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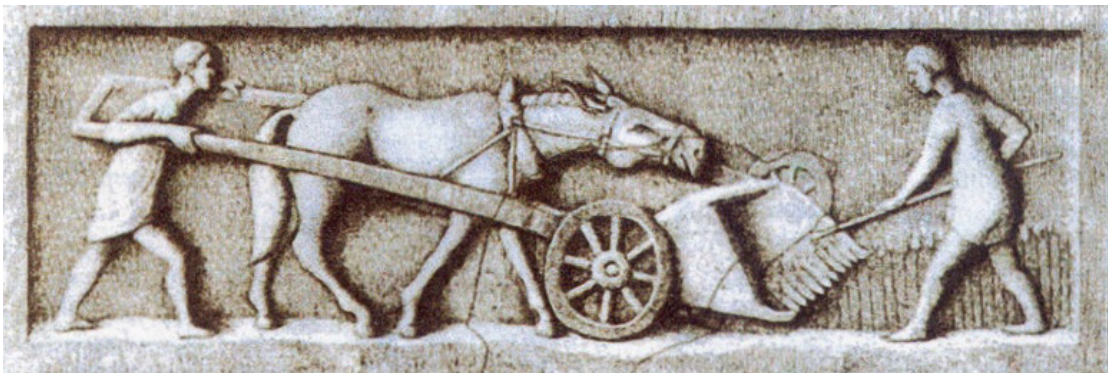

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Editorial

How I edited an agricultural paper once

The title was adopted from the marvellous short story of Mark Twain, published in 1870 by The Galaxy: an illustrated monthly magazine edited in New York. The humorous story summarizes the adventures of a journalist who is appointed by an agricultural paper temporarily. The regular editor of the paper was going off for a holiday, and someone accepted the terms he offered, and took his place. He tried to handle the journal in accordance with the general patterns of newspapers. Namely – it does not matter what you write, as long as it is attractive to the readers. The more astonishing the information is the better is the reputation of the periodical. In this belief our man wrote various crazy articles about turnips that should never be pulled from the soil but harvested from trees, or how to hatch the eggs of the guano bird, and why not produce pumpkins instead of gooseberries etc. The readers of the paper soon started to revolt, the poor regular editor had to return from his leave, and our man was fired. At the end of the story there is a real "Mark-Twainian" conclusion. Specific papers in general and agricultural papers in particular should avoid employing journalists having no sufficient information on the matter they write about. This should be left for political parties since they welcome anybody with the philosophy "that the less a man knows the bigger noise he makes, and the higher the salary he commands".

Front page of an original GALAXY magazine from the Dave Thomson collection.

Márton Jolánkai

So, it isn't an easy job neither to produce nor to edit a scientific paper. Columella - Journal of Agricultural and Environmental Sciences has reached a milestone. We are five years old now, and the present issue is just the 10th in our history. Of course it is not (or not *yet*) an anniversary. We don't have to be proud and also we cannot be satisfied with the results of this brief period. However it would be worth to see some of the records of the paper. The foundation of the journal was in 2014. We are completing the fifth volume by now. Ten regular issues and one conference proceedings book have been edited, so far containing 74 full papers and 50 conference papers. These have been selected from among 145 articles. The rest of them were discarded during the reviewing processes. We believe in the wise opinion of a onetime professor of ours according to whom "the value of a paper is determined by the power of the reviewer". During the past five years altogether 105 international reviewers contributed to the success of our work. The magnitude of papers published is 944 pages. The editorial process required some 1500 working hours. All papers are open access published and so they are available on the internet having DOI numbers and the journal is ISSN registered with both hard copies and electronic issues. The journal is indexed and stored by a wide range of repositories and it is a scientific journal approved by the Hungarian Academy of Sciences.

Finally we have to return to the initial question again; what is the mission of a scientific paper nowadays? I believe it is the support of science in general, and the dissemination of scientific results in a controlled, broad and open access way to the public. Our journal, Columella provides a forum for scientific publications in the field of agricultural and environmental sciences. The journal of one of the most ambitious agricultural faculties of Hungary has a task to disseminate novel research results, in favour of creating a better world.

The editors would say thanks to the authors of the present issue, and also welcome the readers who may read, use and broadcast the scientific information compiled. We also do hope that they may become future authors as well.

Márton Jolánkai

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Inoculation with *Septoglomus constrictum* improves tolerance to heat shock in tomato plants

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Abstract: Arbuscular mycorrhizal fungi (AMF) are symbiotic soil fungi colonizing roots of about 80% of vascular plants. This symbiosis enhances the growth and survival of numerous plant species including vegetables; moreover, it offers some other benefits for the plants. This work aimed to study the impact of AMF on tomato plant tolerance to heat shock. Tomato (*Solanum lycopersicum* L.) plants inoculated or not with *Septoglomus constrictum* were placed in a commercial potting media at 26/22°C with 16/8h photoperiod for six weeks, then exposed to normal (26°C for 6h) or high temperature (42°C for 6h). Arbuscular mycorrhizal (AM) colonization rate, level of lipid peroxidation (malondialdehyde – MDA), hydrogen peroxide (H_2O_2) accumulation and antioxidative enzymes in roots and leaves were measured after the stress application. AM colonization rate of mycorrhizal plants was 73% under non-stress conditions and 68% under heat shock conditions while no mycorrhizal colonization found in non-AM treatments. MDA and H_2O_2 content substantially increased in leaves of all plants after exposure to the heat shock. Leaf and root peroxidase (POD), leaf catalase (CAT) and root superoxide dismutase (SOD) activities of mycorrhizal plants were enhanced compared to those in non-AM ones while the activity of leaf SOD and root CAT in mycorrhizal plants remained unchanged. Furthermore, there were significant decreases in MDA and H_2O_2 content in leaves of inoculated plants compared with non-AM ones under heat shock conditions. Our results indicate that AM inoculation can increase stress tolerance against heat shock by enhancing the activity of antioxidative enzymes. Further research is required to understand the mechanisms that contribute to heat tolerance.

Abbreviations: AM, arbuscular mycorrhizal; AMF, arbuscular mycorrhizal fungi; CAT, catalase; FW, fresh weight; MDA, malondialdehyde; POD, peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase.

Keywords: arbuscular mycorrhizal fungi, *Septoglomus constrictum*, heat shock stress, stress tolerance, tomato plants

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Introduction

Due to the effects of global warming, heat stress has become the major challenge for crop production on earth. Heat stress causes anatomical, morphological, physiological, biochemical, and genetic responses *in planta* (Camejo et al. 2005; Chen et al. 2012; Min et al. 2014), diminishing crop yield and quality. When plants are subjected to very high temperatures, severe injuries in the cell, even death, may take place within minutes. These could be ascribed to a devastating collapse of the cellular system (Schoffl et al. 1999). Nevertheless, such cell damages and death may take place merely after plants are exposed to moderately high temperatures in the long term (Wahid et al. 2007). As direct consequences of heat stress, proteins are denatured and aggregated while the fluidity of membrane lipids is elevated in plants. Indirectly or slowly, high-temperature stress causes enzyme inactivation in mitochondria and chloroplast, protein synthesis prohibition, degradation of proteins and membrane integrity loss (Howarth 2005). These damages eventually lead to starvation, plant growth reduction, decreased ion flux, generation of toxic compounds as well as reactive oxygen species (ROS) (Howarth 2005).

ROS consist of peroxides, superoxide, hydroxyl radical, and singlet oxygen. ROS overproduction may result in cell death as a consequence of oxidative stress, such as peroxidation of membrane lipid, causing oxidative damage to nucleic acids (Tanou et al. 2009). Peroxidation of membrane lipid is detected by measuring malondialdehyde (MDA) which is a widely used marker of oxidative lipid injury caused by environmental stresses (Kong et al. 2016). To reduce oxidative damage under temperature

stresses, plants have evolved different antioxidative strategies to detoxify harmful ROS components by non-enzymatic and enzymatic antioxidant defence systems where peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) are main enzymatic ROS scavengers (Wu et al., 2014). SOD is the first defense against ROS (Alscher et al. 2002) due to its ability to catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2 (Wu et al. 2014). Subsequently, CAT and other scavenging enzymes detoxicate H_2O_2 to H_2O and O_2 (Apel and Hirt 2004). POD can also generate and detoxify H_2O_2 in the first and next phase, respectively (Siegel 2003).

Studies on plants under temperature stresses are extensively carried out to develop strategies to deal with the adverse effects of heat stress on crop productivity through breeding heat-tolerant varieties, suitable crop shifts and cultivation practices. Most of them are costly and time-consuming whereas noticeably, using beneficial microbes has been proved as a potential solution to improve plant tolerance to various abiotic and biotic stresses. Arbuscular mycorrhizal fungi (AMF), one of the common soil microbes, can form the symbiotic association with roots of 80% of terrestrial plant species. The application of AMF enhances not only nutrient and water uptake but plant tolerance to abiotic stress (Birhane et al. 2012).

Cabral and coworkers (2016) showed that inoculation of an AM mixture including *Rhizophagus irregularis* BEG140, *Rhizophagus irregularis*, *Funneliformis mosseae* BEG95, *Funneliformis geosporum*, *Claroideoglomus claroideum* in wheat plants mitigated adverse effects of temperature stress at 35°C (day) and 25°C (night) for seven days. In maize, inoculation of *Claroideoglomus etunicatum* reduced relative membrane permeability and MDA concentration in roots and leaves of plants while it increased soluble sugar and proline content in roots but lowered leaf proline content, relative to non-AM plants as exposure to 35°C and 40°C for one week (Zhu et al. 2010). Improved net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), the maximum quantum efficiency of photosystem II (Fv/Fm) together with higher chlorophyll contents in leaves of mycorrhizal maize plants under such heat stress conditions were observed (Zhu et al. 2011). Enhanced biomass of mycorrhizal plants under heat stress conditions was also detected (Zhu et al. 2010; Maya and Matsubara 2013; Matsubara et al. 2014; Maya et al. 2014). AM application triggered the higher activity of SOD, ascorbate peroxidase in whole plants (roots, tubers, and leaves) (Matsubara et al. 2014; Maya and Matsubara 2013) and resulted in heightened leaf ascorbic acid and polyphenol in cyclamen plants after exposure to heat stress (Maya and Matsubara 2013). Similarly, POD, SOD, CAT activities were elevated in roots and leaves in mycorrhizal plants under temperature stresses (Zhu et al. 2010). It is worth mentioning that the level and duration of stress (acute versus chronic) significantly affect plant responses (Tattersall et al. 2007; Pinheiro and Chaves 2011). Previous studies on mycorrhizal plants focused on the chronic heat stresses which usually lasted for one week. Therefore, mechanisms underlying the effect of AM inoculation on the ROS metabolism and antioxidative enzymes of host plants under heat shock representing acute stress remain unknown.

Tomato (*Solanum lycopersicum* L.) is a main vegetable crop in the world, widely cultivated optimally in agricultural areas with temperatures between 20◦C and 30◦C. Tomato is a primary dietary component in different countries because it contains a rich source of vitamins, antioxidant compounds, minerals, sugars, providing significant nutritional value for the human. Nevertheless, tomato productivity is substantially decreased by abiotic stresses (Schwarz et al. 2010).

The objective of this study was, hence, to contribute to the understanding of the effect of AM inoculation on ROS metabolism and the antioxidative activity in tomato plants under heat shock (42°C for 6h). *Septoglomus constrictum,* distributed around the world (Opik et al. 2010), was chosen as the fungal inoculant in our study. A degree of lipid peroxidation (estimated by MDA) and H_2O_2 accumulation, together with POD, SOD, CAT activity in leaves and roots of mycorrhizal tomato plants and non-AM tomato plants under heat shock were examined.

Material and methods

Tomato (*Solanum lycopersicum*) seeds, cultivar MoneyMaker, were soaked in 2.5% sodium hypochlorite for 20 minutes, then washed with

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distilled water five times and placed on wet papers in Petri dishes for germination for three days at room temperature. Germinated seeds were put in plastic pots (0.5-lit volume) with 0.5 kg of the sterile mixture of sand and soil (4:1, v/v). The loamy soil with pH 7.1, 1.61% organic matter, N 15.6 mg kg^{-1}, available P 36 mg kg^{-1}, available K 60 mg kg^{-1} (Duc et al., 2017) was used.

The experiment consisted of 12 plants without AM inoculation and 12 plants inoculated by *Septoglomus constrictum* (formerly *Glomus constrictum* Trappe.). The AM inoculum was cultured in the sterile sand with *Zea mays* as host plants for four months. Thirty grams of the AM inoculum (27 spores g^{-1}) were utilised for each pot in AM treatment while plants in non-AM treatment were added the same amount of autoclaved inoculum and 3 ml aliquot of a filtrate (< 20 µm) of the AM inoculum in order to provide a general microbial population free AMF propagules. Pots were put randomly in a growth chamber (EKOCHIL 1500), and the pot positions were changed weekly. Growing conditions, 26/20°C with 16/8 hours photoperiod, light intensity 600 µmol m^{-2} s^{-1} and 60% humidity were applied. Pots were watered twice and fertilized with Long Ashton nutrient solution (Hewitt, 1966) with low phosphorus level (3.2 µM $Na_2HPO_4.12H_2O$) once a week. After six weeks of plant growth, heat shock was carried out by transferring six non-AM plants and six AM plants to 42°C for 6h (Zhou et al. 2014) whereas six plants without AMF and six mycorrhizal plants were kept under non-stress conditions. Then all leaf and root samples were collected for further analysis.

Assessment of arbuscular mycorrhizal colonization

Root samples were washed by tap water and cleaned before staining according to Vierheilig et al. (1998). AM colonization was examined by visual inspection of fungal structures under a stereomicroscope at x 100 magnification. AM colonization rate was determined by the gridline intersect method (Giovanetti and Mosse 1980).

Measurement of hydrogen peroxide accumulation and oxidative damage to lipids

The H_2O_2 content was determined by the method of Alexieva et al. (2001). Shortly, leaf samples (500 mg) were ground with a cold 0.1% (w/v) trichloroacetic acid (TCA) (5 ml), subsequently, centrifuged at 12,000×g at 4°C for 15 min. The reaction mixture consisted of 100 mM potassium phosphate buffer (pH 7.0, 0.5 ml), 1 M KI (1 ml) and the leaf extract supernatant (0.5 ml). The reaction occurred in the dark for 1h. Its absorbance was recorded at 390 nm.

The leaf lipid peroxidation was estimated by the method of Heath and Packer (1969). In detail, leaf samples (200 mg) were ground in 0.1% TCA (5 ml), then centrifuged at 10,000×g for 5 min. A 1 ml of leaf supernatant was mixed with 20% TCA (4 ml) containing 0.5% 2-thiobarbituric acid (TBA). Then the mixture was heated at 95°C for 15 min and immediately cooled. Absorbances of the mixture at 532 nm and 600 nm were recorded for MDA estimation. The content of MDA was estimated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Measurement of antioxidant enzymatic activities

Leaf and root samples (500 mg) were frozen in liquid nitrogen and ground with 50 mM Tris-HCl buffer pH 7.8 (3 ml) containing 1 mM Na_2EDTA and 7.5% (w/v) polyvinylpyrrolidone K25. Then, crude extracts were centrifuged at 10,000 x g for 20 minutes at 4°C. The supernatants were collected to examine enzyme activities by U-2900 UV-VIS spectrophotometer (Hitachi). Soluble protein contents were estimated according to the method of Bradford (1976).

The activity of peroxidase (POD, EC 1.11.1.7) was tested according to Rathmell and Sequeira (1974). A 2.2 ml reaction mixture included 10 µl plant extract, 12mM H_2O_2 (100 µl), 50 mM Guaiacol (100 µl) and 0.1 M sodium phosphate buffer (pH 6.0). Changes in the absorption at 436 nm for 5 minutes were recorded. The activity of POD was presented by the changes in absorbance per mg protein per minute.

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was examined by the method of Beyer and Fridovich (1987). A 2 ml 50 mM phosphate buffer (pH 7.8) containing 55 µM NBT, 2 mM EDTA, 9.9 mM L-methionine and 0.025% Triton X-100 and leaf extract (20 µl), 1 mM riboflavin (20 µl) was used for the reaction

mixture. Absorbance changes of the reaction at 560 nm were recorded.

The activity of catalase (CAT, EC 1.11.1.6) was assayed by the method described by Aebi and Lester (1984). The reaction mixture (3 ml) consisted of 10 mM H_2O_2, 50 mM potassium phosphate buffer (pH 7.0) and the enzyme extract. Absorbance decreases of the reaction at 240 nm were recorded. CAT activity was presented as absorbance changes per mg protein per minute.

Statistical analysis

All data were tested by two-way factorial analysis of variance (ANOVA) using SAS 9.1 (SAS Institute, Cary, North Carolina). The means were compared at the 5% level by Duncan posthoc test.

Results

No dead plants were observed in our experiment after heat shock treatment. AM colonization rate of plants colonized by *S. constrictum* was 73% under non-stress conditions and 68% under heat shock conditions whereas no AM colonization was observed in plants without mycorrhiza (Figure 1A). Also, heat shock (42°C in 6 hours) did not change the AM colonization significantly although a slight decrease occurred. Under non-stress conditions, both AM and non-AM plants had similar MDA values, however; when plants were subjected to heat shock, MDA levels considerably increased in non-AM plants (by 42%) but not in AM plants (Figure 1B).

In addition, AM plants also showed a significant lower MDA (17% lower) than non-AM plants under heat shock conditions. Similarly, there was no significant difference in H_2O_2 level in AM and non-AM plants under non-stress conditions (Figure 1C). Heat shock increased H_2O_2 level by six-fold in non-AM plants and by over three-fold in AM plants. H_2O_2 accumulation reduced by 40% in AM plants, as compared to the corresponding in non-AM plants under heat shock.

As shown in Figure 2A, POD activity in leaves of mycorrhizal plants was not significantly different from that of non-AM plants under non-stress conditions. Nonetheless, exposure to heat shock substantially increased (61-76%) leaf POD level in plants although AM symbiosis induced a considerable higher (9%) POD activity than plants without AM inoculation. Notably, heat shock substantially heightened (19-26%) SOD activity of leaves in plants but there was

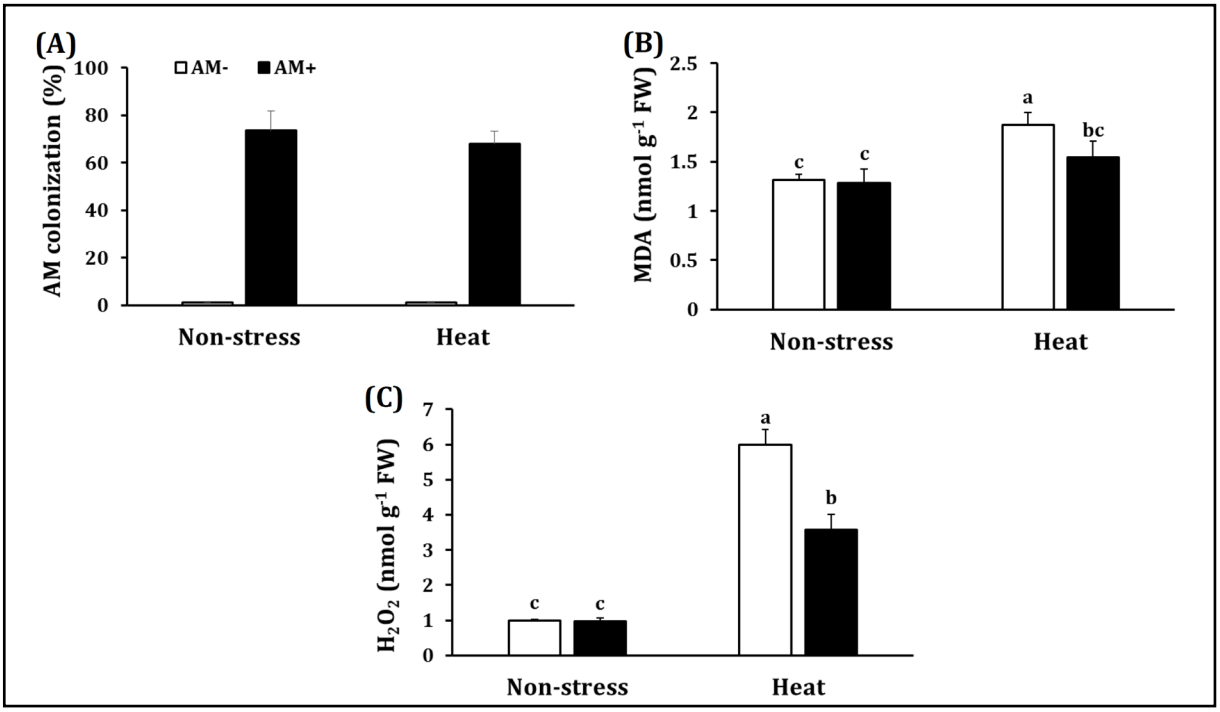

Figure 1. Mycorrhizal colonization rate (A), malondialdehyde (MDA) (B) and H_2O_2 (C) accumulation in leaves of non-AM plants (AM-) and plants colonized by *Septoglomus constrictum* (AM+) exposed to non-stress, heat-shock conditions. Bars present mean ± standard deviation (n = 4). Different letters present significant differences among treatments according to Duncan posthoc test ($P < 0.05$).

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no significant difference in leaf SOD activity between AM and non-AM plants (Figure 2B). Likewise, leaf CAT activity of colonized plants was considerably improved (increased by 18%), in comparison to that of uncolonized plants under heat shock despite no significant differences in this enzyme between both AM and non-AM plants under non-stress conditions (Figure 2C).

In roots, POD activity had the same pattern as in leaves (Figure 2D). Root POD activity in AM and non-AM plants under non-stress conditions did not differ considerably, nevertheless; under heat shock conditions AM plants expressed an improved POD activity (increased by 22%), relative to non-AM plants. In contrast to leaf SOD activity, this enzyme was enhanced significantly (increased by 87%) in AM plants, as compared to non-AM plants under heat stress conditions although no substantial differences between AM and non-AM plants under normal conditions were recorded (Figure 2E). Regarding root CAT activity, no considerable differences between AM and non-AM plants were found under non-stress as well as heat shock conditions although the level of CAT was elevated by 75-102% in plants subjected to the heat stress (Figure 2F).

Discussion

AMF can enhance the host tolerance to temperature stresses in maize (Zhu et al. 2010; 2011), in cyclamen (Maya and Matsubara 2013), in citrus (Wu, 2011), in tomato (Abdel Latef and

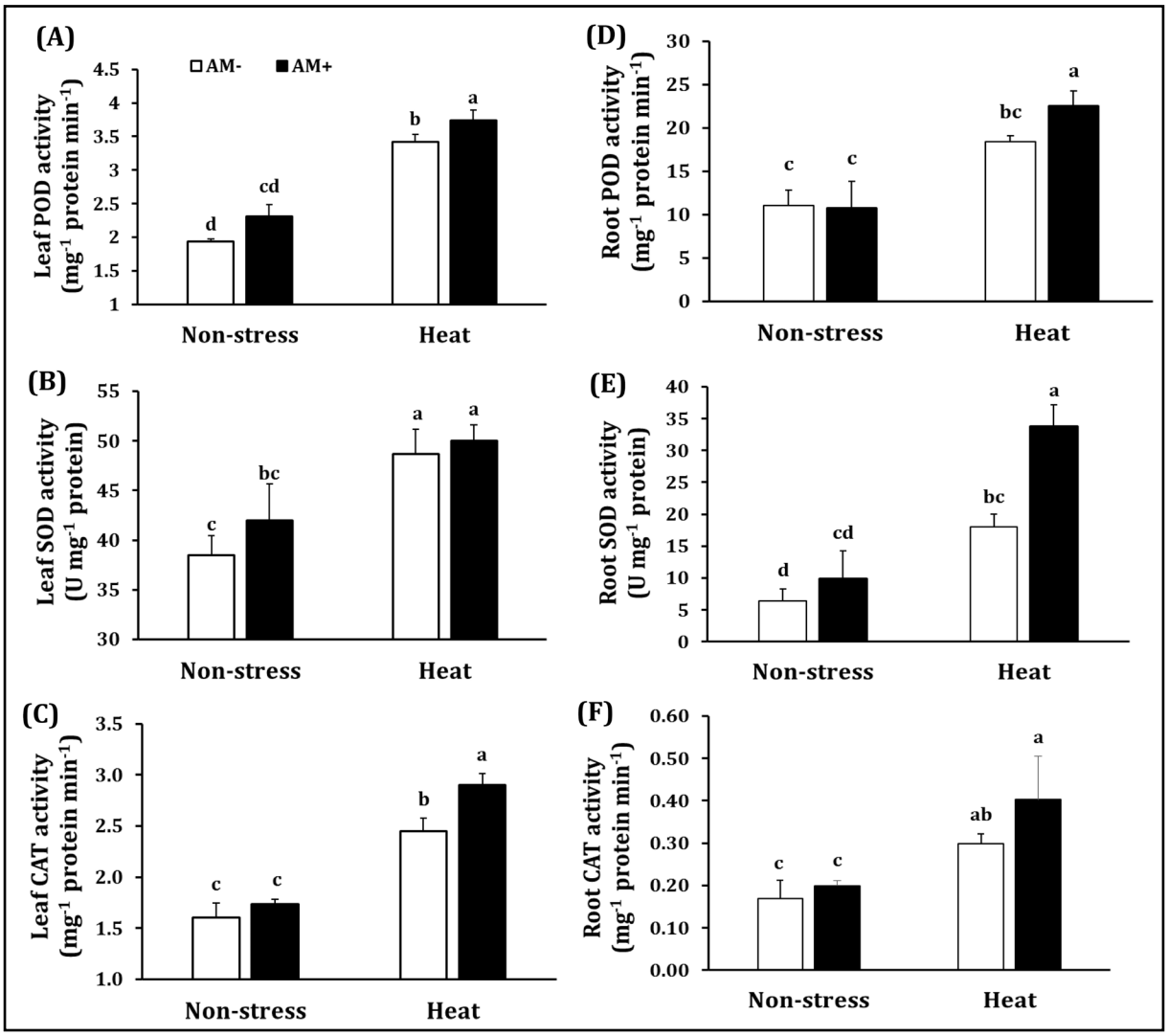

Figure 2. Peroxidase (POD) (A), superoxide dismutase (SOD) (B), catalase (CAT) (C) activity in leaves and POD (D), SOD (E) and CAT (F) in roots of non-AM plants and plants colonized by *Septoglomus constrictum* exposed to non-stress, heat-shock conditions. Bars present mean ± standard deviation (n = 4). Different letters present significant differences among treatments according to Duncan posthoc test ($P < 0.05$).

Chaoxing 2011), however; little information on how AM symbiosis responds to heat shock was explored. In this study, the effect of AM inoculation with *Septoglomus constrictum,* an uncommon AM isolate in previous studies under heat stress, on plant tolerance to heat shock in tomato plants was investigated.

Temperature stresses can negatively impact on the growth and development of AM symbiosis (Zhu et al. 2010; 2011). Earlier studies showed that the development of AMF was inhibited by low temperatures (Zhu et al. 2010) whereas high temperatures influenced detrimentally the activity of AMF (Martin and Stutz 2004) and negative or neutral AM colonization (Compant et al. 2010). In the present study, heat shock had no significant effect on mycorrhizal colonization of tomato plants, which might be due to the length of heat stress applied. This result is in line with the observations in previous studies under heat stress in cyclamen (Maya and Matsubara, 2013), in maize (Zhu et al. 2010).

Extreme temperatures cause unbalanced cellular homeostasis, resulting in overproduction of ROS, membrane lipid peroxidation, and damage plant cells. In this study, we observed that there was an elevated MDA and H_2O_2 accumulation in leaves of tomato plants exposed to heat shock, nevertheless, substantially lower MDA and H_2O_2 contents were found in colonized plants than in plants without AM inoculation, suggesting that the presence of the AMF could alleviate the oxidative stress and peroxidation. The present results concur with observations in tomato plants (Abdel Latef and Chaoxing 2011). The authors illustrated that inoculation of *Funneliformis mosseae* decreased MDA in plants as compared to non-AM plants under cold stress (8°C) for 1 week. Zhu et al. (2010) also showed that maize plants colonized by *Claroideoglomus etunicatum* reduced substantially MDA in roots and leaves when plants were subjected to various temperature stresses (5, 15, 35, 40°C) for one week.

Plant tolerance to high-temperature stress has been linked with an increase in activities of antioxidant enzymes (Sairam et al. 2000). Under heat shock, AM plants showed an enhanced POD, CAT activity in leaves, relative to non-AM plants, which is consistent with lower MDA and H_2O_2 contents observed in leaves of AM plants in our study. In roots, POD and SOD activity in mycorrhizal plants were significantly higher than those in uncolonized plants. These findings suggest that AMF treatment improved the effectiveness of antioxidant systems to protect the host plant against oxidation damage under heat shock conditions. Our results are in accordance with earlier mycorrhiza studies in tomato plants under cold stress (Abdel Latef and Chaoxing 2011), in maize under high-temperature stresses (Zhu et al., 2010). However, leaf SOD and root CAT were not improved in AM plants in our study, which was inconsistent with observations of Abdel Latef and Chaoxing (2011), Zhu et al. (2010) and Maya and Matsubara (2013). The findings may indicate that different AMF isolate could induce differently antioxidant systems in the host plant under heat stress conditions. Similarly, Cekic et al. (2012) reported that pepper plants colonized by *Rhizophagus irregularis* enhanced substantially CAT activity in leaves whereas the leaf SOD level remained unchanged in both AM and non-AM plants when plants exposed to 1 mM NaCl stress.

In conclusion, our results point out that inoculation of AMF, *Septoglomus constrictum,* could enhance tomato plant tolerance against heat shock by decreasing oxidative stress (reduced H_2O_2 and MDA content) and increasing activities of main ROS scavengers such as leaf and root POD, root SOD and leaf CAT. Nonetheless, further studies are necessary to elucidate mechanisms by which AMF influence antioxidant production, proline, photosynthesis, respiration, and water status in plants to better understand their benefits under heat stress for agricultural application.

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In vitro activity of the cinnamon essential oil against the plant pathogen *Septoria melissae* desm.

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Abstract: *Septoria* leafspot caused by the mitosporic fungus *Septoria melissae* Desm. is the most significant disease of lemon balm (*Melissa officinalis* L.). The fungus frequently appears in the plantations and causes serious yield losses or decreases the quality of the drug. At present plant protection of lemon balm is inadequate due to the lack of authorised plant protection products. The essential oil of the cinnamon bark (CEO) has a strong antimicrobial activity proved by several *in vitro* experiments. Therefore the goal of our work was to test antifungal effect of the CEO against the pathogen *Septoria melissae* Desm.

In vitro tests were carried out with three concentrations (0.3%, 0.1%, 0.03%) of the CEO against a Hungarian and a Polish isolate of the fungus. Inhibition of the germination of the conidia as well as the mycelial radial growth was investigated. Supplementary tests were carried out with colonies of the fungi transferred to growth media without CEO after an incubation period of 14 and 35 days on media containing CEO.

Our results showed that all the applied concentrations of CEO have very strong (98.07-100%) inhibitory effect on the mycelial growth of both isolates. Germination of conidia was also blocked on each medium containing CEO. However, the effect of the CEO at 0.03% concentration was reversible. Mycelium began to grow again on media without CEO after transfer.

Based on the results, further investigation of CEO as a potential plant protection product in lemon balm crops is recommended.

Keywords: *Septoria* leafspot, lemon balm, growth inhibition, environmental friendly, medicinal plants

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Introduction

Septoria leafspot caused by the mitosporic fungus *Septoria melissae* Desm. is the most important disease of lemon balm (*Melissa officinalis* L.) (Nagy et Horváth, 2010; Jadczak et Pizoń, 2017; Wielgusz et Seidler-Łożykowska, 2017). This pathogen frequently appears in lemon balm crops and causes serious yield losses expressed by the severe leaf fall as a consequence of the lack of proper pest management. Furthermore, even a moderate infection may highly influence the quality of the drug by decreasing the essential oil (EO) content and modifying the rate of the main compounds (Aulerio et al., 1995; Kowalska et al., 2014). At present, the plant protection of lemon balm is inadequate, due to the strict regulation of the maximum residue levels allowed in herbal products (Kowalska et al., 2014; Bernáth et Zámbori-Németh, 2015). In Hungary, only a few plant protection products are authorised in this crop which cannot provide reliable control of the pathogens (Ocskó et al. 2017).

In the light of the facts mentioned above, the development of new and environmental friendly methods for the protection of lemon balm is essential. EOs are potential substances for this purpose. The antimicrobial activity of these compounds has been confirmed by several *in vitro* and *in vivo* investigations in the past decades. EO of the cinnamom bark (CEO) is one of the most highly investigated EOs in the field of food science. The results of an *in vitro* experiment of Ju et al. (2018) demonstrated the efficacy of the CEO against *Penicillium* and *Aspergillus* species. The results of López et al. (2007) indicate, that *trans*-cinnamaldehyde, the main compound of this oil might be responsible for its antimicrobial activity. Another *in vitro* experiment of Feyaerts et al. (2018) carried out with 175 EOs confirmed the previous

observation. The authors' results showed, that the EOs which contain high amount of aldehydes, had the highest inhibitory effect in vapour phase.

Field trials were carried out by Hochbaum et Nagy (2013) with the combination of the EOs of *Thymus vulgaris* and *Cinnamomum zeylanicum.* Spraying a mixture of the EOs significantly decreased the disease incidence of *Monilinia* blossom blight (*Monilinia laxa*) of apricot and leafcurl (*Taphrina deformans*) of peach in the applied 0.05% and 0.1% concentrations, respectively. The wide spectrum of antimicrobial activity of CEO was also demonstrated by a small plot trial of Kovács et al. (2013). The application of this EO gave the best result against the *Fusarium* head blight (*Fusarium graminearum*, *F. culmorum*) of winter wheat on artificially inoculated plants.

In medicinal plant production only few data are available (e.g. Kovács et Nagy (2014)) about the use of EOs as plant protection product. Therefore, the goal of our recent study was to test the efficacy of the EO of *C. zeylanicum* bark *(Aetheroleum cinnamomi zeylanici corticis)* against the most important pathogen of lemon balm: *S. melissae* Desm.

Materials and methods

Characterisation of the applied essential oil

The investigated CEO was purchased from a commercial company (Aromax Zrt., Hungary). The chemical composition was determined by gas chromatography mass spectrometric method, using an Agilent Technologies 6890N instrument equipped with HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm) and an Agilent Technologies MS 5975 inert mass selective detector. The temperature program was the following: initial temperature 60°C, then by a rate of 3°C/min up to 240°C; the final temperature was kept for 5 min. Carrier gas was helium (1 mL min−1), injector and detector temperatures were 250°C. Split ratio: 30:1. Injected quantity: 0.2 μl (solvent: n-hexane). The percentage composition of the EO was computed from the GC peak areas. Ionization energy was 70 eV. The MS were recorded in full scan mode that revealed the total ion current (TIC) chromatograms (mass range m/z 50–550 uma). The components were identified by linear retention indices that were calculated using the generalized equation of Van Den Dool and Dec. Kratz (1963), and by mass spectra by using NIST MS Search 2.0 library and Adams mass spectra library (Adams, 2007).

According to the GC-MS analysis, the applied EO contains *trans*-cinnamaldehyde in 78.8% (*Table 1.*). Other components of the oil in higher amounts were the followings: *trans*-cinnamyl-acetate (3.6%), *trans*-anetol (2.8%), linalool (2.8%) and eugenol (1.3%).

Origin and maintenance of fungal isolates

Two isolates of *S. melissae* Desm. were used in our experiment. Target pathogens were

Table 1. Chemical composition of the applied cinnamon essential oil according to the GC-MS analysis

Compound	RT	LRI	%
α-Pinene	5.56	938.000	0.6
Benzaldehyde (artificial almond)	6.28	967.000	0.2
ß-Pinene	6.64	980.856	0.1
p-Cimol (para)	8.09	1025.856	1.4
Limonene	8.19	1028.529	1.5
1,8-Cineole	8.38	1033.503	2.3
Linalool	10.76	1097.246	2.8
α-Terpineol	14.55	1189.123	1.2
cis-Cinnamaldehyde	15.73	1217.000	0.1
trans-Cinnamaldehyde	17.72	1264.000	78.8
trans-Anetol	18.51	1283.175	2.8
Eugenol	21.44	1360.822	1.3
α-Copaene	22.03	1376.986	0.04
ß-Caryophyllene	23.68	1419.951	2.5
trans-Cinnamyl acetate	24.80	1448.000	3.6
α-Humulene	25.07	1454.187	0.5
orto-Methoxy Cinnamaldehyde	28.24	1536.000	0.1
Benzyl benzoate	36.83	1773.296	0.1
Total			99.9

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isolated from infected leaves of lemon balm on malt extract agar (MEA). The leaves were collected at Budapest-Soroksár (47°24'08.7"N 19°09'03.9"E) in Hungary (HBS) and at Warsaw-Wilanów (52°09'36.9"N 21°06'08.2"E) in Poland (PWW). The fungal isolates have been maintained on malt extract agar (MEA) at 24°C without light.

Experimental methods to test the antimicrobial properties

Inhibitory effect of CEO on both the *germination of conidia* and the *mycelial radial growth* were tested by the following method.

For the testing of the effect of CEO on the *germination of conidia*, suspension of conidia was prepared by suspending conidial exudates developed on the surface of monosporic cultures of the isolate PWW in distilled water. Concentration of the suspension was determined by haemocytometer. The amount of conidia in the suspension was approximately 1.6×10^6 conidia/ml. Preparation of media carried out according to the followings. CEO was evenly diluted in MEA at the 0.3%; 0.1% and 0.03% concentrations in Petri dishes. Silwet Star wetting agent in 0.02% was also applied in each treatment to improve homogeneity. Conidia growing on medium containing no CEO served as control. Besides, MEA mixed with the wetting agent were also tested. 30µl of conidial suspension was spread evenly on the surface of the media in two replications. The number of germinated conidia was counted 24 hours after inoculation on ten randomly chosen 10^6 µm^2 area of the media. Conidium was considered germinated if measured length of the germ tube was longer than the length of the conidium. Ratio of the germinated and non germinated conidia was expressed in percentage.

For the testing of the effect of CEO on the *mycelial radial growth* preparation of media and the treatments were carried out in the same way as described above. 31 days old monosporic cultures of the slow growing fungus *S. melissae* Desm. were used to obtain adequate amount of inoculum. Mycelial fragments from the margin of the cultures of both origin, HBS and PWW, were placed onto previously prepared media aseptically. The experiment was set up in 10 replicates. The radial growth of mycelium was determined by measuring the diameter of the colonies 0 and 14 days after inoculation. The ratio of inhibition was calculated by the following formula: $PI=([C_A-C_S]-[T_A-T_S])/(C_A-C_S)\times100$, where C_S is the area of the colonies on intact medium at the time of inoculation, C_A is the area of the colonies on intact medium on day 14 after inoculation, T_S is the area of colonies on the treated medium at the day of inoculation and T_A is the size of the culture on the treated medium on day 14 after inoculation. Growth inhibition was expressed in percentage comparing the growth rate of CEO treated colonies with the control ones.

The *survival rate of the treated cultures* was investigated with HBS isolates. After the maintenance of 14 and 35 days of the cultures on medium containing 0.3%, 0.1% and 0.03% CEO, mycelial fragments were placed onto media containing no CEO. The mycelial radial growth of these cultures was measured on the 3rd, 8th and 14th days after transfer. The mycelial growth was calculated by the following formula: $MReg=S_A-S_S$, where S_S is the area of the colonies on intact medium at the time of transfer and S_A is the area of the colonies on intact medium at the time of the assessment. The results of the calculations were expressed in mm^2.

Statistical analysis

Data were analysed by the IBM SPSS Statistics 22 software. Univariate ANOVA (ANOVA) was used to evaluate the significant differences among the inhibitory effect of the investigated CEO in different dilutions. Multivariate ANOVA (MANOVA) was used to detect the significant differences between the growth rates of the transferred cultures during survival rate test. The normality of the residuals was tested according to the Saphiro-Wilk and Kolgomorov-Smirnov tests. If the normality could not be justified by the mentioned analyses, it was verified by the skewness and the kurtosis. Homogeneity of variances was tested by the Levene's method. If the homogeneity assumption was not violated, Tukey *post hoc* test was used to group the genotypes. Otherwise the separation was made by the Games-Howell test.

Table 2. The rate of inhibition of mycelial radial growth of the two *Septoria melissae* Desm. isolates by cinnamon essential oil (CEO) (Legends: The abc letters refer to the significantly different groups according to the ANOVA test)

Treatment	Isolate	
	HBS	PWW
CEO 0.3%	97.68% a	100.00% a
CEO 0.1%	98.07% a	100.00% a
CEO 0.03%	99.65% a	100.00% a
Silwet Star	15.38% b	1.01% b

Table 3. The effect of cinnamon essential oil (CEO) treatments on the germination of conidia of the PWW isolate

Treatment	Conidia	
	germinated	not germinated
CEO 0.3%	0%	100%
CEO 0.1%	0%	100%
CEO 0.03%	0%	100%
Silwet Star	100%	0%
Control	100%	0%

Results and discussion

Inhibitory effect on the germination of conidia and on the mycelial radial growth

Conidia of the PWW isolate did not germinate on any of the treated medium containing CEO (*Table 3.*; *Figure 1.*). The Silwet Star wetting agent did not influence the germination of the conidia.

The CEO achieved a powerful inhibitory effect on the *mycelial growth* of both isolates of *S. melissae* Desm. (*Table 2.*) as well. All applied concentrations showed approximately 100% growth inhibition. Differences among the concentrations were not significant according to statistical analysis. The applied wetting agent showed a very low growth inhibition (1.01-15.38%) of the mycelia of both isolates.

Information about the inhibitory effect of the CEO against *Septoria* species was not found in the international literature. The effect of other EOs (thyme, fennel, rosemary, etc.) on the related fungus *Septoria tritici* (syn.: *Zymoseptoria tritici*) was investigated by Matusinsky et al. (2015). The EO of thyme inhibited mycelial growth by 100%. The strong antifungal activity of CEO was demonstrated by several authors, although against organisms other than *Septoria* spp. In the *in vitro* experiment of Kovács et al. (2013) CEO showed 100% inhibition of the mycelial growth of *Fusarium graminearum*. Another trial carried out with the pure compound *trans*-cinnamaldehyde (the main component of CEO) revealed a strong inhibitory effect on the mycelial growth of both *Aspergillus flavus* (100% inhibition) and *Penicillium islandicum* (91% inhibiton) (López et al., 2007).

Survival rate of the treated cultures

Although a good inhibitory activity of CEO was detected in each applied concentrations, survival rate trials showed that the fungal mycelium was not completely destroyed in all treatments. The transferred mycelial fragments from colonies grown for 14 days on MEA containing 0.03% CEO began to grow on MEA without CEO. The measured growth intensity of these colonies was

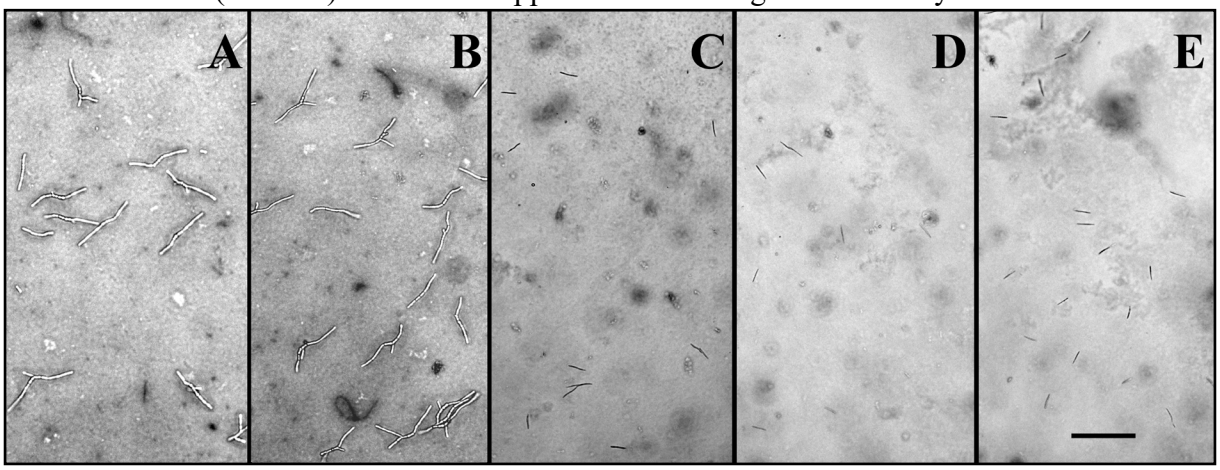

Figure 1. Inhibition of the germination of conidia by cinnamon essential oil (CEO) (Legends: Surface of the media in the following treatments: A – control; B – Silwet Star 0.02%; C – CEO 0.03%; D – CEO 0.1%; E – CEO 0.3%. Scale 100 μm)

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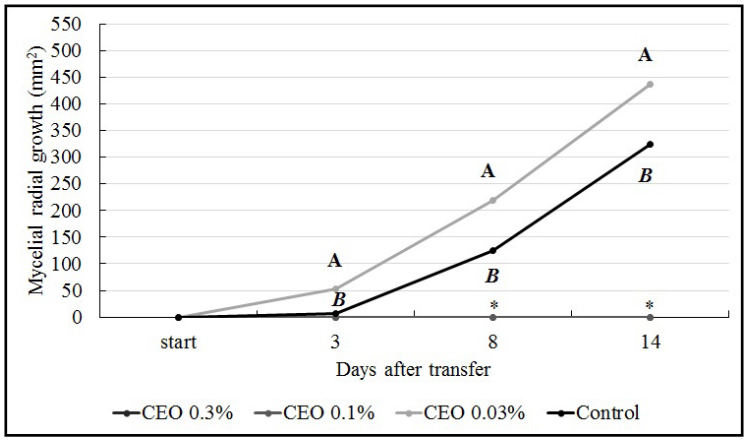

Figure 2. Mycelial radial growth of HBS colonies on MEA transferred after 14 days incubation on CEO containing MEA (Legends: The ABC letters means the significantly different groups in one time according to the MANOVA test. Normal letters belongs to the CEO 0.03% and italic letters to the control. The stars sign the CEO 0.3% and CEO 0.1% treatments where mycelial growth was not observed).

significantly higher than that of the control ones (*Figure 2.*). After a longer incubation period (up to 35 days) on MEA containing 0.03% CEO only a few colonies, which were transferred to MEA without CEO started to grow with very different intensity. This part of the experiment was not evaluated statistically. Colonies transferred from MEA containing higher CEO concentrations (0.1% and 0.3%) were not able to grow on MEA without CEO.

Conclusion

Our experiment showed that the investigated CEO had a powerful *in vitro* inhibitory effect on conidial germination and the mycelial growth of both *S. melissae* Desm. isolates. In accordance with the results of the *in vitro* experiment of Kovács et al. (2013) carried out with the wheat pathogen *Fusarium graminearum*, CEO had approximately 100% growth inhibition even in the lowest (0.03%) concentration. However, in our experiment this CEO concentration caused only a reversible inhibition and was not lethal to the mycelium of *S.melissae* Desm. Treated isolates managed to grow on MEA with no CEO after transfer.

According to Gutiérrez et al. (2010) EOs break the integrity of the cytoplasmic membrane, which leads to the collapse of fungal cell. CEO might have the same effect on the conidial cell or on the membrane of the germ tube of *S. melissae* Desm.

Based on our results CEO applied in 0.1% and 0.3% concentrations can be a potentially efficient plant protection product used for the control of *S. melissae* Desm. However, the practical application needs further investigations including *in vivo* trials.

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Preliminary results of a pot experiment with the combined effects of a terrestrial isopod species (*Porcellionides pruinosus*, Brandt 1833) and organic mulching on tomato

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Abstract: Organic mulching practice has a beneficial effect on soil life. Increased soil activity may in turn lead to increased soil fertility. To study the effect of terrestrial isopods and organic mulch on potted tomatoes we measured the growth, vitality and yield of plants. Half of the pots had the surface of the soil covered with a mulch of equal quantity of walnut (*Juglans regia*), elm (*Ulmus minor*) and maple (*Acer platanoides*) leaves; and half of them had the soil uncovered. 20 *Porcellionides pruinosus* (Brandt, 1833) individuals were introduced to every second pot. There were four treatments in 10 replicates: (1) I+M+ (isopods, mulching); (2) I+M- (isopods, no mulching); (3) I-M+ (no isopods, mulching) and (4) I-M- (no isopods, no mulching). To determine the microbial activity of the soil, fluorescein diacetate hydrolysis activity was measured. The fluorescein diacetate concentration was higher in the "combined" treatment than in the "isopods only" treatment, and in the "mulch only" treatment. Regardless of treatment, overall microbial activity figures were lower after the experiment than their respective starting values. The "combined" treatment significantly increased the generative growth of tomatoes. The number of flowers was significantly higher, and thus significantly more tomato was harvested when compared to the "control" treatment. Our preliminary results indicate the beneficial effects of *P. pruinosus*, because its presence had an advantageous influence on tomato yield.

Keywords: Oniscidea, soil fertility, woodlice, soil cover

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Introduction

Higher habitat and ecological diversity in a home-scale horticulture decreases the pressure from pests (Philpott 2013). Tomato production has an increasing trend both in the total area of production and the amount of harvest worldwide (FAOSTAT data, 2018). Tomato is typical to most home vegetables patches as well, with most of the gardeners cultivating more than one variety.

Consumers believe that landraces are nutritionally more valuable (Casals et al. 2011). While this argument has not yet been proven in all cases, genetic variability differs among various landraces and depends on selection pressure (Passam et al. 2007).

For our study we selected a Hungarian landrace (Dány) with the preconception in mind that landraces are better adopted to local conditions. We wanted to find a method that has the potential to increase the success of home-scale tomato production, to increase the amount of yield and the vitality of soil at the same time. In our study we were looking for providing production conditions that are relatively less experienced with yet are low on the budget and energy demand.

The benefits of using natural mulches are manifold. Suppressing weeds, preserving soil moisture and therefore, stabilizing soil structure are just some of them. One has to mention that besides contributing to the production of healthy, marketable crops, good mulching practice provides suitable conditions for beneficial organisms, making the use of mulch a factor of key importance within the complexity of crop protection (Mancinelli et al. 2015, Diver et al. 1999). Mancinelli et al. (2015) found that organic mulching, irrigation and fertilization decreased CO_2-emission and increased C-storage of the soil. There are many soil-dwelling living organisms involved in nutrient cycles to maintain soil fertility. Terrestrial isopods hide under dead plant matter, using it as shelter and food source at the same time (Stachursky 1968). Woodlice are also known for manipulating the soil: to consume decaying organic matter and release it back as faeces to the soil again. Within their intestinal tract, decaying materials are broken down to small, organic molecules, which are now available for the soil bacteria and fungi for the processes of immobilization, mineralisation and humification. This is how isopods have an indirect influence on the microbial activity of

the soil (Zimmer and Topp 1999; Lavelle et al. 2006; Vilisics et al. 2012). *Porcellionides pruinosus* was found the most abundant species in compost heaps (Farkas and Vilisics 2013).

The positive effect of mulching has been well documented in recent times (Campiglia et al., 2011, Shirgure, 2012, Kumar, 2014), and the beneficial effects of faecal pellets of woodlice and millipedes on the growth of test plants were proven in pot experiments (Gere 1956; Farkas et al. 2017).

Therefore we expected mulching to increase the microbial activity of the soil, and tomato yield; and by adding (not faecal pellets but living individuals of) woodlice to the system, to further increase the beneficial effects of mulch. We also predicted that the lack of mulch would make woodlice leave the pots, and that in those pots there would be no significant change in either the microbial activity of the soil or in the yield.

Methods

Selection of plants and test animals

For our purposes, the regional cultivar Dány was used. This determinate, mid to late season variety is mainly for fresh consumption. It has a moderate growth vigour, and a strong foliage rejuvenation (ÖMKi 2015). The growing period for this variety was observed to start late March to early April with sowing, and to end mid to late September with the final harvests. Tomato seeds were sown in Florimo® general potting soil (*Sphagnum* moss peat, flat peat, organic humus, composted cattle manure, clay, fertilizer mixture, with a pH of 6.4±0.5). The plants were grown in greenhouse until the age of six weeks, when the seedlings were transplanted one by one into a pot. There were 40 pots with the same soil, one seedling per pot. Thereafter Isopods were placed into the pots with or without leaf litter.

Our test animal was Porcellionides pruinosus. Besides its simple availability and synanthropic lifestyle, this isopod became our test species because it responds well to various environmental circumstances and is easy to rear among artificial conditions.

Porcellionides pruinosus individuals were collected from the Regional Waste Management Center of Pusztazámor by handsorting. The animals were bred and kept at the Institute of Plant Protection of Szent István University. For identification we used the taxonomic key of Farkas and Vilisics (2013).

Experimental design

The study started on 24 May 2016 in the experimental area of the Plant Protection Institute of the Szent István University in Hungary (SZIE NVI) in a partially covered glasshouse and it was terminated on 23 September 2016, after the growing period. Before distributing the same amount of potting soil („Original soil") into every pot, the enzymatic activity of the soil was measured with *fluorescein diacetate*

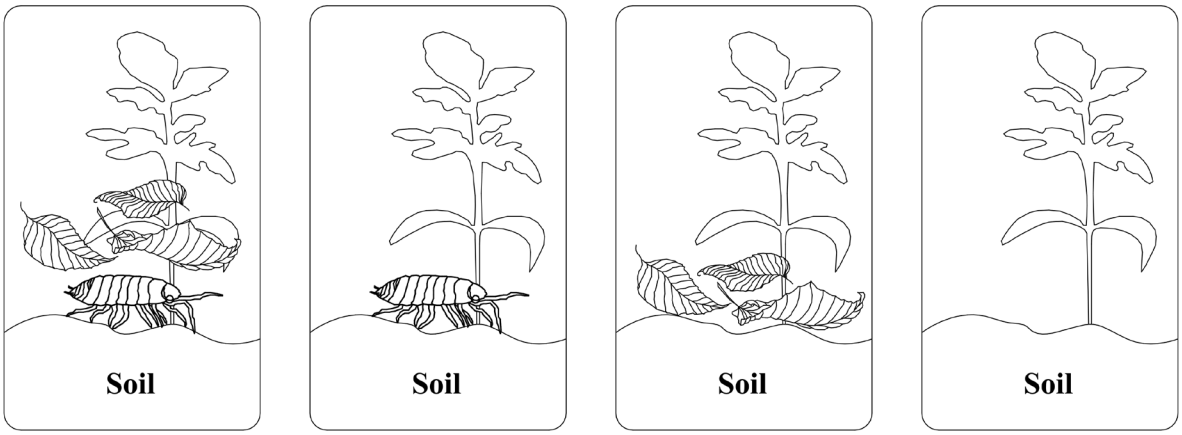

Figure 1. Pot experiment with four different treatments: studying the effect of the addition of mulch and isopods, using a tomato landrace variety as test plant

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(*FDA***)** *hydrolysis assays* te ts. There were four treatments in 10 replicates as follows: (1) I+M+ (isopods, mulching); (2) I+M- (isopods, no mulching); (3) I-M+ (no isopods, mulching) and (4) I-M- (no isopods, no mulching) (Fig 1.).

Placement of Porcellionides pruinosus individuals and mulch

In each I+ pot, 20 specimen of *P. pruinosus* adults (with an average body weight of 228.5 mg) were placed. We chose to not differentiate between genders as this type of selection would have hurt the individuals, but gravid females were excluded. The number of individuals, adults and juveniles were counted at the completion of the experiment. Mulch material was collected from the surrounding areas: the mulch mixture contained walnut (*Juglans regia*), elm (*Ulmus minor*) and maple (*Acer platanoides*) leaf litter of local trees in a ratio of 1:1:1. Every mulched pot was covered with an average of 39.6 g of leaf matter. The soil of the pots was sieved at the end of the experiment, and the number of adult and juvenile isopods was recorded.

Soil microbial activity

To measure and monitor the microbial activity of soil, we followed the method of Schnürer and Rosswall (1982). Each pot was sampled before the experiment and at the end of the experiment again by taking 2 g of soil. Samples were incubated for two hours at 24°C with 10 ml sodium-phosphate buffer (60mM, pH= 7.6) and 100 µl of FDA (2 mg/l). The rotary shaker was set at 300 RPM. After two hours the reaction was halted by the addition of acetone (10 ml). The solution was sieved, and the amount of hydrolysed FDA was measured. Absorbance values were obtained by using a Haca DR/2000 spectrophotometer at 490 nm. Calibration curves were drawn using solutions of known fluorescein concentration, and thus, the amount of fluorescein obtained from our samples during the two-hour period was calculated. Each test was replicated ten times. The values were calculated for one gram of dry soil as mg of fluorescein.

Parameters of tomato

Both the tomatoes and their respective pots were sampled every fortnight during the growing season. The number of flower buds, flowers and leaves were recorded. Indicators of maturity: the number of fruits, their weight were also measured. The number of flower buds, flowers and fruits, and the total weight fruits per plant were regularly monitored. Root weight was measured at the end of the growing season.

Statistical analyses

Levene's test for homogeneity of variance, ANOVA with Tukey's posthoc test, Kruskal-Wallis test with Mann-Whitney pairwise comparisons and Welch test (PAST® program: Hammer et al 2001) were used for statistical analyses.

Results

Soil microbial activity

The fluorescein concentration values (µg/ml) of treatments revealed that the addition of mulch material resulted in higher microbial activity (Fig. 2) ($F=14.35$, $df=21.77$, $p=7.015\times10^{-6}$).

Regardless of the presence of isopods, there was a significant difference between treatments with or without mulch, that is, between treatmentsww

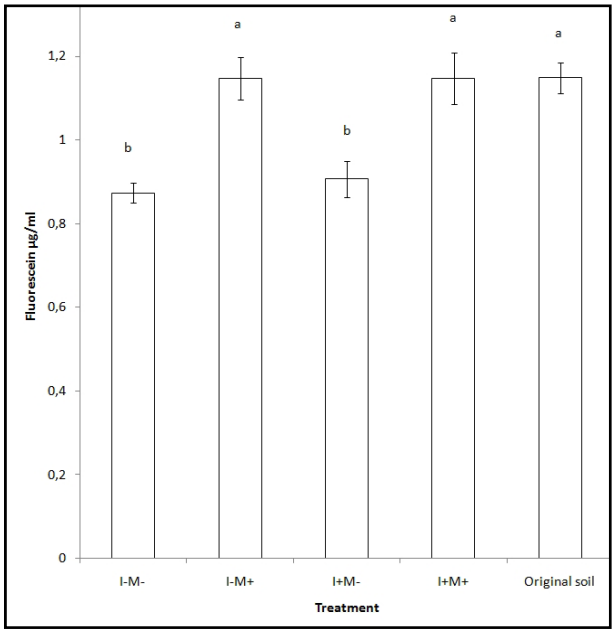

Figure 2. Total soil microbial activity by fluorescein concentrations (µg/ml) in a tomato pot experiment with mulch and the isopod *Porcellionides pruinosus* [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; ANOVA, Tukey's posthoc test]

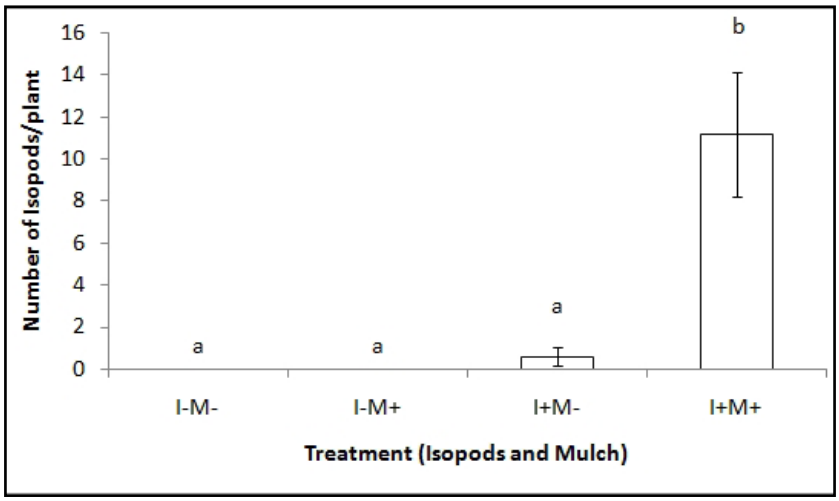

Figure 3. The number of *Porcellionides pruinosus* individuals at the end of a tomato pot experiment where isopods and mulch were both added to the soil [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; Kruskal-Wallis test, Mann-Whitney pairwise comparisons]

I-M- and I-M+; and between treatments I+M- and I+M+. The fluorescein values measured for the two no-mulch treatments (I-M- and I+M-) were also significantly lower than the initial values (Original soil) measured prior to the pot experiment. Statistical analysis confirmed that the original microbial activity of the soil was not significantly different from the values obtained in treatments with mulch (I-M+ and I+M+) but was significantly higher than those without mulch (I-M-) and (I+M-) (Annex 1).

Porcellionides pruinosus and mulch

The number of *Porcellionides pruinosus* individuals at the end of the experiment was lower than initially, but mulching had a beneficial effect on number of *P. pruinosus* individuals (Fig.3., Annex 2) ($H=17.42$; $p=3.40\text{x}10^{-6}$). The number of juveniles were also recorded, but due to low recapture no reliable conclusions were drawn from these data.

Parameters of tomato

Number of flower buds, flowers and leaves

No significant difference was found in the maximum number of flower buds and leaves between the treatments (Annexes 3-4) There was a significant difference however, in the maximal number of flowers ($F=5.246$, $df=19.83$, $p=0.008$; CI95% and average I-M- 0.682 and 3.9, I-M+ 0.787 and 5.5, I+M- 0.889 and 4.5,

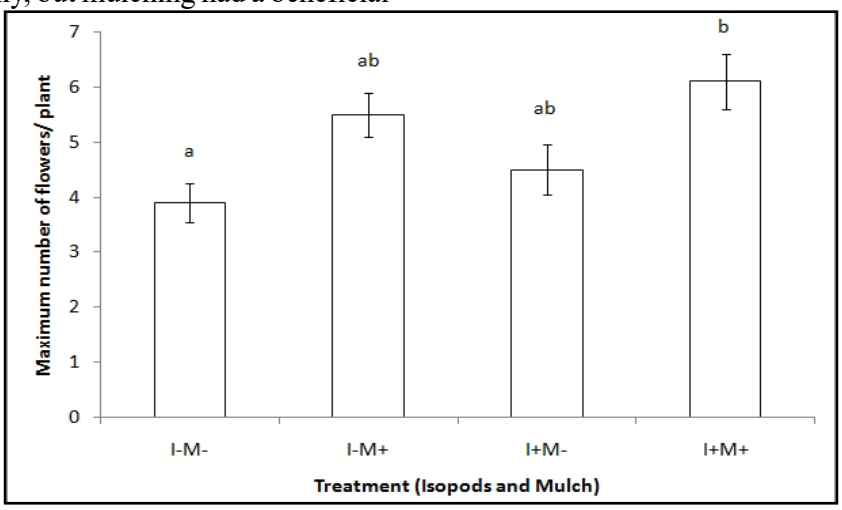

Figure 4. The maximum number of tomato flowers in a pot experiment studying the effect of *Porcellionides pruinosus* and mulch [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; ANOVA, Tukey's post hoc test]

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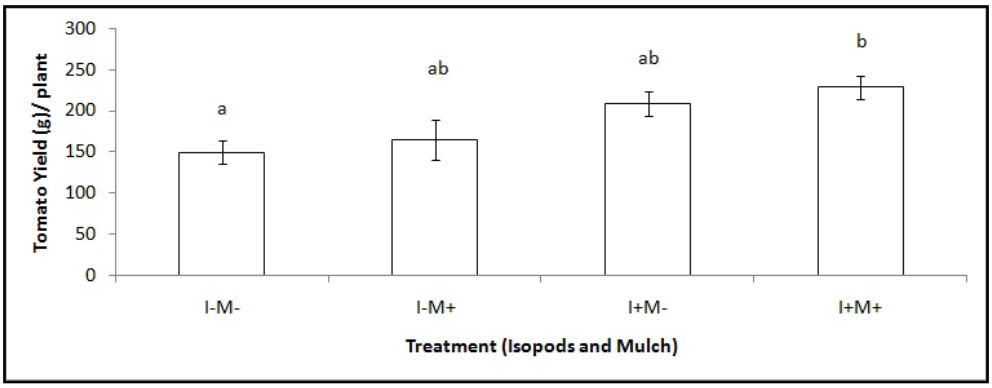

Figure 5. Tomato yield according to the presence or lack of a woodlice species, *Porcellionides pruinosus* and mulch in a tomato pot experiment [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; ANOVA, Tukey's post hoc test]

I+M+ 0.989 and 6.1; Tukey's pairwise I+M+ 0.005) (Fig. 4, Annex 5).

When only isopods were added (I+M-), the decreasing trend in the number of flower buds and flowers was slower than in any of the other treatments.

The number of fruits, their weight and root weight

None of the treatments had a significant effect on the number of fruits per plant (p=0.079).

Neither the addition of isopods, nor the addition of mulch alone had a significant effect on fruit weight, but isopods and mulch together had a recognizable effect on tomato yield (F=5.78. df=19.76. p=0.005) (Annex 6).

The effect of adding isopods to pots on tomato yield is recognizable (p=0.013).

On the other hand, no significant difference was recorded when only mulch was added (M+). According to these results, the difference between the yield of tomato plants treated and those not treated with isopods was statistically not proven. None of the treatments alone had a significant effect on the number of fruits harvested, but there was a significant dissimilarity between the effect of the "combined" (I+M+) and the "control" (I-M-) treatment on the weight of the yield (Fig. 5).

Measuring the dry and fresh matter content of the roots revealed that none of the treatments had a significant influence on the growth of the root system (p = 0.486). Yield (fruit weight per plant) kept increasing continuously as the season wore on. The increase was the highest with the "mulch only" (I-M+) treatment; and its values were followed by the values of the "combined" treatment (I+M+) (Fig. 6).

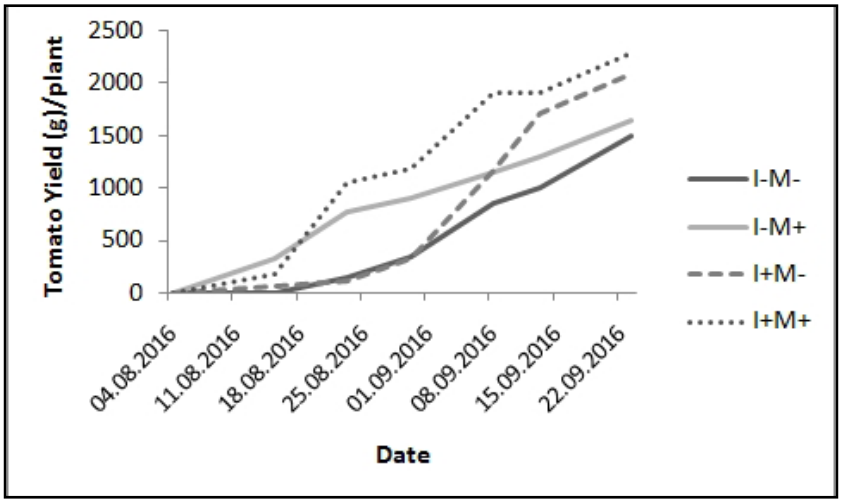

Figure 6. Cumulative tomato yield growth during the experiment with four treatments (I=Isopod, M=Mulch) in a tomato pot experiment

Discussion

Adding mulch alone did not have a significant effect on tomato yield, but a combination of mulch and isopods did. While Tuf and Tufová (2005) claimed that soil macroinvertebrates, due to their role in the ecosystem, increase soil fertility, we only recorded a significant effect of macroinvertebrates when combined with mulch.

Since the type and origin of mulch materials may have an impact on production indicators (Zribi et al. 2015), based on our previous studies (Fehér et al, 2017), we now decided to use organic mulch and in particular, the locally available leaf litter: an even mixture of walnut (*Juglans regia*), elm (*Ulmus minor*) and maple (*Acer platanoides*). Similarly to our experiment, Förster et al. (2006) also mulched with a various mixture of forest leaves, but in their set up faecal pellets of millipedes and woodlice were also added to crops, while we preferred to use the organisms themselves, which were found useful by Tantachasatid et al. (2017) in their experiment. Our prediction, based on the results of Förster et al. (2006) was proven: soil microbial activity declined in the absence of mulch in our treatments. Without a proper organic cover, soil was more prone to desiccation, its microbial activity decreased, and it also failed to provide isopods with habitat and shelter.

Within this limited timeframe of our present experiment mulch material did not have the time to enhance the nutrient content of the soil. We believe mulch provided a habitat and a shelter for our isopods as was found by Diekötter et al (2010). Similarly to a natural environment, isopods were not confined: they had the ability to leave the pots, and we assume that they hardly ever found their way back to the same, or to any pot. Where there were none at the beginning, none was found at the termination of the experiment either. No new isopods settled to pots, and migration was low, probably because it was hindered by the mulch leaves. In the end the number of juveniles was measured but it is not a reliable data because the sex ratio was not determined before the experiment.

Individuals of *Porcellionides pruinosus* contributed to leaves being decomposed and buried into the soil, although the contribution was definitely smaller than that of the dung beetle, also considered an ecosystem engineer (Johnson et al. 2016). This raises the question: would the impact of isopods on soil and plant have been higher had the isopods been introduced in considerably larger numbers. If so, is there an optimum number of individuals to be introduced. Is this optimum amount different for each isopod species, for soil types, and in general, what influence ecological conditions have on this data? To answer these questions, further studies are needed.

Conclusions

Our preliminary results indicate the beneficial effects of the combined application of leaf litter mulch and *Porcellionides pruinosus* on tomato yield.

The observation that isopods were found only in pots where they were introduced at the beginning of the experiment may suggest that these animals do not leave their micro-environment when the conditions were favourable for their survival. One may thus suspect that the useful influence of isopods is only present when the conditions favoured by these arthropods are created within the course of crop production protocol, that is, when mulching is part of the management.

We propose to test this innovative system in larger scale experiments. The biomass quantity of woodlice in our experiment was not remarkable enough and more experiments with higher isopod density are suggested.

We consider treating production areas with mulch and isopods a preliminary step in elaborating a new element of technology in crop production. We contemplate the possibilities of expanding our studies to a larger scale, to arable lands. At present, *P. pruinosus* is already a marketable item: it is reared mainly as prey (e.g. http://www.ebay.co.uk/itm/40-Isopods-*Porcellionides-pruinosus*-Dart-frog-Newts-and-Salamander-food-culture-/171520772125, https://www.insecte.org/forum/viewtopic.php?t=134763, https://argiope.se/ovogram/**).** We suggest a series of detailed studies to take other steps towards the commercial sale of isopods to enhance soil fertility.

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Annexes

Annex1: **Soil microbial activity**

	I-M-	**I-M+**	**I+M-**	**I+M+**	**Original soil**
Average	0.8736	1.1477	0.9068	1.1473	1.1486
Deviation	0.075020294	0.1602	0.13805297	0.1943	0.115313293
SE	0.0237235	0.0506	0.04365618	0.0614	0.036465265
CI 95%	0.046497205	0.0993	0.08556454	0.1204	0.071470606
One-way ANOVA					
	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	0.802225	4	0.200556	9.891	7.892E-06
Within groups:	0.912425	45	0.0202761		
Total:	1.71465	49			
omega^2:	0.4156				
Levene's test for homogeneity of variance. based on means: p(same) =	0.7645				
Based on medians: p(same) =	0.8287				
Welch F test in the case of unequal variances:	F=14.35. df=21.77. p=7.015E-06				
Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal					
	I-M-	I-M+	I+M-	I+M+	Original soil
I-M-		0.0009404	0.9848	0.0009562	0.0009059
I-M+	6.087		0.004092	1	1
I+M-	0.7373	5.35		0.004165	0.003932
I+M+	6.078	0.008883	5.341		1
Original soil	6.107	0.01999	5.37	0.02887	

Annex2: ***Porcellionides pruinosus*** **and mulch**

Kruskal-Wallis test for equal medians				
H (chi2):	17.42			
Hc (tie corrected):	28.14			
p (same):	3.40E-06			
There is a significant difference between sample medians				
Mann-Whitney pairwise comparisons. raw p-values. uncorrected significance				
	I-M-	I-M+	I+M-	I+M+
I-M-		1	0.1681	0.0002312
I-M+	1		0.1681	0.0002312
I+M-	0.1681	0.1681		0.001151
I+M+	0.0002312	0.0002312	0.001151	

Annex3: Maximum number of flower buds

	Average	CI 95%
I-M-	7.2	1.998778921
I-M+	6.3	2.08756356
I+M-	7.7	1.802267141
I+M+	8.6	1.466702744

One-factor variance analysis

Summary

Groups	*Quantity*	*Sum*	*Average*	*Variance*
I-M-	10	72	7.2	10.4
I-M+	10	63	6.3	11.34444444
I+M-	10	77	7.7	8.455555556
I+M+	10	86	8.6	5.6

Variance analysis

Factors	*SS*	*df*	*MS*	*F*	*p-value*	*F crit.*
Between groups	27.7	3	9.233333333	1.031657356	0.3901503	2.866266
In groups	322.2	36	8.95			
Sum	349.9	39				

Annex4: **Maximal number of leaves**

	Average	CI 95%
I-M-	12.1	1.110646258
I-M+	11.8	0.703648605
I+M-	12.5	0.602332503
I+M+	12.8	1.124016514

One-factor variance analysis

Summary

Groups	*Quantity*	*Sum*	*Average*	*Variance*
I-M-	10	121	12.1	3.211111111
I-M+	10	118	11.8	1.288888889
I+M-	10	125	12.5	0.944444444
I+M+	10	128	12.8	3.288888889

Variance analysis

Factors	*SS*	*df*	*MS*	*F*	*p-value*	*F crit.*
Between groups	5.8	3	1.933333333	0.885496183	0.4578358	2.866266
In groups	78.6	36	2.183333333			
Sum	84.4	39				

One-way ANOVA

	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	5.8	3	1.93333	0.8855	0.4578
Within groups:	78.6	36	2.18333		
Total:	84.4	39			

omega^2: -0.008662

Levene's test for homogeneity of variance. based on means: p(same) = 0.355

Based on medians: p(same) = 0.5658

Welch F test in the case of unequal variances: F=1.004. df=19.41. p=0.4124

Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal

	I-M-	I-M+	I+M-	I+M+
I-M-		0.9685	0.9298	0.7161
I-M+	0.642		0.7161	0.4404
I+M-	0.8561	1.498		0.9685
I+M+	1.498	2.14	0.642	

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Annex5: **Maximum number of flowers**

	I-M-	I-M+	I+M-	I+M+
Average	3.9	5.5	4.5	6.1
Deviation	1.100504935	1.269295518	1.433720878	1.595131482
SE	0.348010217	0.401386486	0.45338235	0.504424865
CI 95%	0.682087492	0.786703056	0.888613078	0.988654568

One-way ANOVA

	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	29.2	3	9.73333	5.246	0.004167
Within groups:	66.8	36	1.85556		
Total:	96	39			
omega^2:	0.2415				

Levene's test for homogeneity of variance. based on means: p(same) =	0.6626
Based on medians: p(same) =	0.7355

Welch F test in the case of unequal variances:	F=5.246. df=19.83. p=0.007889

Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal

	I-M-	I-M+	I+M-	I+M+
I-M-		0.0583	0.7589	0.004985
I-M+	3.714		0.3691	0.7589
I+M-	1.393	2.321		0.0583
I+M+	5.107	1.393	3.714	

Annex6: **Tomato Yield**

	I-M-	**I-M+**	**I+M-**	**I+M+**
Average	149.69	164.88	209.9	228.94
Deviation	44.67739796	75.84869149	46.58826748	44.82772456
SE	14.12823375	23.98546226	15.05652313	14.17577119
CI 95%	27.69082931	47.01064219	29.51024308	27.78400099

One-way ANOVA

	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	41573.9	3	13858	4.647	0.00759
Within groups:	107362	36	2982.27		
Total:	148936	39			
omega^2:	0.2148				

Levene's test for homogeneity of variance. based on means: p(same) =	0.006713
Based on medians: p(same) =	0.009981

Welch F test in the case of unequal variances:	F=5.78. df=19.76. p=0.005236

Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal

	I-M-	I-M+	I+M-	I+M+
I-M-		0.9244	0.08299	0.01304
I-M+	0.8796		0.2704	0.05875
I+M-	3.487	2.607		0.8632
I+M+	4.589	3.709	1.103	

Yield components of winter oilseed rape regard to plant population

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Abstract: The aim of this study was to analyze the yield components of winter oilseed rape depending on the plant population in 2017/2018 growing season. Three plant populatios were considered in the study: 20, 40 and 60 plants m^{-2}. At harvest several yield components were determined: plant height (cm), plant mass (g), height of the first fertile branch (cm), number of fertile lateral branches, number of pods per plant, length of the pod (cm), number of seeds per pod, mass of seeds per pod (g), number of seeds per plant, 1000 grain mass (g) and yield of seeds per plant (g). At the plant population of 40 plants m^{-2} the plants were the highest (153.4 cm), with the highest plant mass (295.3 g) and the number of lateral fertile branches (5.6 branches per plant). Furthermore, the plants from 40 plants m^{-2} had the highest number of pods per plant (716 pieces), the longest pods (6.5 cm) with the largest number of seeds per pod (21.0) and the number of seeds per plant (15 036 seeds). The highest and significant correlation coeficient was found between number of pods per plant and plant mass (r=0.890***) and than between the number of lateral branches and number of pods per plant (r=0.850***). Linear regression showed that for every centimeter increment of pod length the number of seeds increase for 4.8 seeds at 20 and 40 plants m^{-2} and for 5.5 seeds per pod at 60 plants m^{-2}. Furthermore, regression analysis showed that for every centimeter pod length increment the seeds mass per pod increase on average of all plant population for 0.02 g.

Keywords: winter oilseed rape, 2017/2018, plant population, yield components

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Introduction

According to FAOStat data (2018) in the world production of oilseed rape form 2012 to 2016 was on about 35 mil. ha with seed yield of 2.0 t ha^{-1}, whereas in the Europe there was about 25% of world sown area, and it counts on average 8.6 mil. ha with seed of yield 2.9 t ha^{-1} (Table 1). The greater producers in the EU are France, Germany and United Kingdom, while in the world great producers are Canada, China, India and Australia. In Republic of Croatia the average production area of winter oilseed rape was 21 948 ha and seed yield 2.8 t ha^{-1} (Statistical Yearbook of the Republic of Croatia 2017). The importance of rape has thus increased in recent years and today it is cultivated on every continent. Nowadays the cultivation of oilseed rape have a great importance due to its usage as vegetable oil in human diet, but also for the biodiesel production as a renewable source, as a catch crop for green manuring and as a forage crop: nutrition in the form of rape cake and meal (Pepó, 2013; Carré and Pouzet, 2014; Lääniste et al. 2016; Nath et al. 2016; Zając et al., 2016; Novoselec et al. 2018).

Table 1. Production of oilseed rape in the world from 2012 to 2016 (FAOStat, 2018)

	Harvested area (ha)	Seed yield (t ha^{-1})
The greater producers in the Europe		
Europe total	*8 619 138*	*2.9*
France	1 520 668	3.3
Germany	1 355 440	3.9
Russian Federation	993 076	1.1
Poland	873 228	2.9
Ukraine	705 758	2.5
Great Britain	675 303	3.4
The greater producers in the world		
World total	*35 175 387*	*2.0*
Canada	8 322 800	2.1
China	7 537 637	1.9
India	6 085 748	1.2
Australia	2 726 891	1.3

The number of plants per unit area is one of the most important yield components in plant production (Ma et al., 2014; Masarirambi et al., 2012; Pepó and Murányi, 2014; Varga et al., 2015; Li et al., 2017; Varga et al., 2017; Vinze, 2017). According to Balodis and Gaile (2016) plant population in relation to the sowing rate particularly affects the number of pods per plant and seed number per pod. The optimum density or plant population results in mature plants that are sufficiently crowded to efficiently use resources such as water, nutrients, and sunlight, yet not so crowded that some plants die or are unproductive. Thus, the distribution of plants

per unit area is of great importance for yield stability. There are several factors that have influence on the realized plant population like soil properties, seed quality, field germination, sowing time, plant morphology, diseases, pests, seedbeed preparation etc. (Sidlauskas et al., 2003; Kristek et al., 2015; Balodis and Gaile 2016; Kovacevic et al., 2017; Zebec et al., 2017).

The optimum time for sowing winter oilseed rape in Croatia is from 25th August to 10th September. It is therefore sown at 12.5 cm or 25 cm distance between the rows and at the depth from 1.5 cm to 2.5 cm. The recommended seed rate for winter sown oilseed rape in the Croatia is 2.5 – 5 kg ha^{-1}. In Europe oilseed rape is usually sown as winter crop at sown rate around 70 seeds m^{-2} (Roques and Berry, 2016). It can be also sown as a spring crop, when it is sown in higher density as 150 seeds m^{-2} (Lääniste et al. 2016) or 100 – 110 seeds m^{-2} (Leach et al., 1999) for hybrids.

The optimum plant population for oilseed rape hybrids is 30 – 50 plants m^{-2} at harvest (Pospišil, 2013.). On the one hand, in lower plant populations oilseed rape form greater lateral branches, which can somewhat compenstate the lack of plants. On the other hand, larger number of plants per m^{-2} can couse decrease in the steam diameter, thus plants are more sensible to lie down. Leach et al. (1999) indicate that the yield of winter oilseed rape increases if 50-60 plants m^{-2} is achieved. Authors suggested that the productivity of the plant at lower plant population is compensated by increasing the leaf area, multiple lateral fertile branches and with more pods per plant. On the contraty, authors stated that in large plant population, no significant increase in yields was found becouse of the greater possibility of disease in oilseed rape crops. Mendham et al. (1981) shown that plant populations of 20–30 plants m^{-2} produce yields comparable to crops with 70–80 plants m^{-2}, in some instances a crop with only 9–10 plants m^{-2} produced acceptable yields if the plants were healthy and evenly distributed. Diepenbrock (2000) reported that plant population had the greatest effect on yield components of individual plants.

There is a lack of research available on winter oilseed rape yield components in the conditions of Croatia, especially regard to plant population. It is therefore, essential to understand how individual plants interact with each other and the environment and to possibly come up with the ideal crop density levels to optimize yields. The aim of this study was to determine the winter oilseed rape yield components regard to different plant populations in 2017/2018 vegetation.

Material and methods

Field trail was set up in Eastern Croatia (location Beravci) on Family farm «Ivica Anđelić». Winter oilseed rape was sown on 5th September 2017 (hybrid Hybrirock, KWS). One month after sowing (5th November 2017) when plants had about 5-6 leaves, the plant population correction was made to get three different densities: 20, 40 and 60 plants m^{-2}. Each plant population was established in plot size 10 m x 3 m. There were total 171 kg ha^{-1} N, 67 kg ha^{-1} P i 87 kg ha^{-1} K added with fertilization (Table 2). All protection against weeds and pests was made properly.

During the vegetation, the number of leaves per plant were determined in 7 dates (from 5th November 2017 to 5th April 2018). In 2017 the number of leaves represent the average number of leaves per plant counted on 30 individual plants, whereas in 2018 the number of leaves per plant represent the average number of 45 individual plants. Thus, the number of leaves were determined on 270 individual plants.

Besides leaves counting, during vegetation the realized plant population was determined three times for every treatment in 4 replications. The harvest was on 11th June 2018 and therefore from the every plant population (20, 40 and 60 plants m^{-2}) the 15 individual plants were harvested manually. Therefore, the yield components were

Table 2. Fertilization of winter oilseed rape for 2017/2018 vegetation year

Fertilizer	Amount (kg ha^{-1})	N	P_2O_5	K_2O
	5th July 2017 (with soil tillage)			
NPK 0-20-30	200	-	40	60
UREA	100	46	-	-
	5th September 2017 (with sowing)			
NPK 15-15-15	180	27	27	27
	1st March and 12th April 2018 (top dressing)			
KAN	180	49	-	-
KAN	180	49	-	-
	Total	***171***	***67***	***87***

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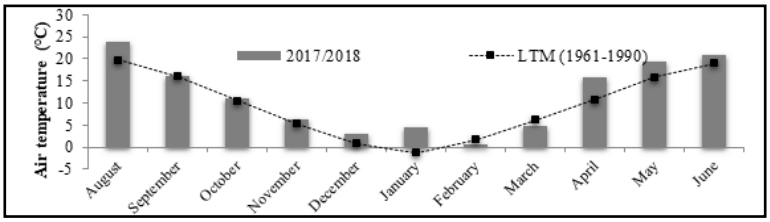

Figure 1. The mean air temperature (°C) in 2017/2018 winter oilseed rape vegetation as compared to the long term mean (LTM = 1961-1990) for Meteorological station Slavonski Brod (Croatian Meteorological and Hydrological Service, 2018)

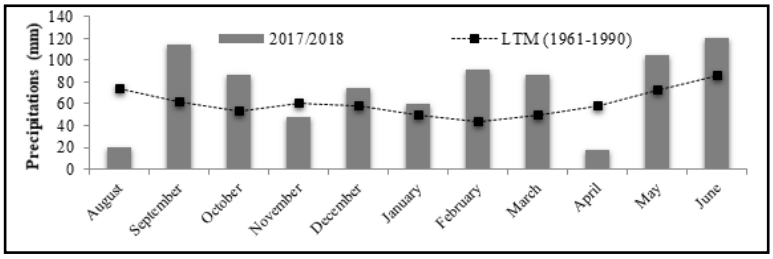

*Figure 2.*The total monthly precipitation (mm) in 2017/2018 winter oilseed rape vegetation as compared to the long term mean (LTM = 1961-1990) for Meteorological station Slavonski Brod (Croatian Meteorological and Hydrological Service, 2018)

determined on 45 individual plants. The plants were marked and afterwards the plant hight (cm), the plant hight to first fertile branche (cm), total number of pods per plant were measured. From every plant 100 individual pods were separated in the paper bag, and than on every pod several measurments were made: the pods length (cm), the number of seeds per pod and mass of seeds per pod. Total of 4500 individual pods were analyzed. Afterwards, the number of seeds per plant, the 1000 grain mass and the yield of seeds per plant (g per plant) were determined.

During vegetation period, form August 2017 to June 2018 (Figure 1) the average air temeprature was 1.9°C higher as compared to the long-term mean (LTM, 1961-1990) (Croatian Meteorological and Hydrological Service, 2018). The winter period was not so cold, so the plants were not destroyed. Even though, in January the mean air memperature was for 4.5°C which was higher for 5.7°C comparing to LTM. During vegetation, there were total 824.1 mm precipitation (Figure 2), which was for 156.8 mm higher as compared to the LTM (667.3 mm). In spring 2018 there was 173.8% higher rainfall in March, and than the lack of rainfall in April (17.7 mm), but towards to winter oilseed rape maturation, in May and June there were higher rainfall and warm air temperatures, thus the harvest of winter oil seed rape and other winter crops in Croatia started about 20 days earlyer than usual.

The diferences between the mean values were calculated in SAS 9.4. with one way ANOVA as plant population as the main factor.

Results

In order to determine the phase of growth for winter period, the number of leaves per plant were determined (Figure 3). Thus, the rosette was in the optimum phase for winter period. Also after winter period there was not extreme decrease of planed plant population (Table 3). The mean plant hight was 138.8 cm (Table 4)

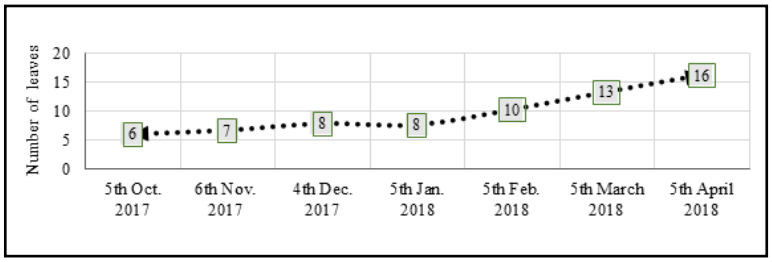

*Figure 2.*The average number of leaves per oilseed rape plant in 2017/2018 vegetation

Table 3. Realized winter oilseed rape plant population

Planed plant population	Date					
	5th November 2017		5th March 2018		11th June 2018	
	Realized plants m^{-2}	% of planed	Realized plants m^{-2}	% of planed	Realized plants m^{-2}	% of planed
20 plants m^{-2}	19	95	18	90	18	90
40 plants m^{-2}	39	98	39	98	39	98
60 plants m^{-2}	59	98	58	97	57	95

Table 4. The plant hight (cm), plant mass (g), hight to first fertile branches (cm), the number of lateral branches of winter oilseed rape at different plant population

	Plant hight (cm)	Plant mass (g)	Hight to first fertile branches (cm)	Number of lateral branches
20 plants m^{-2}	123.7 [c]	112.0 [b]	28.4 [a]	3.5 [b]
40 plants m^{-2}	153.4 [a]	295.3 [a]	21.0 [b]	5.6 [a]
60 plants m^{-2}	139.1 [b]	167.9 [b]	19.1 [b]	4.4 [b]
Average	***138.8***	***191.8***	***22.9***	***4.5***

*The differences between the means within the column are marked with different letter (**abc**) at p<0.05*

Table 5. The number of pods per plant, the pods length (cm), number of seeds per pod and mass of seed per pod (g) of winter oilseed rape at different plant population

	Number of pods per plant	Pods length (cm)	Number of seeds per pod	Mass of seed per pod (g)
20 plants m^{-2}	339 [b]	6.2 [b]	20.4 [a]	0.09 [a]
40 plants m^{-2}	716 [a]	6.5 [a]	21.0 [a]	0.10 [a]
60 plants m^{-2}	462 [b]	6.4 [ab]	20.3 [a]	0.10 [a]
Average	***506***	***6.4***	***20.6***	***0.10***

*The differences between the means within the column are marked with different letter (**abc**) at p<0.05*

Table 6. The number of seeds per plant, 1000 grain mass (g) and yield of seeds per plant (g) of winter oilseed rape at different plant population

	Number of seeds per plant	1000 grain mass (g)	Seed yield per plant (g)
20 plants m^{-2}	6 916 [b]	4.71 [a]	35.0 [b]
40 plants m^{-2}	15 036 [a]	4.65 [a]	85.2 [a]
60 plants m^{-2}	9 379 [b]	4.85 [a]	45.0 [b]
Average	***10 423***	***4.74***	***55.1***

*The differences between the means within the column are marked with different letter (**abc**) at p<0.05*

and it varyed from 123.7 cm (20 plants m^{-2}) to 153.4 cm (40 plants m^{-2}). The highest plant mass (295.3 g) had the plants at 40 plants m^{-2}, and there there were no statistically differences determined for plant mass between 20 and 60 plants m^{-2}. The plants formed 4.5 lateral branches and the mean hight of first fertile branche was 22.9 cm.

At plant population of 40 plants m^{-2} plants formed the higher number of pods per plant (Table 5), but also, at 40 plants m^{-2} the number of seeds per plant and seed yield per plant (Table 6) were the highest, which was significant ($p<0.05$) in comparison with number of pods per plant, number of seeds per plant and seed yield per plant at 20 and 60 plants m^{-2} where the differences was not significant. The average length od pods was 6.4 cm with 20.6 seeds which weighted on average 0.10 g (Table 5). The 1000 grains mass was not significant different between plant population.

In order to determine the relationship between the yield components the Pearson's correlation coeficient was calculated (Table 7). Significant and positive correlation for plant population and plant hight was found (r = 0.385**) and significant but negative correlation was between plant population and the hight to first fertile branch (r = -0.372*). Other yield components (the number of lateral branches, the number of pods per plant, plant mass, pod length, number of seeds per pod, mass of seeds per pod and

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Table 7. The Pearson's correlation coeficient between yield components of winter oilseed rape (N = 45)

	PP	PH	BH	NB	NP	PM	PL	NSP	MS	PS
PP	-									
PH	0.385**	-								
BH	-0.372*	-0.300*	-							
NB	0.229ns	0.625***	-0.465***	-						
NP	0.156ns	0.662***	-0.481***	0.850***	-					
PM	0.179ns	0.666***	-0.467***	0.782***	0.890***	-				
PL	0.246ns	0.500***	-0.378**	0.561***	0.508***	0.415**	-			
NSP	0.001ns	0.247ns	-0.251ns	0.296*	0.335*	0.199ns	0.755***	-		
MS	-0.075ns	0.368*	-0.369*	0.542***	0.619***	0.631***	0.448**	0.481***	-	
PS	-0.073ns	0.373*	-0.368*	0.545***	0.619***	0.631***	0.454**	0.488***	0.757***	-

p< 0.001 ***; p<0.01 **; p<0.05 *; **ns** – not significant)

PP – plant population; **PH** – plant hight; **BH** – hight to first fertile brnch; **NB** – number of lateral branches; **NP** – number of pods per plant; **PM** – plant mass; **PL** – pod length; **NSP** – number of seeds per pod; **MS** – mass of seeds per pod; **SY** – seed yield per plant

seed yield per plant) did not have significant relationship with winter oilseed rape plant population. For other winter oilseed rape yield components, the highest and significant correlation coeficient was found between number of pods per plant and plant mass (r = 0.890***) and than between the number of lateral branches and number of pods per plant (r = 0.850 ***).

Linear regression was also calculated for pod length and number of seeds per pod and pod length and mass of seeds per pod (Figure 4). According to linear regression analysis the equations showed quite similar relationship between the pod length and number of seeds per pod at every plant population. Thus, it was found that for every centimeter increment of pod length the number of seeds increase for about 4.8 seeds at 20 and 40 plants m^{-2} and for about 5.5 seeds per pod at 60 plants m^{-2}. In regression analysis of the mass of seeds per pod (g) and pod length (cm) it was found that for every centimeter pod length increment the mass of seeds per pod increase for 0.02 or 0.03 g.

Discussion

In the period from August 2017 to June 2018, temperatures were higher by 1.9°C than the long term mean (9.6°C) and during this period the precipitation amount was 156.8 mm higher than the long term mean (667.3 mm). The weather conditions were suitable for the development of oilseed rape. According to the data obtained in this study, the planed plant population was not drastically reduced to harvest. It is important to note that in the winter period form December 2017 to March 2018 was not extremely cold and did not affect the plant's population reduction. Zając et al. (2013) state that as a result of very cold winter, oilseed rape plants developed a larger number of lateral branches to compensate for reduced plant population. In this study, high temperatures in April and May led to earlier harvest of oilseed rape, which was about 20 days before the optimal harvest dates (3rd decade of the June).

The plant population is a very important factor winter oilseed rape production. Pospišil et al. (2014) and Diepenbrock (2000) stated that the plant population had the greatest influence on the seed yield and the yield component of winter oilseed rape. Zhang et al. (2012) found that the number of pods per plant and the number of seeds per pod are the most varied yield component of winter of oilseed rape.

The basic yield components of oilseed rape seed are the number of plants per unit area (m^2), the number of pods per plant, the number of seeds per pod and the mass of 1000 seeds. Even though Momoh and Zhou (2001) and Clarke and Simpson (1978) found that in small plant population oilseed rape will increase the number of lateral branches, in this study on

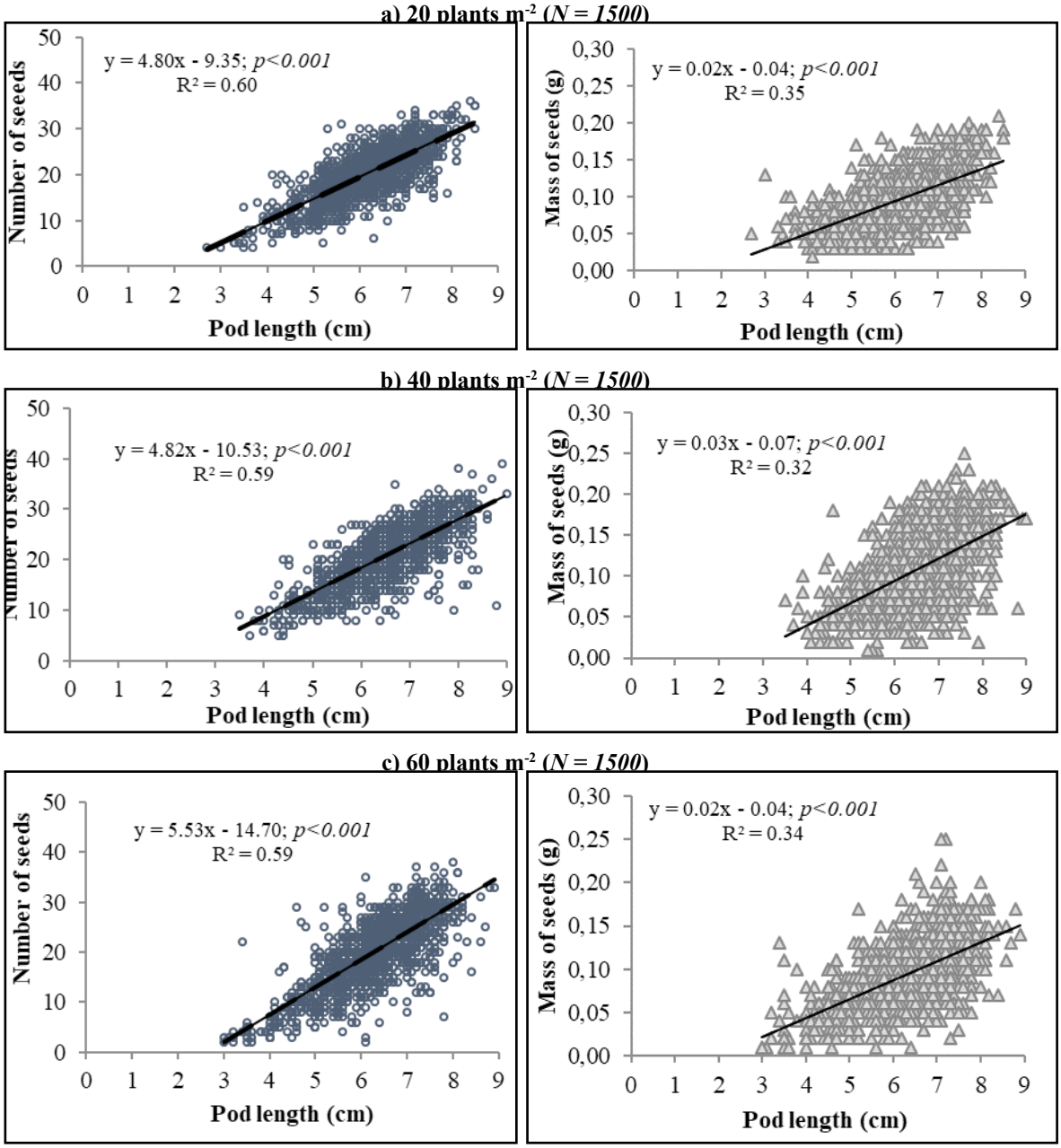

Figure 4. The linear regression of pod length (cm) and number of seeds per pod and pod length (cm) and mass of seeds per pod (g) of winter oilseed rape at three different plant population

the contrary, the smallest plant population (20 plants m^{-2}) did not resulted with highest number of lateral branches (3.5 lateral branches). In our study some relationships between several yield components (parameters of an individual plant productivity) were observed. The number of pods per plant depends on the number of developed pods and aborted pods, which vary depending on different pollination conditions. Abiotic stress like high temperatures during flowering can also affect the number of pods per plant. Angadi et al. (2000) reported that high temperatures at flowering affected yield formation more than high temperature at pod development and that in such conditions *Brassica* sp. could have more pods per plant, but with less seeds due to seed abortion was also more common. According to Pospišil (2013) the number of pods per plant in our agroecological conditions could varyed from 100 to 600. In our experiment the highest number of pods per plant was 716 in plant population of 40 plants m^{-2} and at 20 and 60 plants m^{-2} it was decreased by 52.7% and 35.5%, respecively. Balodis and

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Gaile (2016) stated that the differences in the oilseed rape yield component "pods per plant" showed plant ability to compensate seed yield in cases when plant density was lower, such as due to decreased sowing rate and poor wintering. So, in our experiment this was not confirmed. Based on three year experiment in Croatia, Pospišil et al. (2014) found that the number of pods per pant varyed from 140 (cultivar Ricco, 2009/10 season) to 528 (hybrid Turan, 2011/12 season).

Correlations are important for the breeder in order to associate all the possible valuable features in the newly created genotypes. It was interesting to find that plant hight was positive and extremely significant correlated with the number of lateral fertile branches, number of seeds per plant and pod length (Table 7). Zhang and Zhou (2006) reported that number of seed per pod and 1000 seed mass were positively correlated with seed yield per plant plant. Besides correlatios, the regression analysis can explain prediction and connection between the yield components. Zając et al. (2011) found a high coefficient of determination R^2 = 0.945 between the seed mass per oilseed rape pod and the total mass of the pod, but also authors found that the length of the pod was very poorly correlated with seed mass. This is quite similar to our results, which showed a small coefficient of determination (R^2 = 0.32 to R^2 = 0.35) between pods lenght and seeds mass per pod.

Zając et al. (2011) reported that mean number of seeds per pod is around 19 and that it should be considered that location of particular pod on a plant was also important; more seeds per pod were found in the middle part of plant in comparison with upper and lower parts. Authors stated that the seed number per pod can increase up to 27 seeds on the main stem, in relation to pod location on the plant. Even though it would be interested, in our study, the location of pods on a plant was not observed, but there were no significant difference for aveage number (20.6) of seeds per pod regard to plant population (Table 5). The opposite findings were in Li et al. (2017) study, where with increasing winter oilseed rape plant population, decreased number of seeds per pod. Pospišil et al. (2014) emphasize the genotype differences in number of seeds per pod and between 11 hybrids and 5 cultivars, the cultivar Ricco had the highest number of seeds per pod in two seasons, 30,00 in 2010/11 and 33,41 in 2011/12.

Our study did not shown the significant influence of plant population on 1000 grain mass. This is simmilar to Li et al. (2017) found that with increasing winter oilseed rape plant population there were no significant influence on 1000 grain mass. In study with different nitrogen fertilizer rate, Spitek and Pospišil (2017) did not found significant difference in 1000 grain mass (on average 5.65 g).

Our trial results showed the highest significant ($p<0.05$) seed yield per plant of 85.2 g at 40 plants m^{-2} in comparison with other plant populations (Table 6). Similar results was obtained in the study of Nasiri et al. (2017). Authors include 6 different oilseed rape genotypes (Ahmadi, Okapi, Opera, L72, Karaj1 and SW102) and found that the seed yield at 60 and 80 plants m^{-2} in all varieties was significantly reduced compared to the 40 plants m^{-2}. In the study of different plant population (30, 40, 50 and 60 seeds m^{-2}) by Ratajczak et al. (2017) indicate that the lowest seed yield was obtained at 30 m^{-2} seeds (38.9 dt ha^{-1}), while there was no statistically justified differences between yield of seeds of other sowing density and the yield ranged from 41.0 dt ha^{-1} (40 seeds m^{-2}) to 41.4 dt ha^{-1} (60 seeds m^{-2}). Zhang et al. (2012) also found yield decrement with increasing plant population and they statted that the highest seed yield per plant was at 36 plants m^{-2}.

Conclusion

In this study field trail was set up to analyze the yield components of winter oilseed rape depending on the plant population (20, 40 and 60 plants m^{-2}) in 2017/2018 year. It is obvious in our experiment that, generally the best results were found at 40 plants m^{-2}. Thus, there were the highest plants developed (153.4 cm per plant) with the largest plant mass (295.3 g per plant) and furthermore, plants formed the most fertile side branches (5.6 branches per plant) and the largest number of pods per plant (716 pieces). Also, plants had the longest pods length (6.5 cm) and therefore the largest number of seeds per pod (average 21.0). The highest seed yield per plant of 85.2 g was found at 40 plants m^{-2}, which was 58.9% and 47.2% higher compared to 20 and 60 plants m^{-2}, respectively. Even though this results showed only one oilseed rape season, our results coincides with the recommendations that the winter oilseed rape hybrids should be sown within 30 to 50 plants m^{-2}.

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Calculation nitrogen and sodium budget from lysimeter-grown short-rotation willow coppice experiment

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Abstract: Alternative water resources utilization should take priority over the conventional irrigation water resources (surface and subsurface waters) in the future in Hungary as well, similarly to the global trends because of the climate change impacts. However because of the environmental risks (e.g. nitrate leaching and soil salinization) the reuse of the wastewater require sustainable practices hence the farmers and researchers are responsible for development soil management practices and irrigation principles. Aim of our study was to determine the impact of a wastewater originated from an African intensive catfish farm on the nitrogen budget of the soil-water-plant system in order to evaluate the nitrogen substitution effect and the risk of the nitrate leaching. On the other hand, the aim of the study was to calculate also sodium budget to assess the risk of the sodicity regard to the high sodium concentration of the wastewater. The experiment was conducted at the National Agricultural Research and Innovation Centre (NAIK), Research Institute of Irrigation and Water Management (OVKI) in Szarvas, Hungary. The experiment was set up in the NAIK ÖVKI Lysimeter Station in 2014 with energy willow. During the study (between 03.07.2015 and 21.04.2017) irrigation water quality, rainwater quality, willow N and Na uptake by stems and N and Na leaching was measured. Nitrogen and sodium budget were calculated for two years (2015, 2016) from these data. According to our results the wastewater had high nitrogen content what was able to increase the nitrogen amount in the examined budget however without supplementary fertilizer it could not able to balance the budget only just at W60 treatment (irrigation with wastewater: N concentration 22,7 mg/l). The wastewater had high environmental risk as soil sodicity according to results of the sodium budget.

Keywords: nitrate leaching, salinization, wastewater, rainwater quality

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Introduction

Sustainable soil and water management and use of alternative water resources for agricultural production are one of the key elements of the fight against the consequences of the continuous increase in global population and effects of climate change (Singh 2015). The new water resources play determining role because of the water scarcity in addition to water and energy saving irrigation methods (Francés et al. 2017). The reuse of treated wastewater for irrigation is a suitable practice to close the water cycle in the agro-industrial sector (Vergine et al. 2017).

The climate change affects our country due to the basin character, the unfavourable distribution of rainfall is the consequence of the change of the regional climate patterns. Other example is the decreasing surface water quantity at summer time thereby there is a growing number of water bodies with water scarcity and regions with water stress. This water scarcity is occurred at summer time when the irrigation water demand is the highest (Hungarian River Basin Management Plan 2015). Alternative water resources utilization should take priority over the conventional irrigation water resources (surface and subsurface waters) in the future in Hungary as well, similarly to the global trends because of the global warming and water scarcity.

However, inappropriate management of irrigation with wastewater can cause substantial risks to public health and the surrounding environment (Elgallal et al. 2017). Understanding the processes of nitrate leaching and introduction of deeper-rooted trees or other crops can minimise the ground water contamination (Khajanchi Lal et al. 2015). On the other hand, wastewater usually has a higher concentration of total dissolved solids and major ions and a higher electrical conductivity than fresh water especially in regions with hot climates or it can originate from many sources such as detergents and washing material, the chemicals used during the treatment process and other sources (Elgallal et al. 2017). Removing salts from wastewater for irrigation purposes is prohibitively expensive.

Therefore, there is a need for specific measures and management strategies to prevent and control the effects of salinity and sodicity during irrigation with wastewater (Elgallal et al. 2017).

The energy plantation may provide prosperous opportunity for the wastewater reuse in the future through irrigation (Vermes 2017). Energy willow and poplar have many favourable advantages compared to other plants: long growing season, high evapotranspiration rate, ability to take up nutrients and toxic elements with minimum leaching potential (Isebrands and Richards 2014). According to Ligetvári (2014) the use of sewage can make safer production of energy crops or forests or even not directly consumed agricultural products.

Arable crops can be characterised by low species and breed number of crops with minimum crop rotation (Grónás et al. 2006). To improve the biodiversity and to break the monotony of the landscape, installation of energy plantation would be one feasible prosperous solution. Diversification of arable crops are would be beneficial to decrease the risk of the market and the cost of production (Grónás et al. 2006).

Aim of our lysimeter experiment was to determine the impact of a wastewater originated from an African intensive catfish farm on the nitrogen budget of the soil-water-plant system in order to evaluate the *nitrogen substitution effect* and *the risk of the nitrate leaching*. On the other hand, the aim of the study was to calculate also sodium budget to assess the *risk of the sodicity* regard to the high sodium concentration of the wastewater.

Materials and methods

Experiment site

The experiments were conducted at the National Agricultural Research and Innovation Centre (NAIK), Research Institute of Irrigation and Water Management (OVKI) in Szarvas, Hungary. The experiment was set up in the NAIK ÖVKI Lysimeter Station in 2014 in 64 pieces of 1 m^3 vessels with energy willow. The year 2015 was the second year of the plantation when the irrigation started. The willow clone (no. 77, 82) were selected by the NAIK Forest Research Institute of Püspökladány Experimental Station. Each treatment occurred in 8 replication/ lysimeter. The 1 m^3 lysimeter vessels were filled with disturbed meadow topsoil. The soil was characterised by high clay content (~70-80%), low humus (~2%), lime content less than 0.5%, total soluble salt content less than 0.08%, and pH_{KCl} values between 5.88-6.97 (Kun et al. 2018).

Irrigation treatments

Three different irrigation waters were applied in the experiment. First one was originated from the Oxbow Lake of Körös River (K15, K30, K60 – numbers mean one-time irrigation doses in mm), while the second one was the wastewater (W15, W30, W60) from an intensive African catfish farm in Szarvas. The third irrigation water type (HG60) used for irrigation (only 60 mm doses) was treated wastewater, which was diluted (1:3) with Körös water than gypsum was added to it according to the following equation:

$$x = Sz_e \times E$$

where the x means the gypsum quantity (mg/l or g/cm^3), Sz_e= residual sodium carbonate index (RSC), E= equivalent weight of gypsum (86,1). There were one non-irrigated treatment (Control). The irrigation water quality was describe according to the 90/2008. (VII. 18.) Hungarian decree and FAO guideline as well (Kun 2018).

Sampling method and analyses

The irrigation water sampling occurred in the irrigation periods at every time between 15th April and 30th September according to Hungarian standard (MI-10-172/9-1990). Inorganic nitrogen (ammonium, nitrate, nitrite, total inorganic-N) and sodium was determined according to the Hungarian standards (MSZ EN ISO 11732:2005, MSZ EN ISO 13395:1999, MSZ EN ISO 13395:1999, MSZ 12750-20:1972, MSZ 1484-3:2006, respectively). In 2015 3 replication represents the Körös River and the wastewater quality, in 2016 the number of the replication was 7. In case of diluted and improved wastewater treatment (HG60) the irrigation quality was analysed based on 4 replications. The rainwater was collected at 4 rainfall events (14.06.2016, 20.09.2016, 19.04.2017, 29.06.2017) meaning 4 replications and the same parameters were

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Table 1. The irrigation and the leachate water amounts (mm) in the experiment

	Irrigation water amounts (mm)		*Leachate water amounts (mm)*	
Treatments	***2015***	***2016***	***1st period***	***2nd period***
Körös 15 mm (K15)	195	90	383	23
Körös 30 mm (K30)	390	180	409	106
Körös 60 mm (K60)	780	360	621	532
Wastewater 15 mm (W15)	195	90	463	358
Wastewater 30 mm (W30)	390	180	470	145
Wastewater 60 mm (W60)	780	360	717	313
Diluted wastewater + gypsum (HG60)	720	360	674	676
Control (non-irrigated)	0	0	655	130

Remark: 1st period: between 15.06.2015 and 17.06.2016 2nd period: between 17.06.2016 and 21.04.2017

analysed as from the irrigation waters. The lysimeter is suitable to collect the leachate. The leachate water amounts were measured 33 times between 03.07.2015 and 21.04.2017 for 22 months in 62 lysimeter vessels. (In H15 and HG60 treatments there was 1-1 not-working lysimeter.) The leachate sampling occurred in winter time in both years (16.01.2016, 28.02.2016, 06.02.2017, 08.02.2017) and the analysed parameters were nitrate and sodium according to the methods above. The results represent 2 replications per treatments per the analysed periods.

The willow stem sampling occurred in 2015 at time of the willow harvest (02.12.2015). Harvested stems from each treatment (in 8 replication) were collect and analysed for dry mass, total Kjeldahl-nitrogen and sodium concentration according to Hungarian standards (MSZ-08-1783-1:1983, MSZ EN ISO 5983-2:2009, MSZ-08-1783-5:1983).

Nitrogen and sodium budget calculation and statistical analyses

Based on the leachate analyses results nitrogen and sodium budget was calculated for two periods. First period (2015) was between 15.06.2015 and 17.06.2016 and the second period (2016) was between 17.06.2016 and 21.04.2017. For the calculation the input quantities were: the nitrogen fertilizers, the nitrogen content of the irrigation waters and the rain and the output quantities were the nitrogen amount that was accumulated in the willow stems and the nitrogen losses by leaching. The applied fertiliser amount was 40 kg N/ha in the first period and there were no applied fertiliser in 2016. Input inorganic-N of the irrigation water were calculated by multiplying irrigation water amounts (Table 1). The input inorganic-N of the rainwater calculated by multiplying the precipitation volumes. The applied irrigation water amounts were different in 2015 and 2016 (Table 1), because the weather was different in the experiment years. In the first year the precipitation was 116.8 mm during the irrigation period but in the second year it was 220.6 mm. In 2015 the total precipitation was 400.6 mm and in 2016 it was 632.8 mm. Nitrate leaching loads were calculated by multiplying drainage volumes for each period (Table 1). The output nitrogen by the willow stem was calculated from the dry biomass data in 2015 and 2016 (not published data) and the Kjeldahl-nitrogen of the willow stems in 2015. The subtraction of the input and output N means the nitrogen budget (ΔN values). According to Arronson and Bergström (2001) the estimated ΔN values express the combined effect of mineralization, immobilization, build-up of the soil pool of nitrogen, and gaseous losses. Apart from the gaseous losses the others do increase or decrease the store of the soil nitrogen. So if ΔN has negative sign it means decreasing N-store in the soil and if ΔN is positive, the soil becomes richer in nitrogen. In our experiment the nitrogen content of the willow leaves were did not calculated in the nitrogen budget, due to the difficulty of estimate the leaf mass and partly because it can not be considered as real loss as leaves stay at the soil surface for the degradation processes, hence in our case the ΔN values also expresses some nitrogen in falling leaves at autumn. However, according to Szalai (1968) the leaves contain small amount of nitrogen. The sodium budget calculation was according to the same method above (without the fertilizer).

Table 2. Nitrogen budget of the willow lysimeter experiment in 2015 and 2016

				2015				
	W15	***W30***	***W60***	***HG60***	***K15***	***K30***	***K60***	***Control***
				Input				
Fertilizer (kg/ha)	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
N content of the irrigation water (kg/ha)	43.3	86.7	173.3	76.3	1.3	2.6	5.2	0.0
N content of the rainwater (kg/ha)	6.2	6.2	6.2	6.2	6.2	6.2	6.2	6.3
				Output				
N content of the willow stem (kg/ha)	90.4	122.8	171.2	101.8	58.0	80.1	95.6	35.7
N leaching (kg/ha)	6.1	5.6	15.9	0.6	1.5	0.6	0.3	56.9
Δ N (kg/ha)	*-7.0^{abc}*	*4.5^{abc}*	*32.5^{c}*	*20.1^{bc}*	*-11.9^{abc}*	*-31.8^{ab}*	*-44.5^{a}*	*-46.3^{a}*

				2016				
	W15	***W30***	***W60***	***HG60***	***K15***	***K30***	***K60***	***Control***
				Input				
Fertilizer (kg/ha)	0	0	0	0	0	0	0	0
N content of the irrigation water (kg/ha)	20.8	41.6	83.1	38.2	0.6	1.2	2.3	0.0
N content of the rainwater (kg/ha)	5.9	5.9	5.9	5.9	5.9	5.9	5.9	5.9
				Output				
N content of the willow stem (kg/ha)	72,8	92,4	95,6	53,2	46,6	46,6	41,6	28,5
N leaching (kg/ha)	0.4	5.5	12.3	1.1	0.9	0.4	0.3	8.1
Δ N (kg/ha)	-46,1^{a}	-44,9^{a}	-6,5^{c}	-9,1^{bc}	-40,1^{ab}	-39,5^{ab}	-33,4^{abc}	-22,6^{abc}

Remark: ANOVA was used to determine the significant difference between the treatments. The ^{a}, ^{b}, ^{c} indexes means the homogen subsets of the Tukey's post-hoc test. The negative ΔN values mean the soil (and the willow leaves) contribution to the nitrogen budget.

The statistical calculation was performed in SPSS 22.0 Statistics Software. ANOVA and Tukey's Test was used to determine significant difference between ΔN values and the homogenous subset means no significant difference between the treatments. To determine significant different between ΔNa values Non-parametric, Kruskal-Wallis test was used in both years.

Results

Nitrogen budget

To calculate the N budget three input factor were determined. The N content of the fertilizer, irrigation water and rainwater build up the all input N (Table 2).

The wastewater contained considerably more inorganic-N (2016: 23.1 mg/l, 2016: 22.2 mg/l) than the diluted and improved irrigation water type (2015, 2016: 10.6 mg/l) and the irrigation water from the River Körös (2015: 0.64 mg/l, 2016: 0.67 mg/l). In the wastewater the 99.8% of inorganic-N was in ammonium form. Nitrogen (N) and phosphorous (P) in metabolic waste produced by fish are the origin of most dissolved N and P waste resulting from intensive aquaculture operations (Lazarri and Baldisserotto 2008, Tóth et al. 2016). The ammonia production by fish is primarily dependent on the protein intake and metabolic efficiency of the fish, which is species-specific and is affected by waterborne ammonia levels (Dosdat et al. 2003). In case of the River Körös, the ammonium : nitrate : nitrite rate was 43:50:7. All of them was under the limit values of Decree No. 10 of 2010 (VIII. 18.) VM of the Ministry of Rural Development hence the oxbow lake can be consider as good quality surface water body. The input N content from the irrigation water was depended on the irrigation water amount (Table 1, Table 2).

The inorganic-N concentration of the rainwater was 1.25 mg/l. According to Gelencsér et al. (2012) the average total N concentration of the rainwater is 1.68 mg/l and the main nitrogen forms are inorganic forms. According to Csapák (2009) the ammonium concentration in the rainwater was 0.24 mg/l in NH_4-N, the nitrate was 0.21 mg/l in NO_3-N and there were no nitrite. The input N in the budget from the precipitation was 6.2 kg/ha in 2015 and 5.9 kg/ha in 2016 (Table 2).

Because of the different weather conditions of the experimental years the applied irrigation water amount was twice more in 2015 than in 2016. In 2016 fertilizer were not applied and less irrigation water was used hence the N input in

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the second year were lower than in 2015. In the treatments without waste water (K15, K30, K60 and Control) the fertilise-input was only 13-16%, but in waste and diluted water treatments the fertilise-input even was not more than 36-41% of the total input N in 2015.

Two output factor was used to determine the nitrogen budget: the N content of the willow stem and the N content of the leaching water. The mean N content of the willow stem was 0.51 m/m%, the range was 0.2-0.87 m/m%. According to our results the lowest accumulated nitrogen values of the willow stem was 29 kg N/ha and the highest was 171 kg N/ha (Table 2). According to Dimitriou and Aronsson (2004) the N-uptake of the willow was 110-115 kg N/ha in clay soil and 36-44 kg N/ha in sandy soil. Four willow clones were compared by Curneen and Gill (2014), according to their results the smallest N-uptake was 82 kg N/ha (in case of *Torhild* clone, irrigated with freshwater) and the highest was 262 kg N/ha (in case of *Sven* clone, irrigated with secondary treated wastewater). According to Galbally et al. (2013) the willow N-uptake after treatment with biosolid was 107 kg N/ha and after irrigation with distillery effluent water it was 231 kg N/ha. So our data can show the same order of magnitude then other researchers'.

According to our results the mean nitrogen losses caused by nitrate leaching was 10.9 kg N/ha in 2015 and it was 3.6 kg N/ha in 2016 (Table 2). According to willow lysimeter experiment belongs to Aronsson and Bergström (2001) the highest N leaching was measured in the first year of the willow plantations (clay and sandy soil: 341-140 kg N/ha). After the starting year in the second (43-17 kg N/ha, respectively) and in the third year (3-1 kg N/ha, respectively) the N leaching was negligible. According to Mortensen et al. (1998) also in the first year of the plantation was the highest nitrogen leaching (130-142 kg N/ha) and it was decreased in the second (9-61 kg N/ha) and in the third (0-4 kg N/ha) years.

The subtraction of the input and the output N amount was the result of the nitrogen budget (ΔN). In 2015 in the wastewater treatments were calculated the most favourable ΔN values, in three cases (W30, W60, HG60) it was positive. The lowest values were in case of irrigation with River Körös water and in the Control treatment (mean of the ΔN values for this 4 treatment was -34 kg N/ha). In 2016 the most favourable ΔN values were also in the W60 and the HG60 treatments. All the other treatments had lower values then W60 and HG60 (Table 2).

Sodium budget

To calculate the Na budget two input factor was determined. The Na content of the irrigation water and rainwater build up the all input Na. The wastewater had considerably higher Na concentration (2015: 278 mg/l, 2016: 274 mg/l) than the diluted and improved irrigation water

Table 3. Sodium budget of the willow lysimeter experiment in 2015 and 2016

				2015				
	W15	***W30***	***W60***	***HG60***	***K15***	***K30***	***K60***	***Control***
				Input				
Na content of the irrigation water (kg/ha)	542.6	1085.2	2170.4	945.0	81.5	162.9	325.9	0.0
Na content of the rainwater (kg/ha)	14.1	14.1	14.1	14.1	14.1	14.1	14.1	14.2
				Output				
Na content of the willow stem (kg/ha)	1.0	1.5	1.4	1.2	0.5	0.7	0.9	0.3
Na leaching (kg/ha)	28.4	29.6	65.7	88.4	23.3	33.6	48.7	37.8
Δ Na (kg/ha)	*527.3*	*1068.2*	*2117.4*	*869.5*	*71.8*	*142.7*	*290.4*	*-23.9*

				2016				
	W15	***W30***	***W60***	***HG60***	***K15***	***K30***	***K60***	***Control***
				Input				
Na content of the irrigation water (kg/ha)	247.0	494.0	987.9	472.5	26.0	52.0	104.0	0.0
Na content of the rainwater (kg/ha)	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4
				Output				
Na content of the willow stem (kg/ha)	0,8	1,1	1,0	0,6	0,4	0,5	0,4	0,3
Na leaching (kg/ha)	1.6	11.1	68.1	61.5	5.7	14.3	29.9	6.0
Δ Na (kg/ha)	258,0	495,2	932,3	423,8	33,4	50,7	87,1	7,2

type (2015, 2016: 131 mg/l) and the irrigation water from the River Körös (2015: 42 mg/l, 2016: 29 mg/l). The high sodium content of the wastewater was originated from the fishfarm water source because thermal water is used to provide the high water amount for intensive technology (Tóth et al. 2016). Because of the wastewater quality the input sodium from the irrigation water was very high (200-2200 kg/ha/year), (Table 3).

Two output factor was used to determine the Na budget: the Na content of the willow stem and the Na content of the leaching water. The mean Na content of the willow stem was 53.6 mg/kg, the range was 23.8-161 mg/kg. It means 0.3-1.5 kg/ha/year which is negligible compared to the leaching. According to Jama-Rodzenská et al. (2016) most of sodium (~300 mg/kg) was found in the willow shoots during the first year of the study which decreased in the second year (~200 mg/kg). According to Stolarski et al. (2017) the average Na concentrations of the analysed willow clones were between 80-180 mg/kg. Due to the low ion uptake of the willow the output mean Na values were very low in both years varied between 0.3 and 1.5 kg Na/ha (Table 3).

According to our results the mean Na losses caused by leaching was 44.0 kg Na/ha in 2015 and it was 24.5 kg Na/ha in 2016 (Table 3). According to Stefanovits (1963) the average sodium losses by leaching on clay soil is 9-42 kg/ha/year, in our lysimeter experiment the sodium losses varied between 1 and 125 kg/ha/year. According to Sorrenti and Toselli (2016) in 18-month-lysimeter experiment with 1-year old nectarine the sodium losses by leaching was 224 kg/ha in sandy soil.

The subtraction of the input and output Na amount was the result of the sodium budget (ΔN). In 2015 in the wastewater treatments were calculated the most unfavourable ΔNa values between 527-2117 kg/ha. The prosperous sodium budget was calculated in both years in the treatments with irrigated with River Körös. The lowest ΔNa value were in case of non-irrigated treatment, in the Control treatment in both years (Table 3).

Discussion

The ΔN value of the W60 treatment in 2015 was significantly higher than the other treatments (Table 2). In this case there were the highest N input owing to the nitrogen content of the wastewater. According to our results the wastewater not only does not pose the threat to groundwater nitrate pollution but it could be useful in the irrigation management and foster the sustainable soil management practices also. However according to the European regulation the maximum permissible amount of fertilizer per hectare is 170 kg N. Therefore, the concentration of nitrogen in the wastewater should be taken into account during the application on nitrate sensitive areas (Council Directive 91/676/EEC). There were no significant differences between the ΔN values of the W15, W30, K15 treatments. In these cases the input nitrogen was approximately enough for the plants hence neither the soil nitrogen loading nor the soil exhaustion did not occur. The ΔN value of the K30, K60 and Control was the lowest. In the treatments with Körös River water (K30, K60) the input N was not enough for the soil-water-plant system because the irrigation water did not contain enough inorganic-N hence the ΔN values were negative and it means the soil exhaustion. According to Kenessey (1931) the irrigation should always coexist with nutrient supply, because the water explore the soil nutrient stock and the plants become rich in nutrients but the soil exhaustion can occur. Otherwise in case of the Control treatment the adverse nitrogen budget was caused by high nitrogen losses by leaching. According to Szalókiné and Szalóki (2003) in the soil of non-irrigated treatment, there is more nitrogen (because of the less plant N-uptake) which, during the winter period, results in higher nitrate concentration in leachate waters.

In 2016 (the third year of the plantation) the lowest value was in the treatment W15 and W30, but they were in the same homogenous subset as the K15, K30, K60 and Control (Table 2). Nitrogen balance could be realized only in W60 and HG60 treatment, where the input N and the output N was approximately equal. In 2016 the

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Table 4. Subset of the ΔNa values in the different treatments

					2015			
	H15	***H30***	***H60***	***HG60***	***K15***	***K30***	***K60***	***Kontroll***
H15	-	-541	-1590	-342	456	385	237	551*
H30		-	-1049	199	996**	925*	778	1092***
H60			-	1248	2046***	1975**	1827*	2141***
HG60				-	798*	727	579	893**
K15					-	-71	-219	96
K30						-	-148	167
K60							-	314
Kontroll								-

					2016			
	H15	***H30***	***H60***	***HG60***	***K15***	***K30***	***K60***	***Kontroll***
H15	-	-236	-671	-160	225	208	171	251
H30		-	-435	76	461**	444	407	487***
H60			-	511	896***	878**	842	922***
HG60				-	386	368	331	411**
K15					-	-18	-55	26
K30						-	-37	43
K60							-	80
Kontroll								-

Remark: Results of the Non-parametric, Kurskal-Wallis test. Values (i-j) mean the subset of the columns (i) and the rows (j). (*:p<0.05 **: p<0.01 ***: p<0.001).

higher ΔN values in the control occurred than in the previous year because of the less nitrogen loss by leaching. The lower ΔN values in the W15 and W30 was the results of the lack of nitrogen fertilizer, because the output by the willow stems and the leaching was less than in 2015. According to Arronsson and Bergström (2001) in the third year of the willow plantation the ΔN value was -39 kg N/ha (low N input and irrigation water) and it was -237 kg N/ha (high N input and irrigation water) on clay soil.

For all irrigated treatments the sodium budget was unfavourable but in case of the River Körös the values were significantly lower (Table 3, Table 4). The Na concentration of the River Körös is very low in both years and it makes it suitable for irrigation purposes. According to Ayers and Westcot (1989) above the limit of sodium (3 meq/l) in the irrigation water ion toxicity can occur and the sodium sensitive plant developing can decline. According to the recommendation of the FAO for irrigation water quality under the limit there is no harmful impact of the sodium content in case of sprinkler irrigation (Ayers and Wastcot 1989). According to our results the wastewater and the diluted and improved water did not meet the recommended limit (~12 meq/l and 5.7 meq/l, respectively) but the River Körös (1.8 meq/l) is under the limit. In all irrigated treatments with wastewater the ΔNa values were the highest which means that harmful sodium stay at the system (Table 3).

In both years neither the sodium contain of the stems nor the sodium leaching were not able to increase the output Na amount making the sodium budget more favourable. In the treatments irrigated with wastewater the sodium outputs were higher than in the treatments with River Körös however it was not enough high to reduce the sodium loading during the irrigation period owing to the wastewater quality. According to Nouri et al. (2017) green remediation is the use of vegetation to remove or contain environmental contaminants such as heavy metals, trace elements, organic compounds and radioactive compounds in soil or water and it is suggested to use for treat the soil salinity. In our case the willow did not uptake and accumulate so many sodium ion to balance the sodium budget.

In case of the HG60 treatment the wastewater was diluted and improved by gypsum hence the sodium budget were better than in the W60 (Table 3). However in the HG60 the sodium budget was not auspicious and also truly high hence the soil monitoring is exceptionally important for the sustainable, long-term irrigation. In both year, the ΔN value were significantly higher than in the K60 because the N input was higher

(from the diluted wastewater origin), (Table 2). According to our results the wastewater treatment was sufficient to reduce the sodium loading of the soil-water-plant system compared to the wastewater irrigation in both years and it was able to increase the nitrogen budget.

Summary

Aim of our study was to evaluate the impact of the wastewater irrigation on the nitrogen budget of soil-water-plant system and to assess the environmental risk of the sodium accumulation. The wastewater is originated an intensive fish farm hence it had high nitrogen content what it was able to increase the nitrogen amount in the examined system however without supplementary fertilizer it could not able to balance the budget only just at W60 treatment. The wastewater had high environmental risk as soil sodicity according to results of the sodium budget. Even the treated wastewater require soil monitoring to ignore the sodium accumulation, however the dilution and added gypsum was able to reduce the rate of the sodium accumulation of the wastewater.

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OBITUARY
György FÜLEKY (1945-2018) prominent agrochemist passed away

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Professor Emeritus György Füleky former Head of the Department of Soil Science and Agrochemistry, Director of the Institute of Environmental Sciences, Dean of the Agricultural and Environmental Sciences Faculty, and Deputy Rector of the Szent István University died on May 4 2018 at the age of 74.

György FÜLEKY (1945-2018)

György Füleky was born in Ekecs on February 5, 1945. He continued his primary school, high school and university studies in Budapest. He graduated from the Faculty of Chemistry and Physics at the Eötvös Lóránd University in 1968. Between 1968 and 1983, he worked as a research assistant at the Research Institute of Soil Science and Agricultural Chemistry at the Hungarian Academy of Sciences, and after becoming a candidate for agricultural sciences in 1978 as a senior lecturer. The title of his candidate thesis was “The phosphorous state and easily soluble phosphorus content of soils”. Since 1983, he worked as assistant professor at the Agricultural Chemistry Department at the Agricultural University of Gödöllő. His university positions were chronologically the followings: 1987-1990 Deputy Assistant Professor of Agricultural Sciences; from 1991 Professor at the Department of Soil Science and Agrochemistry; from 1990 to 2008 Head of the Department of Soil Science and Agrochemistry; 1991-1994 Deputy Dean of Education of the Faculty. He awarded the Széchenyi Professorate Felowship between 1998 and 2001. He was the deputy rector of the Szent István University from 2000 to 2003. He functioned as Director of the Institute of Environmental Sciences between 2008 and 2010, and as Dean of the Faculty of Agricultural and Environmental Sciences in 2012.

György Füleky had a wide range of education subjects. Most importantly he taught subjects in graduate courses in agrochemistry, soil management, environmental effects of agriculture, geochemical circuits, land evaluation, environmental reconstruction, and environmental practices. In English language he held courses in agrochemistry, plant nutrition, and land evaluation. In the doctoral training program he was responsible for soil fertility and soil chemistry specializations.

György Füleky designed the thematic of the subjects listed above. He prepared the curriculum for agricultural engineer degree after the political change of regime in 1991 on the Agricultural University of Gödöllő. Nationally, in 1989, he prepared a new curriculum for the Soil Science – Soil Fertility Engineer vocational training what he led till 1998. In 1998 he worked on the curriculum of Soil Science for the Faculty of Civil Engineering. After preparing accreditation of the Agricultural Environmental Management vocational training program he led it till 2006.

In 1993 he developed the doctoral training program for Soil and Agrochemical Fundamentals for Environmental Management and led it till 2000. From 2000 he led the Soil Science, Agrochemistry, Environmental Chemistry subareas of the Environmental Sciences Doctoral School to 2014. Under his leadership 16 aspirants earned the PhD degree.

Additionally György Füleky worked out a curriculum for five year university and three-year college education of the Environmental Engineer vocational training. He developed the curriculum for specialization in Disaster Management of that training. Between 2002 and 2005 he participated in the work of the Agri-Bologna Committee and in the design of the BSc and MSc programs. At Szent István University, he developed curriculums for Environmental Engineer BSc and MSc programs. By the end of 2014, MSc's Disaster Management Engineer's curriculum was also prepared by him.

In 1986, he launched the journal "Bulletin of the University of Agricultural Sciences Gödöllő", which was published from 2000 as "Bulletin of the Szent István University". He was chairman of the editorial board of the periodical until its termination in 2012.

Prof. Füleky organized and edited the proceedings of 10 conferences entitled as "Landscape changes in the Carpathian Basin".

He was the author and editor of "A talaj" (Soil) (1988), "Talajtan" (Soil Science) (1999), "Tápanyag-gazdálkodás" (Nutrition Management) (1999), Korszerű tápanyag-gazdálkodás" (Modern Nutrition Management) (2014) and "Tápanyag-gazdálkodás mezőgazdasági mérnökök számára" (Nutrition Management for Agricultural Engineers) (2014) books and textbooks. He did important editorial work in the production of the monograph EOLSS (UNESCO) entitled as "Cultivated plants as primarily food sources" issued in 2000.

Prof. Füleky presented different social activities, as chairman of the Hungarian Quality Compost Society, whereby he was one of the main organizers of the "Biological Waste Management Conference" organized in 2014 Gödöllő. He was a member of the MTA Soil Science, Water Management and Crop Cultivation committee and the Editorial Board of Agrochemistry and Soil Science Journal.

His research topics covered both basic and applied research and development. György Füleky's most important basic research activity focused on determination of the nutrient supply capacity of soils by plants, by chemical methods and their mathematical description. His research included determination of toxic elements in the soil by the rapid seedling biological test and the hot water percolation (HWP) chemical method. He developed a rapid methods for determining nitrogen content of damaged plant parts and fertilizers. In the framework of his applied research, he primarily intended to monitor the unfavorable or favorable effects of intensive crop production and nutrient management technologies on soil. In the field of environmental management, determination of the natural environment of the past times he concerned with the parallel use of soil and archaeological methods. With this concept he initiated a new research branch in the Hungarian soil research.

He has received numerous professional and scientific awards and publishing prizes for his outstanding work.

György Füleky was demanding and knowledgeable. He communicated with literary variety and inimitable kindness, he was shaded and always personalized. People who watched his words and understood his thoughts learned much from him. He was liked by closer and distant colleagues and students because he was straight, warm-hearted and selfless. Through his knowledge, extensive cultivation and unshakeable honesty, he gained undeniable prestige. Because of his gentle nature, helpfulness and special humor, he also had a unique love by his colleagues.

György Füleky's professional work remains active long and even will be continued by his colleagues and students in Hungary and the Carpathian Basin. We are all grateful for his oeuvre, his educational activities and opening the soil science to co-sciences of archeology and agriculture in historical times in Hungary and neighboring countries in the Carpathian Basin. All of us will miss his dear, helpful personality, cheerful humor and professional wisdom. Colleagues working on soil and agrochemistry disciplines, as well as his former students express a thankful heart and ask for eternal rest.

Source of the graphics

Front cover:
Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber (1987): Die Technik : Von den Anfangen bis zur Gegenwart

Rear cover:
Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours

URBÁNYI Béla, editor-in-chief

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Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.