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The effect of mulching materials on the arbuscular mycorrhiza fungi root colonisation, peroxidase activity, and chlorophyll content in *Lactuca sativa*

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Abstract

Lettuce is one of our most important leaf vegetables that can be cultivated safely in organic farming, which is not only pesticide-free, but also aims to maintain and stimulate the presence of naturally occurring beneficial organisms, such as algae, mosses, bacteria, or arbuscular mycorrhiza (AM) fungi. These organisms are all beneficial for soil life and nutrient decomposition. The positive effects of beneficial microorganisms could be enhanced by mulching which is a widely used practice in organic farming. Mulching may also increase soil nutrient substance after decomposition and inhibit weed growth. In our experiment, we sought to determine the effect of different mulching techniques (alfalfa, rye, black foil) on AM root colonisation, leaf chlorophyll (Chl) content, and on peroxidase (POD) activity in *Lactuca sativa* plants and observe whether there are correlations between these parameters. Results show natural mulching has a positive effect on mycorrhiza fungi root colonisation and therefore lowers the stress in lettuce plant. On the other hand, there was no significant correlation between root colonisation and Chl content. As POD enzymes are directly linked to enzymatic browning, the high colonisation rate of AM may consequently lower post-harvest browning in lettuce.

Keywords: arbuscular mycorrhiza, chlorophyll, *Lactuca sativa*, lettuce, organic farming, peroxidase, stress.

Introduction

Lettuce (*Lactuca sativa* L.) is one of the most important leaf vegetables produced worldwide (Simko *et al.* 2014). Among plant products which are eaten fresh or raw, lettuce is consumed in the largest quantity (Sönmez *et al.* 2017). Lettuce has high water content, so it is very susceptible to drought stress, and this could easily cause significant economic loss for farmers. Plants have numerous mechanisms to adapt or to mitigate various stress conditions, including the accumulation of solutes (proline, total sugars, or soluble proteins) or activation of enzymes such as peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX), or catalase (CAT) (Anjum *et al.* 2016). POD is very widespread in nature. It catalyses more than one reaction and acts on a great number of substrates, beside this it is also relatively heat stable. From plants, soluble POD can be extracted from tissue homogenates with a low ionic strength buffer (Vámos-Vigyázó and Haard 1981, Loaiza-Velarde *et al.* 1997).

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Abbreviations: AM - arbuscular mycorrhiza; Chl - chlorophyll; F% - mycorrhiza fungi colonisation of the root system; OS1 - control treatment; OS2 - alfalfa (*Medicago sativa* L.) mulch treatment; OS3 - rye (*Secale cereale* L.) straw mulch treatment; OS4 - black foil covered treatment; POD - peroxidase; PS II - photosystem II; ROS - reactive oxygen species.
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Plants possess a number of antioxidant mechanisms that protect them from the excessive production of reactive oxygen species (ROS). Antioxidants scavenge free radicals inside cells and prevent or reduce the damage caused by oxidation (Arriaga *et al.* 2020). Under stress conditions, the balance between the generation and elimination of ROS shifts (Smirnoff 1998). The increased accumulation of ROS inside plant cells leads to the degradation of lipids, proteins, DNA, and cell membranes, which eventually causes irreparable damage (Ullah *et al.* 2017).

Apart from the plants own ROS mitigating mechanism repertoire, there are external sources that could also help with ROS elimination. Mycorrhiza fungi are symbiotic fungi that coexist within plant roots and obtain photosynthetic products from the plant, and in return, they contribute to the plants water supply and reduce abiotic stress (Brundrett 1991, Ishii 2018). According to Brundrett (2004) and Smith and Read (2008), mycorrhiza can be divided into three main groups: endomycorrhiza, ectomycorrhiza, and special mycorrhizas such as ericoid, orchid, and subepidermal mycorrhizas. These microfungi are very specific, they cohabit with only one plant species. Ectomycorrhizas have two main types: one is associated typically with angiosperms such as *Eucalyptus*, *Betula*, *Populus*, *Fagus*, and *Shorea* with a Hartig net confined to epidermal cells. The other type is associated with gymnosperms, such as the members of the *Pinaceae* family, where the Hartig net occupies multiple layers of cells in the cortex.

Arbuscular mycorrhiza (AM) is a type of endo-mycorrhizis, where fungi penetrates into the root tissue, colonising many of the individual root cells. The name "arbuscular" derives from the name "arbusculum", which is a characteristic structure that occurs within the cortical root cell. The structure of arbusculum is "tree-like" as it branches into several sections. The enlarged surface area of this structure is well suited for nutrient uptake (Smith and Read 2008).

In agriculture, inoculation of certain crops with AM fungi has a positive effect on plant growth. The size and mass of vegetative parts can be higher, and therefore lead to an increase in yield (Sheng *et al.* 2008). Furthermore, AM can improve the resistance of the crop to biotic (pathogens) and abiotic (drought, salt, heavy metal) stresses (Gosling *et al.* 2006, Hildebrandt *et al.* 2007, Andrade *et al.* 2009, Galván *et al.* 2009). One reason for the beneficial effects could be that the plants with AM symbiosis could reach for otherwise unreachable water and nutrition sources by the external hyphae net system. One example is that the increased phosphorus and potassium uptake through AM directly increases the photosynthetic activity of plants. According to Zuccarini and Okurowska (2008) the photosynthetic activity in colonised than in non-colonised plants could be 2 - 10 times higher. AM fungi also protect the photosystem II (PS II) centre and the photosynthetic apparatus by reducing the detrimental effects of high temperature. AM also increases the chlorophyll (Chl) content and provides a higher photosynthetic efficiency during heat stress (Zhu *et al.* 2011).

AM fungi can also induce resistance in plants, called MIR (mycorrhiza induced resistance). As a result, plants will be more resistant to soil-borne pathogens, such as *Rhizoctonia*, *Fusarium*, *Verticillium*, *Phytophthora*, *Pythium*, or *Aphanomyces* and compensate their negative effects more easily (Jung *et al.* 2012). In vegetable cultivation, the most commonly present arbuscular mycorrhiza (AM) fungi are the members of *Glomeromycota*, such as: *Glomus mosseae*, *Glomus intraradices*, *Acaulospora laevis*, or *Gigaspora gigantea* (Smith and Read 2008).

The number and diversity of soil microbes can be a good indicator of soil life. In agriculture, there are agrotechnical solutions to maintain or even stimulate soil life, which is particularly important in organic farming. Mulches are defined as materials applied to soil surface to regulate soil temperature fluctuation, conserve soil moisture, reduce soil evaporation, increase water infiltration into the soil, and slow down erosion (Ghawi and Battikhi 1986, Adekalu *et al.* 2007, Chalker-Scott 2007, Chakraborty *et al.* 2008). Mulching materials can be classified into three main groups: organic materials (*e.g.*, plant material), inorganic or synthetic materials and special materials. Organic mulching materials derive from organic substances such as agricultural wastes from canopy-, shoot- and leaf-management or materials resulting from harvesting procedures (straw, stalks). Wood industrial wastes, such as sawdust can also be implemented as an adequate mulching material, but residues of grain processing (*e.g.*, rice husk) are also used for mulching. Inorganic mulching materials usually include polyethylene plastic films, which are petroleum based products (Gill 2014), and synthetic polymers (Kyrikou and Briassoulis 2007). Special materials, such as sand or concrete, have also been used for mulching, but very rarely, due to some disadvantages of the materials: sand mulching reduces soil nutrients and concrete mulching is not cost efficient (Kader *et al.* 2017).

Mulching has a significant effect on soil microbiology through moisture and temperature regulation (Moreno and Moreno 2008). Furthermore, organic mulches may increase soil nutrients after decomposition. After crop harvest, organic mulching can work as soil fertilizers, under optimal water and temperature levels. The decomposition of organic matter releases nutrients and minerals, which positively affects the soil quality for the next cropping season (Chalker-Scott 2007) and may increase crop yield (Sinkevičienė *et al.* 2009). Mulching treatments augment the total soil nitrogen content, compared to bare soil (Ren *et al.* 2007). Organic mulching has a positive effect on soil nitrogen content as it increases the nitrogen metabolism by nitrogen fixation (Kader *et al.* 2017). Straw mulching was also found to increase Chl content of crops, but this effect was also present, when plastic mulching was used (Yang *et al.* 2006). It also enhances the biotic activities of earthworms in soil (Lal 1998) and other soil organisms (such as algae, mosses, fungi, or bacteria) that improve the soil structure and quality (Döring *et al.* 2005). For example, an increased content of phosphor- and potassium

bacteria was reported under polyethylene film mulching (Hu *et al.* 1995). Mulches can inhibit weed growth by providing a physical barrier (Teasdale and Mohler 2000), due to this, mulch coverage reduces the germination of several weed species (Appleton and Kauffman 2009). Another way how organic mulches can inhibit weed growth is the release of certain allelo-chemicals (Barnes and Putnam 1987). For example, rye stalk is a widely used organic soil coverage because of its high biomass. Furthermore, rye contains allelopathic components as DIBOA [2,4-dihydroxy-1,4(2H)-benzoxazin-3-one] and BOA [2(3H)-benzoxazolinone] which inhibit the germination, growth, or development of other plants (Barnes and Putnam 1987, Weston 1990). For the same reason, alfalfa is also a suitable mulching material, as it contains secondary allelopathic compounds (White *et al.* 1989), and additionally, alfalfa has large amount of nutrients (Wiens *et al.* 2006). Beside weed suppression, mulching materials can also reduce the occurrence of plant diseases, and can affect the appearance of pests and predator insect species as well (Boyhan *et al.* 2006).

Beside the positive effects, mulch can present some drawbacks. Dessureault-Rompré *et al.* (2020) in an open field experiment, experienced lettuce yield loss under high rye mulching, but there was no significant effect of low rye mulching on lettuce yield. In a separate experiment, Smith *et al.* (2011) experienced no yield loss of soybeans under rye mulch cover. To sum up these experiments, certain mulches may have an allelopathic quality, but the effect on cultivation highly depends on the crop species itself; and with the careful monitoring of certain factors (crop species, the type of organic mulch, seedling or transplant health, proper amount of mulching material, *etc*.) the negative effects could be prevented. In our experiment yield loss was not observed and by the end of the trial the average head mass of the lettuces was 360 g which is suitable for the market.

In order to better understand how certain mulches effect the naturally present AM and its colonisation ratio on *L. sativa*, we have conducted an open-field experiment with three mulching materials (rye, alfalfa, and black foil coverage). Furthermore, we concluded the positive effects of AM on lettuce by measuring inner content parameters and the stress mitigating aspects of AM by peroxidase enzyme activity determination.

Materials and methods

Experiments location and plant material: The experiments were carried out in 2019 in the Sector of Organic Farming of the Experimental Farm of Hungarian University of Agriculture and Life Sciences (MATE) located at Soroksár (47°23'33.0"N, 19°08'53.7"E) in a certified organic farm. Before any experiment was conducted, accredited soil analysis was made at National Reference Laboratory for Plant and Soil Protection (accreditation No.: nah-1-1594/2022). The region Soroksár is categorized as humic sandy soil (pH 7.53, $K_A = 33$). Essential results of the soil analysis are marked in the Table 1 Suppl.

Prior to the experiments, samples were taken from the experimental area from several different plants (leek, onion, spinach, strawberry) to check whether natural mycorrhizal inoculation occur, to avoid using any type of artificial inoculum.

During the experiment, soil moisture, air temperature, and irradiance were measured in every 15 min with a *Parrot* "*Flower Power*" multi measurement data logger. As we had a drop irrigation, the soil moisture was balanced during the whole experiment. The temperature did not fluctuate much, during sampling it ranged between 25 - 30°C. However, irradiance dropped around the 16th of June and fluctuated between 27 000 - 60 000 lx (daily average) until the end of the experiment.

The lettuce cultivar used in the experiments was Voltron (*Rijk Zwaan*, The Netherlands), which is a batavia type lettuce. It is a robust cultivar, optimal for year-round open-field cultivation. The main advantage of this cultivar is the strength against bolting and internal tipburn. Seeds were sown on 26th March 2019 in 6 × 11 sectored plastic trays, each sector was 5 × 5 × 5 cm. The trays were filled with *Latagro KB2* peat moss (pH 5.2 - 6.0). For organic fertilization *Italpollina 4-4-4* manure was used both when the seeds were shown and when seedlings were planted on field 30th April 2019. For each mulch treatment, transplants were placed in 3 twin-row, with spacing of (50 + 15) × 20 cm. Each parcel was about 200 cm long and contained 20 lettuce plants + bordering lettuce plants (~ 15 plants). For treatments of alfalfa and rye straw mulches, mulch material was applied right after planting in 15 cm thickness. In the black foil treatment, the foil was pulled out before the lettuce transplants were placed in it. The control treatment did not have any kind of mulch coverage. Treatments were the following: OS1 - control, OS2 - alfalfa (*Medicago sativa* L.) mulch, OS3 - rye (*Secale cereale* L.) straw mulch, OS4 - black foil. Each treatment was repeated three times. The presence of pathogens and pests was checked before each sampling time. Lettuces were healthy throughout the experiment and did not receive any plant protection treatments.

Mycorrhiza fungi colonization in lettuce roots (F%): During the experiment, root samples were taken four times from five randomly selected lettuce plants per repetition of each experimental trial: 30th April 2019 (developmental stage BBCH14) and 12th (BBCH41), 19th (BBCH45), and 26th (BBCH49) June 2019. For the verification of mycorrhiza colonisation, roots samples were cut off from the lettuce plants and stored in 60% diluted ethanol in 50-ml Falcon tubes in cooled environment until laboratory examination. To visualize AM colonisation, lettuce roots were painted with arbuscular mycorrhiza painting method based on Phillips and Hayman (1970). The principle of the method is the dye bond to the fungal chitin, while the stem cells remain transparent. After cleaning, the samples were cured in a 10% KOH solution at 65°C for 1 h, washed in distilled water and soaked in 10% lactic acid overnight. For staining, roots were soaked in aniline blue for 1 min. The excess paint was washed off with lactic acid. According to Giovannetti and Mosse

(1980) colonisation is measured with a gridline intersect method. The painted and prepared root samples were observed under an *Olympus Szx7* stereomicroscope at 7× magnification. The partial results were calculated further with *MycoCalc* software (Trouvelot *et al.* 1986).

Chlorophyll content: Plant samples were taken three times during the experimental period: 12th (BBCH41), 19th (BBCH45), and 26th (BBCH49) June 2019. Five fresh lettuce heads were collected per treatment, per repetition, and blended to an average sample. The chlorophyll in the lettuce leaves was extracted with an acetone and the amount of pigment in extract was determined spectrophotometrically at 663 and 644 nm. To determine the Chl content Arnon's method (Arnon 1949) was used.

Peroxidase activity: Plant samples were taken three times during the experimental period: 12th (BBCH41), 19th (BBCH45), and 26th (BBCH49) June 2019. Five fresh lettuce heads were collected per treatment, per repetition, and blended to an average sample. POD activity in the leaf tissues was measured spectrophotometrically ($\lambda = 460$ nm) in the presence of H_2O_2 as substrate and ortodianisidine ($\varepsilon = 11.3$) as chromogen reagent after Shannon *et al.* (1966). For the measurement 300 mg plant material (leaf) were used. Samples were homogenized in an ice-cold mortar with 1.2 ml of P-phosphate buffer and were centrifuged at 4°C for 20 min at 13 500 rpm. Hundredfold diluted aqueous solution was made from concentrated (30%) hydrogen peroxide. For the measurement a pH 4.5 Na-acetate buffer was used. Orthodianisidine was diluted in methanol to a concentration of 10 mg ml^{-1}. The first measurement was performed with a blank at 460 nm. Then, for further measurements plant extracts were added to the mixture as follows: 1 500 µl buffer + 30 µl 0.3% H_2O_2 + 20 µl orthodianisidine + 60 µl plant extract = 1 610 µl. The spectrophotometer measured the absorbance at 460 nm in every 10 s for 1 min.

Statistical analysis: The statistical analysis was carried out with *XLSTAT* (*Addinsoft*, New York, USA). Data preparation was started with normality test ($\alpha = 0.05$). As the variables do not follow a normal distribution (P-value < α), non-parametric test was used for further evaluation. Kruskal-Wallis test was run to test whether samples originate from the same distribution and principal component analysis (PCA) with Spearman correlation ($\alpha = 0.05$) was carried out whether there is a correlation between parameters.

Results

Mycorrhiza fungi colonisation in the root system (F%) was measured from all treatments and at all sampling times. There was an increase in the colonisation as time progressed in every treatment (Fig. 1). The lowest values were measured in treatment OS4 (12.95%, 26.44%, 34.52%). The highest values for the first two sampling periods were measured in treatment OS1 (30.10%, 33.76%), but in the sampling on 26th June, OS3 treatment had the highest colonisation rate (54.46%).

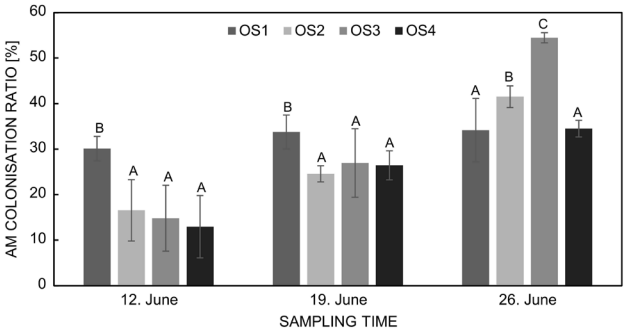

Fig. 1. Rate of mycorrhizal colonization measured under control (OS1), alfalfa (OS2), rye straw (OS3), and black foil (OS4) covered lettuce throughout the experimental period. Means ± SEs, $n = 36$, *different letters* indicate significant differences according to Kruskal-Wallis test, $P < 0.0001$.

Kruskal-Wallis test also showed significant differences between treatments in every period. On 12th and 19th June the measured values were significantly higher in OS1 than in other treatments, on 26th the highest measured value was in OS3 which was significantly higher than in other treatments. By the end of the experiment the AM colonisation in OS1 (34.19%) and OS4 (34.52%) were almost the same. The higher AM colonisation ratios were measured under the natural mulches than under other treatments.

Transplants were checked for mycorrhiza inoculation before being planted to the experimental field, but no inoculation was visible. At the first sampling time (12th June), we could measure different inoculation ratio in every treatment. Every subsequent measurement revealed an elevated growth in AM colonisation (Table 1).

The highest measured F% on 12th June was in OS1 and the root colonisation was significantly higher in 19th June, however after that F% decreased.

In OS2 the root colonisation did not show a significant increase between 12th June and 19th June, however then the largest increase was measured between 19th June and 26th June in this treatment and by the end of the experiment, the second highest root colonisation occurred here.

In OS3 and OS4 treatments, the root colonisation was significantly increased as time progressed. The lowest

Table 1. Growth dynamics of mycorrhiza colonisation measured under control (OS1), alfalfa (OS2), rye straw (OS3), and black foil (OS4) covered lettuce throughout the experimental period (* indicates significant differences according to Kruskal-Wallis test, $P < 0.0001$).

Treatment	12 June - 19 June	19 June - 26 June
OS1	+12%*	+1%
OS2	+48%	+69%*
OS3	+82%*	+102%*
OS4	+104%*	+31%*

measured F% on 12th June was in OS4, but this treatment showed the most remarkable growth in root colonisation between 12th June and 19th June. The highest F% between 19th June and 26th June was measured in OS3. Significant changes in F% are marked in Table 1.

Chlorophyll content was measured in samples from all treatments and at all sampling times. Five lettuce heads were collected per treatment, per repetition, and blended to an average sample. There was a fluctuation in the amount of Chl as time progressed (Fig. 2). At the first sampling time, the highest measured Chl content was in OS2, which was significantly higher compared to the others. For the second sampling time, there was a slight decrease in the Chl content in treatments OS2 and OS4. But there was an increase in treatments OS1 and OS3. In the third sampling time there was a decrease in all treatments, however, the measured value was highest in OS3 and lowest in OS2.

Peroxidase activity was measured in samples from all treatments and at all sampling times. Five lettuce heads were collected per treatment, per repetition, and blended to an average sample. As Fig. 3 shows, there was an increase in the enzyme activity as time progressed. At the first sampling time the highest, almost identical values were measured in OS3 (0.68 U mg^{-1}) and OS4 (0.65 U mg^{-1}) treatments. Then, the highest values were measured in OS4 treatment (1.04 U mg^{-1}, 1.24 U mg^{-1}). On 12th and 19th June the lowest values were found in OS2 (0.31 U mg^{-1}, 0.62 U mg^{-1}) and on 26th June the lowest value was in OS3 (0.96 U mg^{-1}). Kruskal-Wallis test also showed significant differences between treatments every period. On 12th June the POD activity was significantly higher in treatments OS3 and OS4 than in OS1 or OS2. On 19th June every treatment was significantly different from each other, however the highest value was measured in treatment OS4. On 26th June the measured POD activity values were significantly higher in OS2 and OS4 than in OS1 and OS3.

Principal component analysis (Fig. 4) was carried out to test if there was any correlation between POD, Chl, and F% regardless mulching type. On 12th June there was a negative correlation (-0.421) between F%_1 and POD_1 value, which got stronger (-0,533) by the end of

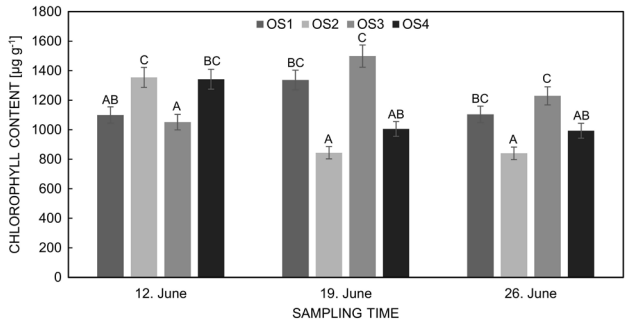

Fig. 2. Chlorophyll content measured in control (OS1), alfalfa (OS2), rye straw (OS3), and black foil (OS4) covered fresh lettuce throughout the experimental period. Means ± SEs, $n = 12$, *different letters* indicate significant differences according to Kruskal-Wallis test, $P < 0.0001$.

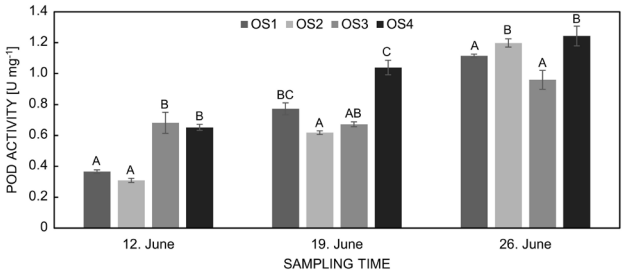

Fig. 3. Peroxidase enzyme activity measured in control (OS1), alfalfa (OS2), rye straw (OS3) and black foil (OS4) covered fresh lettuce throughout the experimental period. Means ± SEs, $n = 36$, *different letters* indicate significant differences according to Kruskal-Wallis test, $P < 0.0001$.

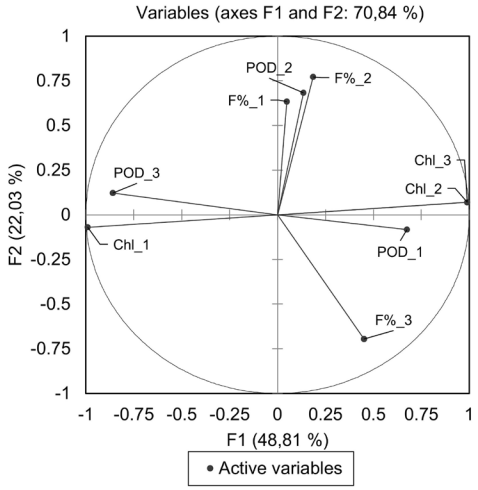

Fig. 4. Principal component analysis (PCA) for POD activity (POD), chlorophyll content (Chl), and mycorrhizal colonisation (F%) on 12th June (_1), 19th June (_2), and 26th June (_3) (Spearman correlation, $\alpha = 0.05$).

the experiment (F%_3 and POD_3). Which means the higher was the mycorrhizal colonisation of the roots, the lower was the POD activity in plants. However, at the second sampling time, there was just a weak positive correlation (0.292) between the two parameters. Regarding the colonisation parameters (F%), the correlation circle also confirms what the Kruskal-Wallis test earlier, that root colonization really increased by the third sampling time.

On 12th June, there was a strong negative correlation (-0.650) between POD_1 and Chl_1. At the second sampling time, there was a weak positive correlation (0.195) between POD_2 and Chl_2. However, for the third sampling time, the negative correlation between POD_3 and Chl_3 was strong (-0.823). This means the higher was the POD enzyme activity in plants, the lower was the Chl content in the leaf.

The Spearman test did not show any significant correlation between F% and Chl for the first and second sampling time, but for the third sampling time, it showed a slight correlation (0.367) between these two parameters (F%_3 and Chl_3).

Discussion

Mycorrhiza fungi are symbiotic fungi that colonize plant roots and obtain photosynthetic products from the plant, and in return, they contribute to water and mineral supply or reduces abiotic stress (Brundrett 1991, Ishii 2018). In our experiment, the highest measured mycorrhizal colonisation on 12th June was measured in OS1. However, after that time, there was a relapse in the root colonisation. As there were no mulching in this treatment, the biggest temperature fluctuation both in soil and leaf area occurred and this could also lead to increased evaporation. In the other treatments the temperature was more stable and this could lead to a more remarkable growth in root colonisation.

The lower F% values were measured every time in treatment OS4. Under black foil, the soil activity could be higher as the increased soil temperature promotes soil microbial activity and speeds up decomposition of organic matter in the soil (Kader *et al.* 2017). This may lead to a lower mycorrhizal colonisation as the plants in these treatments had an easier access to nutrients.

Numerous studies have reported that mycorrhizal inoculation has a positive impact on plants. According to Zhu *et al.* (2011), PS II and the whole photosynthetic apparatus could be damaged at high temperature, but AM fungi could protect it, increase the Chl content and provide a higher photosynthetic efficiency during heat stress. In our experiment, the results concerning Chl content fluctuated. Among treatments, there was a significant increase in OS3 at the second sampling time, but on 26th June, in all treatments Chl content decreased. However, the slightest decrease was measured in OS3. This could be caused by the hot weather (25 - 30°C) in this period, which was above the optimal range for lettuce.

As peroxidase activity might be a stress marker, the higher the values, the higher the stress. The high water content and big leaf area in lettuce causes fast evaporation during sunny and hot weather. Ideal daytime temperature for growth is 20°C (Rubatzky and Yamaguchi 1997) but the measured temperature during the experiment was much higher, which could cause heat stress. Also, black foil covering could further increase the stress; this could lead to higher POD activity in OS4.

Peroxidase can be found in most fruits and vegetables and is connected to enzymatic browning (Vámos-Vigyázó and Haard 1981), which is not favourable for the market. Mycorrhiza colonisation could lower the amount of POD in lettuce and may lower the risk of browning after harvesting. By the end of the experiment, there was a strong negative correlation between the Chl content and POD. Which means the higher was the POD activity in plants, the lower was the Chl content of the leaf. This may again support that photosynthesis is impaired by heat stress.

On the contrary, there was no significant correlation between F% and Chl for the first and second sampling time, and just a slight correlation appeared between the two parameters by the end of the experiment. This contradicts Zhu *et al.* (2011) findings. Mycorrhiza fungi get photosynthetic products (*e.g.*, sugar, vitamins, and other organic substances) from the plant (Ishii 2018), but under stress conditions, they cannot provide the required nutrients, consequently this could lead to a relapse in mycorrhiza fungi colonisation ratio, function, or both. As lettuce plants were under heat stress, mycorrhizas did not get the required photosynthetic products from the plant and this could lead to a relapse in mycorrhizas colonisation ratio or functioning.

Studies also stated that mycorrhizal inoculation has a positive impact on stress responses in plants. However, the fungi must cope with severe weather conditions. As it is a symbiotic connection between the two parties, if one does not function properly, problems may arise in the other. So, in long term, both plants and fungi could benefit this symbiosis, but in the short term, both must first adapt to the arising circumstances. Heinemeyer and Fitter (2004) also stated from their experiment, that when plant and fungus were both exposed to varying temperatures, the impact on the AM fungus was related to different biomass or root growth dynamics.

To conclude, all mulches, even the black foil, had a positive effect on mycorrhiza fungi root colonisation, however, by the end of the experiment, the higher values were measured under the natural mulches. The lowest measured POD activities were in those treatments where natural mulch was used. These mulches did not cause overheating neither in the root nor in the leaf area. Beside this the increase in colonisation was more prominent in these treatments. This could mean that natural mulching has a positive effect on mycorrhiza fungi root colonisation, which leads to lower stress in plants and in longer term, it could lead to a more stable photosynthesis and to a higher tolerance to environmental impacts.

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