNPs,VNPs, SeNPs (at WSMV infected plants) has been observed. This indicates an increase in stress resistance at pre-sowing treatment with these nanoparticles. However, an increase in grain yield was observed only in the variant of the pre-sowing treatment with 3,75 mg/l GeNPs.

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THE INFLUENCE OF THE VARIETY AND DIFFERENT DOSES OF NITROGEN ON THE GRAIN YIELD AND PROTEIN CONTENT IN TRITICALE GRAIN

SUMMARY

Observed through agronomic and economic significance, cereals represent the most important group of field plants, and the protein content in grain is the most important chemical parameter of complex grain quality. The research was conducted in order to determine the influence of the variety and different doses of nitrogen on the grain yield and protein content in triticale grain. The experiment, set up according to a random block system in four replications on eutric cambisol soil in the north of Montenegro, included five varieties of winter triticale (Odyssey, Kg-20, Triumph, Rtanj and Tango).

In addition to the control (variant without fertilization), four more fertilization variants were applied. The results of the research showed that the application of fertilizers has a significant influence on the values of the observed traits on the one hand, but also that the grain yield and protein content in the grain is largely conditioned by the genotype. In this regard, the highest average protein content in the grain had the variety Triumph in both tested years. The protein content of all tested varieties was significantly lower in the control variant compared to the fertilized variants. The highest grain yield in the first year had the variety Tango while the highest yielding variety in the second year was variety Rtanj.

Keywords: Triticale, Fertilization, Variety, Grain yield, Protein content

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INTRODUCTION

Triticale, a hybrid created by crossing wheat and rye, combined the positive characteristics of the parent species. Thus, he inherited the ability to survive strong frosts from rye, and high genetic potential for yield and increased tolerance to diseases from wheat. The method of using triticale is largely conditioned by the characteristics of the variety. In this regard, large and uniform grain varieties with increased content of total proteins in relation to starch, are used for the preparation of concentrated fodder, while varieties that form a large biomass, such as rye, are used to store bulky fodder in the form of silage, hay or fresh masses (Glamočlija et al., 2018).

Thanks to the high content of essential amino acids, certain minerals and vitamins, the grain of triticale can satisfy a significant part of the needs in the diet of domestic animals. What sets triticale apart from other cereals is the higher yield, faster growth in the spring and the possibility of longer use as green fodder because the above-ground mass roughens later compared to rye or oats. In addition to being grown as a pure crop, triticale can also be sown in various mixtures with winter and spring legumes, which further increases its nutritional value (Schwarte et al., 2005).

The quality of triticale is conditioned by genotype, environmental conditions (Salehi and Arzani, 2013) and their interaction and can be improved by the application of nitrogen fertilizers. The application of nitrogen fertilizers in the diet of triticale contributes to the increase of its productivity (Lestingi et al., 2010), at the same time influencing the increase of the content of crude proteins and non-protein nitrogen (Zečević et al., 2009). Increasing the amount of nitrogen affects the improvement of the technological quality of triticale varieties (Zečević et al., 2010). Insufficient supply of soil with nutrients results in a shortening of the period of grain formation and pouring, which negatively affects the yield and grain quality. Nitrogen fertilization has the greatest impact on the protein content of the grain. Increasing the dose of nitrogen fertilizer contributes to the increased accumulation of protein in the grain, while the intake of small doses of nitrogen can reduce the protein content. The nitrogen content in the grain, among other things, depends on the mutual relationship of nutrients in the applied fertilizers. Thus, the use of nitrogen with potassium and NPK - fertilizers with a nutrient ratio of 1:1 increases the protein content in the grain, while the increased share of other nutrients in relation to nitrogen reduces the protein content (Tyrone, 2002; Jelić et al., 2004; Lalevic et al., 2019).

MATERIAL AND METHODS

The study was performed at the Sutivan site, in the north of Montenegro, during two consecutive seasons (2010/11 and 2011/12). The soil type was eutric cambisol. In both years, the mean temperature was higher than 30 yr average (Table 1). The basic processing and pre-sowing preparation of the soil was done using standard method.

The experiment was set up by random block system in four repetitions, with the size of an elementary plot of 6 m^2 (3x2 m), including 5 cultivars (Odyssey, Kg-20, Triumph, Rtanj and Tango) of winter triticale (×Triticosecale Wittmack). The study included the following varieties of fertilizers: unfertilized plot (0 - the control) and four variants of fertilization (a – 60 kg ha^{-1} N, b - 60 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O; c – 90 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O and d - 120 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5, 80 kg ha^{-1} K_2O). Phosphorus and potassium were used in equal amounts (80 kg ha^{-1}) before the sowing period, while nitrogen was used in small amounts before the sowing period, and the rest of the planned amount was used as fertilization at the end of March. Sowing (550 grains per m^2) was done by hand, during the optimal planting period (17.10.2010, 18.10.2011.) with a row spacing of 12 cm.

The samples for analysis were taken at the stage of full maturity of crops. The spikes were hardened, dried and ground to determine the total nitrogen content by the Kjedahl method. Protein content was calculated by multiplying the concentration of total nitrogen in the grain by a coefficient of 6.25.

The obtained results were statistically processed by the method of analysis of variance, where the significance of the difference of mean treatment (fertilization variant) was tested by LSD test using WASP 2.0. statistical package.

Soil and meteorological conditions

Before setting up the experiment, the soil samples from two depths from 0 to 10 cm and from 10 to 30 cm were taken at seeding, air-dried and analized for pH, $CaCO_3$, humus and available phosphorus and potassium.

Samples were analysed in the Agrochemical Laboratory of the Centre for Cereal Grains in Kragujevac. The applied soil testing methods were adopted by the Yugoslav Society for Soil Studies (JDPZ).

The soil on which the experiment was set up is acid (pH H_2O = 5.61-5.53), weakly carbonate (content of total carbonates 2.4-2.44%), quite humus (3.35-3.96%) and poorly supplied with phosphorus (5.12-4.24 mg 100 g^{-1} soil) and potassium (7.5-3.8 mg 100 g^{-1} soil).

The analysis of meteorological conditions during the two - year research showed that the temperature conditions, but also the amount of precipitation had a certain influence on the values of the observed traits in the selected genotypes of triticale included in the research. The amount of precipitation in the second year of the study was 212.8 mm lower than the amount of precipitation measured during the vegetation period in the first year of the study.

In both years of the research, the measured amount of precipitation was lower compared to the multi-year average. The production year 2011/12 was marked by significantly lower amounts of precipitation in October, November and December, in the period of sowing, germination and initial growth of plants compared to the production year 2010/11, but also the multi-year average. A smaller amount of precipitation during the second year of the survey, compared to

the first year and the multi-year average, was recorded during March, April and May.

In contrast to the amount of precipitation, the average air temperatures for the observed period in both years of the research were higher in relation to the multi-year average. Thus, the average air temperature for the period October-July in the first year of the research was 1.7 ° C, and in the second 1.2 ° C higher than the multi-year average (Table 1).

Table 1. Mean monthly air temperature (°C) and monthly rainfall during the growing seasons of experimental years and long term average (1961-1990)

Year	**Months**										**Sum**
	X	XI	XII	I	II	III	IV	V	VI	VII	
	Monthly rainfall (mm)										
2010--	65.3	130.7	147.3	36.0	76.0	30.9	45.6	120.8	33.2	78.8	**764.8**
2011-12	36.2	7.4	54.6	78.7	182.7	56.7	47.6	46.2	34.2	7.7	**552.0**
1961-90	80	115	91	87	68	60	70	76	72	64	**783**
	Average monthly temperatures (°C)										**Average**
2010-11	10.1	8.5	2.0	-0.6	0.9	6.0	10.5	14.5	18.9	21.2	**9.2**
2011-12	9.3	3.2	2.2	-1.7	-3.5	5.9	10.8	15.0	20.7	24.6	**8.7**
1961-90	9.4	4.7	0.2	-1.3	0.7	4.9	9.0	13.3	16.3	17.9	**7.5**

RESULTS AND DISCUSSION

Grain yield is the surest indicator of differences in productivity between varieties and their specificity in terms of mineral nutrition, and therefore the yields achieved have the highest practical value for each variety. Data on the realized yields of the tested varieties during the two - year trial are presented in Table 2.

The data in the table show that the average yield, observed for all examined cultivars and fertilization variants in the second year, which can be considered more unfavorable in terms of meteorological conditions, was significantly very lower compared to the first and amounted to 3.95 t ha^{-1}.

All cultivars achieved the lowest grain yield on the variant without fertilization in both years of testing and it was significantly very lower in relation to the grain yield achieved on the variants that involved the use of mineral fertilizers. Average values show that the use of the highest amount of nitrogen in combination with phosphorus and potassium, in both years, led to the highest yields.

However, the difference in yield between the use of the lowest and highest amount of nitrogen in combination with nitrogen and phosphorus was not statistically significant.

Table 2. Grain yield of winter triticale depending of variety and different doses of nitrogen (t ha^{-1})

Year (C)	Variety (A)	Fertilization variant (B)					Average
		0	a	b	c	d	
2010/11	**Odissey**	3,15	3,85	4,69	4,68	4,97	**4,27**
	Kg-20	2,67	3,62	4,34	4,42	4,45	**3,9**
	Triumph	3,44	4,38	5,27	5,21	5,37	**4,73**
	Rtanj	3,36	4,55	5,26	5,37	5,62	**4,83**
	Tango	3,67	4,71	5,70	5,75	6,05	**5,18**
Average		**3,26**	**4,22**	**5,05**	**5,08**	**5,29**	**4,58**
2011/12	**Odissey**	2,55	3,43	4,08	4,41	4,42	**3,78**
	Kg-20	2,43	3,24	3,79	3,88	4,12	**3,49**
	Triumph	2,90	3,77	4,56	4,53	4,69	**4,09**
	Rtanj	2,92	3,84	4,97	4,83	4,67	**4,25**
	Tango	3,03	3,77	4,49	4,77	4,68	**4,15**
Average		**2,77**	**3,61**	**4,38**	**4,48**	**4,51**	**3,95**
Two year aver.		**3,01**	**3,91**	**4,71**	**4,78**	**4,90**	**4,26**

Anova Table

Source of variation	Degrees of freedom	F cal.	F prob.
Replication	2	-	-
Factor A	4	1162,892	0,000
Factor B	4	37,801	0,000
Factor C	1	16,316	0,000
AxB	16	72,672	0,000
AxC	4	18,757	0,000
BxC	4	25,428	0,000
AxBxC	16	16,127	0,000
Error	98	-	-
Total	149	-	-

LSD	A	B	C	AxB	AxC	BxC	AxBxC
0,05	0,186	0,186	0,118	0,416	0,263	0,263	0,588
0,01	0,246	0,246	0,156	0,551	0,348	0,348	0,779

Our results are similar to those obtained by Piekarczyk et al. (2011) which founded only minor increase in grain yield as a result of the application of nitrogen at the dose of 40 to 160 kg ha^{-1}. As the most productive varieties, Tango in the first year (average yield was 5.18 t ha^{-1}) and Rtanj in the second year of testing (average yield was 4.25 t ha^{-1}) stood out, with the difference in yield between varieties Tango, Rtanj and Trijumf did not show statistical significance. Variety Kg-20 had the lowest average yield in both years of testing. Our results are in accordance with results of Kara and Uysal (2009), Ivanova and Kirchev (2014), Djuric et al. (2015), Biberdzic et al. (2017), Madic et al. (2018), Lalevic

et al. (2020) and Bielski et al. (2020) which pointed out that the level of yield depends largely on meteorological conditions during the growing season and on mineral fertilization (Dumbrava et al., 2016). Also, Kara and Uysal (2009) state that with low moisture, N fertilization will have minor effects on crop yield, since, yield is more limited by moisture than by N supply.

Table 3. Protein content of winter triticale depending of variety and different doses of nitrogen (%)

Year (C)	Variety (A)	Fertilization variant (B)					Average
		0	a	b	c	d	
2010/11	**Odissey**	12,31	15,87	14,68	14,62	13,56	**14,21**
	Kg-20	12,15	15,21	14,50	14,20	13,68	**13,95**
	Triumph	12,78	16,62	15,37	15,10	14,18	**14,81**
	Rtanj	11,87	14,06	13,49	13,55	13,12	**13,22**
	Tango	11,84	13,75	13,43	13,85	13,18	**13,21**
Average		**12,19**	**15,10**	**14,29**	**14,26**	**13,54**	**13,88**
2010/11	**Odissey**	10,72	13,62	13,37	13,32	13,46	**12,89**
	Kg-20	12,53	13,93	13,18	13,06	13,08	**13,15**
	Triumph	12,18	14,65	13,62	13,03	12,81	**13,26**
	Rtanj	11,03	13,06	12,43	12,34	12,28	**12,23**
	Tango	11,12	12,90	12,48	12,12	12,61	**12,25**
Average		**11,52**	**13,63**	**13,02**	**12,77**	**12,84**	**12,76**
Two year average		**11,85**	**14,36**	**13,65**	**13,51**	**13,19**	**13,31**

Anova Table

Source of variation	Degrees of freedom	F cal.	F prob.
Replication	2	-	-
Factor A	4	69,952	0,000
Factor B	4	32,019	0,000
Factor C	1	7,322	0,008
AxB	16	35,895	0,000
AxC	4	30,695	0,000
BxC	4	16,891	0,000
AxBxC	16	25,986	0,000
Error	98	-	-
Total	149	-	-

LSD	A	B	C	AxB	AxC	BxC	AxBxC
0,05	0,340	0,340	0,215	0,760	0,481	0,481	1,075
0,01	0,450	0,450	0,285	1,007	0,637	0,637	1,424

The biological value of triticale is mainly due to the high levels of protein in the grain. The protein content in the grain is a property of the genotype and its inheritance is controlled by factors of a complex nature, and the content largely

depends on environmental conditions (Đekić et al., 2010) and mineral nutrition (Dobreva, 2016).

The data in Table 3 show that the use of mineral fertilizers, in both years of testing, led to a significantly very large increase in grain protein content in all cultivars included in the study. Also, it was noticed that all varieties achieved the highest protein content in the variant of fertilization where only nitrogen was used in the amount of 60 kg ha^{-1}. If we talk about the influence of the variety on the protein content in the grain, we can notice that in both years of research, the highest content of protein in the grain was registered with the variety Triumph. Also, we can notice that all varieties achieved higher protein content in the first year of research, which can be assessed as more favorable in terms of climatic conditions. Our results are in accordance with the results of Alaru et al. (2003), Lestingi et al. (2010) and Wojtkowiak et al. (2013) which pointed out that cultivar was the main factor influencing the content of protein in triticale grain, that weather conditions in the growth period have a lesser impact and nitrogen fertilization being the least important. In accordance with the opinion of these authors fertilization with nitrogen at tillering stage caused an average increase in the protein content in the grain of triticale and the optimal dose of nitrogen for maintaining good quality of triticale was under 60 kg ha^{-1}.

CONCLUSIONS

Higher grain yield as well as protein content in grain, all examined varieties, achieved in the first year, which was marked by moderate air temperatures at the time of grain filling and higher precipitation in the second part of the vegetation. The Tango variety had the highest yield in the first year, and the Rtanj variety in the second year, with the difference in the amount of yield between the mentioned varieties and the Triumph variety not being significant. The Kg-20 variety had the lowest total grain yield. The Triumph variety had the highest protein content in grain in both years of testing.

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RESPONSE OF YIELD AND QUALITY OF TOMATO GROWN IN UNHEATED GREENHOUSE TO IRRIGATION AND FERTILIZATION WITH DIFFERENT RATES

SUMMARY

An experiment was conducted during 2020 on tomato variety Big Beef F1, planted in Haplic chromic luvi soil under drip irrigation with mulching and fertigation in an unheated greenhouse. The aim was to determine how the yield and quality of tomato were affected under full (100% ET_c) and deficit irrigation (60% ET_c) in interaction with four fertilizer rates (120% RDF, 100% RDF, 80% RDF, 0%). The total yield of tomato plants under full irrigation was 25.7% higher than the yield of the tomato plants under deficit irrigation. Full irrigation, in interaction with the fertilization rates 120 % and 100% RDF of NPK, maintained 30% higher yield of tomato than the yield of plants under reduced water supply with the same fertilization rates. The following parameters: total soluble solids, pH, reducing sugars, ascorbic acid content and titratable acidity were examined to assess the quality of tomato fruits. Except pH, all of the quality parameters of the tomato plants, subjected to moderate stress, due to a reduction in the irrigation rate, showed higher values than those of fully irrigated plants. The pH trend was reversed and the parameter had higher values at full irrigation. Analysis of variance (ANOVA) was performed to analyze the effect of irrigation and fertilization with different rates. The effect of fertilization on the yield and quality of tomato was less pronounced compared to the effect of irrigation.

Keywords: *Solanum lycopersicum*, yield, quality, drip irrigation, greenhouse

INTRODUCTION

Modern agriculture faces the challenge, on the one hand, to maintain high yields and crop quality, while increasing water use efficiency, and on the other

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hand, to reduce the amount of pollutants from agricultural activities (Djurović et al., 2016; Hou M. et al., 2017; Tang et al., 2016). Inefficient irrigation and fertilization can compromise yields, degrade crop quality, and provoke the accumulation of nitrates and pesticides in soil and groundwater (Xing Ying-ying et al., 2015).

In Bulgaria tomatoes are the most common crops grown in greenhouses. Although the total greenhouse area in Bulgaria was within 916 ha in 2018, which was only 2.08% of total vegetable area, because of the high intensity of greenhouse production, the share of greenhouse tomatoes is almost 74% of those produced in the country..

Tomato has high water requirements and irrigation is needed during the cultivation. (Patane et al., 2011) In recent years, drip irrigation systems have been widely used in the greenhouses, which allow supply of the needed water for irrigation to the plants directly into the active soil layer without surface and deep water losses, with minimal losses of evaporation and filtration. Together with the drip irrigation, the mineral fertilizers are also applied to increase the yields. This process is known as fertigation. Many studies worldwide in recent years have shown that the management of water and fertilizers applied through the drip irrigation system is a prerequisite for achieving optimal yields of high quality agricultural products. Many studies have focused on the effects of drip fertigation on tomato yield and fruit quality.

The aim of this study was to determine how the yield and quality of tomato, grown in greenhouse were affected under full (100% ETc) and deficit irrigation (60% ETc) in interaction with various fertilizer rates (120% RDF, 100% RDF, 80% RDF, 0%).

MATERIAL AND METHODS

The study was conducted in the Chelopechene experimental field of the ISSAPP „Nikola Poushkarov" in town of Sofia, Bulgaria in an unheated polyethylene greenhouse with dimensions of 7.9 x 53 m and a total area of 420 m^2 during 2020. The experimental field with geographical coordinates: 42 ° 44′22.8″N, 23 ° 28′3.7″E is a part of the Sofia Field, located at 550 m above sea level. This area has continental climate characterized by cold winter. The soil is slightly humus (2.33%) Haplic chromic luvi soil which can be defined as moderate to strong water-permeable with an average filtration capacity.

The object of the study are tomato variety "Big Beef" F1. A two-factor experiment was performed with experimental factors - irrigation (V) and fertilization (T). The factor irrigation was applied in two levels: V1 – full irrigation at irrigation rate estimated by evapotranspiration (100% ETc) and V2 – deficit irrigation (60% ETc). The factor fertilization was applied at four levels: T0 – without fertilizer, T1 – suboptimal fertilization $N_{8.95}$ $P_{11.82}$ $K1_{3.87}$, T2 – optimal fertilization $N_{11.59}$ $P_{15.84}$ $K_{17.74}$, T3 – luxury fertilization $N_{14.50}$ $P_{20.13}$ $K_{21.88}$. The experimental treatments V1T0, V1T1, V1T2, V1T3, V2T0, V2T1, V2T2, V2T3 were arranged according to the method with long plots. Each plot has a surface of

24 m^2 and consisted of twin rows of tomato with a total of 81 plants. They are planted „checkerboard“ at a spacing of 0.6 m and at a distance between rows of 0.5 m.

Irrigation was performed with a drip irrigation system, comprising a command unit and two batteries consisting eight laterals situated next to the each row of tomato. Mulching was applied to further reducing the evaporation. Black polyethylene mulch (UV 15 mic/1.20 m) was used. Immediately after planting the tomatoes in a permanent place, a watering of 1-2 l per plant was carried out to intercept seedlings and next watering 7 days later. Depending on the growth stage of tomatoes, watering was carried out with a frequency of 3-7 days. Deficit irrigation began to apply from the beginning of fruit setting.

The irrigation rate for the fully irrigated treatments was determined by the sum of daily evapotranspiration for the irrigation interval. The microclimate parameters temperature, relative humidity of the air and the solar radiation in the greenhouse required for the calculations of the evapotranspiration using the Penman-Monteith method (Allen et al., 1998) were measured with an automatic weather micro station located in the centre of the greenhouse. The average monthly data of the parameters during the entire growing season from May to September are shown in Table 1. The conditions were favorable for medium early cultivation of tomato.

Table 1. Average microclimatic conditions in greenhouse

Month	Solar Radiation W/m²	Temperature		RH_{mean} %
		Min, °C	Max, °C	
May	132.98	2.64	51.14	57.77
June	169.01	5.26	43.95	66.25
July	190.08	9.34	44.91	60.75
August	168.85	10.47	45.53	63.79
September	144.92	3.99	42.98	55.01

In the autumn 2019, the main plot was fertilized with combined fertilizer $N_{15}P_{15}K_{15}$ at a rate of 450 kg per ha. During the growing season of tomato simultaneously with irrigation, 100% water-soluble fertilizer with 120:100:80:0 fertilizer dose was given in the treatments 9 times at 7 days interval beginning 10 days after transplanting.

Harvesting of tomato fruits begins on the 80th day and continued until the 140th day after transplanting. Average samples of tomato fruits were taken of each treatment in four representative harvests and the physico-chemical analyzes were performed for determination of the following quality paramaters: Total Soluble Solids (TSS) - with moisture analyser Kern; Active Acidity (pH) - with Hanna pH meter; Titratable Acidity (TA) - by titrimetric method with

0,1nNaOH; ascorbic acid (Vitamin C) content - by the Moore method - titration with 2,6-dichlorophenol-indophenol; Reducing Sugars (RS) - by the Shoorl method.

Experimental results were expressed as mean. Data were analyzed by and two-way ANOVA performed for significance level $p \leq 0.05$ using SPSS software, version 19 (IBM Microsoft, New York, USA).

RESULTS AND DISCUSSION

The results of the analysis of the quality of tomato fruits are shown in Table 2. As shown the studied quality parameters of tomato fruits differed significantly in the two irrigation regimes. They were also affected by the fertigation levels. The highest values of the parameters: TSS (9.35%), reduced sugars (4.73%) and titratable acidity (0.44%) were obtained under deficit irrigation and fertigation with 100% RDF and for the parameter ascorbic acid content (41.67 mg%) in fertigation with 120% RDF. Only the maximum value for the pH parameter was at full irrigation without fertigation (4.33%). Minimum values of the parameters reduced sugars (3.71%) and ascorbic acid content (27.87 mg%) were obtained under full irrigation and fertigation with 120% RDF, also for titratable acidity (0.23%) under full irrigation and fertigation with 100% RDF. The values of TSS parameter(6.68%) under full irrigation and the pH parameter (4.09) under deficit irrigation without fertigation were the lowest.

Table 2. Average data of quality parameters of tomato and yield by treatments

Parameter Treatments	Total soluble solids, %	pH %	Reducing sugars, %	Vitamin C , mg %	Titratable acidity, %	Total Yield, t ha^{-1}	Yield per plant, kg
V1T0	6.68	4.33	3.80	30.14	0.28	89.21	2.64
V1T1	7.49	4.24	4.05	28.03	0.30	89.88	2.66
V1T2	6.72	4.27	3.93	29.28	0.23	106.10	3.14
V1T3	6.87	4.27	3.71	27.87	0.24	128.33	3.80
V2T0	7.08	4.09	3.96	34.01	0.31	69.66	2.06
V2T1	8.61	4.19	4.33	34.56	0.35	70.15	2.08
V2T2	9.35	4.19	4.73	37.00	0.44	77.37	2.29
V2T3	8.69	4.22	4.44	41.67	0.38	90.07	2.67

The results of the analysis of variance to establish the statistical differences between the treatments of the experiment in the studied parameters of tomatoes are shown in Table 3. Statistically significant differences of the treatments with different levels of irrigation in terms of TSS parameter, ascorbic acid content, titratable acidity in tomato fruits have been proven. Regarding the ascorbic acid content parameter, they have the lowest level of statistical significance $p < 0.05$,

for the TSS parameter, they have the higher level $p < 0.01$ and for the titratable acidity parameter, they have the highest level $p < 0.001$. The results of the interaction between irrigation regimes and fertigation with different levels for all studied quality parameters are also shown in Table 3. No significant interactions between the applied experimental factors for the quality parameters of tomato fruits have been proven.

Table 3. Quality parameters of tomato and yield as affected by irrigation and fertilization

Parameter **Irrigation Regimes**	Total soluble solids, %	pH %	Reducing sugars, %	Vitamin C , mg %	Titratable acidity, %	Total Yield t ha^{-1}	Yield per Plant kg
V1	6.939	4.276	3.874	28.831	0.261	76.813	2.275
V2	8.433	4.171	4.367	36.812	0.370	103.380	3.060
F-Ratio	9.436	3.508	3.961	6.150	21.289	6.594	6.539
P-Value	0.004	ns	ns	0.019	0.000	0.042	0.043
Fertigation Levels							
T0	6.883	4.211	3.880	32.076	0.294	79.435	2.350
T1	8.048	4.212	4.193	31.295	0.326	80.015	2.370
T2	8.034	4.229	4.333	33.143	0.333	91.735	2.715
T3	7.780	4.242	4.076	34.771	0.310	109.200	3.235
F-Ratio	1.003	0.059	0.520	0.172	0.304	1.016	1.025
p-Value	ns	ns	ns	ns	ns	ns	ns
Interaction VxT							
F-Ratio	2.057	0.226	0.066	0.603	0.826	4.048	4.018
p-Value	ns	ns	ns	ns	ns	0.025	0.026

ns – not significant

The results obtained in the study show that the quality parameters: ascorbic acid content, titratable acidity and reduced sugars at both levels of irrigation for tomato plants with fertigation are higher than the those obtained by Milenković et al., 2018 in experiment with the same tomato variety under conventional application of fertilizers to the soil and shading coatings.

Correlation analysis was performed to establish a relationship between the various quality parameters of tomato. The results showed significant correlations between the studied quality parameters (Table 4).

Table 4: Correlation between tomato quality parameters

Parameter	Vit.C	TSS	pH	TA	RS
Vit.C	1	.778*	-0.48	.821*	.790*
TSS	.778*	1	-0.405	.942**	.970**
pH	-0.48	-0.405	1	-0.489	-0.414
TA	.821*	.942**	-0.489	1	.944**
RS	.790*	.970**	-0.414	.944**	1

* Correlation is significant at the 0.05 level (2-tailed).
**.Correlation is significant at the 0.01 level (2-tailed).

Ascorbic acid content has a significant positive correlation with TSS, TA and RS ($p < 0.05$). On the other hand, Vitamin C parameter, as well as the other quality parameters TSS, TA and RS are negatively correlated with pH. For all parameters the correlation with pH is not significant. There is also a significant positive relationship between TA on the one hand and TSS and RS on the other ($p < 0.01$).

Table 5. Regression equations for the quality parameters

Relationship	**R^2**
Vit. C = 56.364 TA + 15.021	0.674
Vit. C = 0.056 RS + 2.265	0.624
TSS = 0.0642TA - 0.177	0.887
TSS = 2.899 RS - 4.255	0.94
TA = 13.818 TSS + 3.322	0.887
TA = 0.192 RS - 0.476	0.891
RS = 4.633 TA + 2.656	0.891
RS = 0.324 TSS + 1.626	0.94

Using linear regression analysis empirical dependences have been derived between the quality parameters of tomato fruits for which a significant correlation has been established. Table 5 shows the obtained linear regression models as well as the coefficient of determination. The equations are statistically significant at the $p = 0.05$ level. The coefficients in front of the independent variable as well as the free term of the linear regression models are statistically significant at the same level of significance ($p < 0.05$) and higher ($p < 0.01$). The level of significance of the models and their coefficients shows that the obtained regression models correspond well to the experimental data.

Table 5 shows that the relationship between the TSS and reducing sugars in tomato fruits has a high coefficient of determination $R^2 = 0.94$. Literature data are available for the correlation between TSS and reducing sugars, which is due to of reduced water content in tomato (Anthon et al., 2011; Mitova and Dinev, 2011). A high coefficient of determination R was also obtained for the linear equations describing the dependences: titratable acidity from the TSS (R^2=0.887) and titratable acidity from the reducing sugars ($R^2 = 0.891$).

Experimental results for the tomato yield by treatments are given in Table 2. The results of the statistical analysis, showing the influence of irrigation and fertilization with different irrigation and fertilizer rates, are given in Table 3. Significantly higher yields were obtained in the fully irrigated treatments, as the highest yield per plant (3.8 kg per plant) and total yield (128.33 t ha^{-1}) was obtained from tomato plants receiving 100% irrigation rate and fertigation with 120% RDF.

The yield of tomatoes plants under deficit irrigation was lower than those of fully irrigated plants, as the highest yield (90.7 t ha^{-1}) and yield per plant (2.67 kg per plant) was obtained at the application of the maximum fertilizer rate (120% RDF). Therefore, the yield of tomatoes differed significantly depending on the levels of fertigation. The application of fertigation with 120% RDF achieved the highest yield, while applying fertigation with 80% RDF registered a significantly of 10% to 30% lower yield compared to those in higher levels of fertigation.The total yield of tomato plants under full irrigation was higher by 25.7% than the yield of plants under deficit irrigation. Full irrigation, applied with luxury and optimal fertilization, maintained 30% higher yield than the yield of tomato plants subjected to moderate stress due to reduced irrigation rate under same fertilization.

The yield of tomatoes obtained in this study is comparable in size with that obtained by Milenković et al., 2018. In the present study, the yield per plant was lower - 2.28 kg per plant under deficit irrigation and 3.06 kg per plant under full irrigation, while in the experiment of Milenković et al., 2018, the yields of the hybrid Big Beef F1 range between 3.2 and 5.1 kg per plant. The larger number of plants per unit area in the experiment conducted in Bulgaria was the reason for the reducing food area and for more unfavorable light conditions, which have been affected the fruiting of the plants.

CONCLUSIONS

The results obtained from the quality analysis determine the production of tomatoes as such with high quality parameters: TSS between 6.72 and 9.35%; pH, which varies in a small range of 4.09 to 4.33%, titratable acidity with relatively low content - 0.23 - 0.44 %; reducing sugars - 3.71-4.73% and ascorbic acid between 27.87 and 41.67 mg%. With the exception of pH, all others quality parameters of tomato plants subjected to moderate stress due to a reduction in irrigation rate show higher values than those of fully irrigated plants. It can be concluded that deficit irrigation improves the quality of tomato fruits in terms of

TSS, ascorbic acid, reduced sugars and titratable acidity. A significant correlation was found between the studied quality parameters and linear regression equations with high value of the coefficient of determination R^2 were obtained.

The total yield of tomato plants subjected to full irrigation was higher by 25.7% than the yield of plants subjected to deficit irrigation. It has been found that combining irrigation levels with different levels of fertigation affects tomato yield. The results show that the yield in the treatments with the application of the highest fertilizer rate is the highest for both full and deficit irrigation (128.33 and 90.07 t / ha).

Statistical analysis of the experimental data showed that the effect of fertilization on the yield and quality of tomatoes was less pronounced compared to the effect of irrigation.

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PHYLOGENETIC ANALYSIS OF TWO UKRAINIAN ISOLATES OF CUCUMBER MOSAIC VIRUS FROM GLADIOLI GROWN UNDER DIFFERENT AGROECOLOGICAL CONDITIONS

SUMMARY

Cucumber mosaic virus (CMV) is one of the most widespread and harmful viruses infecting gladiolus plants worldwide. The aim of the study was to perform a phylogenetic analysis of two CMV isolates from gladioli grown in different regions of Ukraine. For the first time, 443 nt sequences of the capsid protein (*CP*) gene of gladiolus isolates CMV-Gl-Skv-20 (MW847710) and CMV-Gl-SkvP-20 (MW847714) from Kyiv and Poltava regions, respectively, were submitted to the NCBI GeneBank. Phylogenetic analysis showed that isolates clustered with different phylogenetic subgroups. CMV-Gl-Skv-20 belongs to subgroup IA, and has nucleotide (nt) sequence identity 81.9%-99.27% and amino acid (aa) identity 82.3%-97.6% with isolates from this group. The highest identity of the CMV-Gl-Skv-20 was found to be with Turkish CMVs from *Rapistrum rugosum* TUR83, TUR86 and *Brassica* TUR4 (98.8%-99.3% nt and 97,6% aa), as well as with Australian isolates Ny and 207 from tomato, and banana isolate Cameroon (98.9%-99% nt and 97,6% aa). CMV-Gl-SkvP-20 belongs to subgroup IB and shares 95.8%-100% nt and 96%-100% aa identity with the members of this subgroup. CMV-Gl-SkvP-20 has the highest identity with Ukrainian isolates from cucumber Ukr-1409 and *Echinacea* P-EP-Ukr-19 (99.5%-100% nt and 99.2-100% aa), Chinese pumpkin isolates ZBR, WHR, isolate lu-17-14 from sweet potato and SXCH from *Bupleurum sp.* (98.3-99% nt and 98.4%-99.2% aa), as well as with gladiolus South Korean isolate ABI (98.3% nt and 97.7% aa). The results support the fact that gladioli are affected by CMV not only through the corms, but by vector insects circulating in growing areas.

Keywords: cucumber mosaic virus, gladiolus, coat protein gene, phylogenetic analysis, Ukraine.

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INTRODUCTION

Cucumber mosaic virus (CMV) is one of the most dangerous and widespread plant viruses in the world, which can infect more than 1,200 species of plants from more than 100 families, including economically important crops. Cucumber mosaic virus belongs to the *Bromoviridae* family, *Cucumovirus* genus. Despite that cucumber mosaic virus is characterized by seed transmission for some plant species (Montes *et al.,* 2021), vector transmission is considered more efficient. More than 70 species of aphids (*Aphidoidea*) are able to transmit the virus non-persistently (Tungadi *et al.,* 2017). CMV isolates differ in host range and pathogenicity and divided into three main subgroups: IA, IB and II. Isolates of these subgroups are characterized by uneven distribution in the world. Thus, IA and IB CMVs have tropical and subtropical origin, causing severe disease and significant economic losses. Group II isolates predominate in temperate regions and cause milder symptoms in plants (Berniak *et al.* 2009). Numerous phylogenetic studies of CMV show that subgroup IB mainly includes isolates from Asia and the Middle East, in contrast to subgroups IA and II, which include isolates from different countries and continents, mostly from Europe. Detailed phylogenetic analysis of CMV isolates revealed that subgroup IA originated from subgroup IB. The only obvious differences between the phylogenetic trees based on *CP* and *3a* gene sequences are: i) the degree of branching, which is slightly higher in *3a* tree; ii) the branch lengths, which are longer in the *CP*-based tree, especially for branches leading to subgroups I and II (Roossinck, 2002). Genetic analysis of CMV subpopulations indicates heterogeneity of genetic variation. Asian subpopulations have significantly higher genetic diversity, compared to American ones, and the difference within subgroup I and originating from Asia differ by 7– 12% in sequence arrangement from other subgroup I strains (Shahmohammadi *et al.,* 2019).

Cucumber mosaic virus in gladiolus plantations was found in Argentina (Arneodo *et al.* 2005), the United States (May *et al.,* 1963), India (Dubey *et al.,* 2010), Israel (Gera *et al.* 1990), the Netherlands (Asjes *et al.,* 1997), the Czech Republic (Pokorny *et al.,* 2009), South Korea (Park *et al.* 1998). The virus is revealed on *Gladiolus* plants in Ukraine. Symptoms of CMV infection on gladioli are characterized by leaf chlorotic stripes, mosaics, growth retardation, and 'color break' of the flower (Sovinska *et al.,* 2020). Infection can lead to the loss of cultivar, destruction of gladiolus collections in botanical gardens, as well as to complication of the selection and creation of gladiolus cultivars.

In Ukraine, the cucumber mosaic virus has been detected in agricultural plantations since 1970. However, the study of the prevalence and detailed analysis of cucumber mosaic virus in gladiolus plantations, its molecular characteristics has not been conducted in Ukraine before. The aim of the study was to perform a phylogenetic analysis of two CMV isolates from gladiolus plants grown in different regions of Ukraine.

MATERIAL AND METHODS

Samples collection and visual diagnostics

Initially, sampling of *Gladiolus sp*. variety Victoria Skvyrska was conducted in the autumn of 2019 in the Kyiv region, northern Ukraine. Visual diagnostics revealed symptoms of viral infection on leaves, flowers and corms. Fifteen samples of gladiolus with both virus-specific symptoms and visually healthy plants were selected. Healthy plants that were confirmed by ELISA and RT-PCR have been planted next year in field conditions in the Poltava region (central Ukraine) in the agrocenosis where CMV circulation was revealed (Mishchenko *et al.*, 2021).

Enzyme-linked immunosorbent assay

To determine the presence of viral antigens, the double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used. The analysis was performed using commercial antibodies against cucumber mosaic virus manufactured by Loewe (Germany) in three replicates. Samples of healthy gladioli were used as negative control. Commercial CMV positive control was used (Loewe, Germany). The reaction results were recorded on the Termo Labsystems Opsis MR (USA) reader with Dynex Revelation Quicklink software at wavelength of 405 nm. Samples with absorbance values that exceeded the negative control at least three times were considered positive.

RNA extraction, RT-PCR and sequencing

GeneJET Plant RNA Purification Mini Kit (Thermo Scientific, USA) was used to extract total RNA from gladiolus leaves. Samples of healthy gladioli were used as negative control. RevertAid Reverse Transcriptase (Thermo Scientific, USA) was used for cDNA synthesis. The amplification steps were performed using a Genetic Research Instrumentation LTD thermocycler (United Kingdom). The amplification reactions were set up as follows: initial denaturation for 3 min at 95 °C, followed by 30 cycles of 95 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. The final extension was at 72 °C for 5 min. The primers are expected to amplify DNA product of 500 bp. PCR products were separated on a 1.5% agarose gel with DNA markers CSL-MDNA-100bp (Cleaver Scientific, United Kingdom), and visualized under UV light. The PCR products were purified from the agarose gel using Zymoclean Gel DNA Recovery Kit (Zymo research, USA).

The PCR products were sequenced on a 3130 Genetic analyzer (Applied Biosystems HITACHI) using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Sample analysis was performed using Sequencing Analysis Software v5.2.0. The obtained sequences of capsid protein (*CP*) gene fragments of gladiolus isolates CMV-Gl-Skv-20 from Kyiv region and CMV-Gl-SkvP-20 from Poltava region were submitted to the NCBI GenBank under accession numbers MW847710 and MW847714, respectively.

Phylogenetic analysis

The sequences of Ukrainian cucumber mosaic virus isolates isolated from gladiolus CMV-Gl-Skv-20 and CMV-Gl-SkvP-20 were compared with sequences of 64 CMVs from different countries available in the NCBI GenBank using the

BLAST program. Nucleotide and amino acid sequences were aligned using Clustal W. Phylogenetic tree for the 443 bp fragment of coat protein gene of CMV isolates was constructed in MEGA 10 by the Neighbor Joining method using the Jukes–Cantor model with 1000 bootstrap replicates to estimate the statistical significance of each node. Peanut stunt virus, isolate ER (Ac No NC_002040) was taken as an outgroup. The pairwise nucleotide sequence identity scores between every isolate with each other represented as color-coded blocks using SDT v.1 software (Sequence Demarcation Tool Version 1.1). Multiple amino acid sequences alignment of the CMV isolates coat protein was performed with BioEdit 7.2.5 program.

RESULTS AND DISCUSSION

Gladiolus samples of the Ukrainian variety Victoria Skvyrska were selected in the summer-autumn period of 2019 in the Kyiv region. Gladioli with symptoms of viral infection and visually healthy plants were used in the study (Fig. 1A, 1B, 1C). DAS-ELISA showed the presence of CMV antigens in plants with chlorotic stripes on the leaves and flower ‘color break’. Visually healthy gladioli did not contain CMV antigens.

Fig.1. Symptoms of CMV infection on gladioli var. Victoria Skvyrska: A – ‘color break’ of the flower and chlorotic stripes on the leaves (Kyiv region, 2019); B – flower ‘color break’ (Kyiv region, 2019); C – visually healthy plants (Kyiv region, 2019); D – chlorotic stripes on the leaves (Poltava region, 2020);

In 2020, corms from healthy gladioli were planted in field conditions in the Poltava region, where the circulation of cucumber mosaic virus of IB subgroup was previously revealed on other plants (Mishchenko *et al.*, 2021). In gladioli planted in 2020, symptoms of stripes on the leaves were observed (Fig. 1D). DAS-ELISA and RT-PCR confirmed presence of CMV in these plants. The results of RT-PCR are consistent with the data obtained by DAS-ELISA and demonstrate the presence of CMV in the studied samples of gladiolus leaves

The sowing season of 2020 in Poltava region was characterized by harsh summer with high average daily temperatures, low soil moisture and the predominance of sunny weather. The beginning of the autumn season was

influenced by a severe drought, which lasted until October. Climate changes associated with global warming are closely related to the level of losses from plant diseases because the environment significantly affects plants, pathogens and their vectors (Mishchenko *et al.*, 2017). Such weather conditions are favorable for aphids' reproduction and CMV distribution.

Next step was to compare molecular characteristics of two CMV isolates from gladioli grown in 2019 (Kyiv region) and in 2020 (Poltava region). For the first time, 443 nt fragments of the *CP* gene of gladiolus CMV isolates CMV-Gl-Skv-20 (Ac. No. MW847710) and CMV-Gl-SkvP-20 (Ac. No. MW847714) from Kyiv and Poltava region, respectively, were sequenced and deposited to NCBI GenBank. Phylogenetic analysis showed that isolates of CMV-Gl-SkvP-20 and CMV-Gl-Skv-20 belong to different phylogenetic groups. Isolate CMV-Gl-Skv-20 belongs to subgroup IA (Fig. 2). It has an identity of 81.9%–99.27% by nucleotide sequence (nt) and 82.3% –97.6% by amino acid (aa) with isolates of this subgroup (Fig. 3). CMV-Gl-Skv-20 has the highest identity of 98.8% – 99.3% nt and 97.6% aa with Turkish isolates from *Rapistrum rugosum* TUR83 (LC066509), TUR86 (LC066515) and from *Brassica* TUR4 (LC066500); 99% nt and 97.6% aa identity with banana Cameroon isolate (EU428827); and 98.8 – 98.9% nt and 97.6% aa with Australian tomato isolates 207 (AJ585517) and Ny (CMU22821). Sequences of gladioli CMV isolates from other countries were also included to the phylogenetic analysis. Analysis showed that they are belonging to subgroup IA. The studied fragment of the *CP* gene of the CMV-Gl-Skv-20 isolate does not have a high identity with the gladiolus CMV isolates available in the NCBI GenBank database. Thus, CMV-Gl-Skv-20 has an identity 94.6% nt and 92.1% aa with the gladiolus isolate GPP (AJ131623) from the Netherlands and identity of 81.9% – 82.9% nt and 83%-84.7% aa with isolates from *Gladiolus dalenii* from India Glad-NBRI-10 (KP713797), Glad-NBRI-4 (KP713798).

Phylogenetic analysis of the CMV-Gl-SkvP-20 isolate showed that the isolate belongs to subgroup IB and have the identity 95.8% – 100% nt and 96% – 100% aa with isolates of this subgroup (Fig. 2, 3). CMV-Gl-SkvP-20 has the highest identity of 99.5% –100% nt and 99.2 – 100% aa with Ukrainian isolates from cucumber Ukr-1409 (KT199741) and purple coneflower P-EP-Ukr-19 (MT978189), the identity of 98.3–99% nt and 98.4%–99.2% aa with Chinese pumpkin isolates ZBR (KP710850), WHR (KP710851), isolate lu-17-14 (MK778781) from sweet potato and SXCH (JX993913) from *Bupleurum sp.*, as well as identity of 98.3% nt and 97.7% aa with gladiolus South Korean isolate ABI (L36525).

Comparative analysis of the amino acid sequences of gladiolus isolates CMV-Gl-Skv-20 and CMV-Gl-SkvP-20 with 64 isolates from different countries and hosts was performed. The unique amino acid substitution in the studied sequence of the *CP* gene of the CMV-Gl-Skv-20 isolate was revealed, namely: G → R at the position 96 (Fig. 4).

The analysis of the 443 bp *CP* gene sequence of the CMV-Gl-SkvP-20 isolate has not detected any aa substitutions compared to the sequences available in the NCBI GenBank database (Fig. 4).

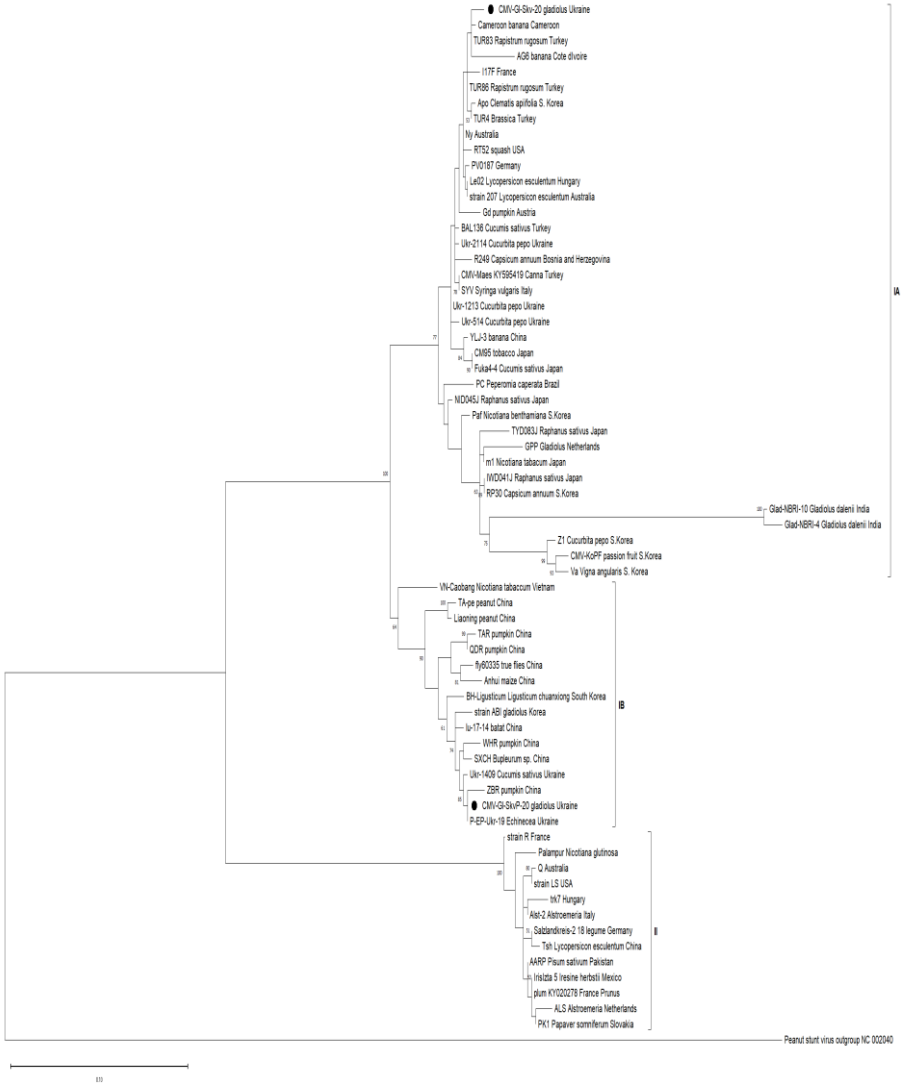

Fig. 2. Neighbor-Joining tree based on nucleotide sequences of 443 bp *CP* gene region of CMV isolates. Jukes-Cantor model was performed. The scale bar shows the number of substitutions per base. Peanut stunt virus (Ac. No NC002040) used as an outgroup. The studied Ukrainian isolates were marked with a dot.

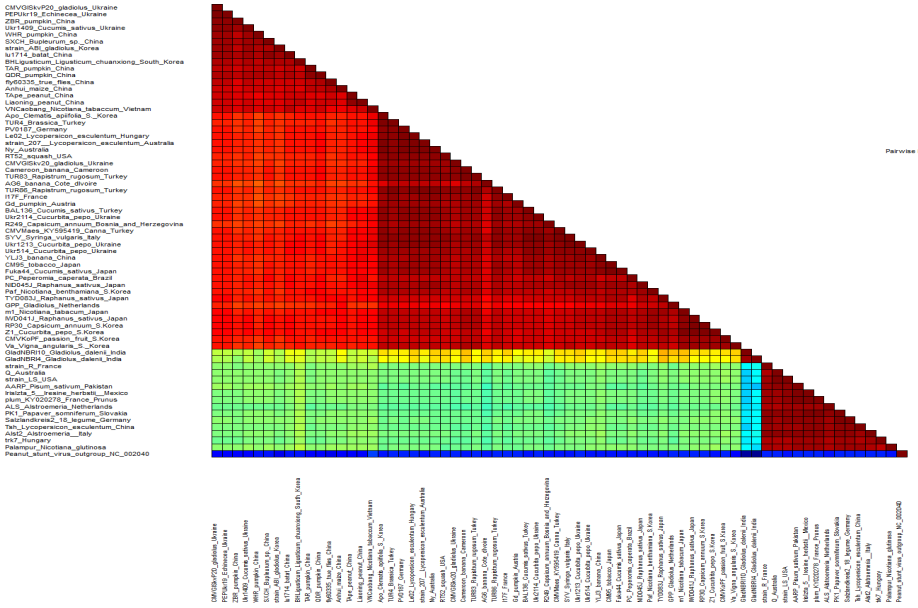

Fig. 3. Graphical representation of pairwise nucleotide identity of 443 bp *CP* region of 64 CMV isolates (with percentage identity scale).

Fig. 4. Comparative analysis of amino acid sequences of CMV-Gl-Skv-20, with other CMV isolates. Numbers on top represent the deduced CP amino acid position. Only the differences are shown.

CONCLUSIONS

Our investigations revealed that CMV-Gl-SkvP-20, belongs to subgroup IB and has the highest percentages of identity with Ukrainian isolates from cucumber Ukr-1409 and purple coneflower P-EP-Ukr-19, the last was studied earlier and originates from the same agrocenosis. In contrast, the isolate CMV-Gl-Skv-20 (Kyiv region, 2019) belongs to subgroup IA, has the greatest identity with the Turkish, Cameroonian and Australian isolates from vegetable plants and bananas. Despite that CMV-Gl-Skv-20 was initially identified as a gladiolus isolate, a comparison with other CMV gladiolus isolates of subgroup IA available in the NCBI GenBank indicates a low similarity with them inside the subgroup. This can be explained by different origin of isolates or/and aa substitution revealed in the CP gene.

Thus, the studied CMV isolates from gladiolus of the same variety, but grown in different regions of the country, belong to different phylogenetic subgroups and therefore have different origin. The isolate from Kyiv region is most similar to foreign CMV isolates, while the isolate from Poltava region is most similar to Ukrainian ones, and the most similar to purple coneflower isolate, which grew in the same agrocenosis with the studied gladioli. These results suggest that gladioli in the Poltava region were infected not by affected corms, but by aphids which transmitted the CMV from vegetable or other crops growing in this agrocenosis.

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COMPETITION BETWEEN SESSILE OAK SEEDLINGS AND COMPETING VEGETATION UNDER A SHELTERWOOD

SUMMARY

The paper presents the results of research on competitive relations between sessile oak seedlings and the most important competing species of woody vegetation, as well as ground flora that grows under a shelterwood, after the preparatory - seed cut of the shelterwood cutting. The research was conducted in sessile oak (*Quecus petraea* /Matt./Liebl.) stand in the area of northeastern Serbia. The studied stand is located at 320 to 350 m above sea level, with a slope up to 25° and western exposure. In terms of phytosociology, the stand is defined as an association of sessile oak with hairy sedge (*Carici pilosae – Quercetum petraeae* B. Jov. 1989). After the preparatory-seed cut, 124 trees per ha remained in the stand, with a basal area of 11.9 m^2/ha and a volume of 129.1 m^3/ha. The obtained results in the paper indicate the competitive relations between sessile oak and the most important competing woody species, as well as the species of ground vegetation. The most common competing woody species are silver linden (*Tilia tomentosa* Moench.) and hornbeam (*Carpinus betulus* L.), while other species (*Fraxinus excelsior* L., *Fraxinus ornus* L., *Crataegus monogyna* Jacq., *Acer campestre* L., *Cornus mas* L.) are represented individually. In addition to a significant presence, competing species of woody vegetation are characterized by very intensive development, which significantly reflects on the presence and development of sessile oak seedlings. In contrast, eight-year-old sessile oak

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seedlings have outgrown blackberries in the studied stand, which no longer competes with them. The obtained results indicate that during the regeneration of sessile oak forests, special attention must be focused on the presence and dynamics of the development of competing vegetation, as well as that the success of the regeneration largely depends on controlling this factor.

Keywords: competition, forest regeneration, sessile oak, shelterwood cutting, seedlings

INTRODUCTION

Natural regeneration of sessile oak forests is one of the most important issues in forestry, considering the presence of these forests, ecological specificity, productivity, as well as the quality of products obtained by their use.

Despite the numerous problems and aggravating circumstances that accompany the process of natural regeneration of sessile oak forests (irregular seed production, competing vegetation, sensitivity to extreme temperatures, etc.), natural regeneration offers numerous advantages over artificial regeneration, which should be practiced only in situations where natural regeneration is lacking. In addition to preserving indigenous genetic diversity (Burczyk et al. 2006), natural regeneration enables the process of natural selection from a large number of individuals during the development of a future stand (Kohler et al. 2020), thus significantly increasing its stability and resistance to negative abiotic and biotic factors.

In situations when the canopy of sessile oak forests is interrupted, if there is a lack of sessile oak seedlings, as well as if the land is weedy or the presence of competing species is too large, it is necessary to carry out auxiliary measures to the natural regeneration of sessile oak forests, which include silvicultural treatments that are focused on the removal of competing species, as well as which involve a combination of natural and artificial regeneration (Stojanović and Krstic, 2000).

One of the key phases that largely determine the outcome of the natural regeneration of sessile oak forests is the phase of the initial development of the young seedlings when its survival is most endangered (Harmer et al. 2005; Annighöfer et al 2015; Krstic et al. 2017; Krstic et al. 2018; Kanjevac 2020; Kanjevac et al. 2021). By regulating the canopy of the stand, i.e., by the controlled addition of light in this phase, the development of the seedlings can be significantly influenced, which indirectly reflects on its qualitative characteristics (Govedar 2006).

Competition by woody species and ground vegetation is one of the key factors that burden the process of natural regeneration of sessile forests and significantly hinders the development of sessile oak seedlings in the first years (Mölder et al. 2019). In accordance with the above, a large number of authors point to the endangerment of sessile oak seedlings in relation to weed species such as *Rubus* spp. (Modrow et al. 2020; Kanjevac et al. 2021), or species that better tolerate shade, such as beech (*Fagus sylvatica* L.), hornbeam (*Carpinus*

betulus L.), silver linden (*Tilia tomentosa* Moench.), etc. (Otto et al. 2009; Ligot et al. 2013; Kanjevac et al. 2020). These species have the potential to outgrow and eventually suppress sessile oak seedlings if light conditions are unfavorable over a long period (Ligot et al. 2013). In this regard, many authors emphasize the importance of removing competing vegetation (especially shoots and root suckers of woody vegetation) during the regeneration process (vön Lupke 1998; Ligot et al. 2013; Kanjevac et al. 2020), as well as pre-commercial thinning after the regeneration process (Ammer and Dingel 1997; Mölder et al. 2019).

Considering the previously stated ascertainments, the aim of this paper is to analyze competitive relations between sessile oak seedlings and competing vegetation that develop under the protection of the parent stand.

MATERIAL AND METHODS

The research was performed in a stand of sessile oak (Quecus petraea / Matt./Liebl.) in the area of northeastern Serbia in the management unit "Ujevac" within the State Enterprise "Srbijašume" Belgrade (44°25´N; 21°52´E). The average annual temperature at the study sites in the reference period 1981-2010 was 10.3°C (17.0 °C in the growing season), and the average annual precipitation was 679 mm (59.4% in the growing season). According to Thornthwaite climate classification (Thornthwaite, 1948), the studied area belongs to the subhumid moist climate (C_2), while according to Lang's classification (Lang, 1915) it also belongs to the subhumid climate i.e., the climate of low-altitude forests.

The studied stand is located at 320 to 350 m above sea level, with a slope up to 25° and western exposure. In terms of phytosociology, the stand is defined as an association of sessile oak with hairy sedge (*Carici pilosae – Quercetum petraeae* B. Jov. 1989). The soil is deep eutric cambisol, formed on neutral and basic eruptive rocks, weakly skeletal, with good properties.

Before performing silvicultural treatments, the total number of trees in the stand ($d_{1.30}$ > 5.0 cm) was 520 per ha, while the basal area amounted to 22.5 m^2/ha, and the total volume to 229.4 m^3/ha, where the share of sessile oak by volume was 63.6%, silver linden (*Tilia tomentosa*) 29.5%, common ash (*Fraxinus excelsior*) 2.7%, hornbeam (*Carpinus betulus*) 2.2%, and service tree (*Sorbus torminalis*) 2.0% (Kanjevac, 2020). After the preparatory-seed cut, 124 trees per ha remained in the stand, with a basal area of 11.9 m^2/ha and a volume of 129.1 m^3/ha, where, in addition to sessile oak trees, individual trees of common ash and service tree remained in the stand after the cutting (Kanjevac, 2020).

The combination preparatory and seed cut was conducted in 2013 when the undergrowth of accompanying tree species (silver linden, hornbeam, common ash) was removed, together with individual dead sessile oak trees. The canopy of the stand after this cut is incomplete (0.5-0.6). An experimental field of 0.25 ha (50x50 m) was set up within the stand, where the characteristics of the number and growth of seedlings (root collar diameter and height) of sessile oak and main competing species were studied on 30 sample plots of 1 m^2. In the autumn of

2017, the shoots and root suckers which appeared after the removal of the undergrowth of accompanying tree species were removed, so that the age of the competing woody species at the time of measurement was 3 years and the seedlings were predominantly of coppice origin. Considering that the majority of seedlings originated from the mast year of 2012, at the end of the vegetation period 2020 the number and growth characteristics of eight-year-old seedlings of sessile oak were studied.

RESULTS AND DISCUSSION

The average number of eight-year-old sessile oak seedlings per square meter was 5.6, the maximum was 11 and the minimum was 0 (Table 1). When it comes to competing species, the most common are silver linden whose average number of seedlings is 1.1 per square meter and hornbeam 0.5 seedlings per square meter, while other species (*Fraxinus excelsior*, *Fraxinus ornus*, *Crataegus monogyna*, *Acer campestre*, *Cornus mas*) are represented by 0.4 seedlings per square meter (Table 1). Overall, the average number of seedlings of competing species per square meter was 2.0, the maximum was 5 and the minimum was 0 (Table 1).

Table 1. The number of seedlings of sessile oak and the main competing species (per square meter)

Stat. paramet	***Quercus petraea***	***Tilia tomentosa***	***Carpinus betulus***	***Other species***	***Σ competing species***
min	0	0	0	0	0
max	11	3	2	2	5
mean	5.6	1.1	0.5	0.4	2.0
std. error	0.65	0.26	0.18	0.15	0.30
cv (%)	54.3	106.2	157.3	199.9	68.3

The average surface presence of blackberries per square meter in the studied stand is 19.3%, while the maximum is 80% and the minimum is 0%.

This indicates a pronounced presence of the main competing species of woody vegetation and ground flora. Kanjevac et al. (2021) also pointed out the pronounced presence of competing species in the regeneration layer of sessile oak forests in the area of northeastern Serbia. Also, a large number of authors point out competing vegetation as one of the key factors that threaten the success of the natural regeneration of sessile oak forests (Kohler et al. 2020).

The average height of eight-year-old sessile oak seedlings in the studied stand is 86.8 cm, while the minimum height is 21.0 cm and the maximum is 262.0 cm (Table 2). The main competing species are characterized by higher average values of height, which for silver linden is 95.5 cm (the minimum height is 43.0 cm, and the maximum is 160.0 cm), and for hornbeam is 125.8 cm (the minimum height is 81.0 cm, and the maximum is 203.0 cm) (Table 2). The blackberry,

which is the most represented of the species of ground flora, is characterized by an average height of 9.2 cm, with a maximum value of height 20.0 cm and a minimum 5.0 cm.

In addition to the fact that the blackberry can outgrow the sessile oak seedlings and thus significantly reduce the available amount of light, blackberry is also known to affect forest regeneration by forming dense thickets that overgrow and eventually press seedlings to the ground under heavy snow (Krstic et al. 2017; Kohler et al. 2020). Blackberry mostly occurs in places with greater canopy openness, i.e., the available amount of light, whereby it prefers deep and moist soils, rich in nutrients (Krstic et al. 2017; Kanjevac et al. 2021). The presence of blackberries is significantly dependent on the initial number of seedlings during the regeneration of sessile oak forests, which is why it is often considered that the initial number of seedlings per unit area is one of the key factors influencing the success of the regeneration of sessile oak forests (Kuehne et al. 2020; Kanjevac et al. 2021).

Table 2. Growth characteristics of seedlings of sessile oak and the most common competing woody species

Element	**Statistical parameter**	**Species**		
		Q. petraea	***T. tomentosa***	***C. betulus***
height (cm)	min	21.0	43.0	81.0
	max	262.0	160.0	203.0
	mean	86.8	95.5	125.8
	std. error	6.06	6.29	14.60
	cv (%)	66.7	34.8	34.8
root collar diameter (mm)	min	2.0	4.0	4.0
	max	24.0	20.0	14.0
	mean	8.0	9.1	9.2
	std. error	0.53	0.77	1.02
	cv (%)	62.9	43.8	35.1

The average value of the root collar diameter of eight-year-old sessile oak seedlings in the studied stand is 8.0 mm, while the minimum value is 2.0 mm and the maximum is 24.0 mm (Table 2). The main competing species are characterized by higher average values of root collar diameter, which for silver linden is 9.1 cm (the minimum is 4.0 mm and the maximum is 20.0 mm), and for hornbeam is 9.2 cm (the minimum is 4.0 mm and the maximum is 14.0 mm) (Table 2).

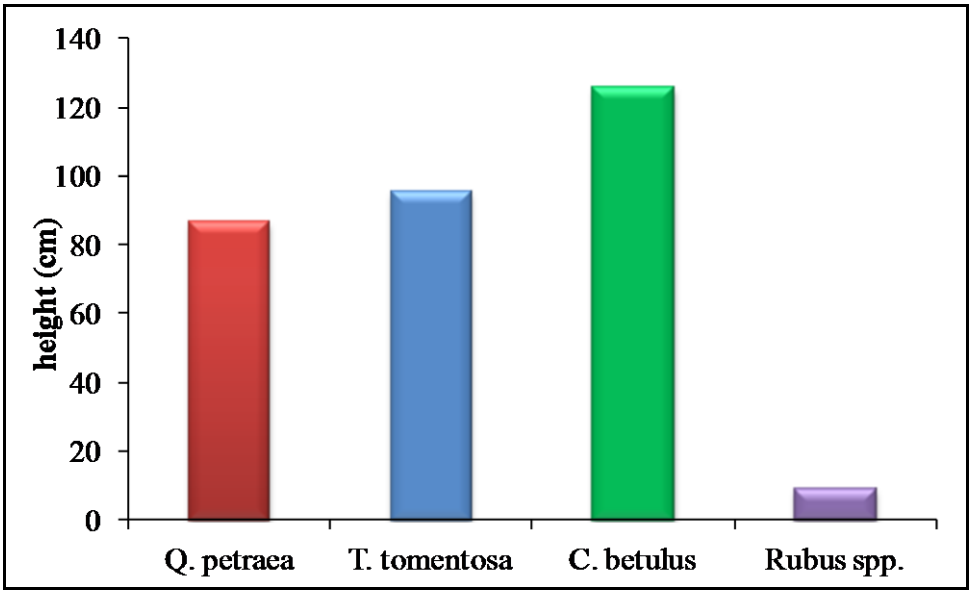
Competition between species and individuals is an ecological mechanism that affects the structure, diversity and functioning of plant communities (Robakowski and Bielinis, 2011). In oak forests, competition in the regeneration layer is a significant variable that controls the regeneration effect of these forests, and it is also known that tree species, shrubs, and ground vegetation interfere with

oak development (Annighöfer et al. 2015). The competition for the light of the seedlings of woody species at an early age is of great importance in silviculture. The competition for light and other ecological factors important for natural regeneration can occur between species or individuals of the same species and significantly depends on the bioecological characteristics of the species, as well as population density, which is one of the key factors when it comes to natural regeneration of forest and seedling development.

Due to the well-known fact that oaks are light-demanding species, any change in the parent stand leads to improved light conditions, which contributes to the development of sessile oak seedlings. However, improved light conditions can affect other species that are considered competitive in the case of oak regeneration.

Accordingly, sessile oak stands cannot be successfully regenerated if the silvicultural intervention is concentrated only on the trees of the parent stand, but it is necessary to apply auxiliary measures that will be concentrated on competing species that have the potential to overcome sessile oak seedlings (vön Lupke 1998; Ligot et al. 2013; Kanjevac et al. 2020, 2021).

Figure 1. Sessile oak seedlings and competing species in the studied stand



Graph 1 The average heights of seedlings of sessile oak and the most common competing woody species and species of ground vegetation

In addition to a significant presence, competing species of woody vegetation are characterized by very intensive development, which significantly reflects on the presence and development of sessile oak seedlings (Figure 1, Graph 1). Numerous authors state that competing vegetation can inhibit the growth of sessile oak seedlings through allelopathy or by reducing the available resources necessary for their growth and survival (Kozlowski et al. 1991; Löf, 2000). Since the eight-year-old sessile oak seedlings in the studied stand are characterized by slower growth compared to the seedlings of competing species, it is necessary to provide an additional amount of light by additional removal of the mature trees (final cut of shelterwood cutting), which will intensify the growth of sessile oak seedlings. In addition, auxiliary measures are needed to regulate the composition of the species in the regeneration layer, which will reduce the negative impact of competing vegetation on the development of sessile oak seedlings.

Accordingly, natural regeneration as an element of the close-to-nature silviculture concept is of immeasurable importance because adequate silvicultural treatments can modify the future structure of forests, influence the interaction between species, encourage wood production, and also encourage the production of numerous ecosystem services (Ammer, 2008).

CONCLUSIONS

Regeneration of sessile oak forests is one of the most important issues in modern forestry, burdened with numerous aggravating factors that have the potential to decisively influence the final outcome.

The obtained results in the paper indicate the competitive relations between sessile oak and the most important competing woody species, as well as the species of ground vegetation. The most common competing woody species are silver linden (*Tilia tomentosa*) and hornbeam (*Carpinus betulus*), while other species (*Fraxinus excelsior*, *Fraxinus ornus*, *Crataegus monogyna*, *Acer campestre*, *Cornus mas*) are represented individually. The number of eight-year-old sessile oak seedlings in the studied stand is 56.000 individuals per ha, while the number of seedlings of all competing species is 20.000 individuals per ha. The average surface presence of blackberries per square meter in the studied stand is 19.3%.

In addition to a significant presence, competing species of woody vegetation are characterized by very intensive development, which significantly reflects on the presence and development of sessile oak seedlings. In contrast, sessile oak seedlings have outgrown blackberries in the studied stand, which no longer competes with them.

The obtained results indicate that during the regeneration of sessile oak forests, special attention must be focused on the presence and dynamics of the development of competing vegetation, as well as that the success of the regeneration largely depends on controlling this factor.

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MONITORING THE REGENERATION OF NATIVE FOREST FRAGMENTS IMPACTED BY FIRE

SUMMARY

Forest fires have increased a lot in Brazil in recent years, causing air pollution, increased soil erosion processes with production of sediments, emission of greenhouse gases and overloading public health services, with severe environmental and socioeconomic losses. Therefore, the development of new analysis and mapping technologies for areas affected by fires is necessary and essential in this context of accentuated expansion of forest fires. Research monitoring flights were performed with an unmanned aerial vehicle to obtain RGB aerial images of a fragment of native forest affected by fire in July 2019. Thus, subareas were defined to define mapping patterns that can serve as algorithms for remote detection of areas affected by fires. Afterwards, land use were generated to monitor the evolution of the natural process of regeneration of the forest fragment. The studies identified that 0.77 ha was affected by the fire and that from August to November of the same year, the area was completely regenerated, and all remnants of the forest fire were eliminated. Thus, the work explains a strategic alternative for reducing, combating, and monitoring forest fires. Furthermore, it also contributes as a tool for the preparation of recovery plans for areas impacted by forest fires.

Key words: Monitoring; Remote Sensing; Environmental Planning; Regeneration; Nature Conservation; Brazil.

INTRODUCTION

Global warming has increasingly raised the concerns of both state administrations and the scientific community in recent decades (Stefanidis &

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Alexandridis, 2021). Climate change and global warming are expected to affect natural hazards like soil erosion, increased sedimentation and depositions in water reservoirs, flooding, and wildfires worldwide (Hysa *et al*., 2021; Spalevic *et al*, 2020; Hysa *et al*., 2020; Chalise *et al*, 2019; Spalevic *et al*, 2013). Forest fire is understood as any uncontrolled fire that affects vegetated areas, because of natural or anthropogenic influences, which negatively impacts animal and plant species and human lives. When criminal or without proper management, it causes negative socioeconomic and environmental effects (Biazatti *et al*., 2020).

Increased air pollution, reduced soil fertility, increased rates of soil erosion and runoff are among the harmful effects of forest fires. In Brazil, fires are the main threats to environmental preservation areas and are most often premeditated by economic interests and, to a lesser extent, caused by acts of vandalism, recreational activities and the negligence of public control and inspection agencies (Tebaldo *et al*., 2013).

Fire risk varies with environmental conditions and characteristics of vegetation types. In other words, monitoring fires in a continental country like Brazil represents an arduous and extremely complex task, due to the logistical difficulties of the territorial extension and the diversity of forest formations. According to Bizatti *et al*. (2020), most forest fires are caused by human activity. These activities can be related to criminal acts or not. However, the impacts triggered by fires cause damage to the landscape and its elements (Eugenio *et al*., 2016).

Recently, in Brazil, wave of fires was recorded that endangered the Brazilian biomes in a generalized way, especially at the Cerrado (Savanna), the Pantanal and the Amazon. Such events are increasing more and more in the Brazilian territory, not only promoting environmental devastation at different scales, but also accelerating and accentuating climate change, negatively affecting the soil water regime and causing water scarcity, which generates insecurity and socioeconomic and environmental uncertainties (Sarra and Mülfarth, 2021).

The widespread use of geographic information systems and geoprocessing and remote sensing techniques allowed advances in methods, like images from unmanned aerial vehicles - UAV (Sestras *et al*, 2021; Felix *et al*., 2021; Sestras *et al*, 2020), for mapping natural phenomena (Kalehhouei *et al*., 2021; Sestras *et al*, 2019; Stefanidis *et al*, 2021). Thus, these technologies are fundamental tools for the development of socioeconomic and environmental management plans, and even for the prevention and combat of forest fires and promoting the economic and environmental sustainability of populations dependent on forest resources. Thus, in countries with continental dimensions, such as Brazil, promoting the use of geotechnologies is essential for the successful mapping, prevention and monitoring of natural phenomena and disasters (Girona-García *et al*., 2021).

The objective of this work was to present, in the degradation scenario of the main Brazilian biomes, the patterns for detecting forest fires in images obtained by the UAV and to monitor the regeneration of native forest in an environmental preservation area affected by fires.

MATERIAL AND METHODS

The study was carried out in a permanent preservation area of 2.81 ha in the Santa Clara Unit of the Campus Headquarters of the Federal University of Alfenas. The area belongs to the Córrego do Cemitério hydrographic sub basin, a tributary of the Furnas Hydroelectric Power Plant reservoir (Figure 1). The sub basin is located at the urban perimeter of the Alfenas Municipality, southern Minas Gerais State, South-eastern Brazil.

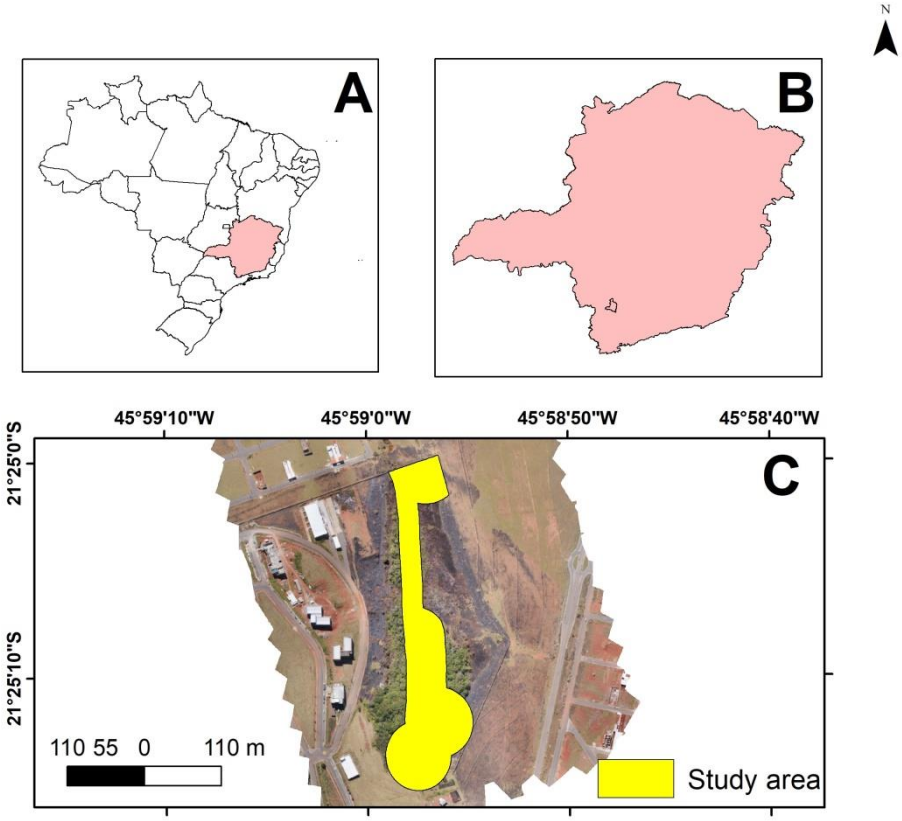

Figure 1. Location of the study area: A- Brazil and Minas Gerais; B- Minas Gerais and Alfenas; C- RGB Image of the study area.

The geological framework is constituted by garnet-biotite-gneisses and biotite gneisses with quaternary soil covers, with fluvial deposits of gravel, sand, and clay (Pinto *et al.*, 2020). The soils are mainly composed of Latosols and Indiscriminate Floodplain Soils (Marangon *et al.*, 2017). The climate, according to Köppen's classification, is Tropical Mesothermal (Cwb) (Sparovek *et al.*, 2007), with two well-defined seasons: hot and humid summer and dry and cold winter.

The dominant riparian vegetation in the permanent preservation area belongs to the Atlantic Forest biome, which has a history of intense devastation

since the beginning of the occupation of Brazilian territory, in the 16th century (Carvalho *et al.*, 2007). Specifically, the area comprises the presence of at least three mapped springs, with three 1st and one 2nd order channels (Marangon *et al.*, 2017).

To obtain the images of the study area by the fires, Drone Phantom 4 with an RGB sensor, a Sony EXMOR camera, which captures images in real colours, was used. The flights were carried out in early August, September, and November 2019 at 100 m altitude and with 40% frontal and lateral image overlap.

The flights were planned and executed in Pix4DCapture software. The images obtained were processed using Structure-from-Motion (SfM) software Agisoft Photoscan Professional® v.1.2.7. A reconstructed mesh surface was derived from the point cloud using the automatic point classification procedure based on maximum angle, maximum distance, and pixel size. Then the image was orthorectified and georeferenced (Panagiotidis *et al.*, 2017).

Then, maps of land use and occupation inside the preservation area were made using ArcGIS 10.5 software. Manual classifications were carried out for the months of August, September, and November 2019. Then, area calculations were made for the respective classifications. Finally, the variation of land use and occupation in the period was evaluated, to verify the evolution of the regeneration of the area affected by the fire (Parsipour *et al.*, 2019).

Three sites in the impacted area were selected as fire mapping standards. These sites also served as control points for diagnosing the evolution of vegetation regeneration. Such places were grouped in a figure and evaluated according to the scientific literature on the subject (Cesljar *et al*., 2021; Ganaie *et al.*, 2021).

Finally, the environmental and socioeconomic impacts of forest fires were evaluated, with emphasis on the adoption of strategies to combat and control clandestine and/or criminal fires in Brazil.

RESULTS AND DISCUSSION

This research produced the land use and occupation map in the area affected by the fire for the months of August, September and November 2019, the dimensions of land uses for the months studied, the rates of variation of land uses in the period, and fire mapping patterns.

Figure 2 illustrates three areas that, due to their unique characteristics, can contribute to remote detection methods for areas affected by fires. The figures were grouped and organized into three classes, A, B and C, to facilitate the interpretation of the obtained features.

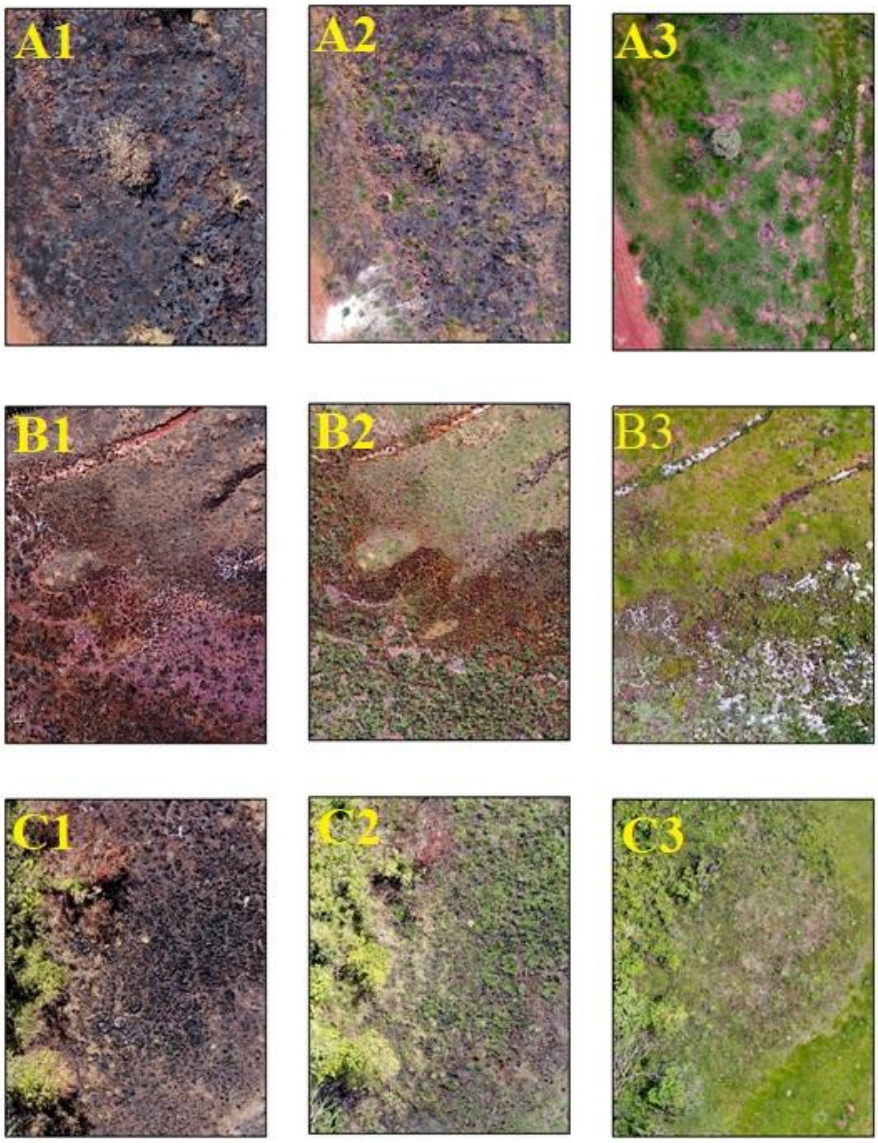

Figure 2. Areas impacted by fires. A1: Countryside area affected by fire in August 2019; A2: Countryside area affected by fire in September 2019; A3: Countryside area affected by fire in November 2019; B1: Floodplain area affected by fire in August 2019; B2: Floodplain area affected by fire in September 2019; B3: Floodplain area affected by fire in November 2019; C1: Forest fragment edge area affected by fire in August 2019; C2: Forest fragment edge area affected by fire in September 2019; C3: Forest fragment edge area affected by fire in November 2019.

In Figure 2 (A1, A2 and A3 and C1, C2 and C3) the area had a very rapid regeneration process and that was recorded for the period of just three months, no fire scars were identified. The full recovery of the landscape after just three months could indicate the possibility of using controlled forest fires for landscape management. Even in absolutely controlled conditions, it is necessary to follow safety procedures, such as specialized technical monitoring, prior study of the species exposed to the procedure and the presence of fire brigades with training and equipment to avoid extreme events of loss of fire from control conditions.

The areas B1, B2 and B3 (Figure 2) illustrate the floodplain impacted by fire. Visual analysis of the images allows the identification of wetlands in B1 and B2 in brown and orange tones, indicating that the fire contributed to promote erosive processes and sediment transport from springs and drainage channels. Thus, as vegetation regeneration evolves, the water regains its colourless visual appearance.

Forest fires cause loss of soil and water quality, in addition to reducing the quantity and diversity of microorganisms, reducing macro fauna, and also causing direct effects in the increase in sediment production, as pointed out by Girona-García *et al.* (2021).

The recent fires in the Amazon region are criminal and difficult to monitor and control due to their extent and access difficulties. In this context, any combat and inspection action is compromised when there is no interest of the different levels of government in articulating actions to combat fires and their adverse effects, contrary to world public opinion (Girona-García *et al*., 2021). Thus, the absence of public and inspection policies exposes the population to risks arising from fires, such as poisoning, severe lung diseases, especially in children and the elderly, and burns (Ignotti *et al*., 2007). In periods of recurrent fires, such as recent ones in Brazil, hospital admission rates increase and burden the public health system. In 2020, in particular, the COVID-19 pandemic began, which further increased the pressure on the public health system (Henderson, 2020). This theme of forest fires is recurrent and has been debated for many years (Ignotti *et al*., 2007), with no prospects for improving this situation so far. Historically, governments have not taken the necessary measures for economic agents to comply with environmental legislation. The Amazon biome is increasingly threatened by the absence of effective public policies, whose consequences threaten life on the planet and the future of humankind.

The monitoring of land use and occupation in the period is illustrated in Figure 3. It was possible to identify the areas impacted by fire within the permanent preservation areas. Such information is essential for the preparation of environmental recovery plans. As shown in Figures 3A, B and C, during the period there was a gradual reduction in the areas affected by the fire.

In the short period covered, the formation of new forest fragments was not identified. However, regeneration areas were identified, mainly in November (Figure 3C), after a short period of rain between September and October.

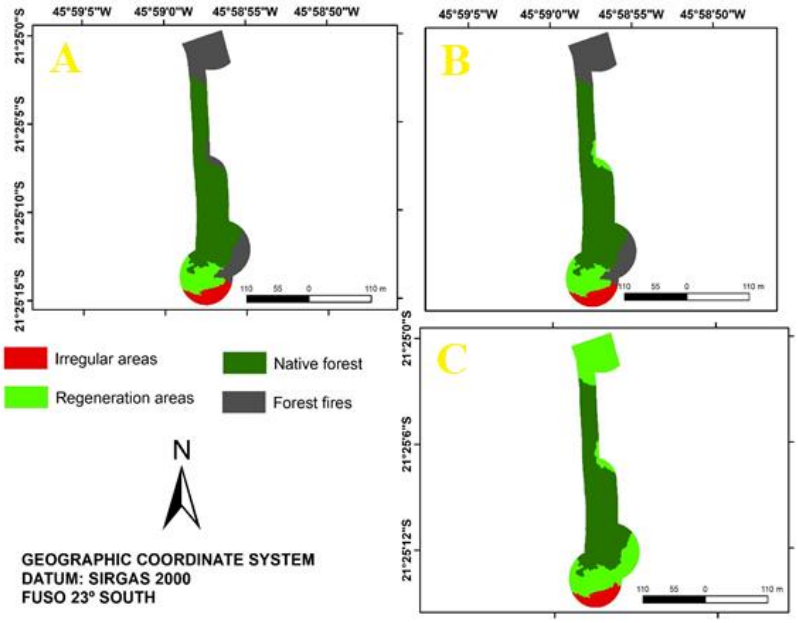

Figure 3. Land use and occupation maps in an permanent preservation area affected by fire. A: August; B: September; C: November.

The distribution of the areas of the respective land uses show that the most expressive land use is the Native Forest, which did not change over the period. Then, the most expressive use identified were the areas burned, which were reduced to zero between August and November 2019 as illustrated in Figure 2 and 3. In contrast to the reduction in areas burned, there was an increase of the areas of regeneration. During the period covered, there was no change in the areas classified as irregular, as they are occupied with infrastructure within the permanent preservation area.

Over the years, forest fires have devastated Brazilian biomes. Effective public policies to combat and prevent forest fires are practically non-existent and the lack of inspection and punishment for legal infractions end up encouraging clandestine forest fires. Management and inspection agencies are unable to adequately fulfil their role due to lack of infrastructure, specialized technical staff and investment in the development of firefighting policies (Sobrinho and Ramos Júnior, 2020).

In this context, about 90% of fires in Brazil are forest fires, mainly in the management of pasture areas for agricultural production, with direct ecological damage to Brazilian biomes (Sobrinho and Ramos Júnior, 2020). Among those most affected by forest fires, we can highlight the Cerrado (Savanna), Pantanal and Amazon Biomes. Theses biomes are strategic for the maintenance of biodiversity and good results in environmental quality indicators (Sobrinho and Ramos Júnior, 2020).

This intense conversion of forest to pasture and agricultural areas has a direct impact on the emission of greenhouse gases from the carbon state in the region's biomass and soils. The Amazon region, as highlighted by Fearnside (2018), has significant carbon stocks that can be released as greenhouse gases when the native forest is converted for any other use. Despite uncertainties in carbon stock estimates, it is necessary to expand research on the impacts of converting native forest to other uses.

Fires larger than 50 ha grew from 32% in 2016 to 46% in 2019 in southwestern Amazonia, suggesting the authorities' inefficiency or lack of interest in enforcing environmental and forest fire fighting laws. Private properties accounted for 1/5 of the burned areas (Silva *et al.*, 2021).

According to Silva *et al*. (2021), to prevent deforestation and forest fires, authorities should promote strategic actions and allocate human and material resources for environmental surveillance and law enforcement. Thus, the authorities should organize a forest fire management plan in vulnerable areas aiming at environmental sustainability in the Cerrado (Savanna), Caatinga, Amazon and Atlantic Forest biomes.

In addition, it is necessary to seek socioeconomic development that reflects the advantages of maintaining and expanding the native forests of Brazilian biomes, favoring a society based on the provision of environmental services and abandoning the outdated vision of the colonizer, which is a predatory and inconsistent vision with main challenges posed by climate change (Fearnside, 2018).

CONCLUSIONS

The equipment used fulfilled the objective of being useful for detection of areas affected by fires, allowing the identification of mapping patterns that can be used as a reference for automated mapping.

The visual analysis of the images allowed us to conclude that fires directly impacted the quality of the water, erosion processes and sediment production.

The methodological procedure used made it possible to monitor the evolution of land use in the area affected by fire and to monitor the development of the initial process of regeneration of the burned area.

Strategic actions by the state administration are necessary and urgent to fight clandestine forest fires and to monitor the application of Brazilian environmental legislation.

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Nebi BİLİR [1]

GROWTH PERFORMANCES IN PLANTING AND SEEDING AREAS OF TAURUS CEDAR (*CEDRUS LIBANI* A. RICH.)

SUMMARY

Planting and seeding are the main tools in successful forest establishment. It is well discussed based on global climate change and sustainable forestry recently. Seedling height, diameter at base, height increment of last growth period of planting areas established by two-year containerized seedlings and seeding areas established by sowing 13 kg pure seeds per ha were compared at sixth-year afforestation of Taurus Cedar (*Cedrus libani* A. Rich).

Averages of seedling height, diameter at base, height increment of last growth period were higher in planting than seeding. For instance, averages of seedling height were 150.4 cm in planting, and 81.6 cm in seeding. Beside, number of seedling was higher in planting (1767/ha) than seeding (1300/ha) at measurement period. Significant differences were found among sampled areas within planting and seeding areas, and between planting and seeding areas for the characteristics. Positive and significant ($p<0.05$) relations were found between the pairs of seedling height, diameter at base and height increment in both areas. Planting could be preferred to seeding in suitable sites in forestry practices.

Keywords: Afforestation, Forest establishment, Planting, Seedling, Sowing.

INTRODUCTION

According to inventory of United Nations Food and Agriculture Organization, there were 3.9 billion ha forest area in whole world, while an average of 4 million ha forest were disposed per year between 1990 and 2015 (FAO, 2015). Its wide distribution and global importance are getting highlights the protection of present forests and conversion of degraded areas. However, many environmental and biological factors such as climate, edaphic, geographic, afforestation method and species could be effective in the protection and conversion. Afforestation method and species can be intervened factors by forestry practices in these factors. These methods have

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advantages and disadvantages over each other. For instance, seeding can be performed for the species which has abundant and cheap seed.

In the study, afforestation methods are compared based on Taurus cedar (*Cedrus libani* A. Rich) which has limited studies. The species is classified as one of the economically important tree species for Turkish forestry and the "National Tree Breeding and Seed Production Programme" (Koski and Antola, 1993) because of its valuable wood properties such as light, soft, highly durable, special colour, smell and of being easily processed by hand and machine tools (Bozkurt *et al*., 1990), and large natural distribution, and high adaptation ability to different environmental conditions (i.e. potential tree species to resistance climate change). However, 48% (235229 ha) of forest of the species is unproductive (Anonymous, 2020) similar to general Turkish forest area. But together with its mixed stands, extremely degraded stands, and bare karstic lands occurred after degradation of Taurus cedar, range of this species covers an area of over 600 000 ha in Turkey (Saatcioglu, 1976; Boydak and Calikoglu, 2008). Forest establishment including afforestation, reforestation, artificial regeneration, rehabilitation and private plantation are the most important way in conversion of unproductive forest and potential area to productive (Bilir and Gulcu, 2015). Afforestation by planting and seeding is a fundamental tool for the establishment of new forests on barren landscapes and restoration of degraded forests ecosystems (Caliskan and Boydak, 2017). For instance, 85 899 ha reforestation was established by broadcast seeding method on bare karstic lands resulted from the destruction of Taurus cedar between 1984 and 2006 (Boydak and Calikoglu, 2008). About half a million seedling was produced for Turkish reforestation of the species in last decade (Anonymous, 2020). Beside, Taurus cedar have also planted in other countries for commercial forestry purposes such as in Italy (Fusaro, 1990), in Bulgaria (Tsanov *et al*., 1990), in Argentina (Ottone and Carloni, 1990). However, limited study was carried out to examine on the success of planting and seeding methods (e.g., Boydak and Ayhan, 1990; Boydak, 2003; Ayan *et al*., 2017; Ozel *et al*., 2018) in Taurus cedar. Besides, growth performances of the Taurus cedar in the planting and seeding areas have not been compared to select better reforestation method, yet. Therefore, in this study, growth performances in the planting and seeding sites were compared in order to contribute the present and future practices in forest establishment of the species.

MATERIAL AND METHODS

Planting and seeding sites were sampled from sixth-year afforestation established by seeds of seed stand (36°31'43" N latitude, 32°46'50" E longitude, 1750 m altitude) of the species at southern part of Turkey (Table 1, Figure 1). Data of seedling height (**H**), diameter at base ($\mathbf{D_o}$) and height increment of last growth period (**HI**) was collected from three planting (**P**) and three seeding (**S**) areas which each of them was 300 m^2. The data was collected from sixth-year afforestation to minimize planting and seeding shock of first years. The planting areas were established by 3x1.5 m spacing and two-year containerized seedlings,

while seeding areas were established by sowing 13 kg pure seeds/ha by routine governmental practices such as soil treatment, tending, dilution, re-seeding/planting in first three years.

Table 1. Geographic details of the planting and seeding areas

Method	Area	Latitude (N)	Longitude (E)	Altitude (m)	Aspect
Planting	**P1**	37°12′41″	31°59′10″	1865	West
	P2	37°14′40″	31°59′31″	1830	West
	P3	37°26′58″	31°46′42″	1210	West
Seeding	**S1**	37°13′30″	31°54′22″	1605	East
	S2	37°13′26″	31°56′34″	1720	West
	S3	37°14′38″	31°57′22″	1725	West

There could be many biological and other environmental factors on success performances of forest establishment. The sampled areas were selected from limited afforestation area of the species to minimize effect of these factors (i.e., similar soil characteristics, temperature, rainfall) based on the purpose of the comparison of afforestation methods in the study. Average slope and soil texture across the sampled areas was 15% and sandy loam at calcareous bedrock. Monthly climatic data of the area for last decade was given in Table 2 (URL, 2019).

Figure 1. Sampled areas from planting (left side) and seeding (right side)

Table 2. Monthly climatic data of the area for last decade

Characteristics	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Average temperature (°C)	-0.3	1.0	5.4	10.9	15.6	20.1	23.5	22.9	18.6	12.4	5.7	1.4
Average max. temperature (°C)	4.6	6.6	11.8	17.5	22.2	26.7	30.2	30.0	26.3	20.0	12.4	6.0
Average precipitation	33.7	23.8	26.3	38.6	41.3	20.9	7.4	5.2	11.3	32.8	37.1	41.0

The sampled area was classified by Erinc's Precipitation effectiveness index (Im) which was used widely in estimation of climate type (Erinc, 1965) as:

$$Im = P/Tom \quad (1)$$

Where, P was the annual total precipitation (mm) and Tom was the annual average maximum temperature (°C).

The sampled area was semi-arid according to Precipitation effectiveness index (Im=17.6).

The sampled areas of planting and seeding were compared for the growth characteristics by following model of multiple analyses of variance (MANOVA) using SPSS (SPSS, 2011).

$$Y_{ijk} = \mu + P_i + S_j + P(S)_{i(j)} + e_{ijk} \quad (2)$$

Where Y_{ijk} is the observation from the k^{th} seedling of i^{th} sampled area of j^{th} method (planting or seeding), μ is overall mean, P_i is the effect of the i^{th} sampled area, S_j is the effect of j^{th} method, $P(S)_{i(j)}$ is the effect of interaction between method and sampled area, e_{ijk} is random error.

Sampled areas were grouped by Duncan's multiple range test (Duncan, 1955). Relation among the characteristics was estimated by Pearson' correlation at SPSS (SPSS, 2011).

RESULTS AND DISCUSSION

Characteristics

Planting showed higher growth performances than seeding for the seedling height, diameter at base and height increment of last growth period (Table 3). Averages of height, diameter at base, and height increment of last growth period were 150.4 cm, 38.6 cm and 35.5 cm in planting site, and 81.6 cm, 26.2 cm and 21.7 cm in seeding site, respectively. Averages of height were 43 cm at three years, 116 cm at six years, and 201 cm at nine years in a planting site originated from seed stand of the species (Bilir, 2004). Averages of height and root-collar diameter of three-year seedling were 20.8 cm and 4.1 mm in the species (Demirci and Bilir, 2001). Averages of height and diameter at base were found 498 cm, 724 cm and 313 cm, and 15.2 cm, 19.3 cm and 7.1 cm in three planting sites which were 12, 12 and 10 years in the species, respectively (Bilir *et al*., 2018).

Annual height growths were 25.1 cm at planting and 13.6 cm in seedling areas in the present study while it was found 41.5 cm, 60.3 cm and 31.3 cm in three planting areas by Bilir *et al*. (2018), and 22.6 cm by Ozel *et al*. (2018). It showed that annual height growth was lower in seeding than planting in the present and early studies. However, there could be many environmental (e.g., preparing of site, spacing, climatic, edaphic) and biological (e.g., provenance, species) effects in success and growth performance of forest establishment (Yazici and Turan, 2016; Ayan *et al*., 2017; Yazici, 2018). Boydak (1996) reported that the species increased the growth performance fast after five or six years in natural stands of the species.

Table 3. Averages ($\bar{x}$) and standard deviation (SD) of the characteristics and Duncan's tes results for the sampled areas

Area	N*	Height (cm)		Diameter at base (mm)		Height increment (cm)	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
P1	40	137.7^{b**}	42.4	37.7^{c}	6.8	31.5^{bc}	14.3
P2	51	133.6^{b}	40.7	36.0^{c}	6.1	34.0^{cd}	14.1
P3	68	170.6^{c}	50.7	41.2^{d}	7.4	38.9^{d}	15.2
Mean		150.4	48.6	38.6	7.2	35.5	14.9
S1	36	83.0^{a}	34.7	23.8^{a}	8.2	28.1^{bc}	15.6
S2	45	74.5^{a}	27.3	27.5^{b}	8.2	13.0^{a}	8.5
S3	36	88.9^{a}	31.8	26.8^{ab}	6.8	26.1^{b}	14.3
Mean		81.6	31.5	26.2	7.9	21.7	14.5
Overall		121.2	54.2	33.3	9.7	29.6	16.2

*; Number of individuals of sampled areas, **; Means with the same letter in the columns are not significantly different.

There were also large differences among sampled areas within planting and seeding and between planting and seeding areas for the characteristics. Averages of height varied between 74.5 cm (S2) and 170.6 cm (P3). Average of height increment of last growth period was three times higher in P3 (38.9 cm) than S2 (13 cm) (Table 3). The differences were also supported by the results of analysis of variance showing significant ($p \leq 0.05$) differences among sampled areas and between planting and seeding. Planting showed 84.3% higher height performance than seeding. It was 27.7% at planting areas (P2 and P3), and 19.3% at seeding areas (S2 and S3) for height (Table 3). Beside method x area interactions were found to be significant ($p \leq 0.05$) for the characteristics. Similar results were also reported among planting sites of the species (Bilir *et al*., 2018; Ozel *et al*., 2018). Planting areas had larger variation than seeding sites for H and HI based on standard deviation. And also, sampled areas were more homogenous for H than D and HI based on results of Duncan's multiple range test (Duncan, 1955) (Table 3). Beside, number of seedling was higher in planting (1767 seedlings/ha) than seeding (1300 seedlings/ha) at measurement period. Numbers of seedling were reported 6000 /ha on sunny slopes and 14000/ha on shady slopes at reforestation areas established by seeding of the species at the end of fifth vegetation period

(Boydak and Calikoglu, 2008). The results emphasized that importance of afforestation method, and local forestry practices.

Table 4. Correlation coefficients between the pairs of for seedling height (H), diameter at base (D) and height increment (HI) for the polled sampled areas

Methods	Traits	H	D_o
P		**-**	
S	**H**	**-**	
P&S		**-**	
P		**.869***	**-**
S	**D_o**	**.831***	**-**
P&S		**.897***	**-**
P		**.819***	**.663***
S	**HI**	**.739***	**.401***
P&S		**.814***	**.650***

*; correlation is significant at the 0.05 level.

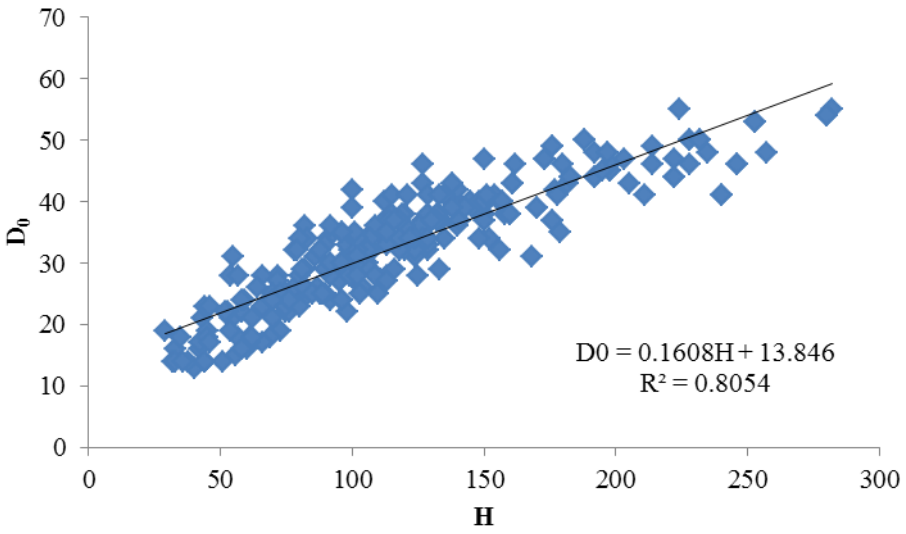

Figure 2. Relations between height (cm) and diameter at base (mm) for the areas of planting and seeding

Relations between the pairs of characteristics

Positive and significant ($p<0.05$) relations were found between the pairs of seedling height, diameter at base and height increment in all sampled areas (Table 4). Relation between height and diameter at base was also shown for the areas of planting and seeding in Figure 2. Similar relations between height and diameter were also reported by Bilir (1997 and 2004), Yazici (2018), Ozel *et al.* (2018), and Bilir *et al.* (2018) in the species.

CONCLUSIONS

Results of the study showed that planting could be preferred to establish fast stand to seeding. Differences of growth performances among sampled areas within areas emphasized importance of local forestry practices.

Significant relations between the pairs of characteristics could be used in future studies, and forestry practices such as forest tending and comprehensive determination of stand performance. Future performances of these areas or different study areas could be investigated for the more accurate conclusions.

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DIAMETER STRUCTURE OF THE STAND AND TIME OF PASSAGE: AN ANALYSIS IN PRIMEVAL FORESTS JANJ AND LOM IN REPUBLIC OF SRPSKA (BOSNIA AND HERZEGOVINA)

SUMMARY

The research was carried out in primeval forest stands of beech, fir and spruce (*Piceo-Abieti-Fagetum illyricum*) on brown dolomite soils (Janj) and dominantly shallow limestone soils (Lom). The diameter structure is typical for primeval forests with the participation of large trees in which physiological weakening is visible, especially in the Lom primeval forest. At the same time, the stands are characterized by the specifics of the transition from the terminal to the initial development phase, with a very pronounced lower floor of the stands where beech and fir dominate. Significant differences in the size of the diameter increment are characteristics for fir, which in the Lom primeval forest has a significantly higher diameter increment at the same diameters than in Janj. The current diameter increment of fir in Janj is 2.3 mm/year and in Lom 4.5 mm/year. The average current thickness growth of spruce in Janj is 0.2 mm higher on average than in Lom and amounts to 2.9 mm/year. The current thickness growth of beech in Lom is significantly higher (4.9 mm/year) than in Janj (2.3 mm/year). The average time of passage is the highest for fir in the Janj primeval forest and for the whole stand is 45 years, and the lowest for spruce in the Lom primeval forest 22 years. Beech trees in the lowest diameter classes have a significantly shorter time of passage than fir and spruce. Fir in the Janj forest has an average relative age of 85 years higher than in Lom. Spruce shows the largest difference in relative age between primeval forests in thinner diameter classes, and unlike fir, the difference in age decreases with increasing diameter.

Keywords: stand structure, time of passage, diameter increment, primeval forests

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INTRODUCTION

Old growth forests cover is only around 3% of EU forested land and being of paramount importance (Barredo et al., 2021). Old-growth stands have developed for a long period of time without relevant human impact. Structural characteristics of old-growth forests represent a worthy model for management and structuring of managed forests (Motta et al., 2015; Vasic et al., 2018; Chivulescu et al., 2019; Curovic et al., 2020). Mixed coniferous and deciduous forests in the area of beech, fir and spruce forests in Bosnia and Herzegovina occupy 716.200 ha. In these forests of production character, the average volume is 309.95 m3/ha. The average current volume increment is 7.91 m3/ha. Management is often based on a selection-tree system, and the obsolescence of the offspring, the reduction of the canopy, and the lack of care measures are the basic problems in management. Former notions of natural harmony contained in the permanent selective structure of primeval forests and the pursuit of a similar situation in commercial forests have become a dangerous unnatural creation without the ability to self-sustain.

Due to different intensities of felling and creation of different conditions for tree canopy development in commercial forests, diameter increase is very variable, and in primeval forests the transition of trees from lower to higher diameter classes mainly depends on spontaneous natural processes of different development phases, habitat conditions and tree species. The time of passage means the average number of years required for all trees of a diameter class to grow the entire width of the same and move to a higher diameter class (Miletić, 1950). Knowing the time of passage is of special importance for forest management because its knowledge determines the average time dynamics of tree diameter changes, which is reflected in the horizontal structure of stands. In addition, the time of passage is important for the proper selection and care of the crop trees. By removing diseased and trees of poor phenotype, we reduce the time of passage (Govedar, 2005). The average time of passage of individual diameter classes is almost equal at higher tree thicknesses.

In the selective forest, the width of the rings usually increases with the age of the trees, and from a certain diameter it is maintained at the same amount. In this phenomenon of permanent maintenance of rings at the same width, and therefore of equal diameter increment, certain legality can be seen, confirmed for both fir and spruce, the types of trees most suitable for selective management (Hufnagl, 1939). However, deviations from this regularity may occur because in stronger diameter classes there may be a re-increase in the time of passage in fir and spruce in better habitat conditions, which is caused by the presence of usually obsolete trees of poorer vitality (Flury, 1932). If the sum of the time of passage (relative age) is shorter, it indicates more favorable habitat conditions, so this parameter may additionally affect the productivity assessment. This paper aims to determine the average time of passage of trees from lower to higher diameter classes as well as their relative age.

MATERIAL AND METHODS

The research was carried out in the Janj and Lom primeval forests in mixed stands of fir, spruce and beech, which phytocoenologically belong to the *Piceo-Abieti-Fagetum illyricum* community. The Janj primeval forest reserve was set aside in 1954 as a facility for scientific research, with a total area of 295 ha. In September 2021, it was declared as a natural world heritage site by UNESCO. This natural asset on the territory of the municipality of Šipovo is protected by the highest category Ia, according to the classification of the International Union for Conservation of Nature (IUCN). The Janj old-growth forest reserve is located on the Stolovaš mountain, between 44°07′ and 44°10′ north latitude and between 17°15′ and 17°17′ east longitude. The primeval forest is located between 1.180 and 1.510 meters above sea level (Fig. 1). The Lom old-growth forest reserve was protected in 1956 as a strict nature reserve in which economic activity was prohibited. It is located between 44º27′ and 44º28′ north latitude and between 16º27′ and 16º30′ east longitude. The reserve belongs to the massif of the mountain Klekovača, on the Lom mountain. The altitude amplitude is from 1.250 to 1.522 meters above sea level.

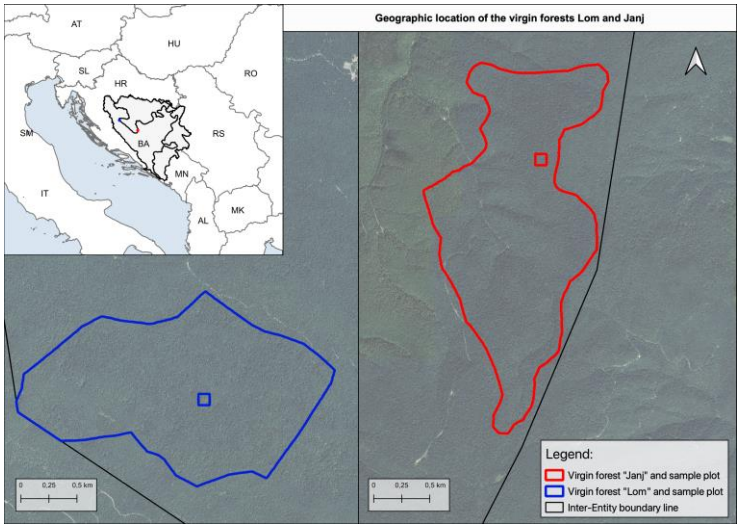

Figure 1. The geographic location of the sample plots

According to the ecological-vegetative regionalization of BiH (Stefanović et al. 1983), the stands belong to the area of the inner Dinarides, the western Bosnian, limestone-dolomite area. The Janj primeval forest is dominated by dolomite, and the Lom primeval forest by a limestone geological base and typical soils of calcomelanosol, calcocambisol and luvisol have developed on them. The research area is dominated by the mountain perhumid climate which is characteristic of the inner Dinarides. According to the classification system, the stands are different concerning the degree of stoniness, which is much more pronounced in the Lom primeval forest and can mainly be classified into two

types of forests (Ćirić et al., 1971): mixed beech, fir and spruce forests on brown dolomite soils (Janj) and forests on dominantly shallow limestone soils (Lom).

In each stand, there is one experimental area of 100x100 m. All diameters and heights of all trees with diameters larger than 5.0 cm were measured. To determine the dependence of tree heights (h) on their diameters (d), the Prodan function was used:

$$h = d^2/(a + ad + ad^2) + 1.30$$

Determination of site class by tree species was performed using site classes dispositions for fir, spruce and beech that are valid in BiH (Drinić et al. 1980). To determine the site class, the assumption was used that only tree heights thicker than 50 cm are favorable for it. In each diameter class, wood samples were taken from 5 fir, spruce and beech trees using a Pressler drill. The dependence of the current diameter increment of fir and spruce on the diameter was determined by applying a second-order parabola. The average times of passage were determined by diameter classes and tree species by the direct method based on the taken samples. The average time of passage of the entire stand is determined by the formula:

$$T_{sast.} = \frac{\sum n_i t_i}{\sum n_i};$$

$T_{sast.}$ – the average time of passage of the entire stand,
n_i – number of trees in the i - th diameter class,
t_i – the average time of passage of trees of the „i“ diameter class.

RESULTS AND DISCUSSION

Diameter structure

The main feature of the diameter structure of trees in primeval forests is the presence of very thick ancient trees of a specific phenotype (Govedar et al. 2018). The total number of trees in Janj is 588 per ha, and in the Lom 548 per ha. The ratio of mixture to the number of trees in the Janj stand was fir (41.4%), beech (35.6%), spruce (23.0%), and in Lom beech (47.6%), fir (35.4%) and spruce (17.0%). The analysis of the diameter structure shows several generations of trees in both primeval forests (Fig. 2). Beech dominates in the lower diameter classes, and the low presence of spruce is caused by insufficient ingrowth, so the spruce failed to develop into the category of trees above the taxation limit. Intensive natural regeneration of beech and dominance in thinner diameter classes in primeval forests usually occurs after fires and destruction by winds (Tregubov, 1941; Šafar, 1955), which causes the appearance of structural forms that are characteristic of stands with fast and short regeneration (Klepac, 1963). Such structural forms characterize primeval forests that are in the initial phase of development where the natural competitive power of beech dominates. Abundant

regeneration of beech in places of the broken canopy and at the same time its predominance in thinner diameter classes is a natural phenomenon that accompanies the development of primeval forests in the Dinarides, so it is "a hasty conclusion about the change of species" (Fukarek, 1965). The oldest generation of trees consists mainly of ancient very developed and tall fir and spruce trees of a specific phenotype. In these trees, there is a visible physiological weakening (especially in the Lom primeval forest) and signs of disease from various species of rot *Armillaria mellea*, *Fomes annosus* and others.

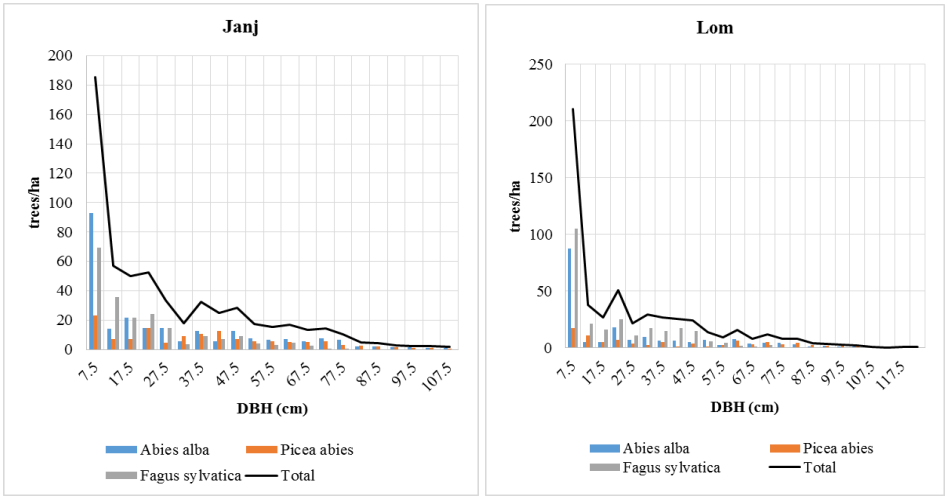

Figure 2. Diameter distribution of trees in primeval forests Janj and Lom

In the category of the largest trees in both primeval forests, the representation of fir and spruce is approximately equal, and beech trees are not represented in the category of the thickest trees in stands. According to the classification of Korpel (1995), stands still have more characteristics of the ingrown stage and a more pronounced initial phase of development within this stage. The basic characteristics of developmental phases (duration, structure, spatial distribution, etc.) depend, among other things, on the length of life of tree species that build a primeval forest, which is why it is necessary to know the times of passage and relative age of trees. Analyzing the diameter structure of stands in Janj and Lom primeval forests, it can be concluded that the stands are characterized by the specifics of the terminal and initial phase, with a pronounced lower floor of the stand where beech and fir trees dominate and the upper floor where dominate ancient fir and spruce trees.

Height curves and stand site class

The height curves in primeval forests do not have a typical esoid shape, and the point of inflection in all curves is located to the right of the Y-axis near the coordinate origin. In the primeval forests, graphically expressed functional dependence of height on diameter represents the height curve of development,

because it is subject to changes in a longer period (Milin, 1965; Stamenković, 1974). It was determined that the main tree species belong mainly to the second site class (Tab. 1). A slower flow of curves occurs when the tree comes to higher light and then the tree yields in height, and intensifies the diameter increment (Rozenbergar et al. 2007), and this is characteristic of one-season stands of low growth (Drinić, 1956). The investigated stands are of normal and very good overgrowth, so their height growth is still intense, especially in spruce trees. Fir in Lom reaches higher heights at the same diameters, so it belongs to a better quality (Fig. 3). Medium-thick and thick trees are on average about 3.0 m higher in height in Janj than in Lom. These differences in heights did not cause differences in site class, so spruce in both primeval forests belongs to the II site class. Earlier research related to the Klekovača primeval forest indicates that up to a diameter of 30 cm there are no significant differences in the heights of beech, fir and spruce, and in the primeval forest on Grmeč these differences are not observed up to 20 cm in diameter (Tregubov, 1941; Miletić, 1950). However, our research indicates that there are significant differences in the heights of these tree species in thinner trees and that fir and spruce trees belong mainly to site class II, which was found in previous research in the same primeval forests (Drinić, 1956; Keren, 2015). Statistical indicators show a relatively strong dependence of tree heights on their diameters with high coefficients of determination (Table 1).

Table 1. Basic statistical indicators of height curves for stands Janj and Lom

Primeval forest	Tree species	$h = d^2/(a + ad + ad^2) + 1{,}30$					
		a	b	c	R^2	Se (m)	Site class
Janj	*Abies alba*	5.5046	0.6630	0.0202	98.5	1.14	II
	Picea abies	9.5387	0.2340	0.0228	89.6	2.86	II
	Fagus sylvatica	- 0.9180	0.7823	0.0220	75.6	3.01	III
Lom	*Abies alba*	6.8039	0.4840	0.0197	71.4	4.37	I
	Picea abies	4.5473	0.6313	0.0200	83.7	3.88	II
	Fagus sylvatica	- 1.2380	1.0639	0.0158	79.0	4.07	II

a,b,c – parameters of function; R^2 – coefficient determination; Se – statistical error

In the Janj primeval forest, the height growth of fir is smaller than the height growth of spruce up to 37.5 cm, after which it is larger and the difference is approximately constant and averages about 0.2 m. In the Lom primeval forest, the height growth of fir trees is higher than spruce trees, but with increasing diameter, this difference decreases, so that with a diameter of about 67.5 cm, it is insignificant and the growths are almost equal. Fir in Lom reaches higher heights than spruce. With thinner diameter classes, no significant difference in heights is observed.

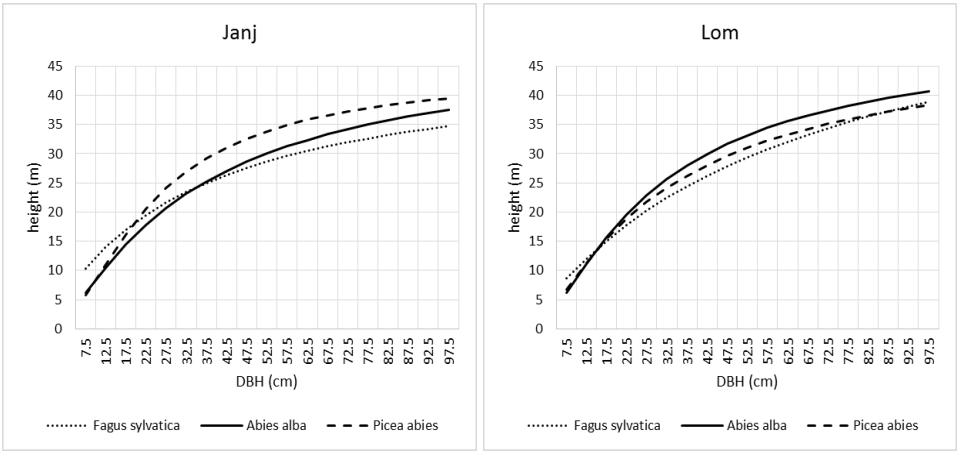

Figure 3. Height curves of fir, spruce and beech in primeval forests Janj and Lom

Both types of trees, according to the course of height curves, indicate the possibility of intensifying the height growth of thick trees of great age.

The appearance of strong height gain in thinner diameter classes and a sharp weakening in the middle ones was noted in works in primeval forests in Switzerland (Schütz, 1999). Fir trees that have not died after a long period of shading in Lom can use direct light, so smaller groups of trees that are laterally shaded by neighboring trees are excreted. That is why fir trees get an energetic increase in height and pass into the upper layer, and part of the trees remains shaded. It is a period of the intensive social ascent of trees and stratification of the stand, which is especially pronounced in Lom. Lom primeval forest is characterized by pronounced rockiness, fractures and falls of trees with a dominance of the terminal development phase (aging and decay) in contrast to Janj, where this development phase is much less represented and the stand has a larger overgrowth (Govedar, 2002). The lower heights of thinner fir trees are caused by the long stage of vegetation. Then the trees use mostly diffuse light. Later, during growth, thick fir trees in Lom reach the highest heights on average and they are larger than fir trees in Janj by about 2.3 m, and in the thickest trees (> d1.30 = 100 cm) the differences in height are even over 4.0 m, which reflected on the site class. For fir, these differences between Janj and Lom continuously increase with increasing diameter, while for spruce in the middle diameter classes they reach a maximum, and then decrease.

Diameter increment and time of passage of fir and spruce trees

In terms of the size of the current diameter increment, fir reacts the most concerning the stand condition and habitat conditions. The dependence of the current diameter increment on the diameter is expressed by a second-order parabola (Tab. 2) with a clearly expressed maximum function for fir and spruce trees (Fig. 4). Beech in both primeval forests has a greater increment of diameter in thinner trees (up to 32.5 cm) than fir and spruce. Significant differences in

terms of the size of the diameter increment occur with fir, which in the Lom primeval forest has a significantly higher diameter increment at the same diameters than in Janj. Large differences in diameter increment for fir are caused by differences in site classes, and for beech by a larger number of trees in the Janj primeval forest and their greater shade. The size of the current diameter increment of fir in Janj is on average 2.3 mm/year, and in Lom 4.5 mm/year. The average current diameter increment of spruce in Janj is 0.2 mm higher than in Lom and amounts to 2.9 mm/year. The current diameter increment of beech in Lom is significantly higher (4.9 mm/year) than in Janj (2.3 mm/year). It is characterized by a large diameter of increment in old, thick spruce trees, which is reflected in the increase in volume increment. The good physiological strength of spruce is indicated by the research results on the mountain Ljubišnja, also in beech, fir and spruce forests, where spruce can have an intensive increase in height and volume even at the age of more than 150 years (Čurović and Spalević, 2012).

Table 2. Basic statistical indicators of diameter increment dependence on diameters

Primeval forest	Tree species	$id = a + ad + ad^2$			
		a	b	c	Se (m)
Janj	*Abies alba*	-0.371743	0.104912	-0.000686	1.7
	Picea abies	-0.404252	0.128397	-0.000965	1.6
	Fagus sylvatica	1.454500	0.087000	-0.001000	1.4
Lom	*Abies alba*	-0.516537	0.142412	-0.000778	2.7
	Picea abies	0.726143	0.070432	-0.000485	1.1
	Fagus sylvatica	2.394100	0.013400	0.000300	2.3

a,b,c – parameters of function; R^2 – coefficient determination; Se – statistical error

Beech in the Lom primeval forest tends to increase sharply in thickness with increasing diameter, which is caused by a significantly higher relative share of thinner than thicker trees. Fir as a highly sciophilic species (Kimmins, 1997; Stojanović and Krstić, 2008) is characterized by periods of vegetation stagnation (Pantić et al. 2011). Earlier research has indicated periods of stagnation and vegetation lasting over 200 years (Banković, 1981; Banković et al. 1994). This period can last up to 330 years in continuity, which was determined at the locality "Goč-Gvozdac" in Serbia in a mixed beech and fir forest with a stable selection structure (Pantić et al. 2015). In the period of growth stagnation, the current diameter increment was extremely low, less than 1.4 mm/year, and height increment less than 0.8 m/year. Oscillations during the growth of the spruce tree were determined in the research of the Lom primeval forest, where a spruce tree, 99.0 cm in diameter and 39.0 meters high, was analyzed (Govedar, 2005). It was found that the tree with minor oscillations had an average diameter increment in a constant and fairly uniform increase until 220 years of age, after which its values until the end of the analyzed period (420 years) remain uniform and amount to

0.23 mm/year. Based on the graphically leveled diameter increment of fir and spruce (Fig. 4) in the investigated stands, the obtained values were used to calculate the time of passage by diameters classes, by dividing the width of the diameter class (50 mm) by the size of the diameter increment for the thickness of the tree of a given diameter increased by 2.5 cm.

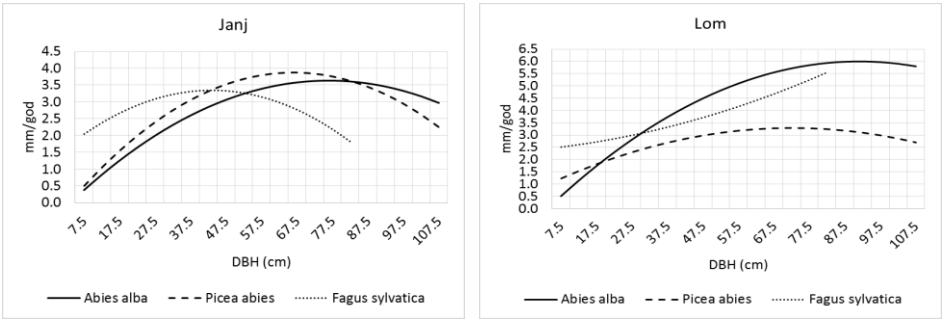

Figure 4. Dependence of diameter increment of trees on the diameter

This means that the time of passage from class dx to class dx + 2.5 is equal to the quotient between the width of the diameter class and the size of the ordinate of the increment of diameter for the abscissa d_x+2.5 read from Figure 4. The average times of passage in the Janj primeval forest for all three tree species are longer in all, and especially in the lower diameter classes (Fig. 5). This is caused by higher overgrowth, i.e., a larger number of trees, especially fir and spruce in the Janj primeval forest. The laid course of the time lines of the passage of fir and spruce in both primeval forests at larger diameters indicates an average uniformity of growth of thick trees.

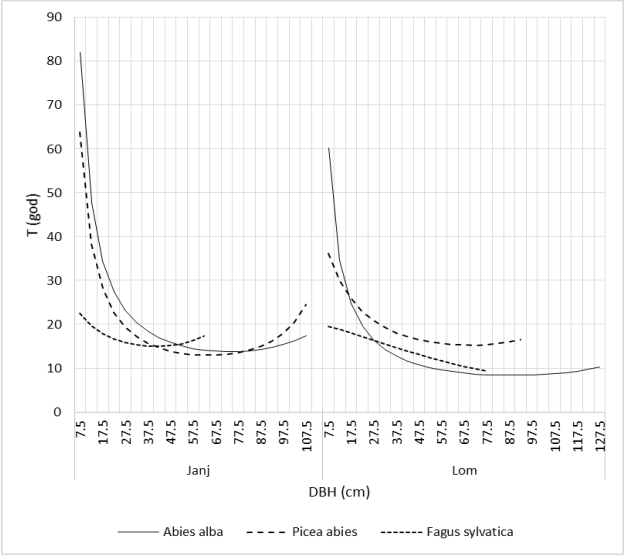

Figure 5. The average times of passage of trees

The average time of passage of fir trees in the Janj primeval forest is the highest and for the whole stand is 45 years and the lowest for spruce in the Lom where is 22 years. Otherwise, in both stands, the time of passage of fir is about 15 years longer than in spruce trees. Beech trees in the lowest diameter classes have significantly shorter times of passage than fir and spruce. Research on Goč has indicated that beech always achieves a "currently optimal" value of the length of the time of passage earlier, i.e., in a thinner diameter class, than fir (Milin, 1961). In the case of trees thicker (over 50 cm), in addition to management treatments, the influences of other environmental factors are more pronounced than in the case of thinner trees. Therefore, the times of passage of thicker trees may be a better indicator for determination of the site class, but certainly not the only one. Also, previous research on Goč in different stands of fir and beech, states that the time of passage is a parameter of low indicative ability and that as such cannot be used alone in the process of production differentiation, but in combination with several other indicators (Jović et al. 1991). Based on the time of passage of the strongest diameter classes ($D_{1.30}$ > 50 cm), in commercial forests, a bound cutting cycle is determined, which is suitable for the construction of the normal condition of stands and the determination of management goals. However, the cutting cycle in the case of old-growth forests in forest reserves cannot be described as in the case of forests of economic character, but it can be a good indicator for the time dynamics of the management of diverse forests. Starting from the tax limit (DBH = 5.0 cm), the relative ages of trees whose diameters are equal to the averages of the diameter classes were determined (Fig. 6).

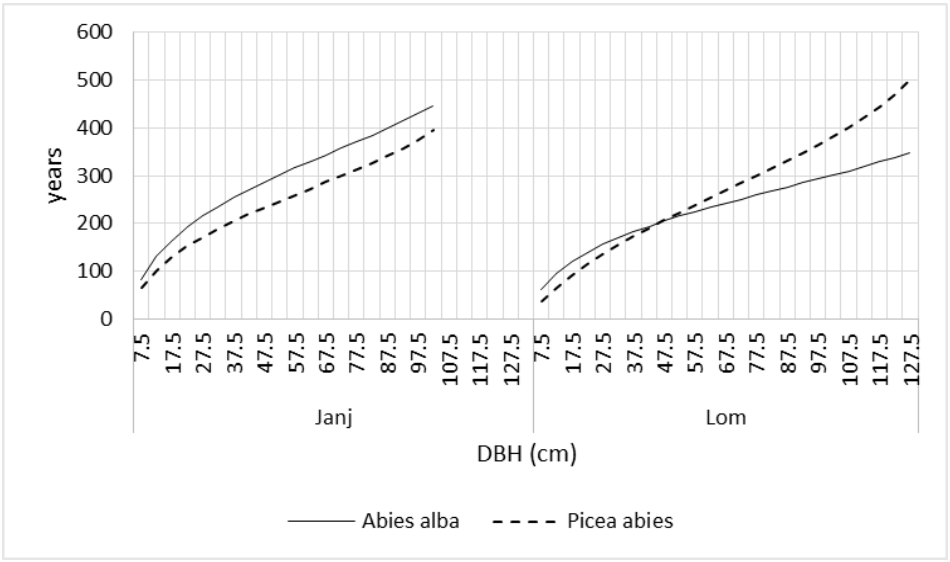

Figure 6. Relative ages of fir and spruce trees in primeval forests

It is evident that trees with a diameter of 7.5 cm have a relative age equal to the time required for a tree with a thickness of 5.0 cm to reach a diameter of 7.5 cm. The following values of relative ages of trees by diameter classes were calculated as cumulants "below", i.e., by successively adding appropriate times of passage to already calculated relative ages. The relative age of the strongest diameter class, under equal other conditions, can be a good indicator for assessing the internal strength of the stand as well as the strength and speed of material renewal (Miletić, 1950; Govedar et al. 2019). It is characteristic that the relative age of fir trees in Janj is higher in all diameters classes than in spruce trees. In the Lom primeval forest, the relative age of thinner fir and spruce trees is almost the same and equalizes in the middle diameters classes, after which the age of spruce trees increases sharply with increasing thickness. The fir in the Janj primeval forest is on average 85 years older than in Lom, and with the increase in diameter, it is constantly increasing. However, spruce shows the largest difference in relative age between primeval forests in thinner diameters classes and, unlike fir, the difference in age decreases with increasing diameter. Fir in primeval forests can reach an age over 500 years (Mayer, 19767; Moro, 2007).

CONCLUSIONS

The main feature of the diameter structure of trees in the studied primeval forests is the presence of ancient trees of large dimensions and very specific phenotype. The analysis of the diameter structure shows that in the lower diameter classes dominate beech with a small presence of spruce, which is caused by insufficient ingrowth. The oldest generation of trees consists mainly of highly developed and tall fir and spruce trees, and beech trees are not represented in the category of the thickest trees in stands. It has been determined that the main tree species belong mainly to the second site class. Fir in Lom reach higher heights at the same diameters, so they belong to a better quality. With thinner diameter classes, no significant difference in heights is observed.

Both types of trees, according to the course of height curves, indicate the possibility of intensifying the height growth of thick trees of great age. Thick trees, especially fir and spruce, are characterized by a uniform average increment of diameter. The average time of passage of fir trees in the Janj primeval forest is the highest and for the whole stand is 45 years, and the lowest for spruce in the Lom primeval forest where is 22 years. Otherwise, in both stands, the time of passage of fir is about 15 years longer than for spruce trees. Beech trees in the lowest diameter classes have significantly shorter times of passage than fir and spruce. The fir in the Janj primeval forest is on average 85 years older than in Lom, and with the increase of diameter, it is constantly increasing. However, spruce shows the largest difference in relative age between primeval forests in thinner diameter classes, and in contrast to fir, the difference in age decreases with increasing diameter. The average time of passage shortens with increasing diameter class, but thicker trees are not always the oldest. Since times of passage

and relative ages are a good indicator of the internal strength of stands, they can be used to assess the degree of acceleration of production in stands.

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EFFECT OF BUNCH LOAD ON THE QUALITY OF CARDINAL GRAPE VARIETY

SUMMARY

Our research aims to evaluate the effect of bunch load variation per unit area on the quality of Cardinal table grape variety. The experiments were carried out during three vegetation seasons (2014 – 2016) on Cardinal grape variety grown on pergola training system, with a planting distance of 2.5 m x 2.5 m, in Gevgelija-Valandovo vine growing region, the Republic of Macedonia. After the blooming phenophase, the number of bunches (crop load) was reduced. Three bunch thinning variants were applied: V_1 (thinning to 3 bunches/m^2), V_2 (thinning to 4 bunches/m^2) and V_3 (thinning to 5 bunches/m^2). They were then compared with the standard (S_T) where no thinning of bunches was applied.

Research results show that bunch load in Cardinal variety has a significant impact on bunch and berry mass, transportability, maturation dynamics and packed grape quantity and that the V_1 variant, with the lowest bunch density per m^2, has the largest individual bunch and berry mass (502.3 g and 8.83 g respectively), while the S_T variant yielded the smallest bunch and berry mass (451.7 g and 7.93 g respectively). We got similar results on grape transportability marked by berry firmness and berry adherence strength. The largest quantity of total harvested grapes was obtained in the V_3 (2.22 kg/m^2) and S_T (2.19 kg/m^2) variants, while the smallest quantity of total harvested grapes (1.47 kg/m^2) was obtained in the V_1 variant. The largest quantity of packed grapes (1.87 kg/m^2) was obtained in the V_2 variant, and the smallest in the V_1 variant (1.44 kg/m^2).

Keywords: table grapes, bunch thinning, bunch mass, berry mass, quantity of packed grapes

INTRODUCTION

Cardinal is a well-known table grape variety, common in all major table grape producing regions. It is a leading, very early maturing table grape variety in

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Macedonia. It is quite popular and sought after on the market, however, its production is specific. Millerandage and coulure are common. Ampelotechnical practices, mainly pinching and bunch thinning, are necessary to prevent these side effects (Bozinovic, 2010). Excessive load often results in delayed and uneven bunch maturation on the vine (Prculovski, 2019).

Summer pruning technique has a significant effect on yield and fruit quality in table grape production. It improves the microclimate in the canopy, ensures good and timely grape maturation and creates less suitable conditions for the development of pathogens (Di Lorenzo et al., 2011). By using canopy management techniques, we can influence the position and amount of leaves, shoots and fruits in order to obtain a desirable arrangement in the space and to reduce excessive shading and overloading the vine with bunches (Dry, 2000). The most significant techniques used during summer pruning are: shoot and bunch pinching, which affect the distribution of photoassimilates between leaves and bunches (Mota et al, 2010); defoliation, which regulates the air temperature, solar radiation frequency, and improvement of grape colour and maturation (Mandelli et al, 2003); and finally, bunch thinning, which regulates yield and improves grape maturation and quality (Prculovski, 2019).

The effect of these practices depends on several factors, such as the timing of implementation, its intensity, and so on. Excessive pinching or excessive shoot and bunch thinning can lead to crop yield and fruit quality reduction (Dardeniz et al., 2008). At the moment, we can find a lot of data on the effect of the timing and the bunch pinching method on yield and fruit quality in a large number of table grape varieties (Dardeniz, 2014; Akin and Coban, 2016 etc.), but there is insufficient data on the bunch thinning method and its effect on the production and technological characteristics of the grapes. The aim of our study is to determine the impact of the applied bunch thinning, which is to determine the ideal load for Cardinal variety under the conditions of the Gevgelija - Valandovo vineyard in order to obtain the largest possible quantity of extra-grade grapes without prolonging the maturity time.

MATERIAL AND METHODS

Our trial was carried out at the production plantations of "VV Vizba Valandovo," Valandovo locality of Gevgelija -Valandovo vine growing region. The research was conducted in three consecutive years, from 2014 to 2016, on Cardinal variety, grown on pergola training system with a planting distance of 2.5 m x 2.5 m. The plantations are 15 years old and are in full fruiting. Three bunch thinning variants were used, as follows:

1. V_1 – leaving 3 bunches per m^2
2. V_2 – leaving 4 bunches per m^2, and
3. V_3 – leaving 5 bunches per m^2.

Comparisons were made with the standard (S_T), in which all ampelotechnical practices were implemented, except bunch thinning, so in standard (St) variant all clusters remained.

Proper pruning, which means leaving four canes with six buds and two spurs with two buds per vine, and agrotechnical and ampelotechnical practices were implemented uniformly for each of the above variants, including the standard. Crop (bunch) load was reduced 7-14 days after blooming phenophase. The effect of bunch thinning on crop yield, harvest dynamics, bunch and berry mechanical composition and properties, quantity of packed grapes, and sugar and acid content was studied. Grapes were harvested twice. The first harvest took place when most bunches exceeded 15^{o} Brix. The second harvest took place after 10 days and the grapes that were not mature sufficiently or did not meet packing requirements were classified as residue.

Both bunch and berry mechanical composition, specifically bunch and berry average mass (g), and bunch and berry mechanical properties, in terms of berry firmness (g/cm^2) and berry adherence strength (g/berry), were examined. Total yield was set in two categories: packed grapes (kg/m^2) and residue (kg/m^2). The packed grapes quantities of the first and the second harvest (kg/m^2) depended on the harvesting dynamics. Regarding the chemical composition of the grapes, the content of sugars and total acids were analysed.

Mean values, standard deviation, and coefficient of variation were calculated based on the results. Analysis of variance (ANOVA) was applied to test our hypotheses while the mean values were compared using the LSD test.

RESULTS AND DISCUSSION

Based on the results and their analysis, it can be confirmed that the number of bunches left per vine influences the production and technological potential of Cardinal variety. The highest average bunch and berry mass for the years studied was recorded in the V_1 variant, where 11% higher bunch and berry mass was recorded compared to the standard (Tab. 1). In the V_2 variant, bunch mass increased by 10% while berry mass increased by 6% compared to the standard. In the V_3 variant, an increase of 3% in bunch mass and 2% in berry mass was recorded and the results showed a statistically insignificant difference compared with the bunch and berry mass of the standard variant.

Studying the effects of bunch thinning on bunch and berry mass in Seyval Blanc, Berkey et al. (2011) found that the impact of these treatments differed between years probably due to the influence of the management of a previous cycle on the subsequent ones, identifying bunch management in the previous production cycles as the cause. The effect of this practice is evident in several studies across the varieties which pointed to increases in bunch and berry mass when smaller bunch densities per plant are adopted (Ivanišević et al., 2020; Karoglan et al., 2014; Bubola et al., 2011; Kavoosi et al., 2009; Somkuwar and Ramteke, 2010; Gil et al., 2013). This is due to the greater availability of photoassimilates directed to bunches in vines with lower bunch density. Thinning intensity, however, should be suitable for the growing conditions and cultivar characteristics, as high bunch thinning intensity may reduce yield (Fanzone et al., 2011; Avizcuri-Inac et al., 2013).

Table 1. Cardinal variety bunch and berry mass

	Bunch mass Variant				**Berry mass** Variant			
Year	S_T	V_1	V_2	V_3	S_T	V_1	V_2	V_3
2014	479,0	528,0	519,0	508,0	8,9	9,6	9,3	9,1
2015	484,0	536,0	528,0	485,0	8,3	9,2	8,8	8,4
2016	392,0	443,0	439,0	407,0	6,6	7,7	7,2	6,8
Mean $\bar{x}$	451,7a	502,3b	495,3b	466,7	7,93	8,83	8,42	8,11
Index	100	111	110	103	100	111	106	102
SD	51,7	51,5	49,0	52,9	1,21	1,02	1,10	1,16
CV	11,5	10,3	9,9	11,3	15,3	11,6	13,1	14,3
LSD 0.05	15,50				0,20			
0.01	23,48				0,30			

*Significant deviation at p = 0.05 level against the standard for the property
The values in each column, marked with different letters, differ significantly from each other at the p = 0.05 level

Regarding the bunch and berry mechanical property, as an important segment on which the transportability and the storability of table grapes depend, we investigated the berry firmness and berry adherence strength. Table 2 shows the data from the three-year trials in relation to these two parameters in all variants tested.

The berry firmness ranged from 1536 g/cm^2 in the standard variant to 1800 g/cm^2 in the V_1 variant, which is 17% higher than the standard. The average berry firmness during the trial in the V_2 variant was 1670 g/cm^2, which is 9% higher than in the S_T variant, while in the V_3 variant we found that the berry firmness was 1563 g/cm^2, which is 2 % higher than the standard.

The berry adherence strength ranged from 405 g/berry in the standard variant to 460 g/berry in the V_1 variant. The V_2 variant had an average berry adherence strength of 441 g/berry, while the V_3 variant had an average berry adherence strength of 419 g/berry. The percentage increase in the berry adherence strength of the variants compared to the standard (S_T) ranged from 3% (V_3) to 14% (V_1).

In our study, a statistically significant difference at the p = 0.05 level in both the berry firmness and berry adherence strength was only observed in the V_1 variant compared to the S_T variants. We determined that the berry firmness and berry adherence strength of all studied variants of Cardinal variety are within varietal characteristics, which was confirmed by the studies of several authors in the region and they range from 1.400 to 1.800 g per berry firmness and 300-600 g per berry adherence srength (Roičev, 2012; Bozinovik et al., 2010; Žunić and Garić., 2017). Perez et al. (1998) reported in their study that lower bunch density per vine may lead to greater berry firmness.

Table 2. Cardinal variety bunch and berry mechanical properties

		Variant				Index			
	Year	S_T	V_1	V_2	V_3	S_T	V_1	V_2	V_3
Berry firmness	2014	1 610	1 820	1 710	1 640				
	2015	1 600	1 900	1 800	1 650				
	2016	1 400	1 680	1 500	1 400				
	x̄	1 536a	1 800b	1 670ab	1 563ab	100	117	109	102
	SD	118,5	111,4	153,9	141,5				
	CV	7,7	6,2	9,2	9,1				
Berry adherence strength	2014	410	456	433	423				
	2015	430	530	500	450				
	2016	375	393	391	385				
	x̄	405a	460b	441ab	419a	100	114	109	103
	SD	27,8	68,6	55,0	32,7				
	CV	6,9	14,9	12,5	7,8				

**Significant deviation at p = 0.05 level against the standard for the property.*
The values in each column, marked with different letters, differ significantly from each other at the p = 0.05 level

According to them, lower bunch density provides greater availability of carbohydrates and other molecules and certain minerals that, when incorporated into the berry cell walls, give the berries greater firmness. On the other hand, De Souza Leão and Coelho De Lima (2017) found no relationship between bunch density and berry firmness.

Certain differences between variants were found in the sugar and acid content. The sugar content (figure 1) ranged from 160 g/l in the S_T variant to 170 g/l in the V_1 variant. Statistically significant differences compared to the standard variant were detected in both V_1 and V_2 variants, where the sugar content was higher by 6% and 3% respectively compared to the standard.

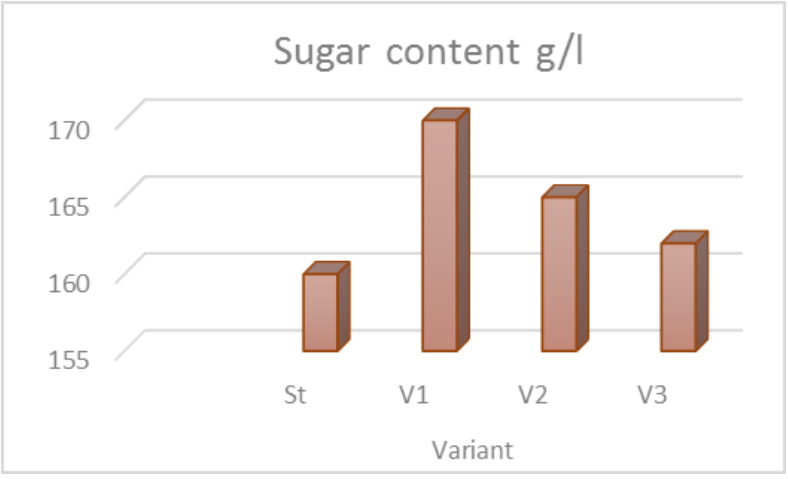

Figure 1. Sugar content by variants in cardinal varieties

The acid content was very variable, changing from year to year, in all variants. It varied from 5.46 g/l in the S_T variant to 6.11 g/l in the V_3 variant (graph 2), and no correlation was observed between the bunch thinning and total acid content.

According to international standards, the minimal sugar concentration in table grapes may vary from 14.0 to 17.5 °Brix depending on the variety (Maia et al., 2014). The sugar and acid contents, as components directly affecting the quality of grapes besides the varietal characteristics, depend on a number of factors: environmental conditions, crop load, time of harvest, application of agrotechnical and ampelotechnical practices, etc. (Avizcuri-Inac et al., 2013). Kavoosi et al. (2009), studying the effect of bunch density in Ascari variety, suggested that decreasing the number of bunches significantly increased the sugar content, sugar/acid ratio, and must pH. Results similar to these were reported in different cultivars by several authors (Dokoozlian et al., 1995; Ezzahouani and Wiliams, 2003; Kunihisa et al., 2003). They also concluded that this influence varies greatly from year to year.

On the other hand, De Souza Leão et al. (2017), investigating the effect of bunch thinning on sugar and acid content in Sugraone variety, did not determine any impact or relation between these elements.

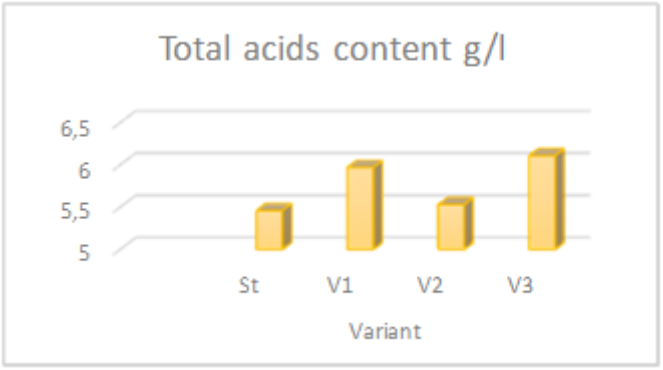

Figure 2. Total acid content by variants in Cardinal varieties

Contrary to the sugar content, where some correlation with bunch thinning was identified in most studies, no such correlation was identified for the content of total acids in most tests (Somkuwar and Ramteke, 2010; Pastore et al., 2011; Miele and Rizzoni, 2013).

Based on the results, we can conclude that the amount of total grapes harvested is closely related to the number of grapes per m^2 (Table 3). The greater the number of bunches, the higher the total yield. However, the quantity of packed grapes increases to a certain load and then starts to decrease, i.e. the quantity of packed grapes decreases and the quantity of residue increases (grapes for processing).

The largest quantity of total grapes harvested was recorded in V_3 (2.22 kg/m^2) and S_T (2.19 kg/m^2), and the smallest in V_1 (1.47 kg/m2). The largest quantity of packed grapes was obtained in V_2 (1.87 kg/m^2) and the smallest in V_1 (1.44 kg/m^2). The quantity of residue (grapes that do not meet the minimum criteria for the category of packed grapes) was 24.9% in S_T and 21.4% in V_3 of the total harvested quantity, while in V_1 and V_2, a significantly lower percentage was statistically recorded: 2.0% and 4.1% respectively of the total harvested quantity.

Table 3. Effect of bunch density on yield of the studied Cardinal variety

Type	Year	Variant				Percent			
		S_T	V_1	V_2	V_3	S_T	V_1	V_2	V_3
Packed grapes kg/m^2	2014	1,65	1,42	1,85	1,82	76,7	95,3	95,9	79,1
	2015	1,74	1,5	1,95	1,88	76,7	99,3	97	81
	2016	1,55	1,39	1,83	1,55	71,8	99,3	94,8	75,6
	$\bar{x}$	1,65b	1,44a	1,87c	1,75b	75,1	98	95,9	78,6
	SD	1,0	0,6	0,6	1,8				
	CV	5,8	4,0	3,4	10,0				
Residue kg/m^2	2014	0,5	0,07	0,08	0,48	23,3	4,7	4,1	20,9
	2015	0,53	0,01	0,06	0,44	23,3	0,7	3	19
	2016	0,61	0,01	0,1	0,5	28,2	0,7	5,2	24,4
	$\bar{x}$	0,55c	0,03a	0,08a	0,47b	24,9	2	4,1	21,4
	SD	0,057	0,035	0,020	0,031				
	CV	10,4	115,5	25,0	6,5				
Total kg/m^2	2014	2,15	1,49	1,93	2,3				
	2015	2,27	1,51	2,01	2,32				
	2016	2,16	1,4	1,93	2,05				
	$\bar{x}$	2,19c	1,47a	1,96b	2,22c	100	100	100	100
	SD	0,067	0,059	0,046	0,150				
	CV	3,04	4,00	2,36	6,77				

*Significant deviation at p = 0.05 level against the standard for the property

The values in each column, marked with different letters, differ significantly from each other at the p = 0.05 level

The yield in all Cardinal variety variants is within the range of varietal characteristics (15-25 t/ha) supporting the data published by several authors (Avramov and Žunić, 2001; Žunić and Garić, 2017; Roičev, 2012; Božinović, 2010).

Kavoosi et al. (2009), studying the effects of bunch thinning, found a decrease in total yield but also an improvement in both the fruit quality and the balance between yield and quality. Berkey et al. (2011), examining the impact of this practice on Seyval Blanc production, found that the impact differed between

years due to the conditions in the year of production. Multiple study data show that high bunch thinning intensity may decrease yield significantly (Fanzone et al., 2011; Avizuri-Inac et al., 2013).

Table 4. Effect of bunch density on maturation dynamics of the studied Cardinal varieties

Harvest	Year	Variant				Percent			
		Ст	B_1	B_2	B_3	Ст	B_1	B_2	B_3
First harvest kg/m²	2014	1,42	1,31	1,6	1,48	86,1	92,3	86,5	81,3
	2015	1,48	1,34	1,65	1,55	85,1	89,3	84,6	82,4
	2016	1,35	1,24	1,64	1,39	87,1	89,2	89,6	89,7
	x̄	1,42b	1,3a	1,63c	1,47b	86,1	90,3	86,8	84,5
	SD	0,065	0,051	0,026	0,080				
	CV	4,6	4,0	1,6	5,4				
Second harvest kg/m²	2014	0,23	0,11	0,25	0,34	13,9	7,7	13,5	18,7
	2015	0,26	0,16	0,3	0,33	14,9	10,7	15,4	17,6
	2016	0,2	0,05	0,19	0,16	12,9	10,8	10,4	10,3
	x̄	0,23b	0,11a	0,25b	0,28b	13,9	9,7	13,2	15,5
	SD	0,030	0,055	0,055	0,101				
	CV	13,0	51,6	22,3	36,6				
Total kg/m²	2014	1,65	1,42	1,85	1,82				
	2015	1,74	1,5	1,95	1,88				
	2016	1,55	1,39	1,83	1,55				
	x̄	1,65b	1,44a	1,87c	1,75bc	100	100	100	100

*Significant deviation at p = 0.05 level against the standard for the property
The values in each column, marked with different letters, differ significantly from each other at the p = 0.05 level

Maturation process dynamics of bunches within the vine and, at the same time, maturation of the berries within the bunch is of great importance for the table grapes. The tendency for very early and early maturing varieties is to obtain as much packed grapes as possible in the first harvest. Our research shows that bunch thinning affects the maturation index and maturation time (Table 4), which has been confirmed in the studies by several authors (Ozer et al., 2012; Silvestri et al., 2017).

The largest quantity of packed grapes was obtained in V_2 in the first harvest (1.63 kg/m^2), while the smallest was in S_T (1.3 kg/m^2). The percentage share of the first harvesting was from 84.5% (V_3) to 90.3% (V_1). The amount of

packed grapes in the second harvest varies from 0.11 kg/m^2 (V_1), to 0.28 kg/m^2 (V_3).

Bunch thinning in Cardinal variety in the first harvest showed statistically significant differences in the quantity of packed grapes in the V_1 and V_2 variants compared to the V_2 and S_T variants.

CONCLUSIONS

Bunch thinning in the period of 7-14 days after blooming showed a significant increase in average bunch and berry mass, better transportability, due to higher berry firmness and berry adherence strength, greater quantity of packed grapes, as well as better maturation dynamics. In Cardinal variety, the best results in relation to these parameters were obtained in the V_2 variant (density of 4 bunches per m^2).

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FORECASTING TIME SERIES CHANGE OF THE AVERAGE ENHANCED VEGETATION INDEX TO MONITORING DROUGHT CONDITION BY USING TERRA/MODIS DATA

SUMMARY

Drought condition is a natural disaster that has caused economic and social damages considerably including a shortage of consuming water, and has been a hindrance to the agricultural production and industrial development. Currently, the drought condition tends to be more severe in Yasothon Province of Thailand, thus affecting plantation in the area. The purpose of this study was to monitor the drought condition of Yasothon Province, which is located in the Northeastern region of Thailand, by using Enhanced Vegetation Index (EVI) data during 2010 – 2019 obtained from Terra/Modis Satellite, and by studying the change in time series from the average EVI during 2010 – 2019, for the forecast in 2020 – 2022 by using moving averages method and exponential smoothing method in order to compare the differences between the original data of average EVI and the data of average EVI adjusted by smoothing algorithm using RMMEH method. Statistics used in examining the forecasting accuracy were MAD and MAPE. It was found from the study that MAD and MAPE of the forecast of the original average EVI and the average EVI adjusted by using RMMEH method were slightly different in that the average EVI adjusted by using RMMEH method and forecasted by moving averages method was best accurate. In addition, according to the time series change forecast of EVI, it was found that the original average EVI and the EVI which was smoothed by RMMEH method of the forecasting year during 2020 – 2022 by using moving average method and exponential smoothing method were very low in each year, indicating that the drought would occur in the future.

Keywords: Enhanced Vegetation Index (EVI), Remote Sensing, Drought, RMMEH method

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INTRODUCTION

Drought condition is a natural disaster found in many areas and has caused huge damage and had greatly hindered the national development of Thailand. The areas affected by drought condition often face a shortage of consuming water and water supply used for farming which relies heavily on rainwater (Laosuwan, et al., 2016). When there is insufficient amount of water, the agricultural yields decrease, thus causing a lack of agricultural products and processed products for domestic consumption and for exporting. The drought condition in the Northeastern region of Thailand has long been taking place and has been considered as the serious problem mainly due to a shortage of rainfall, and other factors including the soil water retention, underground water source, and the use of land.

The drought is caused by a lack of rainfall during rainy season and the irregularity of raining time between June to July (Rotjanakusol & Laosuwan, 2018). In addition, according to the analysis into rainfall pattern in terms of space and time, it was found that there would be no rain after the latter half of September as well since the area cannot be accessed by the Southwestern monsoon; and if in any year there is no tropical cyclone moving through such area, the drought would turn out to be more serious. Main consequences from the drought include a lack of consuming water, water supply used in various activities especially in farming. Farming is the main career of people living in Northeastern region; therefore, a lack of water does cause substantial impact on the cultivation thus decreasing the crop yields leading to the economic, social, and environmental problems, as well as health issue such as the epidemic disease that occurs during the dry season (Rotjanakusol & Laosuwan, 2019a).

The severity of drought condition depends on many factors including physical factor, ecological factor, and human activities factor. Therefore, the rainfall is regarded as the significant factor to be used when finding the relationship pattern with the spectral index and to study the duration of rainwater that affects plantation (Mongkolsawat et al., 2001; Dutta, et al., 2015; Gomasathit et al., 2015). The relationship between the rainfall and the spectral index is a key variable used to determine the drying area (Thavorntam et al., 2015; Lines et al., 2017; Nistor et al., 2018). The phenological change of plants in dry area could appear as the reflection of electromagnetic wave differently that could be seen by satellite data (Tucker, 1979; Peters et al., 2012; Seekaw et al., 2014; Uttaruk & Laosuwan, 2018). However, in studying drought condition, data have to be adjusted for the suitability of use and for the increased efficiency in showing more clearly whatever being studied. The data adjustment of this sort is called Index (Vrieling et al., 2014; Klisch & Atzberger, 2016; Uttaruk & Laosuwan, 2017; Rotjanakusol & Laosuwan, 2019b). The spectral index which is associated with the physical characteristics of plants and amount of water in plants and in soil can identify the drought condition (Kogan, 2001; Fan et al., 2015; Li et al., 2015; Uttaruk & Laosuwan, 2020a) for example, in the calculation for Enhanced Vegetation Index (EVI), the results of such can identify the growth and fertility of

plants in each climatic condition duration (Season) very well (Holben & Justice, 1981; Matsushita et al., 2007). Currently, the study of natural resources is performed by using remote sensing technology used with the application of satellite data in the study. The aim to apply this technology is to reduce time spent in physical exploration in the actual area. Nowadays, a lot of satellite data, both low and high spatial resolution, are used (Uttaruk & Laosuwan, 2020b; Prohmdirek et al., 2020; Auntarin et al., 2021; Piamdee & Laosuwan, 2021). The study of time series forecast is to study the changing pattern of data that have occurred from the past until the present time (Gu, 2007) the extent of data change depends on the pattern of data to be studied (Furtuna et al., 2015), which is the same as natural resources data. Therefore, in studying the time series forecast of EVI, it is to make quantitative comparison; the time series forecast is a quantitative forecast where there are both linear and non-linear methods each of which has its own limitation. The study of change in EVI data to be used in time series forecast is performed using linear method (Li et al., 2014).

Consequently, in this study, EVI was used to study the drought condition together with the time series forecast, by studying the change from the average EVI obtained from Terra/Modis Satellite during 2010 – 2019, in forecasting for 2020 – 2012. In this forecast, the average EVI data adjusted by smoothing algorithm method was used by using RMMEH method (Jin et al., 2013) from Terra/Modis satellite data to be used in the forecast, by applying Moving Averages Method and Exponential Smoothing Methods

MATERIAL AND METHODS

Moving Averages Method

The moving average is a smoothing tool for the convenience in monitoring the trend. Basically, the average would help spread the abnormalities out of the moving averages data used to predict the future. The moving averages method pattern is as shown in Equation 1.

$$Y_{t+1} = \frac{Y_t + Y_{t-1} + Y_{t-2} + ... + Y_{t-N+1}}{N} \quad (1)$$

Where;

Y_{t+1} = prediction value over time t + 1

Y_t = data value in the current period (t)

N = number of data used to mean

Exponential Moving Average

This is the method for data forecast with the same principle as the weighed moving average which is quite complicated; the error occurring from the forecast is used to adjust the next average for more accuracy, however, it is easily applied. The pattern of exponential moving average is as shown in Equation 2.

$$\hat{Y}_{t+1} = aY_t + (1-a)\hat{Y}_t \quad (2)$$

Where;

$\hat{Y}_{t+1}$ = the new smoothing or the value for the next forecast

a = the constant smoothing modifier (0 <a <1)

Y_t = the new data value or the actual value of the time series t

$\hat{Y}_t$ = the old smoothing or forecast period t

Enhanced Vegetation Index (EVI)

This is the method for solve the problem of the response of light reflection from soil and climate. It is reported that EVI is more associated with the spatial index of leaves surface than normalized differences vegetation index. And according to the past study, it was found that EVI is more sensitive to parameters in area where there is more density of leaves than Normalized Difference Vegetation Index (NDVI). The pattern of EVI is as shown in Equation 3.

$$EVI = G \times \frac{NIR - RED}{NIR + (C_1 \times RED - C_2 \times BLUE) + L} \quad (3)$$

Where;

EVI = Enhanced Vegetation Index

G = Gain Factor

NIR = percentage of reflection in the near infrared spectrum

RED = percentage reflection in the visible red spectrum

BLUE = percentage reflection in the visible blue spectrum

C_1 C_2 = coefficient for dust particles in the atmosphere

L = correction factor for the soil

Adjusting EVI data by smoothing algorithm

This is the technique in adjusting data by smoothing algorithm, which is called RMMEH to be used in adjusting EVI from Terra/ Modis Satellite; the procedures are as follows: (1) Finding M_t is to find the average EVI of the selected year. The pattern in finding M_t is as shown in Equation 4; (2) finding R_t or the median from 5 periods of time which are in close proximity. The pattern in finding M_t is shown as in Equation 5, and (3) finding EVI_{RMM_t} is to find maximum value from EVI, M_t, and R_t of EVI of the studied year. The pattern in finding EVI_{RMM_t} is as shown in Equation 6.

$$M_t = \frac{(EVI_{t-1} + EVI_{t+1})}{2} \quad (4)$$

Where;

M_t = mean of the EVI over time t

EVI_{t-1} = previous year's EVI of the period t

EVI_{t+1} = next year's EVI of the period t

$$R_t = RM_5(EVI_{t-2}, EVI_{t-1}, EVI_t, EVI_{t+1}, EVI_{t+2}) \quad (5)$$

Where;

R_t = median of all 5 EVI values of time t

RM_5 = median of the EVI value of time t

EVI_{t-1} = previous year's EVI of the period t

EVI_{t-2} = EVI of the previous 2 years of the period t

EVI_{t+1} = next year's EVI of the period t

EVI_{t+2} = EVI of the next 2 years of the period t

$$EVI_{RMM\_t} = MAX(EVI_t, M_t, R_t) \quad (6)$$

Where;

EVI_{RMM_t} = EVI of the period t

M_t = mean of the interval t

R_t = median of the period t

Checking for forecast accuracy

This is the checking for forecast accuracy of moving averages method and exponential smoothing method was performed. In this study, the accuracy was measured in the following ways: (1) mean absolute deviation (MAD): it is to check for the mean absolute deviation of the forecast, which is very useful for the analysis where deviation in the same unit with the original time series data was to be examined. The pattern in finding MAD is shown as in Equation 7; and (2) mean absolute percentage error (MAPE): this can be calculated by taking the actual error in each period to be divided by actual data in such period and then averaging the actual error in percentage. The pattern in finding MAPE is shown as in Equation 8.

$$MAD = \frac{\Sigma |\text{actual values-Forecast}|}{n} \quad (7)$$

Where;

actual values = observation at a particular time

Forecast = forecast value at a particular time

n = number of comparative data

$$MAPE=\left[\frac{\Sigma\left|\text{Actual values-Forecast}\right|\div\text{Actual values}}{n}\right]\times 100 \qquad (8)$$

Where;

actual values	=	observation at a particular time
Forecast	=	forecast value at a particular time
n	=	number of comparative data

The Study area

The study area in this study is Yasothon Province, located at the Northeastern region of Thailand (Figure 1), at the latitude of 15 – 16 °N and longitude of 104 – 105 °E, 128 meters above sea level, with the area of about 4,161.44 km^2, or equal to 0.81 % of total area of the country. Yasothon area is characterized by slope from the west side down to the east side; much of the soil is sandy and salty. At the upper part of the Province, much of it is high plain area alternated by undulating area, and some areas consist of forests and small mountains.

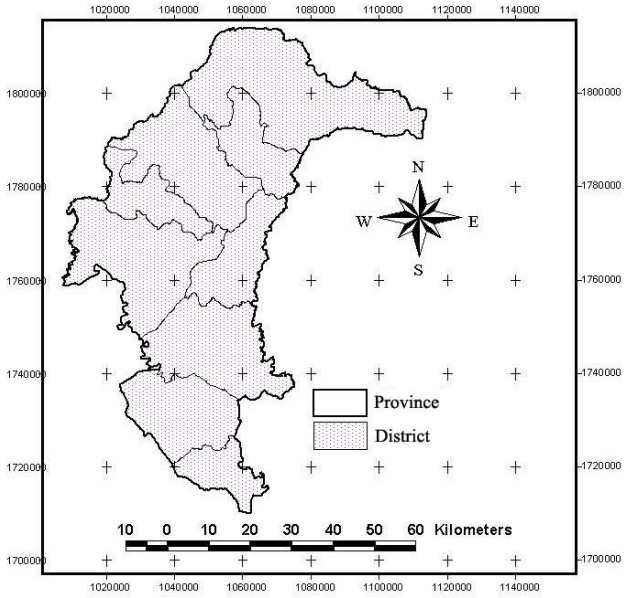


Figure 1. Yasothon Province

Data from satellite

Terra/ Modis Satellite is designed with the aim to follow and monitor natural resources data with swatch of about 2,330 km, with spatial resolution of 250 m to 1000 m, with 36 band recording system, and data from areas all over the world can be recorded within 2 days. In this study, MOD13Q1 product was used to cover the studied area during 2010 – 2019.

Methodology

For the benefit of brevity of the presentation of methodology used in this study, the conclusion was made by using research framework as shown in Figure 2 with the brief procedures as follows: (1) analyze EVI from Terra/ Modis Satellite data in the year of 2010 – 2019, and the average EVI could be analyzed and found by using Equation 3; (2) adjust the average EVI by using smoothing algorithm using RMMEH method from Equation 4, 5, and 6, respectively; (3) predict the land use in terms of time of EVI and EVI index where smoothing algorithm was applied already by using RMMEH method and by using moving averages method and exponential smoothing method; and (4) check for accuracy and compare the results of the study.

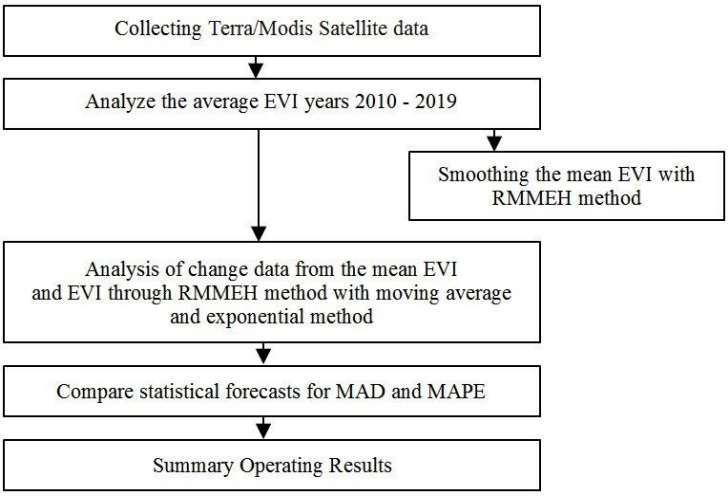


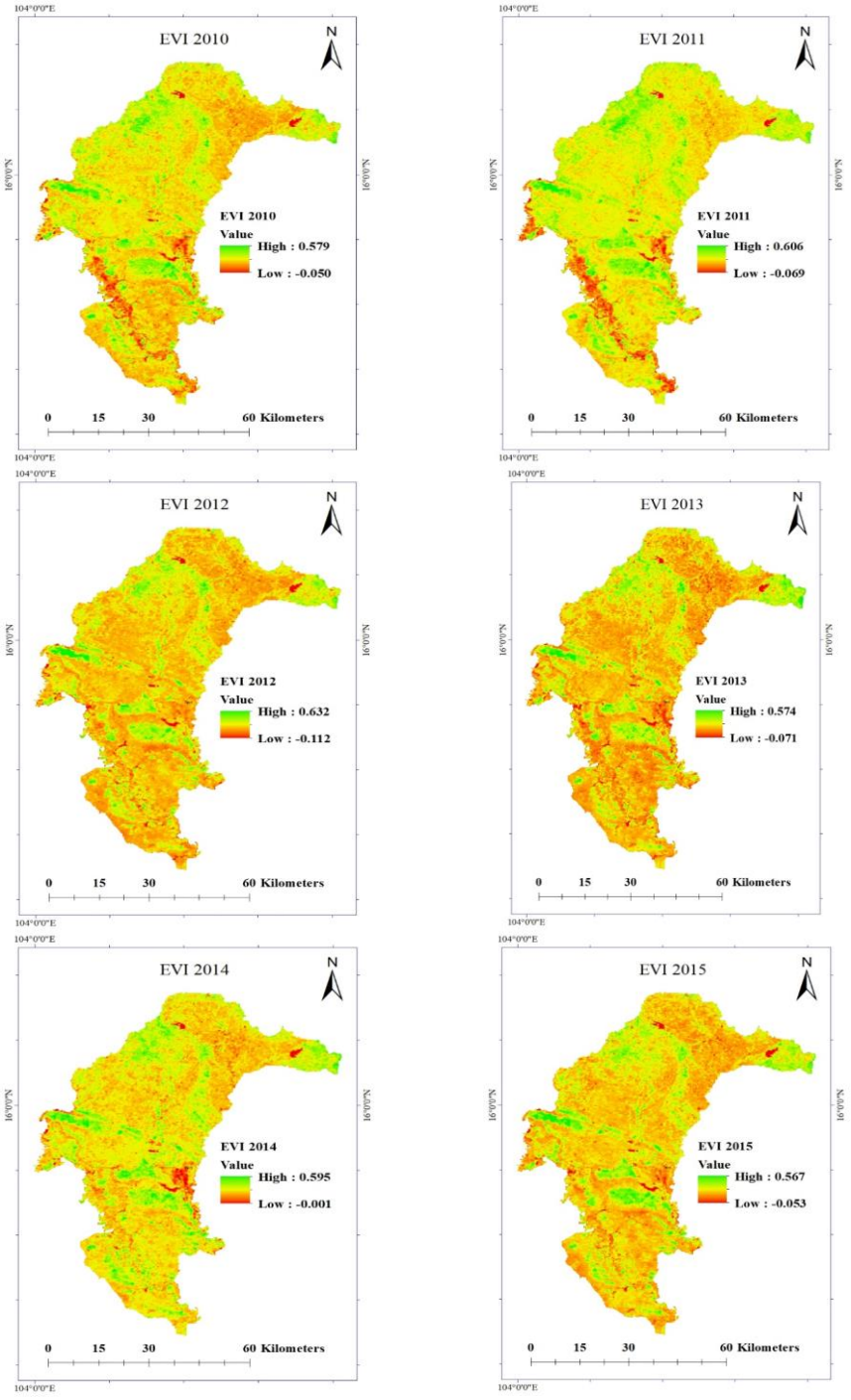
Figure 2. Research framework

RESULTS AND DISCUSSION

In time series change forecast of the average EVI to examine the drought condition from Terra/Modis Satellite data during 10 years (2010 – 2019) in Yasothon Province, the results of the study were as follows:

Result from finding EVI

According to the study during 10 years (2010 – 2019) (Figure 3 and Table 1), it was possible to identify the differences of quantity and fertility of plants in the area; EVI was -1 to 1. Generally, green plants would have EVI of 0.2 – 0.8. According to Table 1, it could be concluded that the maximum value, the minimum value, means, and standard deviation of EVI during 2010 – 2019 in each period revealed different EVI between -1 to 1; such figure was consistent with the theory. The means could identify the condition of plants in the area or the drought condition in each period of time; the minimum means was 0.120 in 2011, and the maximum means was 0.139 in 2016.


EVI 2010
EVI 2010
Value
High : 0.579
Low : -0.050
EVI 2011
EVI 2011
Value
High : 0.606
Low : -0.069
EVI 2012
EVI 2012
Value
High : 0.632
Low : -0.112
EVI 2013
EVI 2013
Value
High : 0.574
Low : -0.071
EVI 2014
EVI 2014
Value
High : 0.595
Low : -0.001
EVI 2015
EVI 2015
Value
High : 0.567
Low : -0.053
N
0 15 30 60 Kilometers
104°0'0"E
16°0'0"N

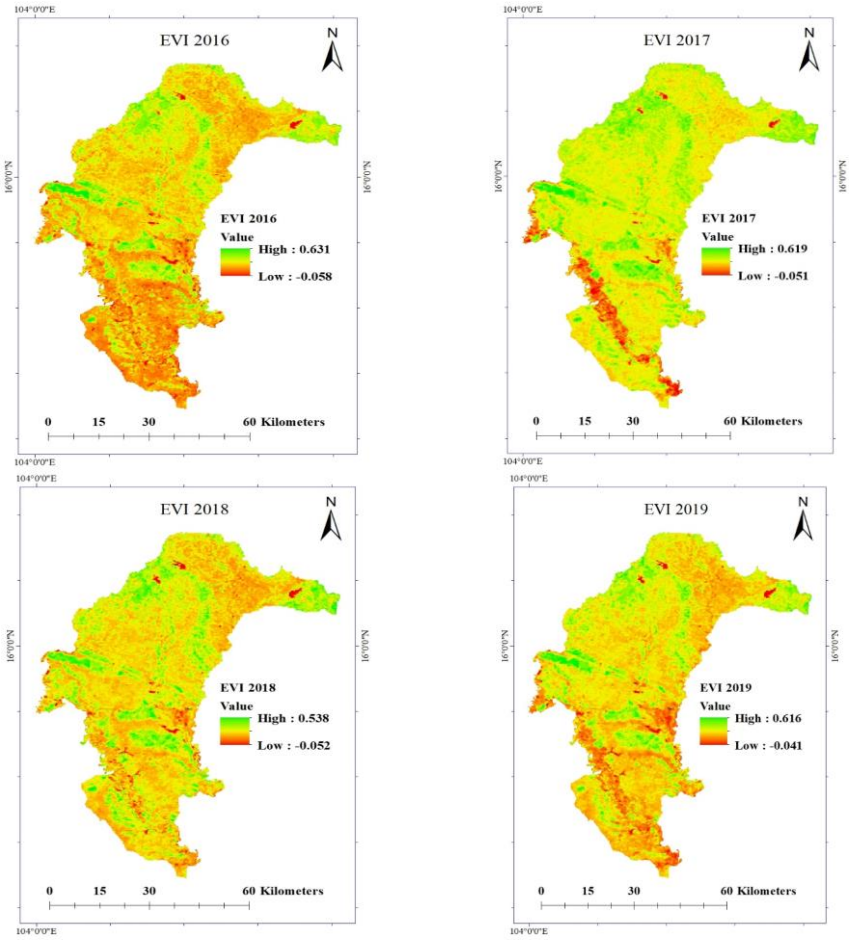


Figure 3. Spatial analysis of EVI during 10 years

Table 1. Minimum, Maximum, Mean, Standard Deviation of EVI

Year	Minimum	Minimum	Mean	Standard Deviation
2010	-0.050	0.579	0.123	0.155
2011	-0.069	0.606	0.120	0.154
2012	-0.112	0.632	0.133	0.168
2013	-0.071	0.574	0.125	0.158
2014	-0.001	0.595	0.130	0.164
2015	-0.053	0.567	0.131	0.165
2016	-0.058	0.631	0.139	0.176
2017	-0.051	0.619	0.130	0.166
2018	-0.052	0.538	0.123	0.157
2019	-0.041	0.616	0.138	0.176

Result from time series change analysis

For the results of the analysis into the average EVI from Terra/ Modis Satellite data with smoothing algorithm by using RMMEH method, EVI, M_t, R_t, and EVI_{RMM_t} of the average EVI during 2010 – 2019 could be shown as in Table 2.

Table 2. the average EVI with smoothing algorithm using RMMEH method

Year	EVI	M_t	R_t	EVI_{RMM_t}
2010	0.123	0.124	0.124	0.124
2011	0.120	0.128	0.124	0.128
2012	0.133	0.122	0.128	0.133
2013	0.125	0.131	0.128	0.131
2014	0.130	0.128	0.130	0.130
2015	0.131	0.134	0.131	0.134
2016	0.139	0.130	0.130	0.139
2017	0.130	0.131	0.130	0.131
2018	0.123	0.130	0.130	0.130
2019	0.138	0.126	0.130	0.138

The result of time series forecast from the original average EVI that had Smoothing Algorithm by RMMEH method

The results of time series forecast from the original average EVI that had Smoothing Algorithm by RMMEH method were shown in Table 3 and Table 4. It was found that the original average EVIs of the forecasted years which were in 2020 – 2022 by using moving averages method were 0.130, 0.130, and 0.132, respectively, and by using the exponential smoothing method, were 0.126, 0.126, and 0.126, respectively.

Table 3. The time series forecast from the original average EVI

Year	EVI	Moving Average	Exponential Smoothing
2010	0.123		
2011	0.120		0.123
2012	0.133		0.122
2013	0.125	0.125	0.123
2014	0.130	0.126	0.123
2015	0.131	0.129	0.124
2016	0.139	0.128	0.124
2017	0.130	0.133	0.125
2018	0.123	0.133	0.125
2019	0.138	0.130	0.125
2020		0.130	0.126
2021		0.130	0.126
2022		0.132	0.126

Table 4. The time series forecast from the average EVI pass Smoothing Algorithm by RMMEH method

Year	EVI_{RMM_t}	Moving Average	Exponential Smoothing
2010	0.124		
2011	0.128		0.124
2012	0.133		0.124
2013	0.131	0.128	0.125
2014	0.130	0.130	0.125
2015	0.134	0.131	0.125
2016	0.139	0.132	0.126
2017	0.131	0.134	0.126
2018	0.130	0.134	0.126
2019	0.138	0.133	0.127
2020		0.133	0.128
2021		0.134	0.128
2022		0.135	0.128

Besides, it was found that the average EVI that had smoothing algorithm by using RMMEH method of the forecasted years which were 2020 – 2022 by using moving averages method were 0.133, 0.134, and 0.135, respectively, and by using exponential smoothing method, were 0.128, 0.128, and 0.128, respectively. From the forecast of the original average EVI that the moving averages method was closer to the original average EVI than the exponential smoothing method. The results of forecast of the average EVI that had smoothing algorithm by using RMMEH method were found that the moving average was closer to the EVI that had smoothing algorithm by RMMEH method than the exponential smoothing method.

Checking for accuracy of the forecast

The results from checking for the accuracy of the forecast by both methods to see which forecasting techniques were of least errors, from 1) MAD and 2) MAPE; the less MAD and MAPE, the better the forecast. The results from checking for the accuracy of the forecast as mentioned could be shown in Table 5. Table 5 showed that errors from both methods were very little and in close proximity. It was found from the forecast that data that passed smooth algorithm by RMMEH method using moving average were best accurate and better than by using the original average EVI.

Table 5. The results from checking for the accuracy

Methods	EVI		RMMEH	
	Moving Average	Exponential Smoothing	Moving Average	Exponential Smoothing
MAD	0.00542	0.00722	0.00357	0.00733
MAPE	4.107 %	5.420 %	2.650 %	5.472 %

According to results of time series change forecast of the average EVI from Terra/Modis Satellite data to examine the drought condition during 10 years (2010 – 2019) in Yasothon Province and by comparing the results between the original EVI and data that passed smoothing algorithm by RMMEH method, with the use of both methods including moving average method and exponential smoothing method, it could be concluded as follows:

(1) To classify the level of drought conditions from EVI during 10-year period, in each year it was possible to make a comparison; if EVI is lower than the means which is 0 to -1, it means that the drought condition is highly severe; if EVI is approaching near 1, it means the vegetation is fertile thus no occurrence of drought condition or little drought condition. According to this study, it can be seen that during 10-year period, the average EVI in each year was very low, suggesting clearly that there was the occurrence of drought condition in the area. And it was found that in 2011, the drought condition was higher than that in other years in the 10-year period since the average EVI was equal to 0.120 which was very close to 0.

(2) From the analysis of Table 2 showing RMMEH method used in adjusting the average EVI, it was found that the average EVI adjusted by RMMEH method was higher than the original EVI, suggesting that RMMEH method could reduce the variable of primary data suitably.

(3) It was found from the comparison of the forecast by checking for accuracy of forecast using MAD and MAPE, it was found that the MAD and MAPE of the forecast of the original average EVI and the average EVI adjusted by RMMEH method were slightly different, and the average EVI adjusted by RMMEH method with the forecast by moving average was best accurate.

(4) It was found from the time-series change forecast of EVI that the original EVI and the EVI that had smoothing algorithm by RMMEH in the forecasted years during 2020 – 2022 by using moving average and exponential smoothing had very low means in each year, suggesting that the drought condition will occur in the future.

Many researchers have applied satellite data in combination with vegetation indices such as NDVI SVI and EVI to monitor drought. For example, the study titled "Performance of Smoothing Methods for Reconstructing NDVI Time-Series and Estimating Vegetation Phenology from MODIS Data" by (Cai et al., 2017), the study titled "Remote Sensing Based Drought Monitoring in the Middle-part of Northeast Region of Thailand" by (Rotjanakusol & Laosuwan, 2018), the study titled "An Investigation of Drought around Chi Watershed during Ten-year Period using Terra/Modis Data" by (Rotjanakusol & Laosuwan, 2019). All three researches mentioned above can be concluded that using satellite data together with vegetation indices can effectively monitor drought.

Additionally, in this study similarly found that the result was in the same direction with other research such as A Novel Compound Smoother—RMMEH to Reconstruct MODIS NDVI Time Series (Jin & Xu, 2013), Normalized Difference Vegetation Index Times Series Change Forecasting Using. Moving

Averages Method and Exponential Smoothing Methods (Sangpradid, 2017). Both studies concluded that when applying the data of average EVI adjusted by smoothing algorithm using RMMEH method, the results were more accurate.

CONCLUSIONS

Drought in Thailand straight affects the agriculture and water source since Thailand is the country where the people do farming particularly growing rice; moreover, Thailand is classified as one of the largest field of rice production in the world. Regularly, the drought in Yasothon Province that straight affects agriculture happens in rainy season where the rainfall is missing for an extended period of time. In this research, the focus was on forecasting time series change of the average enhanced vegetation index to monitoring drought condition by using Terra/MODIS data. From this study it can be concluded that the adjustment by smoothing the average EVI using RMMEH method can be applied with other methods also. The adjustment of the average EVI by RMMEH could help decrease the variance of data; and the map of level of drought condition created by using EVI data of Yasothon Province of Thailand during 10-year period showed clearly the drought areas. Therefore, if the plan is prepared in advanced before the situation, it will efficiently help alleviate and handle the impact from the drought.

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EFFECT OF NANOPARTICLE METAL SOLUTIONS ON CHUFA (*CYPERUS ESCULENTUS* L.) ROOT AND SEEDLING GROWTH

SUMMARY

Nanoparticles of trace elements are easily included in the metabolic cycles of plants. Zinc and molybdenum are important components of enzymes, but even at low concentrations can have phytotoxic effects. Treatment of chufa tubers with microelements increases the viability of tubers, but at certain concentrations of nanoparticles can negatively affect the growth processes at the germination and early vegetative stages. Effect of treatment of tubers with nanoparticle solution of molybdenum, zinc oxide and zinc citrate at a concentration of 20, 40 and 60 ppm on the growth of roots and seedlings was established on 3rd and 10th days after emergence. The positive effect of all treatments at a concentration of 20 ppm was found, but higher concentrations caused different effects on root growth. Zinc citrate and molybdenum nanoparticle solution at a concentration of 60 ppm adversely affect the weight of the root, leaves and root length. Pre-sowing treatment with zinc citrate is indirectly more effective than zinc oxide nanoparticles against root growth at concentrations of 20 and 40 ppm but inhibits it at 60 ppm. Treatments with zinc oxide nanoparticles are more stable regardless of concentration. Short-term effects on root growth processes indicate the prospects of increasing drought resistance and other mechanisms of physiological adaptation.

Keywords: molybdenum, zinc, raw weight, root length, pre-seed treatment

INTRODUCTION

Chufa (*Cyperus esculentus* L.) is a drought-resistant crop with a high oil content in the tubers (Mokady and Dolev, 1970). High oil content in the reproductive organs can adversely affect the viability of the tubers (Pascual *et al.*, 2000; Makareviciene *et al.*, 2013). Micronutrients in a colloidal and nanoparticle form increase the sowing suitability of seeds and vegetative organs, but their

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effect has been little studied in non-traditional crops (Farooq *et al.*, 2012). Nanoparticles are characterized by high absorption capacity and can be included in physiological cycles in a short time. NPs have a strong influence on the formation of elements of productivity in crops under foliar application (Tului *et al.*, 2021), but their effect in the pre-seeds is manifested much earlier. Seed treatment by solutions of NPs can increase plant resistance to fungi and viral diseases, stimulate the growth of roots and stems (Bohdan *et al.*, 2021).

The most important micronutrients are also heavy metals, and their excess can cause phytotoxicity (Taran *et al.*, 2016). Treatment with different nanoparticle metal solution have a long-term effect of gradual growth of the chlorophyll index, dry matter accumulation and other important parameters of productivity (Son'ko *et al.*, 2013; Islas- Valdez *et al.*, 2020). New materials require the establishment of optimal parameters for their use, as the phytotoxicity of different compounds varies and depends on the species of plants (Singh *et al.*, 2013). Pre-sowing treatment of tubers with nanoparticle solution is poorly understood because each plant species has its own ways of including micronutrients in metabolic cycles.

Purpose of this study is to establish the optimal rates of highly active nanoparticle solutions of ZnO, zinc citrate and Mo NPs. Chufa has a relatively low threshold of phytotoxicity to nanometals, in particular various forms of zinc, because the concentration of 60 ppm already has a negative effect on growth processes compared to control without treatment (Honchar *et al.*, 2021). Molybdenum is an important element for plant growth because it is part of many enzyme systems, including those that counteract oxidative stress (Barker and Pilbeam, 2015). Molybdenum is low-toxic to plants (Marschner, 2012), but according to recent studies, even a concentration of 100 ppm inhibits growth processes in *Oryza sativa* (Sharma *et al.*, 2020) and 250 ppm in *Solanum tuberosum* (Mushinskiy and Aminova, 2019). Morphologically and physiologically, chufa (family *Cyperaceae*) differs from cereals and dicotyledonous plants, so studies of the effect of nanometals on growth processes as a component of productivity are important.

MATERIAL AND METHODS

Experimental conditions

Laboratory tests were performed on tubers of chufa variety Pharaoh. Weight of 1 tuber was 1.14±0.02 g. Tubers of chufa had planted in perlite. Air temperature during germination was +22 °C. Chufa plants cultivated on a 12-hour light/12-hour dark cycle in controlled conditions in January of 2021. Preparation of nanoparticle solutions and laboratory research were performed in the laboratory of plant material quality in National University of Life and Environmental Sciences of Ukraine (Kyiv, Ukraine).

Tubers were treated with solutions of nanoparticles (zinc oxide nanoparticles – ZnO NPs; zinc citrate; molybdenum nanoparticles – Mo NPs) with a concentration of 20, 40 and 60 ppm on the day of planting. Variant without

nanoparticle treatment (only water) was a control. Rate of consumption of the stock solution (500 mg of nanoparticle per litre) to pre-sowing treatment was 0.4 (20 ppm), 0.8 (40 ppm) and 1.2 (60 ppm) litre per tonne of tuber. Working solution to pre-sowing treatment was 10 litre per tonne of tubers.

Sampling

Mass of roots and leaves was determined on the 3rd and 10th day after seedling emergence. Ten plants (n=10) from each variant were selected. Root system and leaf surface were weighed separately, the weight of the tuber was not taken into account. Root length was established as maximum length.

Statistical analysis

Suitability of the sample for statistical analysis was evaluated according to the Shapiro-Wilk test. Effect power on 3rd day and 10th day was assessed by two-way ANOVA. Difference between the variants was assessed by Fisher`s LSD post-hoc test at 5% probability.

RESULTS AND DISCUSSION

Root and leaf weight at 3rd day after emergence

Seed treatment with a solution of nanoparticles has a positive effect on the growth of the root system at a concentration of 20 ppm, but pre-sowing treatment with zinc citrate was more effective at 3rd day after emergence (Fig. 1). Root weight in the treatment with zinc citrate in concentration 20 and 40 ppm does not differ significantly from each other, but at 60 ppm there is a significant inhibition of root growth (root weight is significantly lower than the control variant without treatments).

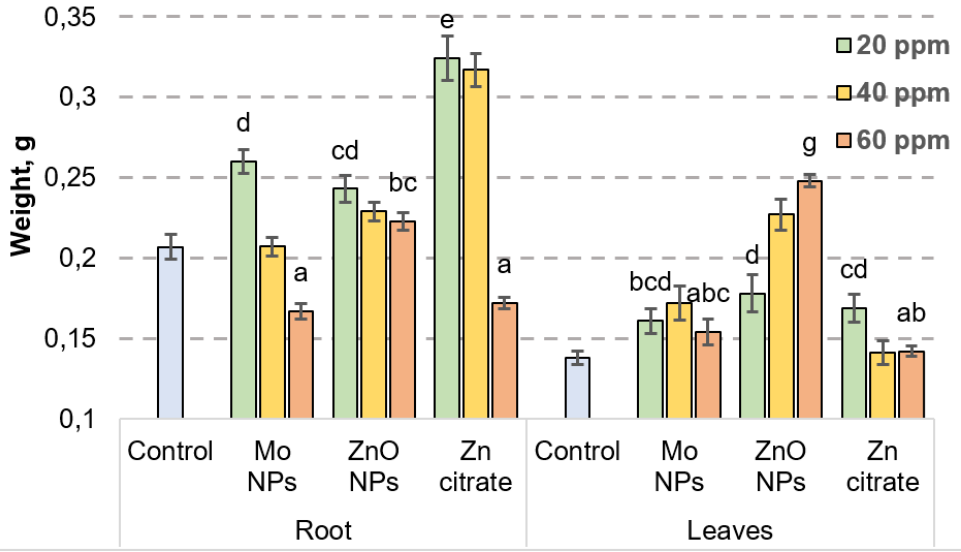

Figure 1. Root and leaf weight (g) depend on concentration of nanoparticle solutions in pre-seed treatment, 3rd day after emergence (Means followed by the same letter do not differ by the Fisher`s LSD test at 5% probability)

Treatment with ZnO NPs gives a smaller increase in percentage, but phytotoxicity is not manifested even at concentrations of 60 ppm, however, a significant increase is only at concentrations of 20 and 40 ppm. Mo NPs treatment has a higher contrast, because root weight increases at 20 ppm, but it significantly decreases at 40 ppm and an even smaller root mass is formed by treatment at 60 ppm.

Formation of leaf mass compared with roots had a different response to pre-sowing treatment with nanoparticle solutions on 3rd day after emergence. Leaf weight in treated variants with Mo NPs increased by 11.6–24.6 percent compared with the control one without a significant difference between the different concentrations. Pre-sowing treatment with ZnO NPs significantly increases the leaf weight and with increasing concentration the positive effect increases too. Zinc citrate has a much smaller effect on leaf mass. Significant increase in this indicator was at a concentration of 20 ppm and there was no significant difference compared with control variant at an increase to 40 and 60 ppm at 3rd day after emergence.

Treatment with ZnO NPs has a positive effect at concentrations up to 9 μM (equivalent 0.7 ppm), while simple zinc oxide is toxic to dicotyledonous plants in same concentration (Singh *et al.*, 2013). Vigor and germination stay high even at a concentration of 80 ppm ZnO NPs in monocotyledons (Esper Neto *et al.*, 2020). Phytotoxicity of zinc oxide nanoparticles in chufa may be at a much higher concentration than the studied ones.

Root and leaf weight at 10th day after germination

Root weight has a more stable response to the concentration of the nanoparticle solutions at the 10th day after emergence (fig. 2).

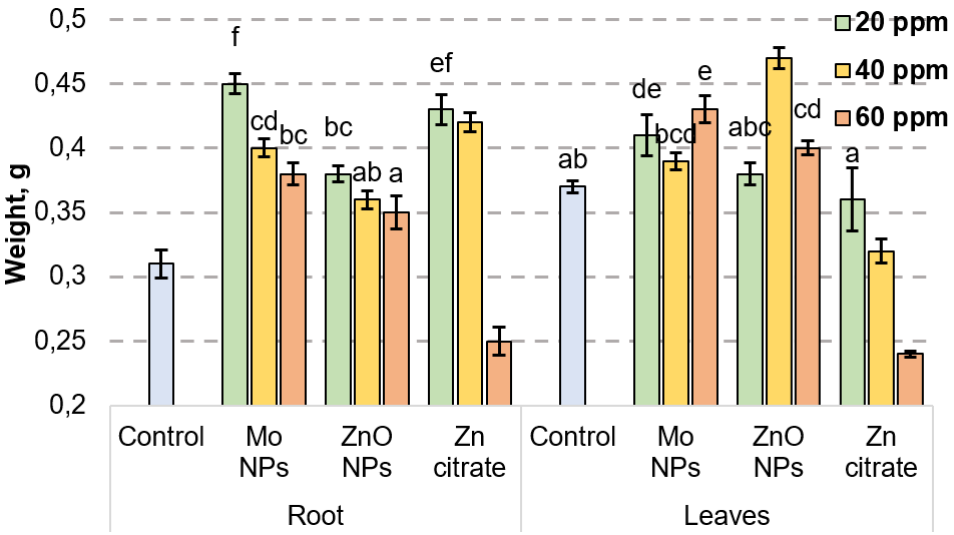

Figure 2. Root and leaf weight (g) depend on concentration of nanoparticles solution in pre-seed treatment, 10th day after germination (Means followed by the same letter do not differ by the Fisher`s LSD test at 5% probability)

The most effective treatment is Mo NPs solution at a concentration of 20 ppm. Root weight is 0.45 g in this variant, while it is 0.31 g in control variant. Root weight was 0.40 g at a concentration of 40 ppm, and it decreased insignificantly with increasing concentration to 60 ppm. Long-term effect of treatment with ZnO nanoparticles can be positive, but has species and varietal specificity (Batsmanova *et al.*, 2020). Root mass significantly exceeded the control variant under treatment with ZnO NPs. It increases in concentration from 20 to 40 ppm and root mass was significantly reduced at 60 ppm. Phytotoxicity of zinc citrate on the growth of the root system remains for a long period of time. Reason for the phytotoxicity of zinc citrate may be its greater digestibility (Montanha *et al.*, 2020).

Leaf growth after germination is significantly slowed down, the manifestation of phytotoxicity is more stable. Leaf weight in treated variants with Mo NPs and ZnO NPs does not fluctuate significantly in percentage, then a significant inhibition of leaf growth occurs in treated variants with zinc citrate with increasing concentrations to 40 and 60 ppm.

Plant weight

Plant weight on the 3rd day after emergence is a marker of efficiency the utilization of tuber spare substances (fig. 3). Treatment of tubers with ZnO NPs significantly increases the plant weight with increasing concentration from 20 to 40 ppm, but the difference between 20 and 60 ppm is insignificant. At the same time, pre-sowing treatment with zinc citrate with increasing concentration forms less plant weight and significant inhibit the growth processes at 60 ppm. Decrease in plant weight is gradual with increasing concentration of Mo NPs and growth processes are inhibited more at 60 ppm (same to zinc citrate, 60 ppm).

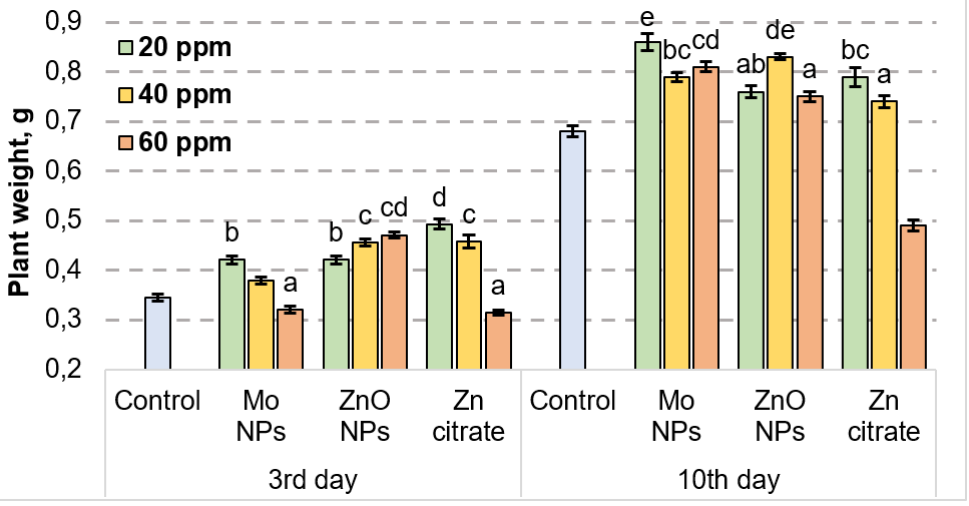

Figure 3. Plant weight (g) depends on concentration of nanoparticles solution in pre-seed treatment, 3rd and 10th day after germination (Means followed by the same letter do not differ by the Fisher`s LSD test at 5% probability)

Plant weight in almost all nanoparticle solutions and their concentration effectively affect the plant weight, compared with the variant without treatment at 10th day after emergence. Only the variant of zinc citrate at a concentration of 60 ppm had a negative effect on plant weight at this period. Elevated concentrations of zinc citrate in the plant can provoke long-term phytotoxicity (Flis *et al.*, 2016).

Root length

Root length depended on the type of solution and its concentration (table 1). There was no significant difference in molybdenum NPs treatments between concentrations of 20 and 40 ppm on 3rd and 10th day after emergence, but there was growth inhibition at a concentration of 60 ppm (root length did not differ from the control variant). Pre-sowing treatment with Mo NPs can increase the length of roots in many plants, but certain species are more sensitive (Chhipa, 2017).

Treatment with ZnO NPs significantly increased root length, and there was no significant difference in different concentrations at 3rd day after emergence. Root growth slowed and variant with treatment at concentration of 60 ppm had shorter roots than 20 and 40 ppm ones at 10th day after emergence.

Treatment of chufa tuber with zinc citrate at a concentration of 60 ppm significantly inhibited root growth, which was manifested in a shorter root length (10.7 cm) than without treatments at 3rd day after emergence. Root length was less at a higher concentration, but at the maximum concentration still exceeded the control variant at 10th day after emergence. This indicates that phytotoxicity has begun to decrease.

Table 1. Root length depends on concentration of nanoparticle solutions in pre-seed treatment.

Solution	Concentration, ppm	3rd day	10th day
Control (water)	–	13.3^{ab}	13.6^{a}
Mo NPs	20	15.0^{cd}	18.6^{cd}
	40	14.5^{bc}	19.3^{d}
	60	12.2^{a}	13.8^{ab}
ZnO NPs	20	16.0^{cde}	20.1^{de}
	40	16.1^{de}	19.6^{d}
	60	15.5^{cd}	17.2^{c}
Zn citrate	20	17.3^{e}	21.6^{e}
	40	16.3^{de}	18.6^{cd}
	60	10.7	15.4^{b}

Means in column followed by the same letter do not differ by the Fisher`s LSD test at 5% probability.

Root length in treated variants with ZnO NPs in chufa began to decrease at a concentration of 60 ppm, while the concentration of 80 ppm had a positive effect in corn (Esper Neto *et al.*, 2020).

Correlation between root length root, leaf and plant weight

Concentration of the studied solution had a negative correlation with root weight on the 3rd and 10th day after emergence ($p<0.01$), but this effect was much weaker and significant only at the level of $p<0.05$ in treated variants with ZnO NPs (table 2).

Table 2. Linear correlation coefficients

Solution	Root weight	Leaf weight	Plant weight	Root length
3rd day after emergence				
Mo colloidal	–0.89**	-0.10^{ns}	–0.87**	–0.65**
Zn colloidal	–0.38*	0.72**	0.67**	-0.14^{ns}
Zn citrate	–0.81**	–0.45*	–0.88**	–0.78**
10th day after emergence				
Mo colloidal	–0.76**	0.21^{ns}	–0.42*	–0.67**
Zn colloidal	–0.41*	0.18^{ns}	-0.09^{ns}	–0.53**
Zn citrate	–0.83**	–0.72**	–0.89**	–0.78**

* $p<0.05$; ** $p<0.01$; ^{ns} – no significant.

Relationship between concentration of nanoparticle and leaf weight varied depend on type of solution and observation time. The increase in concentration had a positive correlation with leaf weight on 3rd day, but there was no such effect on 10th day. This indicates that zinc from ZnO NPs is involved in improving the reutilization of tuber spare substances for leaf formation during germination. The concentration of molybdenum nanoparticles was not significantly related to leaf weight at 3rd and 10th day after emergency, because Mo do not take part in leaf formation processes in early stages, but important during active dry mass assimilation (Rana *et al.*, 2020). Negative correlation between zinc citrate concentration and leaf mass may be occurred by toxicity caused by increased "non-profit" zinc, which is not included in biological cycles (Ullah *et al.*, 2019).

Plant weight had a negative correlation with increasing concentration of zinc citrate and Mo NPs on 3rd and 10th day after emergence. Plant weight increased at a higher concentration in treated variants with ZnO NPs with on 3rd day, but the effect of concentration had no statistically significant effect on 10th day. Short-term positive reactions to ZnO nanoparticle treatment are typical of a wide range of organism (Rajput *et al.*, 2018).

Effect of concentration on the maximum root length was negative in most treatment variants on 3rd and 10th day, exclude ZnO NPs at 3rd day, when this correlation was insignificant.

The positive effect on biometric parameters at different concentrations was caused the ability of zinc nanoparticles to reduce overall phytotoxicity, inducing over expression of antioxidant defense enzymes (Venkatachalam *et al.*, 2017). However, the negative effect of increasing the concentration of zinc may be manifested at lower rates than in other plants (Liu *et al.*, 2016).

Nanoparticle treatment of tubers can affect the architecture of the root system, changing the ratio of roots of different lengths and inducing the growth of many short hairs. Root weight was increased with increasing concentration of ZnO NPs, but its length decreased, which is a consequence of these processes. The obtained results are consistent for other crops with a fibrous root system, as nanoparticles also have a positive effect on the growth of secondary roots (Estrada-Urbina *et al.*, 2018).

CONCLUSIONS

Pre-sowing treatment of tubers with metal nanoparticle solutions has different effects on the growth of roots and seedlings. Molybdenum nanoparticle solution is characterized by negative effects on root and seedling growth at a concentration of 40 ppm on the 3rd day after emergence, but short-term phytotoxicity can be overcome, and the treatment efficiency is better than in the control variant on the 10^{th} day after emergence. Negative effect of Mo NPs at concentration of 60 ppm on the growth of the root system requires further study. ZnO NPs in a concentration from 20 to 60 ppm has a positive effect on the weight of the plant, root and stem, root length, but the most effective for a combination of different indicators is the treatment at a concentration of 40 ppm. Pre-sowing treatment with zinc citrate has more phytotoxic effect on leaf growth than other NPs solutions. Negative effect is observed at a concentration of 60 ppm on the 3^{rd} day after emergence, and slightly weakens on the 10^{th} day under zinc citrate treatment. Pre-sowing treatment with ZnO NPs is highly effective at a concentration of 20–60 ppm, while zinc citrate has a stable effect only at 20 and 40 ppm.

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THE GENUS TURCOGAMMARUS G. KARAMAN & BARNARD 1979 (FAM. PONTOGAMMARIDAE) IN MONASTERY HILANDAR REGION (GREECE) (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 323)

SUMMARY

First member of freshwater Crustacea Amphipoda, *Turcogammarus spandli* S. Karaman, 1931 (Fam. Pontogammaridae), is discovered and described from the region of Monastery Hilandar on Mount Athos on Halkidiki peninsula, northern Greece. This species is redescribed, figured and variability of its taxonomical characters are given. The review of all known members of genus *Turcogammarus* G. Karaman & Barnard, 1979 and key to the species of this genus are presented. Taxonomical position of genus *Turcogammarus* within family Pontogammaridae is discussed.

Keywords: taxonomy, Amphipoda, Pontogammaridae, *Turcogammarus spandli*, freshwater, Hilandar Monastery, Greece

INTRODUCTION

Hilandar Monastery is one of the twenty Eastern Orthodox monasteries on Mount Athos in northern Greece, and the only Serbian monastery there. It was founded in 1198 by Stefan Nemanja (Saint Symeon) and his son Saint Sava. It is situated on northern side of Mount Athos, on Athos peninsula of Halkidiki peninsula, Aegean Sea, Northern Greece. This monastery has strict tradition that bars women from stepping into Mount Athos. As this part of Halkidiki peninsula is practically isolated and needs the permissions to visit them, the biodiversity of this region is poorly known.

The fauna of Amphipoda of entire Mount Athos was never studied. During my visit to monastery Hilandar in September 1996 with some other Academicians of Academy of Sciences and Arts of Montenergo and Academy of Sciences and Arts of Serbia, I have a short opportunity to collect the amphipods from the brook near Monastery, presented in this work.

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MATERIAL AND METHODS

The material was preserved in 70% ethanol. The specimens were dissected using a WILD M20 microscope and drawn using a camera lucida attachment. All appendages were temporarily submersed in a mixture of glycerin and water for study; dissected body-parts were fixed in Liquid of Faure and covered by thin cover glass.

Some morphological terminology and setal formulae follows Karaman`s terminology (Karaman G., 1969) for mandibular palpus article 3 [A= A-setae on outer face; B= B-setae on inner face; C= additional submarginal C-setae on outer face; D= lateral marginal D-setae; E= distal long E-setae]. All illustrations were inked manually.

Term "setae" and "spines" are used based on its shape, not origin. The investigations were provided based on morphological, ecological and zoogeographical studies.

TAXONOMICAL PART

Order AMPHIPODA
Suborder SENTICAUDATA
Family PONTOGAMMARIDAE Bousfield, 1977

Genus *TURCOGAMMARUS* Karaman, G. & Barnard, J.L., 1979: 137

Typus generis: *Turcogammarus turcarum* (Stock, 1974).

Species: *Turcogammarus aralensis* (Uljanin, 1875), *setosus* (Schäferna, 1914), *spandli* (S. Karaman, 1931), *turcarum* (Stock, 1974).

TURCOGAMMARUS SPANDLI (S. Karaman, 1931)
Figures 1-8

Ostiogammarus spandli S. Karaman, 1931: 45, fig. 2; S. Karaman, 1934: 219; S. Karaman, 1953: 49, 57;

Dikerogammarus spandli Birstein, 1945: 519, 520; Carausu, Dobreanu & Manolache, 1955: 54; Barnard, J.L., 1958: 48; Straškraba, M., 1967: 204;

Pontogammarus spandli Schellenberg, 1937: 267; Karaman, G., 1971: 36, figs. 39-55;

Obesogammarus spandli Stock, 1974: 83;

Turcogammarus spandli Karaman, G. & Barnard, J.L., 1979: 137; Barnard, J.L. & Barnard, C.M.1983: 170; Sket & Hou, 2018: suppl. 2: 6.

MATERIAL EXAMINED: **GREECE:**

S-5422= Monastery Hilandar, Halkidiki peninsula, Agio Oros, Mt. Athos, torrent near Monastery, 24.9.1996, many exp. mixed with one juv. exp. *Niphargus* sp. (leg. G. Karaman);

S-1439= Volvi Lake, 60 km E of Thessaloniki, coast, northern Greece, 24.4.1962, 14 exp. (leg.?Kosswig);

S-1454= Drama, spring "Varvara", northern Greece, many exp., 20.10.1942 (leg. Buresch);

S-4200= Drama, 20.10.1942, 9 exp. (leg.?);

G-266= Greece 266, loc?, 6 exp. (leg. ? K. Lindberg);

SLIDES: holotype, Dervent near Thessaloniki, northern Greece.

DIAGNOSIS OF SPECIES

Body moderately stout, mesosomal segments flat or last mesosomal segment with dorsal longitudinal elevation or tooth; metasomal segments 1-3 with more or less developed dorsomedian elevation obtusely triangular or toothed (hooked). Urosomal segments 1-2 with dorsomedian tubercle bearing spines and setae; urosomal segment 3 flat, with dorsolateral groups of elements (spines and setae) and dorsomedian surface with 2 short setae. Telson incised nearly to the basis. Epimeral plate 1 nearly subrounded, epimeral plates 2-3 pointed, all plates setose. Antenna 1 shorter, peduncle inflated, accessory flagellum 3-4-articulated. Antenna 2 remarkably setose, flagellum longer than last peduncular article; eyes reniform.

Mouthparts nearly *Gammarus*-like, labium without inner lobes. Mandible palpus 3-articulated, with A, C, D and E-setae. Maxilla 1 palpus left and right asymmetric; Maxilla 2 inner plate with marginal and submarginal row of setae; maxilliped plates obtuse distally, palpar article 4 with slender spines at inner margin. Coxae 1-4 long, coxa 4 with ventroposterior lobe, coxae 5-7 much shorter than coxae 1-4, bilobed. Gnathopods 1-2 dissimilar, with *Gammarus*-like propodus bearing median palmar spine in males; dactylus of pereopods 3-7 with one inner marginal seta near basis of the nail.

Pereopods 3-4 similar, densely setose, pereopod 4 smaller than 3. Pereopods 5-7 dissimilar: article 2 of pereopods 5 and 7 lobed, that of pereopod 6 unlobed, all with densely setose posterior margin of article 2 and bearing facial setae. Pleopods with 2 retinacula, peduncles remarkably setose. Uropod 1 rami equal, uropod 2 inner ramus longer than outer one. Uropod 3 inner ramus short; outer ramus 2-articulated, densely setose, second article very short. Telson incised nearly to the basis. Sexual dimorphic characters developed.

DESCRIPTION

MALE 12.8 mm [Hilandar]: Body moderately stout, mesosomal segments smooth; metasomal segments 1-3 with dorsomedian longitudinal subrounded carina more or less elevated in the middle (fig. 1B). Urosomal segments 1-2 with dorsomedian tubercles provided with 2 spines and 6-7 setae as

long as or longer than spines; urosomal segment 3 flat, provided on each dorsolateral side with group of 3 strong spines and 3 setae (fig. 1C), in the middle of segment 3 appears 2 short setae (1C). Urosomal segment 1 without ventroposterior spine near basis of uropod 1-peduncle but with one bunch of short ventromarginal setae (fig. 1C).

Epimeral plate 1 nearly subrounded, with poorly marked ventroposterior corner-seta; anterior and ventral margin with numerous simple setae, along posterior convex margin with row of shorter setae (fig. 4A). Epimeral plates 2-3 strongly pointed; epimeral plate 2 with row of ventral long setae and row of shorter posterior marginal setae; outer surface covered with bunches of facial setae. Epimeral plate 3 less setose than plate 2, along ventral margin with row of single or paired longer setae, along posterior margin with row of several shorter setae; facial setae absent (fig. 4A).

Head with short rostrum and angular lateral cephalic lobes, ventroanterior sinus well developed; eyes reniform, as long as or slightly longer than diameter of first peduncular article of antenna 1 (fig. 1A).

Antenna 1 almost reaching half of body-length; peduncular articles 1-3 progressively shorter (ratio: 52:30:20); first article inflated, longer than broad (ratio: 52:25), bearing 2-3 bunches of distal setae not exceeding diameter of article itself (fig. 1E); article 2 slightly tapering distally, bearing distal and several lateral setae (the longest setae exceeding diameter of article itself); article 3 narrowed, with 2 lateral and 2 distal bunches of setae (the longest setae exceeding diameter of article itself). Main flagellum rather longer than peduncle, with 20 slender articles bearing short marginal setae. Accessory flagellum 4-articulated, longer than last peduncular article of antenna 1 (fig. 1E).

Antenna 2 moderately slender, nearly as long as antenna 1 (in living animals); peduncular article 3 short, rather inflated, provided with ventrodistal bunch of long setae and 2 bunches of short dorsal setae (fig. 1F). Peduncular articles 4-5 of nearly equal length; article 4 ventral margin with 2 groups of setae longer than diameter of article itself, dorsal margin with 5 bunches of short setae; article 5 ventral margin with 4 bunches of long setae (the longest setae exceeding diameter of article itself), dorsal margin with 3 bunches of shorter setae. Flagellum relatively slender, 10-articulated, nearly as long as peduncular articles 4-5, dorsal margin with bunches of short setae, ventral margin with bunches of long setae much longer than diameter of article itself; calceola absent; antennal gland cone short (fig. 1F).

Mouthparts nearly *Gammarus*-like. Labrum broader than long, tapering distally (fig. 2A). Labium broader than long, outer lobes subrounded distally, inner lobes absent (fig. 2B).

Mandibles with triturative molar. Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth accompanied laterally by 8 strong rakers (fig. 2D). Right mandible with 4-toothed incisor, lacinia mobilis bifurcate, serrate, accompanied by 7 rakers (first raker rather shorter and less pectinate than other rakers (fig. 2C).

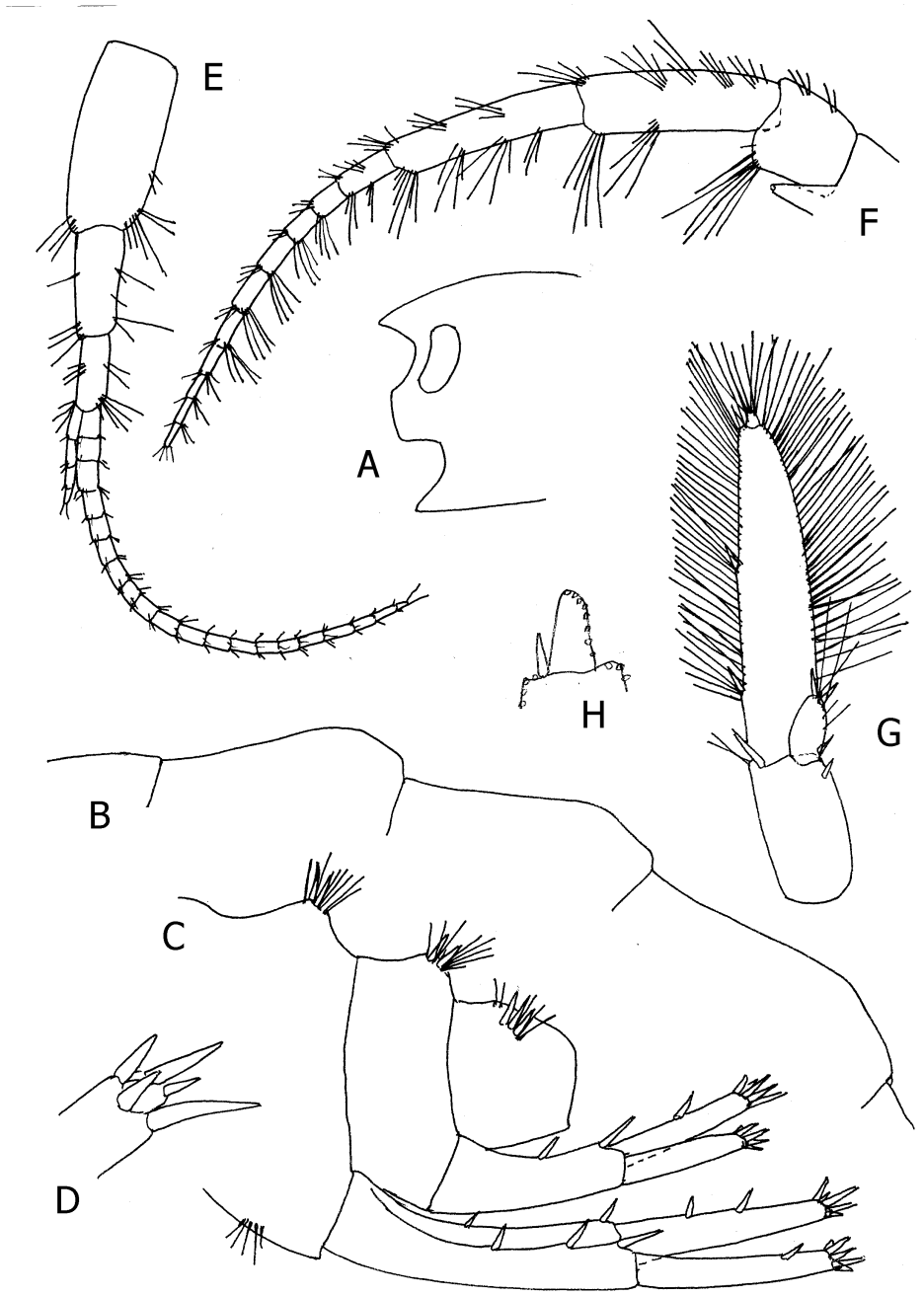

Fig. 1. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, male 12.8 mm: A= head; B= metasomal segments 1-3; C= urosome with uropods 1-2; D= tip of uropod 2 inner ramus; E= antenna 1; F= antenna 2; G= uropod 3 (all setae plumose); H= distal article of uropod 3 outer ramus (setae omitted).

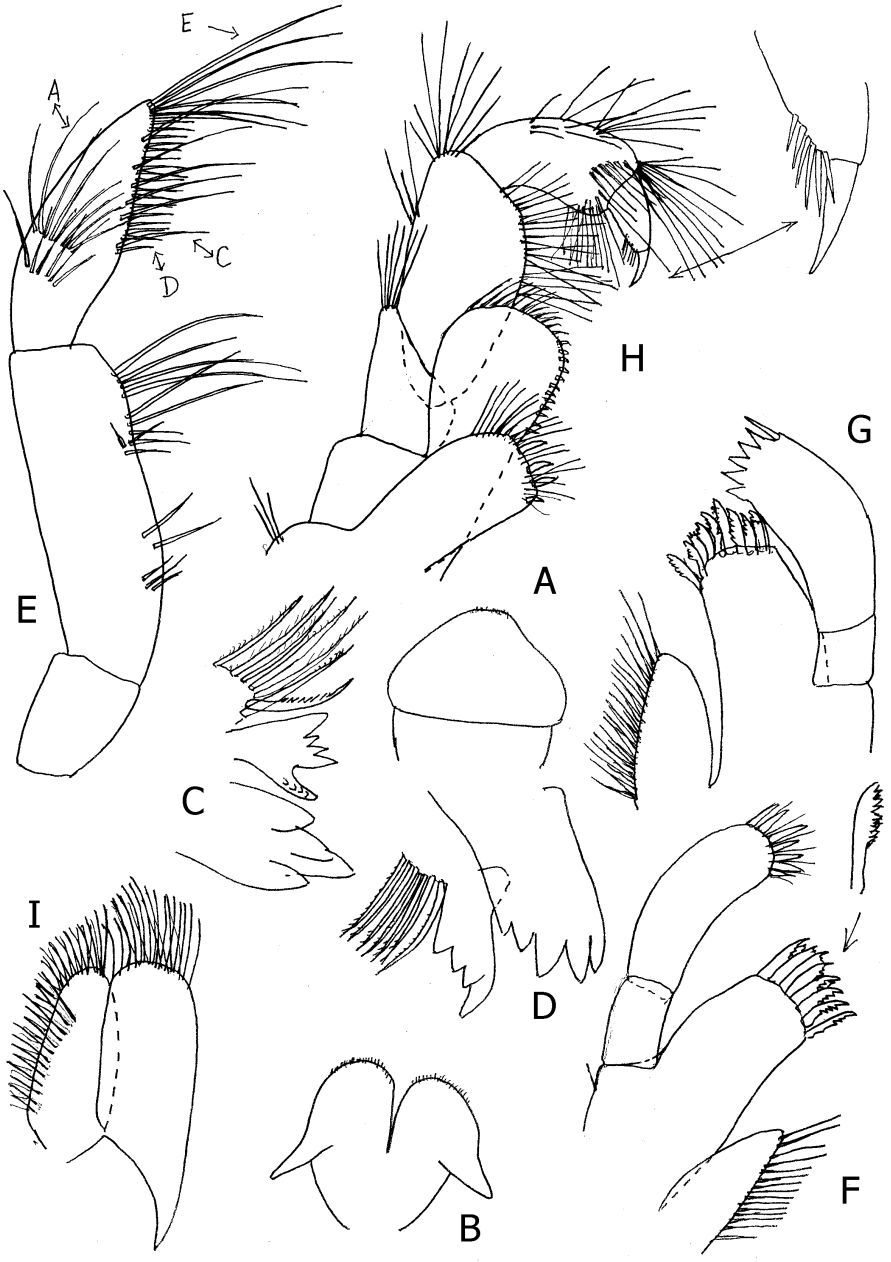

Fig. 2. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, male 12.8 mm: A= labrum; B= labium; C= right mandible, incisor and lacinia mobilis with rakers; D= left mandible, incisor and lacinia mobilis with rakers; E= mandibular palpus, outer face [A= outer facial A-setae; C= outer facial submarginal C=setae; D= marginal D-setae; E= distal E-setae]; F= left maxilla 1; G= right maxilla 1; H= maxilliped; I= maxilla 2;

Palpus similar in both mandibles, 3-articulated: first article naked; second article with nearly 16 strong setae; third (distal) article poorly shorter than second one, subfalciform, with nearly 20 marginal D-setae, 6 submarginal C-setae and 6-7 distal long E-setae (fig. 2E); on outer face 2 transverse rows of A-setae (14 setae) (fig. 2E), B-setae absent.

Left maxilla 1: inner plate triangular, with row of marginal setae at mesial side; outer plate with nearly 9-10 pectinate spines; palpus more narrow, with 5 spines mixed with 5-6 stronger setae (fig. 2F). Right maxilla 1: inner and outer plate like these in left maxilla; palpus broader, with 5 distal strong teeth and one corner spine (fig. 2G).

Maxilla 2: both plates longer than broad, with subrounded distal margin; inner plate slightly smaller than outer one, along mesial margin with 2 rows of setae (marginal and submarginal), distal margin of both plates with numerous setae (fig. 2 I).

Maxilliped: inner plate short and broad, not reaching outer tip of first palpus article, provided with 4 distal spines mixed with several short setae and several setae at distoexternal tip (fig. 2H). Outer plate strong, broad, not exceeding half of second palpus article, at mesial margin with row of short spines, at tip with several spine-like setae. Palpus article 2 at outer margin with 2 bunches of long setae, inner margin with row of numerous long setae; palpus article 3 outer margin with 4 bunches of longer setae, inner margin with numerous distointernal and distal setae; article 4 (dactylus) outer margin with one median short seta, inner margin with 5 slender spines behind nail (fig. 2H).

Coxae 1-4 relatively long. Coxa 1 longer than broad (ratio: 70:40), poorly dilated distally, subrounded distal margin with numerous long setae (fig. 3A). Coxa 2 longer than broad (ratio: 73:42), tapering ventrally, ventral subrounded margin with numerous long setae (fig. 3D). Coxa 3 much longer than broad (ratio: 82:42), slightly tapering distally, ventral subrounded margin with numerous setae (fig. 4B). Coxa 4 longer than broad (ratio: 80:60), ventral convex margin almost straight in the middle, with numerous long setae, ventroposterior lobe well developed (fig. 4D).

Coxae 5-7 much shorter than coxae 1-4, bilobed, ventral margin subrounded. Coxa 5 broader than long (ratio: 50:35), anterior narrowed lobe with 2-3 distal setae, posterior lobe with 5-7 marginal strong setae (fig. 5A).

Coxa 6 smaller than coxa 5, broader than long (ratio: 45:27), anterior lobe with bunch of marginal setae, posterior lobe with row of strong marginal setae (fig. 5B).

Coxa 7 shallow, much broader than long (ratio: 44:23), anterior lobe small, with one lateral bunch of setae, posterior larger lobe with numerous strong marginal setae (fig. 5C).

Gnathopod 1 rather smaller and unequal than gnathopod 2; article 2 posterior margin with long setae, anterior margin with longer and shorter setae. Article 3 with distoposterior bunch of setae (fig. 3A). Article 5 triangular, much shorter than propodus (ratio: 28:49), anterior margin with distal setae, posterior

margin with numerous setae. Propodus slightly egg-shaped, longer than broad (ratio: 113:58), posterior margin with 6 transverse rows of setae, anterior margin scarcely setose, with distal setae (fig. 3B). Palm inclined almost to the half of propodus-length, irregularly serrate, without distinct medial excavation, outer face with 3-4 corner and one median palmar spine mixed with several long setae, on inner face 2 subcorner short R-spines (fig. 3C). Dactylus with one median seta at outer margin (fig. 3B), and 2-3 short setae near nail.

Gnathopod 2: article 2 posterior margin with row of long setae, several proximal setae at anterior margin; median and distal part with row of short setae; article 3 with distoposterior bunch of setae. Article 5 much shorter than propodus (ratio: 32:65), posterior margin with bunch of distoanterior setae and numerous setae (fig. 3D). Propodus remarkably longer than broad (ratio: 120:77), with nearly parallel lateral margins, posterior margin with nearly 11 transverse rows of setae, only single setae at anterior margin and bunch of shorter distal setae (fig. 3E). Palm inclined nearly 2/5 of propodus-length, irregularly serrate, defined on outer face by 3-4 corner single spines mixed with single setae, and one median palmar spine mixed with several long setae, on inner face by 2 short subcorner R-spines (fig. 3F). Dactylus with one median seta at outer margin (fig. 3E) and 1-2 setae near nail.

Pereopods 3-4 moderately stout. Pereopod 3: article 2 anterior margin with proximal long and distal shorter setae, posterior margin with several bunches of long setae; article 3 anterior margin with bunch of median setae and distoposterior bunch of distal setae (fig. 4B). Articles 4-6 of different length (ratio: 45:33:37): article 4 with slightly produced anterior tip, and with 4 anterior marginal bunches of long setae; posterior margin with numerous long setae (the longest setae exceeding diameter of article itself). Article 5 with distoanterior long setae and 4 posterior marginal bunches of long setae. Article 6 with 2 bunches of anterior marginal shorter setae, posterior margin with 7 single short spine mixed with single longer setae (the longest setae exceeding diameter of article itself). Dactylus shorter than article 6 (ratio: 15:37), strong, with one strong seta at inner margin and one slender lateral seta near basis of nail (fig. 4C), nail shorter than pedestal (ratio: 21:34).

Pereopod 4 rather similar to pereopod 3 but slightly smaller; article 2 pilosity like that in pereopod 3; article 3 with 1-3 short anterior and posterior median marginal setae and bunch of long posterior distal setae. Articles 4-6 of different length (ratio: 38:30:33). Article 4 distoanterior tip slightly produced, 3 bunches of anterior marginal setae (fig. 4D), posterior margin with 4 bunches of long setae (the longest setae exceeding diameter of article itself). Article 5 with anterodistal longer setae, posterior margin with nearly 4 bunches of long setae (longer than diameter of article itself). Article 6 anterior margin with 2 bunches of setae, posterior margin with 7 single short spines mixed with single long setae. Dactylus shorter than article 6 (ratio: 15:33), inner margin with setae like these in pereopod 3.

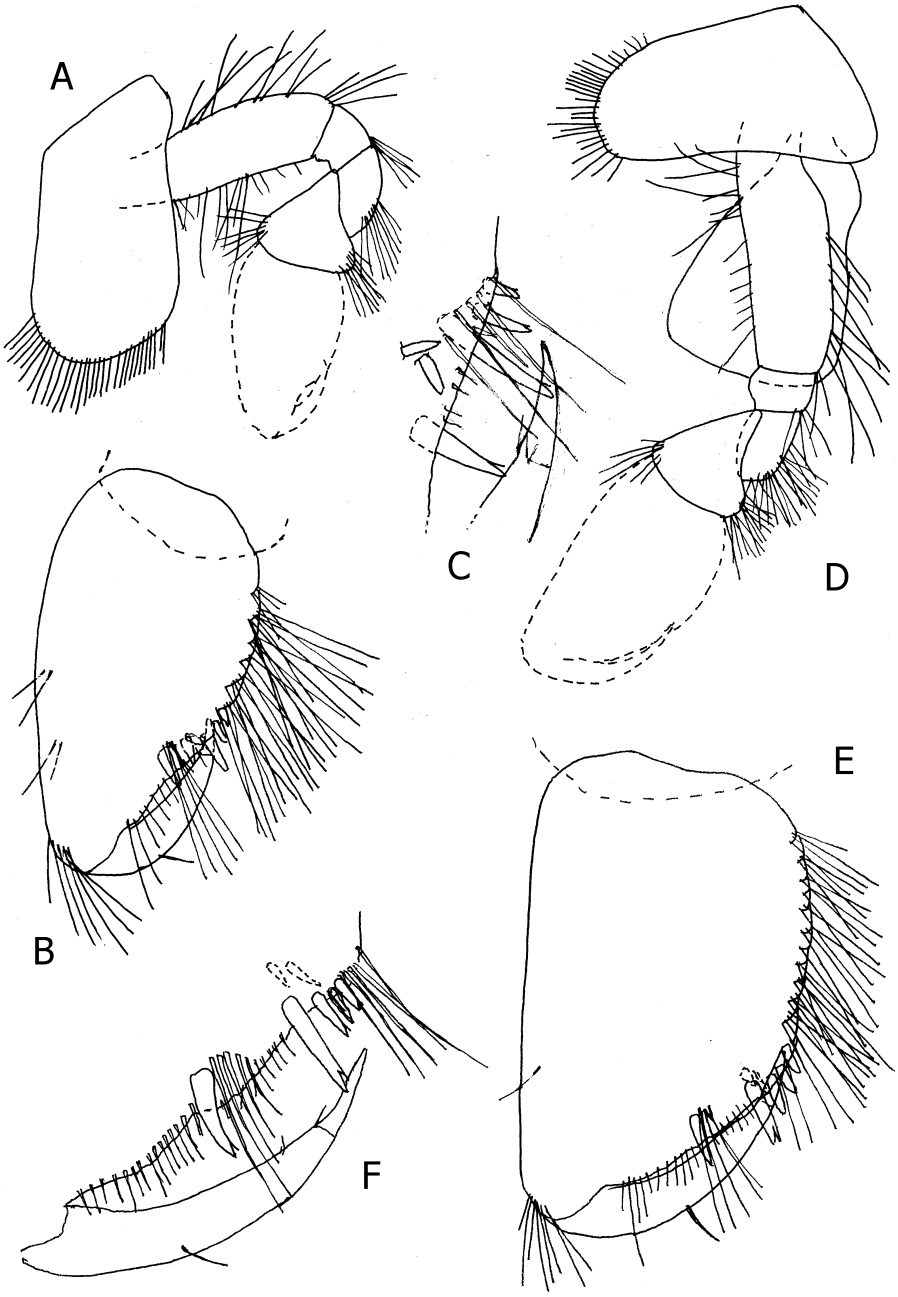

Fig. 3. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, male 12.8 mm: A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1-propodus, inner face; D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2-propodus, outer face.

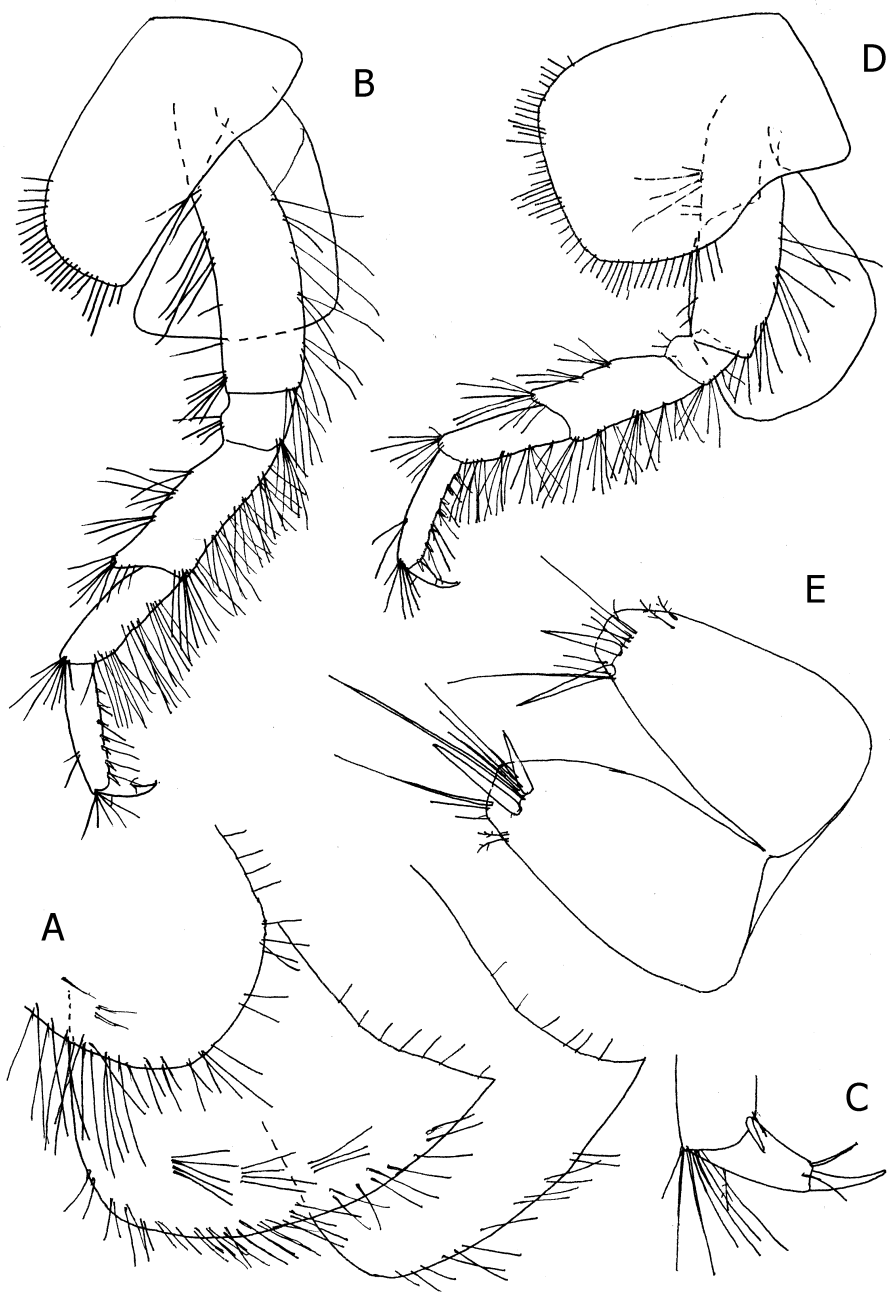

Fig. 4. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, male 12.8 mm: A= epimeral plates 1-3; B-= pereopod 3; C= dactylus of pereopod 3; D= pereopod 4; E= telson.

Pereopods 5-7 moderately stout. Pereopod 5 slightly shorter than pereopods 6-7: article 2 dilated, longer than broad (ratio: 65:23), anterior convex margin with 5-6 single spines mixed with setae as long as or longer than spines, posterior crenulated margin straight in the middle, with row of densely sitting setae, ventroposterior lobe visible; inner face with groups of setae, outer face naked (fig. 5A). Article 3 with anterior distal setae, posterior margin naked. Articles 4-6 of different length (ratio: 40:47:51); article 4 anterior margin with 3 median bunches of setae (most of them as long as diameter of article itself) and distal bunch of setae mixed with one spine (the longest setae exceeding diameter of article itself); posterior margin with 3 groups of spines and setae; article 5 along anterior margin with 3 bunches of long setae and spines, at posterior margin 2 bunches of setae and single spines. Article 6 anterior margin with 6 pairs of short spines often mixed with single seta, posterior margin with 4 bunches of long setae. Article 6 shorter than article 2 (ratio: 51:65). Dactylus much shorter than article 6 (ratio: 20:51) with pilosity like that of pereopod 7.

Pereopod 6 slightly longer than pereopod 5. Article 2 longer than broad (ratio: 77:45), tapering ventrally, outer and inner surface with numerous single or bunches of setae; anterior margin with 6 single spines mixed with several setae each, 2 bunches of setae in proximal part; posterior crenulated margin with dense row of longer setae, ventroposterior lobe absent (fig. 5B). Article 3 with anterodistal setae, posterior margin with bunch of median setae. Articles 4-6 of different length (ratio: 45:57:60); articles 4 and 5 along anterior margin with 4-5 bunches of longer setae (the longest setae exceeding diameter of article itself); article 4 with 2 posterior bunches of setae mixed with single spines; article 5 at posterior margin with median bunch of longer setae and distal setae and spines. Article 6 anterior margin with 6 single of paired short spines and 5 bunches of longer posterior setae. Article 2 shorter than article 6 (ratio: 77:60). Dactylus shorter than article 6 (ratio: 20:60), its pilosity like that in pereopod 7.

Pereopod 7 rather different than pereopods 5-6; articles 4-6 shorter than these in pereopod 6. Article 2 longer than that of pereopods 5-6, large, ovoid, longer than broad (ratio: 88:57), with well developed ventroposterior subangular lobe (fig. 5C), anterior margin with 6 single spines mixed with several setae usually longer than spines; 2-3 bunches of setae in proximal part; posterior convex, finely crenulated margin with dense row of longer setae (fig. 5F); outer face with row of 7-9 bunches of setae (fig. 5C), on inner face appear several groups of 1-3 setae (fig. 5D). Article 3 with distoanterior and medioposterior setae. Articles 4-6 of different length (ratio: 47:50:57). Articles 4-5 with 4 groups of anterior spines mixed with long setae, posterior margin with 2 bunches of setae and single spines. Article 6 with 6 single or paired short anterior spines, posterior margin with 5 bunches of long setae. Article 6 remarkably shorter than article 2 (ratio: 57:88). Dactylus shorter than article 6 (ratio: 20:57), strong, inner margin with long seta, one lateral seta near basis of nail, outer margin with one median plumose seta (fig. 5E), nail shorter than pedestal (ratio: 23:48).

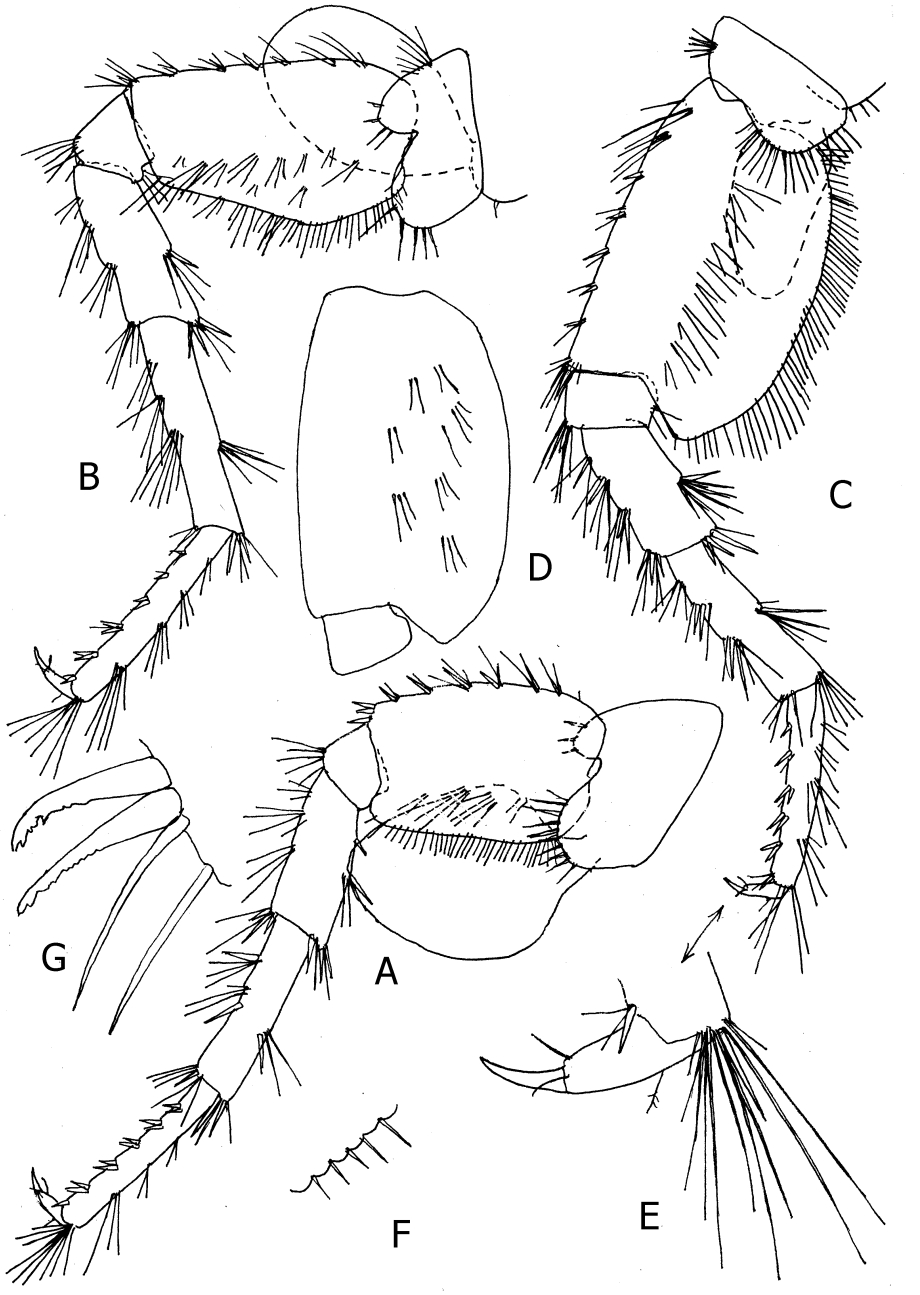

Fig. 5. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, male 12.8 mm: A= pereopod 5, outer face; B= pereopod outer face; C= pereopod 7, outer face; D=article 2 of pereopod 7, inner face (marginal setae omitted); E= pereopod 7 dactylus; F= posterior margin of pereopod 7 article 2; G= retinacula.

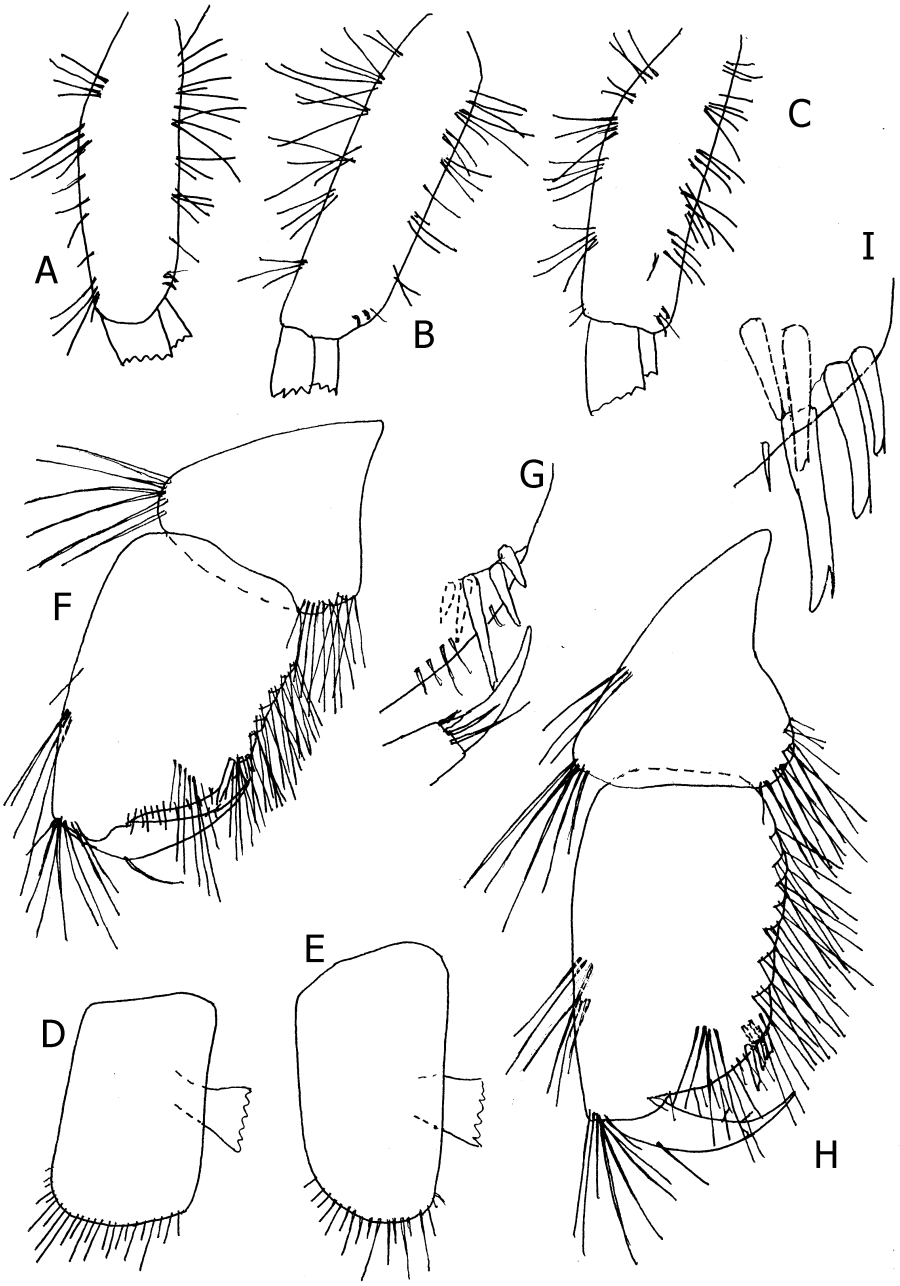

Fig. 6. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, male 12.8 mm: A= pleopod 1; B= pleopod 2; C= pleopod 3.
Female 8.6 mm: D= coxa 1; E= coxa 2; F= gnathopod 1, articles 5-6, outer face; G= distal corner of gnathopod 1-propodus, outer face; H= articles 5-6 of gnathopod 2, outer face; I= distal corner of gnathopod 2-propodus, outer face.

Pleopods 1-3 with 2 retinacula, often mixed with 1-2 setae (fig. 5G). Peduncle of all pleopods with numerous long setae along anterior and posterior margin (figs. 6A-C).

Uropod 1: peduncle longer than rami, with dorsoexternal row of 3 spines and dorsointernal row of 2 spines (fig. 1C); inner and outer ramus of equal length, provided with 1-2 lateral and 5 distal short spines.

Uropod 2: peduncle with single lateral and distal spines; inner ramus longer than outer one, with one lateral and 5 distal short spines; outer ramus with one lateral and 5 short distal spines (fig. 1C, D).

Uropod 3 biramous; peduncle longer than broad, with single distal spines; inner ramus scale-like, much shorter than peduncle, bearing 2 distal spines and 2-3 long setae. Outer ramus 2-articulated: first article long, much longer than peduncle (ratio: 92:41), outer and inner (mesial) margin densely covered with plumose setae, mixed on outer margin with 3 single spines (fig. 1G); second article very short, not exceeding diameter of first article and provided with distolateral plumose setae (fig. 1 H).

Telson slightly broader than long (ratio: 92:83), incised nearly to the basis, lobes tapering distally, with 2 distal spines mixed with several short and long setae; a pair of short plumose setae sitting subdistally at outer margin (fig. 4E).

Coxal gills moderately large, dilated, more or less ovoid, appear on gnathopod 2 and pereopods 3-7 (figs. 3D; 4B, D; 5A, B, C).

FEMALE 8.6 mm with 22 eggs in marsupium (Hilandar)

Body rather smaller and more stout than that in male. Mesosomal and metasomal segments like these in male. Urosomal segments 1-2 with dorsal conical tubercle bearing 2 spines mixed with several setae; urosomal segment 3 flat, on each side with dorsolateral 2-3 spines and single setae, medially with 2 setae. Urosomal segment 1 without ventroposterior spine near basis of uropod 1-peduncle but with bunch of ventromarginal setae. Epimeral plates1-3 like these in male but plates 2-3 less pointed (fig. 7F).

Head and eyes like these in male. Antenna 1 remarkably shorter than half of body-length; peduncular articles 1-3 rather stout, progressively shorter (ratio: 53:27:16), articles with several setae (the longest setae reaching diameter of articles themselves) (fig. 7A), main flagellum 18-articulated, scarcely setose; accessory flagellum 3-articulated, longer than last peduncular article. Antenna 2 and mouthparts like that in male.

Coxae rather similar to these in males. Coxa 1 longer than broad (ratio: 61:40) slightly dilated distally (fig. 6D), with numerous long marginal setae. Coxa 2 longer than broad (ratio: 72:40), slightly tapering ventrally, bearing numerous long distal setae (fig. 6E). Coxa 3 longer than broad (ratio: 80:44), slightly tapering ventrally, with numerous marginal setae (fig. 8B). Coxa 4 longer than broad (ratio: 83:65), with numerous marginal setae, ventroposterior lobe well developed (fig. 8D).

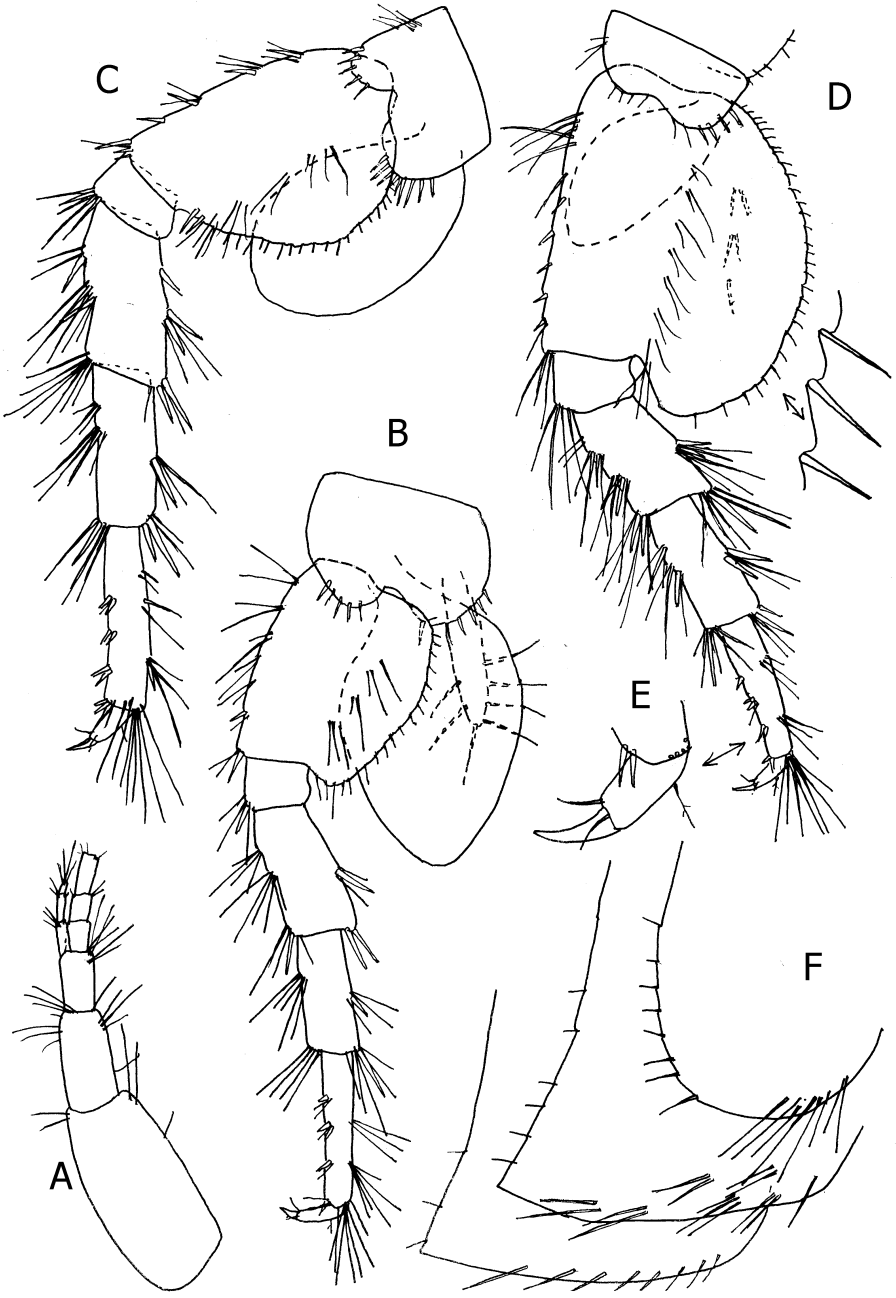

Fig. 7. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, female 8.6 mm: A= antenna 1; B= pereopod 5' C= pereopod 6; D-E= pereopod 7; F= epimeral plates 1-3.

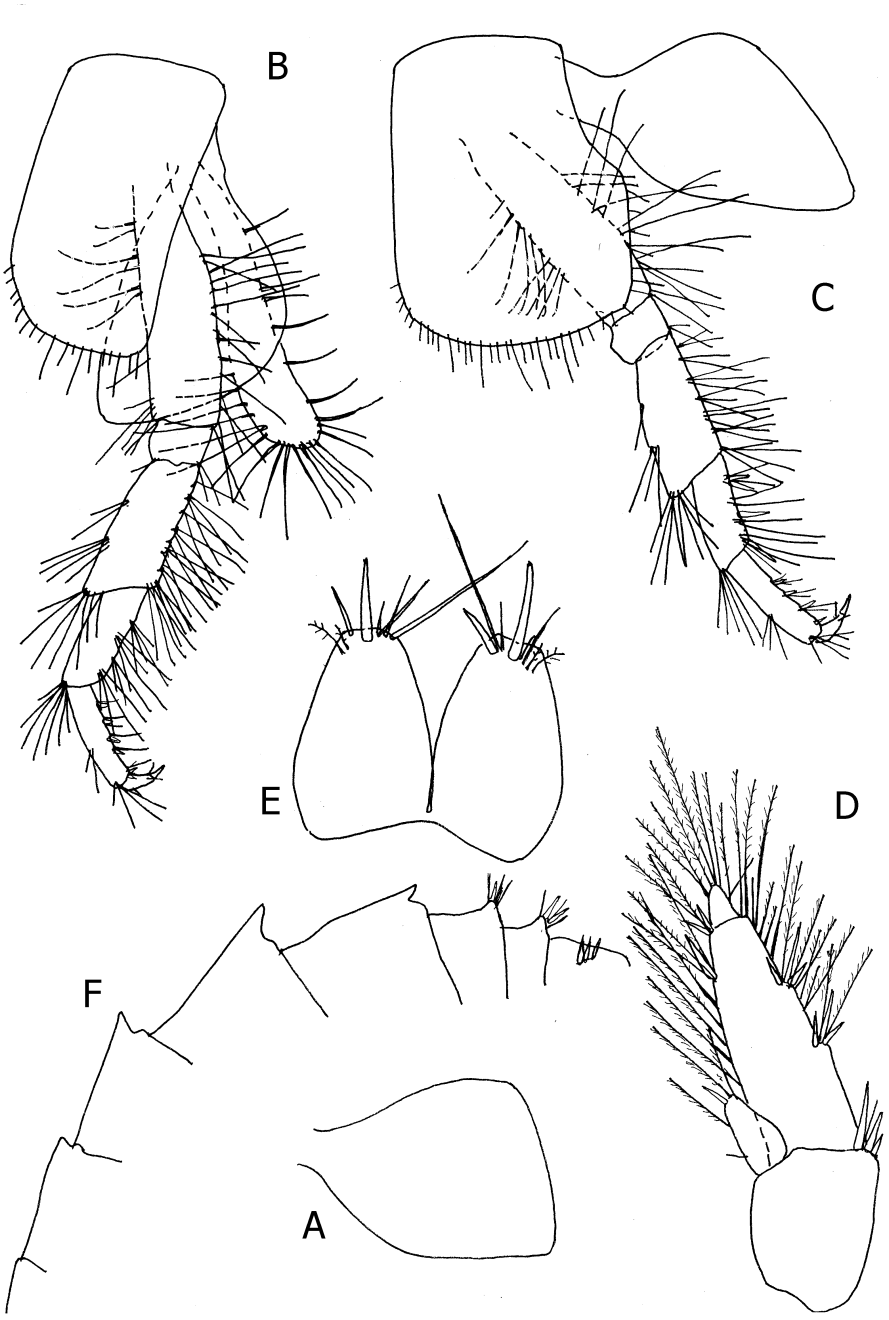

Fig. 8. *Turcogammarus spandli* (S. Karaman, 1931), torrent near Hilandar Monastery, female 8.6 mm: A= gnathopod 2 gill; B= pereopod 3; C= pereopod 4; D= uropod 3; E= telson. F= metasome and urosome of **male 6 mm from Drama** (S-1454).

Coxae 5-7 shallow, bilobed, lobes subrounded ventrally. Coxa 5 broader than long (ratio: 51:35), both lobes with row of marginal spine-like setae (fig. 7B). Coxa 6 broader than long (ratio: 42:30), both lobes with row of spine-like setae (fig. 7C). Coxa 7 broader than long (ratio: 41:21), both lobes with row of spine-like setae (fig. 7D).

Gnathopods dissimilar, smaller than these in male. Gnathopod 1: articles 2-4 like these in male; article 5 shorter than propodus (ratio: 65:85), at both margins with numerous long setae. Propodus nearly subquadrate, longer than broad (ratio: 85:57), slightly tapering distally; posterior margin with 6-7 transverse rows of setae, anterior margin with 2 median and distal group of setae (fig. 6F). Palm slightly convex, inclined nearly 1/3 of propodus-length, outer face in the middle with row of 6-7 long setae (spine absent), corner with 3 unequal spines mixed with single setae (fig. 6G), inner margin with 2 unequal subcorner R-spines. Dactylus poorly exceeding posterior margin of propodus, one median seta at outer margin and 2-setae at lateral margin near basis of the nail, and one ventral marginal seta (fig. 6F).

Gnathopod 2 distinctly larger than gnathopod 1; articles 2-4 like these in males. Article 5 triangular, shorter than propodus (ratio: 80:92), both margin with numerous setae (fig. 6H). Propodus more quadrate, longer than broad (ratio: 92:58), posterior margin with nearly 7 transverse rows of setae. Palm slightly convex, inclined nearly ¼ of propodus-length, outer face with median group of long setae (spine absent) and 3 strong unequal corner spines, inner face with 2 unequal subcorner R-spines (fig. 6 I). Dactylus poorly exceeding width of propodus, outer margin with one median seta, inner margin with 2 setae near basis of the nail and one short marginal seta (fig. 6H).

Pereopods 3-4 stout. Pereopod 3: both margins of article 2 with long setae; articles 4-6 of different length (ratio: 40:30:30); articles 4-5 posterior margin with several bunches of setae longer than diameter of articles themselves, anterior margin with 3 bunches (article 4) or one bunch of long setae (article 5). Article 6 posterior margin with 6 short single spines mixed with single longer setae (fig. 8B). Dactylus shorter than article 6 (ratio: 18:29), inner margin with one strong seta, like that in male, and one lateral seta near basis of nail.

Pereopod 4 pilosity and dactylus like that in pereopod 3. Articles 4-6 of different length (ratio: 39:26:30) (fig. 8C).

Pereopod 5: pilosity like that in male; article 2 short, longer than broad (ratio: 57:45), anterior margin with several groups of shorter setae, posterior convex crenulated margin with numerous short setae, inner face with several groups of setae, outer face without facial setae; ventroposterior lobe developed (fig. 7B). Articles 4-6 of different length (ratio: 36:34:43); anterior margin of articles 4-5 with 4 groups of long setae mixed with single stronger spines, posterior margin with 2 groups of strong spines mixed with single longer setae. Article 6 anterior margin with 4 groups of 1-2 short spines, posterior margin with 3 bunches of long setae. Article 2 longer than article 6 (ratio: 57:43). Dactylus

much shorter than article 6 (ratio: 18:43), with one longer seta at inner margin and one lateral seta near basis of nail.

Pereopod 6: article 2 longer than broad (ratio: 75:52), tapering ventrally, without lobe; anterior margin with 5 single spines mixed with 1-3 short setae, posterior convex crenulated margin with numerous marginal setae, on face appear several groups of setae (fig. 7C). Articles 4-6 of different length (ratio: 45:43:49). Articles 4-5 at both margins with bunches of long setae mixed with single strong spines; article 6 anterior margin with 4 groups of short spines, posterior margin with 4 bunches of long setae and 1-2 spines. Article 2 longer than article 6 (ratio: 75:49). Dactylus shorter than article 6 (ratio: 21:49), inner margin with one seta and one lateral seta near basis of nail.

Pereopod 7: article 2 almost ovoid, longer than broad (ratio: 87:70), with well developed ventroposterior lobe, anterior margin with row of single spines mixed with single short setae, posterior crenulated margin with numerous shorter setae, both faces with several bunches of setae (fig. 7D); articles 4-6 nearly of similar length (ratio: 38:37:38); articles 4-5 at anterior and posterior margin with single strong spines mixed with bunches of long setae (the longest setae exceeding diameter of articles themselves). Article 6 anterior margin with 4 groups of short spines, posterior margin with 3 groups of long setae. Article 2 longer than article 6 (ratio: 70:38). Dactylus much shorter than article 6 (ratio: 19:38), inner margin with one seta and one lateral seta near basis of the nail (fig. 7E), nail shorter than pedestal (ratio: 22:30).

Pleopods 1-3 with 2 retinacula, pilosity of peduncles like that in male.

Uropods 1-2 like these in male. Uropod 3 shorter: peduncle poorly longer than broad, with 3-4 distal spines; inner ramus short, scale-like, with several lateral and distal plumose setae. Outer ramus 2-articulated: first article along both margins with numerous long plumose setae mixed with single spines (fig. 8D); second article very small, shorter than diameter of first article and provided with lateral and distal plumose setae (fig. 8D).

Telson broader than long (ratio: 75:60) incised almost to the basis; each lobe with 1-2 distal spines mixed with 4-5 unequal setae (fig. 8E); usually one distal seta is very long; a pair of short plumose setae are attached subdistally on outer margin of lobe.

Gills on gnathopod 2 and pereopods 3-6 large, more or less ovoid (figs. 7B, C; 8B, C), these of pereopod 7 rather smaller (fig. 7D).

Oostegites narrow, with long marginal setae, appear of gnathopod 2 to pereopod 5 (figs. 7B, 8B).

VARIABILITY

Accessory flagellum with 3-4 articles. The pilosity of facial and marginal setae on pereopods rather variable, but always remarkably setose. Gnathopod 1 propodus in male along posterior margin with 4-6 transverse groups of setae, palm with 3-4 corner spines on outer face, and 2 subcorner R-spines on inner face.

Gnathopod 2 propodus along posterior margin with 8-11 transverse rows of setae; palm inclined, irregularly serrate, with one median and 3-4 corner unequal spines on outer face, and 2 subcorner R-spines on inner face.

Epimeral plates 2-3 usually more pointed in males than in females, epimeral plate 1 nearly subrounded, with more or less marked ventroposterior corner seta, plates 2-3 without distinct corner seta.

The shape of dorsal surface of body very variable: the most number of specimens from Hilandar are with moderately elevated metasomal segments 1-3 with subrounded longitudinal keel, but some specimens are with poorly developed dorsal metasomal elevation, and some specimens almost without dorsal elevation of metasomal segments. The specimens from Drama and Thessaloniki region are usually with hooked metasomal elevation, although some specimens have low metasomal elevation without hooked tip, like these from Hilandar. Only scarce number of specimens have last mesosomal and all three metasomal segments with distinct dorsomedian hooked tooth (some specimens from Drama) (fig. 8F).

Large variability of dorsal surface of mesosomal and metasomal segments in specimens of this species indicated limited validity of this morphological character as taxonomical distinguish character. The similar situation is in *Gammarus roeselii* Gervais 1835-complex, where also large variability of dorsal carina on last mesosomal and all three metasomal segments are present in the specimens of same or different localities (Karaman, S. & Karaman, G., 1959). Further molecular-genetical and morphological studies, including breeding experiments of all these population, will probably help to understand the taxonomical validity of these characters in recognition of single taxa and its reproductive isolation.

Urosomal segments 1-2 in all specimens are with distinct conical dorsal elevation bearing 1-2 spines and various number of setae; urosomal segment 3 always with 2 dorsolateral groups of spines and setae, and usually 2 short median setae.

Telson with 1-2 distal spines mixed with several setae of various length.

LOCUS TYPICUS: torrent near Dervent (Thessaloniki reg., Greece).

LOCALITIES CITED: GREECE:

Karaman, S. 1931: locus typicus;

Karaman, G. 1971: Volvi Lake; Drama; spring Varvara near Drama;

Sket & Hou 2018: Thessaloniki; Apollonias Stream near Thessaloniki; Mikri Volvi Lake; Xanthi, Stathmos, Nestos brook;

Straskraba 1967: Thessaloniki.

New localities: Torrent near Hilandar Monastery, Halkidiki peninsula, Greece.

***TURCOGAMMARUS ARALENSIS* (Uljanin, 1875)**

Gammarus aralensis Uljanin, 1875: 2, pl. V, figs. 15-19; Sowinsky, 1894: 15, pl. 1, figs. 1-9, pl. 2, figs. 10-18;

Obesogammarus aralensis Stock, 1974: 79, 93;

Pontogammarus aralensis Dershavin, 1912: 15*;* Mordukhai-Boltovskoi et al., 1969; 466, pl. XII, fig. 1;

Pontogammarus (Gammarus) aralensis Martynov, 1924: 214;

Pontogammarus cf. *aralensis*: Sket & Hou, 2018, suppl. 2: 6;

Dikerogammarus aralensis Birstein, 1945: 519; Barnard, J.L., 1958: 48; Plotnikov et al., 2012 (2017): 6, 11, fig. 6; Plotnikov et al., 2021: 12;

Turcogammarus aralensis Barnard, J.L. & Barnard, C.M., 1983: 170; Zonn et al., 2009: 179; Palatov, 2018: 152; Copilas-Ciocianu & Arbaciauskas, K., 2018: 286, fig. 1F;

Dikerogammarus haemobaphes (part.) Stebbing, 1906: 459.

LOCUS TYPICUS: Among *Zostera* in bay Sary-chaganack, coast of Aral Lake, Kazakhstan.

LOCALITIES CITED: Regions of Black Sea, Caspian Sea, Azov Sea, Aral Sea:

Uljanski 1875: locus typicus.

Sowinsky 1894: Aral Sea, coast of southern bay of Nikolai Island. He presented various figures of this species.

Dershavin 1912: Delta of Volga River.

Martynov 1924: Don River; Aral Lake; vicinity of Rostov; Charkhal Lake (Uralsk reg.); Mouth of Volga River.

Mordukhai-Boltovskoi et al. 1969: waters of lower Don River; Aral Sea and several bays of Caspian Sea; probably also in salt lagoons and limans of Azov Sea.

Zonn et al. 2009: Balakhandsky relics in Aral paleobasin (Amudarya paleobasin, from the time of hyperhaline Balakhandsky Lake that existed 5.3-3.5 mil. years ago);

Plotnikov et al. 2012 (2017); 2021: Aral Sea (Caspian areal). They mentioned disappearance of *T. aralensis* from the Aral Sea according the disappearance of lake-water;

Copilas-Ciocianu & Arbaciauskas, 2018: Kazakhstan: Kulsary, Zhem River [lat./longitude: 47.051/ 54.060];

Palatov 2018: lower part of Yakornaia River (Krasnodarskii region); water streams of Novy Afon (eastern Black Sea region);

Sket & Hou 2018: Odesskaia Oblast, Katlabuh Lake, Ukraine.

TURCOGAMMARUS SETOSUS (Schâferna, 1914)

Dikerogammarus setosus Schâferna, 1914: 1-5, 2 pls.; Schellenberg, 1937: 267; Birstein, 1945: 519; Barnard, J.L., 1958: 48;

Pontogammarus setosus Martynov, 1924: 214; Barnard, J.L. & Barnard, C.M., 1983: 170;

Obesogammarus setosus Stock, 1974: 83.
Turcogammarus setosus Palatov, 2018: 21.

LOCUS TYPICUS: springs at mouth of river Aras near Ordubad, in area of Caspian Sea, Azerbaidzan)

LOCALITIEX CITED:

Schâferna, 1914: locus typicus.

Palatov 2018 cited it for Aralis region of Araks valley, eastern Zakavkazie.

***TURCOGAMMARUS TURCARUM* (Stock, 1974)**

Obesogammaru turcarum Stock, 1974: 87, figs. 2-6; Ozbek, 2011: 450;
Turcogammarus turcarum Karaman, G. & Barnard, J.L., 1979: 137; Barnard, J.L. & Barnard, C.M., 1983: 170; Palatov, 2018: 248 (=error: *turcatum)*.

LOCUS TYPICUS: fountain at Little Mount Ararat, directly at the Turkish-Iranian border (Agri prov.), Turkey;

LOCALITIES CITED:

Stock 1979: locus typicus.

Palatov 2018: consider this species as relict of Kura-Araksinsk lowland (Azerbaidzan).

Ozbek 2011: Anatolia.

DISCUSSION

The genera *Turcogammarus*, *Obesogammarus*, *Dikerogammarus* and *Pontogammarus* are rather similar genera although distinct to each other. Probably just because of this fact, species belonging now to the genus *Turcogammarus,* have been often mentioned in different genera by various authors.

G. Karaman & Barnard (1979) regarding problem of genera belonging now to the family Pontogammaridae and its genus *Dikerogammarus* Stebbing, 1899, mentioned:" To clarify this genus and *Obesogammarus* Stock (1974), we validate the usefulness of urosomal knobs as a generic character and remove *Pontogammarus spandli* S. Karaman (1931) and *Obesogammarus turcarum* Stock (1974) from *Obesogammarus* to a new genus, *Turcogammarus*. *Dikerogammarus* thus receives all other species with urosomal knobs and *Obesogammarus* remains free of knobbed species. The species to be relegated to *Turcogammarus* differ from *Obesogammarus* in the presence of urosomal knobs and from *Dikerogammarus* in the strong setosity on the anterior coxae and on article 2 of pereopods 5-7 ".

As most of *Turcogammarus* species are only partially described and for this reason seems rather similar to each other, the key to the species of this genus is only conditionally composed.

KEY TO THE SPECIES

1. Pereopod 7 article 2 in males with very short posterior marginal setae; setae on epimeral plate 2 relatively short. (epimeral plates 2-3 sharply pointed) ..... *ARALENSIS* (Uljanin, 1875)
-- Pereopod 7 article 2 in males with longer posterior marginal setae; setae on epimeral plate 2 long and more numerous 2
2. Epimeral plate 2 densely setose, epimeral plate 3 scarcely setose (metasomal segments not elevated dorsally) ..... *SETOSUS* (Schäferna, 1914)
---Epimeral plate 2 only moderately setose, epimeral plate 3 with row of ventral long setae (epimeral plates 2-3 moderately pointed) 3
3. Metasomal segments more or less kieled. Tubercle on urosomal segments 1-2 with 1-2 distal spines and setae; Telson with some long distal setae mixed with spines; setation of pereopods rather shorter; epimeral plates 2-3 in males strongly acute..... *SPANDLI* (S. Karaman, 1931)
-- Metasomal segments not keeled; Tubercle on urosomal segments 1-2 with 3-4 distal spines and setae. Telson with all short setae mixed with spines; setation of pereopods rather stronger; epimeral plates in males moderately pointed.....*TURCARUM* (Stock, 1974)

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PREVALENCE AND HISTOPATHOLOGICAL EFFECTS OF PARASITIC COPEPOD *LERNAEA CYPRINACEA* IN ESTUARINE FISHES FROM MEDITERRANEAN REGION OF TURKEY, WITH A NEW HOST RECORD

SUMMARY

Lernaeid copepod *Lernaea cyprinacea* L., 1758 has been reported on the skin and the external surfaces of fins from three freshwater fish hosts: endemic species *Alburnus baliki* Bogutskaya, Küçük & Ünlü, 2000 and *Pomatoschistus anatoliae* Engin & Innal, 2017 and on native species *Chelon ramada* Risso, 1827 sampled from Karpuzçay Creek Estuary, located in the Mediterranean region of Turkey. The highest prevalence of infection (7.5%) was reached in *C. ramada*, and the mean intensity was the same (1 parasite/fish) in all three host species. *P. anatoliae* (Gobiiformes: Gobiidae) was found as new host record for ectoparasite *L. cyprinacea*. Histopathological examination of the sections made in all analyzed fish hosts revealed chronic granulomatous inflammatory reaction and infiltration of lymphocytes, histiocytes and eosinophils around the parasite attachment sites.

Keywords: copepod, estuary, fish host, histopathology, parasite

INTRODUCTION

Most wild fish populations are vulnerable to metazoan parasites (Dezfuli *et al.* 2021). One of the most important enemies of fishes, the parasitic copepods could damage their hosts by the attachment mechanisms and feeding activities (Alaş *et al.* 2015). So far as known, it was determined that the non-host specific copepod *Lernaea cyprinacea* Linnaeus, 1758 or anchor worm can infect native and introduced fish in Turkey, being recorded on freshwater species more in lakes and ponds rather than in river creek and streams (Ahnelt *et al.* 2018; Koyun and Atici 2018).

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L. cyprinacea is the causative agent of lernaeosis, a disease with serious pathogenic lesions reported in parasitized fish (Innal et al., 2017; Urku and Onalan, 2018). Even edible fish infected with cyclopoid parasites are undesirable to anglers and not consumed (Raissy *et al.* 2013; Koyun and Atici 2018; Hua *et al.* 2019).

This ectoparasite has been found infesting over 40 freshwater fishes in Turkish inland waters, predominantly Cypriniformes, including the following species: *Alburnus baliki, Barbus xanthos, Capoeta capoeta, Carassius carassius, Carassius gibelio, Cyprinus carpio, Chondrostoma nasus, Chondrostoma beysehirense, Cyprinion macrostomus, Oxynoemacheilus anatolicus, Pseudophoxinus egridiri, Pseudophoxinus burduricus, Pseudorasbora parva, Pseudophoxinus zekayi, Squalius cephalus* and *Tinca tinca* (Koyun and Atici 2018; Innal, 2020).

The member of Cypriniformes order of teleost fishes, *Alburnus baliki* Bogutskaya, Küçük & Ünlü, 2000 was distinguished as a new species from Manavgat River system in southern Turkey, where is considered regional endemic (Bogutskaya *et al.* 2000; Giannetto and Innal 2021). According to IUCN Red List, it was assessed as endangered species, with a decreasing population trend (Barrios *et al.* 2014; Innal and Giannetto 2020). The Antalya bleak was studied concerning the morphometric measurements (Mangit, 2014), age and growth parameters (Innal and Gülle 2019) or phylogeography (Bektas *et al.* 2020), however with respect to ichthyoparasitofauna, data remain scarce. The copepod *Lernaea cyprinacea* was detected before on *A. baliki* in Karpuzçay Creek (Innal, 2020). Also, the acanthocephalan *Pomphorhynchus laevis* specimens were identified in this host in streams discharging into Antalya Bay (Aydoğdu *et al.* 2011).

Pomatoschistus anatoliae Engin & Innal, 2017 is a gobiid fish described from the estuary of Göksu River on the Mediterranean coast of the Anatolia. To the best of authors' current knowledge, there are no registered information concerning the biometry or parasitofauna of this fish species.

The mugilid host *Chelon ramada* Risso, 1827 syn. *Liza ramada* or the Thinlipp grey mullet was recorded in Beymelek Lagoon, Antalya (Uysal *et al.* 2008) and all coasts of Anatolian brackish waters (Çiçek *et al.* 2015). Grey mullets are recognized as fish species with economic importance because of the high quality of the their flesh (Reis and Ateş 2020). Worldwide, several parasite species of Myxosporea, Monogenea, Digenea, Acantocephala and Crustacea were recorded in this fish species (Merella and Garippa 2001; Ragias *et al.* 2005; Öktener, 2014; Polinas *et al.* 2021). The copepod *Lernaea cyprinacea* was detected before on *Chelon ramada* (http://www.marinespecies.org).

Although a lot of parasitological observations were made in fishes inhabiting the freshwater systems, there is still a paucity of data regarding the occurrence of lerneid species in brackish water. Thus, this study was aimed to diagnose the presence of a well-documented crustacean affecting the health of its hosts, namely the opportunistic copepod *Lernaea cyprinacea* among native fish

from Karpuzçay Creek Estuary, one of the brackish water systems of the Mediterranean coast of Turkey.

MATERIAL AND METHODS

Overall, 140 fish individuals belonging to three species were analyzed: 60 *Alburnus baliki* Bogutskaya, Küçük & Ünlü, 2000 (Antalya bleak), 53 *Chelon ramada* Risso, 1827 (Thinlipp grey mullet) and 27 *Pomatoschistus anatoliae* Engin & Innal 2017.

Fish samples were taken between November 2014 and April 2019 using a shore seine net (10 m long and 2 m high; 1.2 x 2 mm mesh size) from Karpuzçay Creek (36°42'56.84" N 31°33'00.95" E; Figure 1), which flows into the Antalya Gulf (Mediterranean Sea, Turkey).

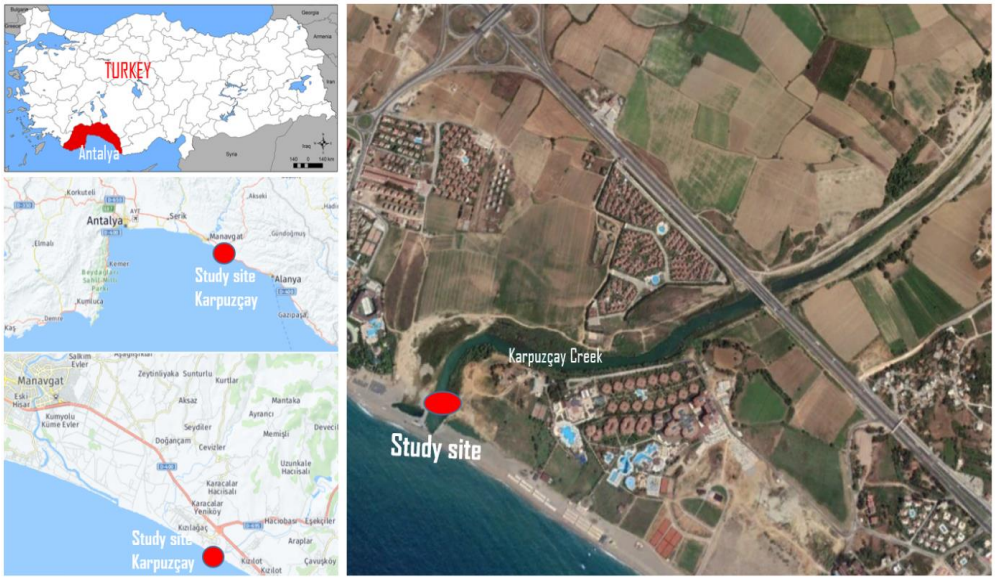

Figure 1. Map of the study site

Immediately after collecting, fish samples were transported in plastic bags to the Burdur Mehmet Akif Ersoy University Ichthyology Laboratory. Each fish individual was measured for total length (TL) to the nearest 0.1 cm, and weighed (W) to the nearest 0.1 g.

Fish species were examined with a dissecting microscope for the presence of ectoparasites. The females of parasitic copepod *L. cyprinacea* were identified according to Bauer (1987). Prevalence and intensity of infection were computed (Bush *et al.* 1997).

The taxonomic names and scientific classification for fish species herein were made in accordance with FishBase (Thomson, 1990; Engin and Innal 2017; Fricke *et al.* 2020).

During the necropsy, samples of fish skin lesions were collected at the site of parasitic infection. For histopathological assessment, the whole body of small fish individuals was transversally cut and fixed in 10% neutral formalin solution. Parasite attachment areas were selected and skin samples were prepared by an automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany). The tissues of fish were embedded in paraffin, and 5 μm serial sections were acquired using a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). Subsequent, histopathological sections were stained with hematoxylin and eosin (HE) and examined under 40X magnification of a light microscope. Morphometric evaluation and microphotography were performed using the Database Manual cellSens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

RESULTS AND DISCUSSION

The highest prevalence of infection was reached in native species *Chelon ramada*, followed by *Pomatoschistus anatoliae* and *Alburnus baliki*, two fish species endemic to Turkey (Table 1) (7.5%; 3.7% and 3.33%, respectively). The mean intensity recorded was same in all three analyzed species (1 parasite/fish).

The copepod parasites were collected from the skin and external surfaces of the fins belonging to the fish hosts (Figure 2).

Widespread in Turkey, the copepod ectoparasite *Lernaea cyprinacea* is considered a possible threat to the endemic fish stocks, such as *Alburnus baliki* (Innal, 2020).

Table 1. Occurence of *Lernaea cyprinacea* in host species from Karpuzçay Creek Estuary and biometric values of fish species examined (N=total number of fish individuals, N'=number of infected fish, P=prevalence of infection, I=intensity of infection, O=origin, E= endemic, N=native)

Species	Order	Family	O	Range of TL (cm)	Range of W (g)	N	N'	P(%)	I
Alburnus baliki Bogutskaya, Küçük & Ünlü, 2000	Cypriniformes	Leuciscidae	E	4.2-10.8 (mean 6.4)	0.6-15.7 (mean 2.69)	60	2	3.33	1
Chelon ramada (Risso 1827)	Mugiliformes	Mugilidae	N	1.6-15.9 (mean 4.27)	0.018-40 (mean 2.53)	53	4	7.55	1
Pomatoschistus anatoliae Engin & Innal 2017	Gobiiformes	Gobiidae	E	2.1-4 (mean 2.92)	0.074-0.558 (mean 0.227)	27	1	3.7	1

Figure 2. *Lernaea cyprinacea* (arrows) on the body hosts: A. *Chelon ramada*; B. *Alburnus baliki*; C. *Pomatoschistus anatoliae*

In Karpuzçay Creek Estuary, the highest infection prevalence value was found in *Chelon ramada* (7.5%) and the lowest in *Alburnus baliki* (3.3%). Despite the total number of *P. anatoliae* was reduced (27) comparing with the other two sampled fishes, the prevalence of infection was higher than in the case of *A. baliki* represented herein by numerous fish individuals (60). This may suggest that Antalya bleak has a vulnerability to parasitic copepods. In addition, *P. anatoliae* represent a new host record for the occurence of *Lernaea cyprinacea*, therefore future investigations would be necessary for elucidate the aspects of host-parasite interactions.

Difference values for prevalence and intensity of infection depend on various factors, such as season, sample size, feeding habits, sex ratio, length range or physico-chemical parameters (Barson *et al.* 2008; Hossain *et al.* 2018 Innal, 2020; Khalid *et al.* 2021).

In *Lernea cyprinacea* infection, hemorrhagic and ulcerative skin lesions are common findings (Mirzaei, 2015; Innal *et al.* 2017; Urku and Onalan 2018). The gross pathology noticed in this study were similar to other reports concerning the effects of lerneosis in teleost fishes

Microscopically, severe pathological findings were reported in all analyzed fish hosts, in areas where copepod *Lernaea cyprinacea* was attached. Numerous parasites were commonly localized in the abdominal regions and skeletal muscle (Figure 3).

At the histopathological examination, lesions causing ulcers were also found in the epidermis and dermis of the fish hosts. Characteristic chronic

granulomatous inflammatory reaction was observed in all attachment sites of *L. cyprinacea*.

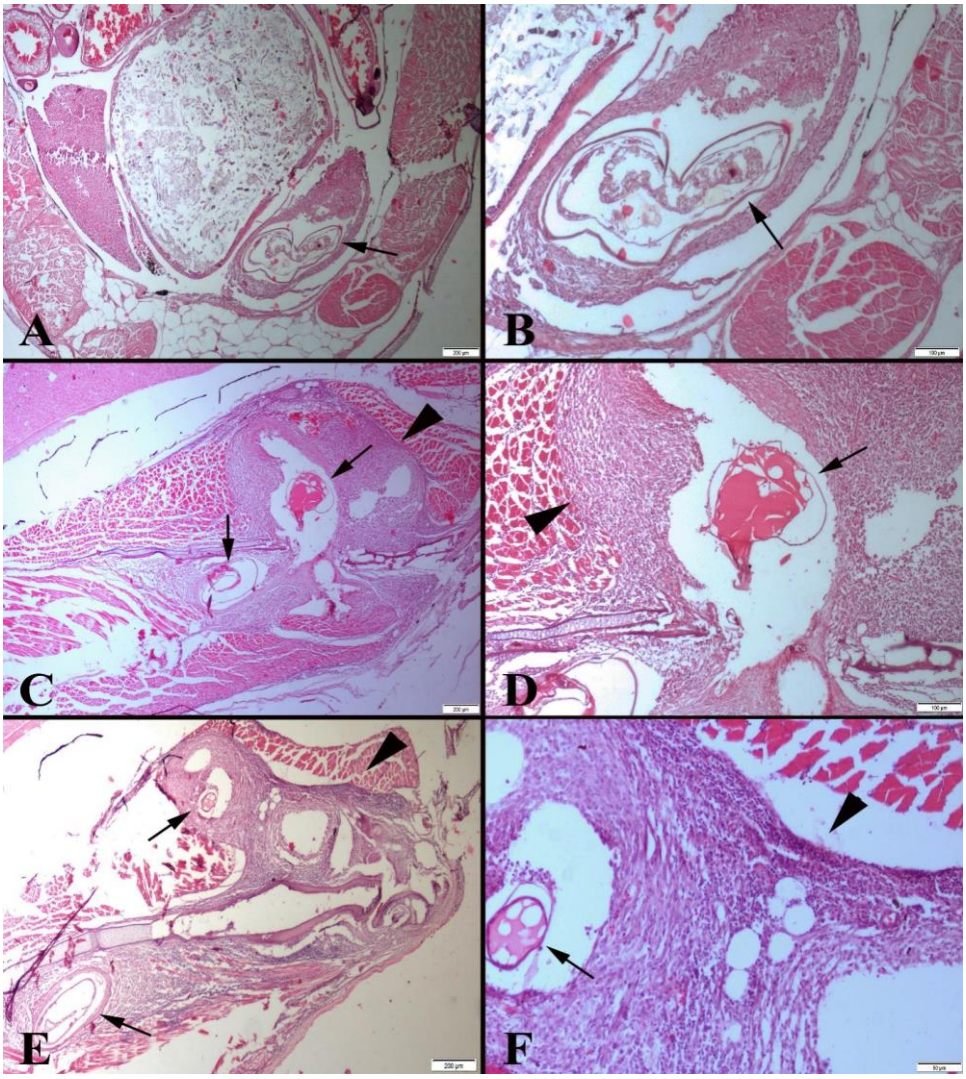

Figure 3: *Lernaea cyprinacea* related histopathological findings in the fish hosts. (A) Granulomatous reaction around the parasite (arrow) localized abdominal cavity, HE, Bar=200µm. (B) Higher magnification of the granuloma, HE, Bar=100µm. (C) Parasites (arrows) localized skeletal muscle and inflammatory reaction around the parasite (arrow head), HE, Bar=200µm. (D) Higher magnification of the granuloma, HE, Bar=100µm. (E) Parasites (arrows) localized skeletal muscle covered by diffuse granulomatous reaction (arrow head), HE, Bar=200µm. (F) Higher magnification of the inflammatory reaction (arrow head) around the parasite (arrow), HE, Bar=50µm.

Mainly lymphocytes, histiocytes, and eosinophils were infiltrated as a response to the parasite presence in affected site. Commonly lesions in abdominal cavity were covered by fibrous tissue. Chronic granulomatous myositis and dermatitis were common findings. In the damaged areas were noticed edema, congestion, and small areas of hemorrhage. Histopathologically parasitic infections generally cause a granulomatous inflammatory response and capsule formation around the parasite, together with melanization and inflammatory cell infiltrations for to isolate and destroy them in fishes (Torres *et al.* 2002; Dezfuli *et al.* 2007; Feist and Longshaw 2008). Sometimes encapsulation can be associated with hemorrhage in the tissues immediately surrounding the encapsulated parasite reported if the excessive number of parasites exists (Ogawa *et al.* 2004). In cases of muscle parasites, migration stages can cause in mechanical destruction of the musculature (Baturo, 1980).

CONCLUSIONS

To the best of our knowledge, this survey reports the first microphotographs of the affected tissues by copepod *Lernaea cyprinacea* in *Chelon ramada*, *Alburnus baliki* and *Pomatoschistus anatoliae.* According to IUCN Red List Status, *C. ramada* is categorized as LC (least concern), *A. baliki* as EN (endangered), while *P. anatoliae* was not yet evaluated (Freyhof 2014; Freyhof and Kottelat 2018). Due to the fact that the Antalya bleak is an endangered species, further studies are needed in order to assess the aspects regarding its epidemiology in natural populations. Moreover, the economic importance of mugilids such as *C. ramada* requires that future research should be focused on assessing the losses induced by lerneid parasites as well as applying good fishery management practices.

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In memoriam

dr Zora Vučinić
(1944-2021)

Višedecenijski naučnik radnik Biotehničkog (Poljoprivrednog) instituta, a današnjeg Biotehničkog fakulteta, istaknuti stručnjak u oblasti zaštite bilja, dr ZORA VUČINIĆ ili samo naša draga ZORA, preminula je 03.11.2021. godine u 78. godini života.
Rođena je 18. aprila 1944. u Bitolju (NR Makedonija). Gimnaziju je završila u Skoplju, a Poljoprivredni fakultet, Odsjek za zaštitu bilja u Novom Sadu 1968. godine. Doktorsku disertaciju iz oblasti fitopatologije odbranila je na Poljoprivrednom fakultetu u Novom Sadu 1991. godine.

U Zavodu za zaštitu bilja Poljoprivrednog instituta počela je da radi 1970. godine, i tu provela cijeli svoj radni vijek do odlaska u penziju 2009. godine. Kao prva žena u nauci u oblasti zaštite bilja u Crnoj Gori prošla je sva istraživačka i naučna zvanja, počev od pripravnika, istraživača saradnika / asistenta, preko naučnog saradnika i višeg naučnog saradnika, pa do naučnog savjetnika.

Veoma plodan naučni rad u oblasti zaštite bilja, prije svega u oblasti mikologije, bakteriologije, biološke borbe, nematologije realizovala je kroz mnogobrojne naučne projekate među kojima su bili i međunarodni (SAD, Italija) i objavljivanjem više desetina naučnih radova u referentnim međunarodnim časopisima i stručnim publikacijama. Boravila je na tromjesečnim specijalizacijama iz zaštite bilja u Francuskoj i Vageningenu (Holandija), jednomjesečnoj specijalizaciji na bolestima maslina u Grčkoj, jednomjesečnoj specijalizaciji iz biološke borbe protiv patogenih organizama u SAD, i učestvovala na raznim kongresima i simpozijumima (SAD, Španija, Izrael, Maroko, Italija, Njemačka i zemlje regiona). Bila je i član naučnih i organizacionih odbora većeg broja nacionalnih i međunarodnih naučnih i stručnih simpozijuma i kongresa.

Dr Zora Vučinić je tokom četiri decenije rada ostavila neizbrisiv trag u sveukupnom razvoju poljoprivredne nauke u Crnoj Gori i institucije u kojoj je provela svoj radni vijek. Posebno je snažan pečat ostavila na razvoju i uspostavljanju naučnih kapaciteta Centra za zaštitu bilja čiji je bila dugogodišnji rukovodilac. Njen rad u Centru obilježio je zapošljavanje velikog broja mladih istraživača i razvoj skoro svih specijalnosti zaštite bilja. Nastavila je i unaprijedila ono što je započeo akademik Milorad Mijuković, kroz uspostavljanje

fitosanitarne laboratorije koja i danas funkcioniše onako kako je ona zamislila, u okviru Centra za zaštitu bilja. Zahvaljujući njenoj viziji razvoja Centra za zaštitu bilja i nesebičnoj podršci koju je davala svojim mladim saradnicima danas su oni u svojim specijalnostima ostvarani stručnjaci i prepoznati kao nosioci razvoja cjelokupnog sektora za zaštitu bilja u Crnoj Gori. Njena vrata bila su otvorena za svakoga, kome je trebao razgovor, savjet, podrška, a naročito za mlade ljude i po tome će ostati upamćena među svima nama koji radimo u ovoj instituciji.

Tokom radnog vijeka dr Zora Vučinić je obavljala brojne funkcije. Bila je pomoćnik direktora za nauku i istraživanje u više mandata, od 1996. do 2007. godine. Glavni i odgovorni urednik naučnog časopisa Poljoprivreda i šumarstvo bila je u periodu od 1993. do 2004. godine. Dr Zora Vučinić je bila član Komisje za početnu akreditaciju studijskih programa iz oblasti poljoprivrede 2005. godine i član strukovnog Vijeća za prirodne i tehničke nauke Univerziteta Crne Gore u dva mandata. Rukovodilac Centra za zaštitu bilja bila je u periodu od 1997. do odlaska u penziju 2009. godine. Bila je predsjednik Savjeta Biotehničkog Instituta, predsjednik Komisije za biotehničke nauke i član Savjeta za nauku i tehnologiju Ministarstva prosvjete i nauke RCG, kao i član više stručnih Komisija kako saveznih tako i nacionalnih iz oblasti zaštite zdravlja bilja i svuda je ostavila neizbrisiv trag. Među kolegama zaštitarima na prostoru nekadašnje Jugoslavije bila je visoko poštovana i cijenjena, ne samo zbog stručnih i naučnih kvalifikacija nego i zbog svoje plemenitosti i ljudskosti na koju niko nije mogao ostati ravnodušan.

Neka joj je vječna slava i hvala

Prof. dr Snježana Hrnčić

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- ACKNOWLEDGMENTS

If received significant help in designing, or carrying out the work, or received materials from someone who did a favour by supplying them, their assistance must be acknowledged. Acknowledgments are always brief and never flowery.

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