PHILIPP AGRIC SCIENTIST
Vol. 102 No. 3, 238-246
September 2019

ISSN 0031-7454

# The Response of Tobacco to Impact of Arbuscular Mycorrhizal Inoculation and Application of Some Micro and Macronutrients

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**To study the response of tobacco to the impact of arbuscular mycorrhizal inoculation and the application of some micronutrients and macronutrients, an experiment was conducted in Marivan City, northwestern Iran during the 2014–2015 growing season. The experiment used split plot in a randomized complete block design with three replications. The main factor was no inoculation and inoculation by arbuscular mycorrhiza (*Glomus intraradices*) while subplots used foliar application of some micronutrients and macronutrients in five treatments: control (distilled water), zinc- boron- (micronutrient), potassium- (macronutrient), and zinc + boron + potassium salts. The results showed that the number of leaves and the leaf dry weight in the middle leaves, and the relative water content in the upper leaves of tobacco were significantly affected by mycorrhizal inoculation. Mycorrhizal inoculation resulted in increased number of leaves and leaf dry weight in the middle leaves and increased relative water content in the upper leaves. Foliar application of micronutrients and macronutrients significantly affected the leaf dry weight in the lower leaves, and the number of leaves and leaf dry weight in both the middle and upper leaves. Mean comparison of the lower leaves showed that the maximum value of leaf dry weight was achieved by foliar application of zinc salts. Also, in the middle leaves, the maximum number of leaves was achieved by application of boron salt; leaf dry weight by application of zinc salt; and finally in the upper leaves, the maximum number of leaves was achieved by application of zinc salt, and leaf dry weight by foliar application with potassium salt.**

Key Words: foliar application, macronutrients, micronutrients, mycorrhizal inoculation, tobacco

Abbreviations: AMF – arbuscular mycorrhizal fungi, RWC – relative water content, SPAD – Soil Plant Analysis Development

## INTRODUCTION

For maximum yield, quality, and profitability of tobacco leaves, there is a need to formulate different fertilization programs (Hoyos et al. 2015). Bozhinova (2016) reported that phosphate application significantly increased plant height, number of leaves per plant and dimensions of the middle leaves of tobacco and that the level of phosphate fertilization slightly affected potassium content in the middle and upper leaves. Phosphate influences root development, plant growth, and the quality of the first leaves, and improves the color and quality of the leaves and accelerates growth and maturity of tobacco (Hoyos et al. 2015). Mycorrhizal symbiosis can enhance plant growth and therefore reduce the need for phosphate-based fertilizers (Roy-Bolduc and Hijri 2010). Arbuscular mycorrhizal fungi (AMF) contribute to plant phosphate nutrition by increasing mineral nutrient availability and enhancing plant nutrient uptake (Almagrabi and Abdelmoneim 2012; Miller 2000), optimizing phosphate solubilization from mineral phosphate and increasing plant growth and microbial activity in the rhizosphere of plants (Duponnois et al. 2005), improving the efficiency of nutrient uptake, increasing plant resistance to pathogens and abiotic stresses (Roy-Bolduc and Hijri 2010) and increasing nitrogen, phosphorus, potassium and sulfur concentrations and certain trace elements such as zinc and copper in the rhizosphere (Mohamed et al. 2014).

On the other hand, micronutrients such as iron-, zinc- and boron-salts are essential for different biological functions that might be attributed to yield and fruit quality (Shoeib and El Sayed 2003; Noorisadegh et al. 2015) and also increased resistance to disease and insect pests and improved drought tolerance (Tariq et al. 2007). Sufficiency ranges of potassium, boron and zinc in mature tobacco leaves are approximately 2.5–4%, 18–75 ppm and 20–60 ppm, respectively.

Boron deficiency is a widespread agricultural problem globally, resulting in yield and quality loss of many crop species including tobacco (Shorrocks 1997). Among the micro-nutrients, boron fertilizer tends to increase the uptake of nitrogen, phosphorus, potassium, copper, iron, zinc ions, and the yield of cotton (Ahmed et al. 2011). Also, it increases crop growth (Marschner 1995); it is necessary in various plant physiological and biochemical processes (Shelp 1993; Blevins and Lukaszewski 1998), increases dry matter yield of tobacco (Lopez-Lefebre et al. 2002), enhances photosynthetic activity of leaves and dry matter in peanut (Duyingqiong et al. 2002), conveys sugar across membrane, is also involved in cell differentiation and auxin metabolism (Mahler 2010), and enhances the grain yield of wheat (Wroble 2009).

Zinc also plays an important role in the production of biomass, grain yield, quality and quantity of safflower oil (Kaya and Higgs 2002; Cakmak 2008). Maralian (2009) reported that foliar application of Zn- and Fe-salts improved grain yield and related traits in wheat.

Potassium is a major mineral element necessary for the growth and quality of tobacco leaves in terms of color, texture, sugar content, nicotine, and combustibility (Gurumurthy and Vageesh 2007; Yang et al. 2007). Miner and Tucker (1990) found that potassium is the most important element for tobacco, followed by calcium, nitrogen, magnesium, and phosphorus. Potassium improves the quality of tobacco leaves by increasing the burning rate and heat retention capacity (Miner and Tucker 1990).

Foliar spray of micronutrients is more effective to control deficiency problem compared with soil application (Torun et al. 2001). Hence, this study was conducted to increase the productivity of tobacco leaves by mycorrhizal inoculation and to increase the efficacy of macronutrients and micronutrients by foliar application.

## MATERIALS AND METHODS

An experiment was carried out using a split-plot based on a randomized complete block design with three replications at a farmer's tobacco field in Marivan City, northwestern Iran (35° N and 46° E, at 1324 m above mean sea level, average annual precipitation of 750 mm) during the 2015 growing season. The main factor was no inoculation and inoculation by arbuscular mycorrhiza (*G. intraradices*) and subplots were foliar application of some micro and macronutrients in five treatments: control (distilled water), zinc-, boron-, potassium-, and zinc + boron + potassium-salts. Sterilized and coated seeds of tobacco (*Nicotiana tabacum* cv. Burley 21) were obtained from the Iranian Tobacco Administration. The seeds were germinated in a float tray system in the greenhouse 2 mo before field planting. At the end of May, seedlings were transplanted in the main field at 40 cm distance between seedlings and 1 m distance between rows. In the mycorrhizal inoculation treatments, prior to transplanting, mycorrhizal powder (3.33 g/p, manufactured by an organic company in Iran) was placed in rows and then covered with 1 cm of soil. The micro and macronutrients were sprayed (2/1000 v/v concentration) 1 mo before harvest of the lower leaves. Watering at regular intervals and appropriate care and weed control were carried out during the various growth stages of tobacco. The irrigation method was furrow system. For higher yield and quality of tobacco leaves, flower topping or removal of the apex of tobacco was done manually and fatty alcohol was used to prevent growth of lateral buds or suckers at 66–75 d after transplanting.

In this study, Soil Plant Analysis Development (SPAD), canopy temperature, and relative water content were measured after spraying during the crop growing period. The SPAD values were obtained from 10-leaf samples of the lower, middle, and upper leaves by using Chlorophyll Meter SPAD–502. Canopy temperature was also read from 10-leaf samples by using Infrared Thermometer CEMDT-8810. The relative water content (RWC) was measured based on the method described by Baslam and Goicoechea (2012) and the equation

$$RWC = 100 \times (FW - DM) / (TM - DM) \quad \text{(Eq. 1)}$$

where FW is fresh weight, DM is dry matter, TM is turgid mass, and TW is turgid weight.

Fresh weight was measured via 10 separate pieces of leaves with 2 cm diameter, which were then placed in an ice tank immediately. Consequently, the pieces were weighed and recorded as fresh weight. To calculate TM, the pieces of leaves were placed in distilled water for 24 h, and finally, after obtaining the excess water, the weight obtained was taken as turgid weight. Also, DM was obtained after drying the pieces of leaves in an oven at 75 °C for 48 h to achieve constant dry weight. At the harvest stage of leaves in the three stages, 66, 91 and 116 d, respectively, after transplant (for the lower, middle, and upper leaves, respectively), two plants from each plot were selected and plant growth parameters such as the number of leaves and leaf dry weight were recorded. Finally, data were analyzed using the experimental design of the SAS 9.1 software package. Trait mean comparisons were done using Duncan's multiple range test at the 0.05 probability level.

## RESULTS AND DISCUSSION

### Canopy Temperature

Canopy temperature was not significantly affected by mycorrhizal inoculation (Table 1). Mycorrhizal inoculation could decrease canopy temperature a bit in the lower and middle leaves of tobacco by inducing water balance in plants due to the possibility of better uptake of water from the soil (Table 2). Probably, if water stress was applied in this study, the greater effect of mycorrhizal inoculation would have been possible. Bakr et al. (2018) found that under full irrigation conditions, inoculation with mycorrhiza reduced the canopy temperature by only 0.2°C, while in the case of irrigation reduced by 50% of the plant requirement, the leaf canopy temperature was decreased by 1.6°C. Bakr et al. found that mycorrhiza could improve root growth, resulting in increased water uptake, thereby reducing the canopy temperature.

Similar results about increased water and nutrient uptake by mycelium of mycorrhizal fungi were obtained by Almagrabi and Abdelmoneim (2012) and Mohamed et al. (2014). Also, the results of foliar application of micronutrients and macronutrients were not significantly different. In this study, the effects of mycorrhizal inoculation and micronutrient and macronutrient application on canopy temperature of the middle leaves were significantly different. In the no inoculation treatment, potassium could reduce the canopy temperature even more (Fig. 1). It seems that this nutrient by increasing the turgid potential in the cells of the leaves, resulting in more water absorption, could reduce canopy temperature, but in the mycorrhizal inoculation treatment, increasing water uptake by mycelium of mycorrhizal fungi could reduce the role of K.

According to Kaur et al. (2018), canopy temperature could decrease by 1 to 2°C as a result of nitrogen application, because nitrogen can create a dense canopy and crop stand. They also found that Zn spraying decreased the canopy temperature by 0.5 to 1°C, which helped the wheat plant to withstand heat stress. They also mentioned the negative relationship between canopy temperature and grain yield.

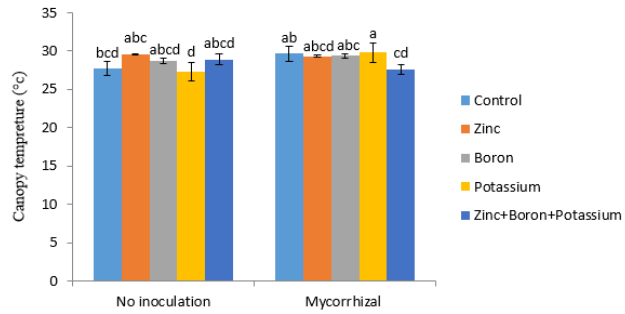


**Fig. 1. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the canopy temperature of the middle leaves of tobacco.**

Eisvand et al. (2018) found that application of nutrient as potassium, zinc and boron reduced canopy temperature as this nutrient had ameliorative effects on bread wheat especially in the late planting dates. Application of nutrients can reduce canopy temperature by increasing the leaf area index, and subsequently evapotranspiration.

Marschner (1995) reported that various environmental factors greatly influenced the assimilation of nutrients by different plant parts. Also, Drossopulos et al. (1994) found that the amount of mineral elements absorbed by a plant was a function of the growth stages, genotypes, agronomic practices and environmental factors.

### Soil Plant Analysis Development (SPAD)

SPAD was not significantly affected by mycorrhizal inoculation. Based on the findings of Mohamed et al. (2014), increase in the availability of necessary nutrient, especially nitrogen, as a result of mycorrhizal inoculation

**Table 1. ANOVA for the response of tobacco when impacts of arbuscular mycorrhizal inoculation and some micronutrients and macronutrients were imparted.**

| Separation of Variables | df | Canopy Temperature | | | SPAD | | | RWC | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lower | Middle | Upper | Lower | Middle | Upper | Lower | Middle | Upper |
| Block | 2 | 0.83$^{ns}$ | 2.04$^{ns}$ | 0.115$^{ns}$ | 3.95$^{ns}$ | 0.88$^{ns}$ | 3.11$^{ns}$ | 8.8$^{ns}$ | 146.35$^{ns}$ | 3.67$^{ns}$ |
| Mycorrhizal | 1 | 1.39$^{ns}$ | 3.55$^{ns}$ | 0.081$^{ns}$ | 1.55$^{ns}$ | 0.471$^{ns}$ | 0.022$^{ns}$ | 12.7$^{ns}$ | 9.95$^{ns}$ | 10.71$^{*}$ |
| Ea | 2 | 0.719 | 1.34 | 5.26 | 0.089 | 11.7 | 0.82 | 7.62 | 30.81 | 0.231 |
| Nutrient application | 4 | 0.59$^{ns}$ | 1.23$^{ns}$ | 0.857$^{ns}$ | 0.831$^{ns}$ | 1.83$^{ns}$ | 2.31$^{ns}$ | 0.34$^{ns}$ | 13895* | 3.11$^{ns}$ |
| Mycorrhizal x Nutrients | 4 | 1.72$^{ns}$ | 3.65$^{*}$ | 0.471$^{ns}$ | 0.721$^{ns}$ | 0.823$^{ns}$ | 3.29$^{ns}$ | 2.39$^{ns}$ | 40.81$^{*}$ | 2.27$^{ns}$ |
| Eb | 16 | 1.49 | 1.06 | 0.75 | 0.997 | 1.45 | 1.97 | 3.41 | 70.16 | 2.34 |
| CV (%) | | 3.99 | 3.58 | 2.9 | 2.51 | 3.96 | 4.35 | 2.57 | 10.95 | 2.29 |

**, * Significant at 1% and 5% level, ns: non-significant
SPAD – Soil Plant Analysis Development, RWC – relative water content

**Table 2. Comparison of the response of tobacco when impacts of arbuscular mycorrhizal inoculation and some micronutrients and macronutrients were imparted.**

| Treatment | | Canopy Temperature (ºC) | | | SPAD | | | RWC (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Lower | Middle | Upper | Lower | Middle | Upper | Lower | Middle | Upper |
| Mycorrhizal | Control | 30.81a | 29.12a | 29.75a | 39.91a | 30.54a | 32.3a | 72.36a | 77.01a | 66.18b |
| | Inoculation | 30.38a | 28.43a | 29.86a | 39.45a | 30.29a | 32.25a | 71.05a | 75.85a | 67.37a |
| Nutrients | Control | 30.67a | 29.42a | 29.91a | 39.9a | 30.51a | 32.12a | 71.45a | 78.39ab | 67.8a |
| | Zinc | 30.14a | 29.02a | 29.33a | 40.19a | 29.63a | 31.44a | 71.79a | 75.94ab | 66.14a |
| | Boron | 30.47a | 28.66a | 30.27a | 39.36a | 30.43a | 33.18a | 71.48a | 83.33a | 66.15a |
| | Potassium | 30.99a | 28.24a | 30a | 36.66a | 31.16a | 32.35a | 72.02a | 70.53b | 67.21a |
| | Potassium +Zinc + Boron | 30.71a | 28.55a | 29.51a | 39.3a | 30.27a | 32.3a | 71.79a | 73.96ab | 66.59a |

Means with letters in each column are not significantly different at 0.05 probability level by Duncan's multiple range test. SPAD – Soil Plant Analysis Development, RWC – relative water content

can affect SPAD due to the role of nitrogen in the chlorophyll structure. In this study, however, mycorrhizal inoculation had no significant effect on SPAD. This result is probably due to high fertility of the soil or the consumption of assimilates by mycorrhiza, reducing growth (Hendrix 1993). According to Magarey et al. (2005), mycorrhizal inoculation can be used to achieve maximum yield with lower levels of P in the soil.

Also, in this study, the effects of micro and macronutrient application on SPAD were not significantly different (Table 1). Micro and macronutrient application had little positive effect on SPAD in the lower, middle and upper leaves of tobacco compared with the control (Table 2). The maximum SPAD number was observed in the lower leaves applied with zinc. Zinc plays an important role in the production of biomass, grain yield, quality and quantity of safflower oil (Kaya and Higgs 2002; Cakmak 2008). Foliar application of Zn and Fe improved grain yield and related traits in wheat (Maralian 2009).

In the middle leaves, K had the best effect on SPAD number. K also had a positive effect on the growth and quality of tobacco leaves in terms of color, texture, sugar content, nicotine, and combustibility (Gurumurthy and Vageesh 2007; Yang et al. 2007; Miner and Tucker 1990).

In the upper leaves, boron application resulted in the maximum SPAD. Duyingqiong et al. (2002) reported that B fertilizer significantly enhanced leaf photosynthetic activity, resulting in more accumulation of dry matter in peanut (*Arachis hypogaea* L.).

Lopez-Lefebre et al. (2002) and Ruiz et al. (1998) reported the effects of B on N metabolism due to a positive influence on enzymatic protein synthesis and also indirectly by promoting the entrance of substrate through plasma membrane to the interior of the cells.

## Relative Water Content

Relative water content (RWC) was significantly affected by mycorrhizal inoculation only in the upper leaves. Inoculation could increase relative water content in the upper leaves of tobacco (Table 2). Mycorrhizal inoculation could increase relative water content by increasing root growth, resulting in more uptake of phosphorus in the soil (Magarey et al. 2005), consequently inducing more water uptake and increasing the RWC and water status in the leaves. Due to more exposure to sunlight and receiving more radiation and consequently more photosynthesis and transpiration in the upper leaves of tobacco, there is greater uptake and transport of water to the leaves as a result of mycorrhizal inoculation.

In a study by Bakr et al. (2018), mycorrhizal inoculation enhanced the stomatal conductance and leaf water potential as a result of stimulated root growth and improved root water uptake due to mycorrhizal inoculation.

In this study, the effects of foliar application of micro and macronutrients on RWC in the middle leaves of tobacco were significantly different (Table 1). Mean comparison of the middle leaves showed that boron application resulted in the maximum RWC. Probably, boron can affect cell differentiation, auxin metabolism, and the conveying of sugar across membranes (Mahler 2010), consequently increasing RWC in the middle leaves.

Boron spraying may have increased osmolites in the cell, as increased leaf proline concentration and RWC has been reported as a result of boron foliar spraying in sesame plant under normal irrigation conditions. However, in low-irrigation conditions, the combination of boron and potassium has been reported to be effective in increasing leaf proline content (Movahhedi Dehnavi et al. 2017). Boron and zinc spraying would cause osmotic adjustment, reducing oxidative damage and saving the plant from cell turgor.

Enhancement of the concentration of soluble sugars by potassium application in tobacco plant has been reported by Bahrami-Rad and Hajiboland (2017) who introduced the soluble sugars as the most valuable osmolytes in comparison with proline and free amino acids in the tobacco plant. They found that potassium application improved the leaf water potential, relative water content and cell turgor.

Interaction of mycorrhizal inoculation and micro and macronutrient application on RWC was significantly different in the middle leaves of tobacco. In the mycorrhizal and non- mycorrhizal treatments, the maximum RWC was found in the control or distilled water (Fig. 2). It seems that the foliar application of water can increase the water potential in cells and also RWC, but in other nutrient applications, it stimulates cell differentiation, cell growth and cell division (Mahler 2010).

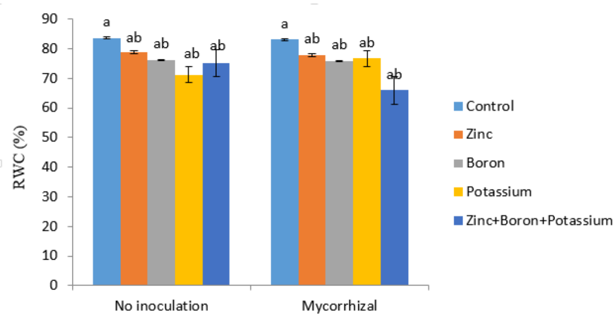


**Fig. 2. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the relative water content (RWC) of the middle leaves of tobacco.**

## Number of Leaves

Mycorrhizal inoculation could significantly affect the number of the middle leaves in tobacco plant (Table 3). Mean comparison of the middle leaves showed the maximum number of leaves as a result of mycorrhizal inoculation. The number of leaves in tobacco is very important in terms of their positive role in leaf yield and consequently farmer's income. In all, a tobacco plant produced about 20–50 leaves in the growing season. Also, the most number and the highest quality of leaves were obtained from the middle leaves in terms of low nicotine percentage in the leaves and higher income. The results of foliar application of micro and macronutrients on the number of the middle and upper leaves were significantly different (Table 3).

Mean comparison of the number of leaves showed that the highest value was achieved through foliar application of B in both the lower and middle leaves and foliar application of Zn in the upper leaves. Ahmed et al. (2011) showed that boron increases uptake of nitrogen, phosphorus, potassium, copper, iron and zinc nutrients. Interaction effects of mycorrhizal inoculation and micro and macronutrient application on this character were significantly different in all leaves. In the lower leaves, the maximum number of leaves was found in the no inoculation treatment and in the treatment applied with potassium (Fig. 3–5). It appears that at the beginning of the growing stage of tobacco, symbiosis between plant and fungi was not yet completed and mycorrhiza consumed some assimilates. In the middle leaves applied with mycorrhizal inoculation and boron, the maximum number of leaves was achieved, and also in the upper leaves with no inoculation and applied with boron, more leaves were produced. It has long been known that an optimum boron level for one species could be either toxic or insufficient for other species (Blevins and Lukaszewski 1998). Finally, due to the great effect of the middle leaves on the yield of tobacco, mycorrhizal inoculation and boron foliar applications are recommended.

## Leaf Dry Weight

Mycorrhizal inoculation had a significant effect on the dry weight of the middle leaves. There was an increase in

**Table 3. ANOVA for the response of tobacco when impacts of arbuscular mycorrhizal inoculation and some micronutrients and macronutrients were imparted.**

| Separation of Variables | df | No. of Leaves | | | Leaf Dry Weight | | |
|---|---|---|---|---|---|---|---|
| | | Lower | Middle | Upper | Lower | Middle | Upper |
| Block | 2 | 0.008 ns | 3.05ns | 0.078ns | 8.68ns | 0.000035ns | 2.15ns |
| Mycorrhizal | 1 | 0.033ns | 46.87ns | 0.075ns | 6.17ns | 0.0009** | 6.03ns |
| Ea | 2 | 0.058 | 0.325 | 0.047 | 0.00001 | 0.000014 | 0.000004 |
| Nutrient application | 4 | 0.112ns | 9.56** | 1.9** | 0.000008** | 0.00077** | 0.00006** |
| Mycorrhizal x Nutrients | 4 | 0.095* | 7.97** | 1.42** | 0.00003** | 0.0013** | 0.00024** |
| Eb | 16 | 0.085 | 0.45 | 0.25 | 0.0000008 | 0.000074 | 0.000004 |
| CV (%) | | 7.18 | 4.31 | 8.19 | 4.46 | 6.32 | 4.54 |

**, * Significant at 1% and 5% level, ns: non-significant the dry weight of the middle leaves of tobacco as a result of mycorrhizal inoculation (Table 4). Increasing the absorption of macronutrients and micronutrients from the soil could probably increase the dry weight of the leaves, a result obtained by other researchers (Mohamed et al. 2014; Munda et al. 2018).

Mycorrhiza can achieve maximum yield with lower levels of P in the soil (Magarey et al. 2005), hence, if pre-sowing of chemical fertilizers was not done in this experiment, there is greater possibility of increased yield. Based on the results of Munda et al. (2018), application of biofertilizer can also improve P availability in the next crop. However, Hendrix (1993) found that a mycorrhizal species reduces the yield of commercial tobacco and is the cause of a poor growth condition called 'tobacco stunt'. Further studies showed that the increase in phosphorus absorption and increasing yield occurred as a result of arbuscular mycorrhizal symbiosis when the soil is not disturbed by tillage. Also, when maize is planted after a non-mycorrhizal crop such as canola (*Brassica napus* L.), mycorrhizal colonization is delayed, reducing early-season P absorption and consequently, yield reductions may occur (Miller 2000). In this study, the previous crop before tobacco was alfalfa (*Medicago sativa* L.), which is well known to fix nitrogen in the soil, and may decrease the mycorrhizal expected efficacy.

In this study, effects of foliar application of micro and macronutrients were significantly different in the dry weight of all leaves of the plant. In the lower leaves, the maximum weight was obtained as a result of applying distilled water and boron. In the middle leaves, the highest dry weight was obtained as a result of boron application, whereas in the upper leaves, the highest dry weight was found in plants treated with potassium foliar application. The same results were found in boron application by other researchers, such as, enhanced grain yield of wheat (Wroble 2009), enhanced dry matter yield, biological yield and increased uptake of nitrogen, phosphorus, potassium, copper, iron and zinc nutrients in cotton (Ahmed et al. 2011), increased cell elongation and division, nucleic acid metabolism and dry matter yield (Shelp 1993; Ruiz et al. 1998). Also, based on the findings of Zhao et al. (2010), low concentrations of potassium in the leaves can reduce the quality of tobacco, hence, concentrations of potassium higher than 2% dry weight are usually essential to the production of upper leaves in tobacco.

Potassium is highly mobile in the phloem and, therefore, a high degree of its reuse is done with re-translocation via the phloem (Marschner 1995). It seems that after flower topping in tobacco, re-translocation and redistribution of potassium occur as a result of leaf senescence and cleavage of the apex (Zhao et al. 2010) as shown in this study. However, in physiological terms, this practice can cause changes in sink-source relations and modify the distribution and translocation of assimilates and mineral nutrients in plants. Interaction of mycorrhizal inoculation and micro and macronutrient application on the dry weight of all leaves was significantly different (Fig. 6–8). It seems that differences in the results are due to different sizes of the leaves (among lower, middle and upper leaves of the plant) and consequently different values of foliar application and different growth stages of the leaves at foliar application time (1 mo before harvest of the lower leaves). Similarly, Lopez-Lefebre et al. (2002) also reported that application of B along with NPK fertilizers increased dry matter.

## CONCLUSION

Mycorrhizal inoculation had a positive and desirable effect on the number and the dry weight of the middle leaves of tobacco, increasing the number and the weight of the middle leaves by 17.44% and 8.46%, respectively, compared with the control. Positive effects of foliar application of micro and macronutrients on the number and the dry leaf weight of the middle and upper leaves of

**Table 4. Comparison of the response of tobacco when impacts of arbuscular mycorrhizal inoculation and some micronutrients and macronutrients were imparted.**

| Treatment | | No. of Leaves | | | Leaf Dry Weight (kg/p) | | |
|---|---|---|---|---|---|---|---|
| | | Lower | Middle | Upper | Lower | Middle | Upper |
| Mycorrhizal | Control | 41.1[a] | 14.33[b] | 6.1[a] | 0.021[a] | 0.13[a] | 0.047[a] |
| | Inoculation | 4.03[a] | 16.83[a] | 6.2[a] | 0.019[a] | 0.141[a] | 0.047[a] |
| Nutrients | Control | 4.08[a] | 15.41[c] | 5.41[c] | 0.021[a] | 0.134[a] | 0.047[b] |
| | Zinc | 4.16[a] | 16.83[a] | 6.91[a] | 0.021[a] | 0.138[a] | 0.048[b] |
| | Boron | 4.83[a] | 16.91[a] | 6.33[ab] | 0.02[b] | 0.145[a] | 0.043[c] |
| | Potassium | 4.08[a] | 14.58[c] | 5.83[bc] | 0.018[c] | 0.144[a] | 0.052[a] |
| | Potassium +Zinc + Boron | 4.16[a] | 14.16[c] | 6.25[b] | 0.02[b] | 0.117[b] | 0.046[b] |

Means with common letters in each column are not significantly different at 0.05 probability level by Duncan's multiple range test.

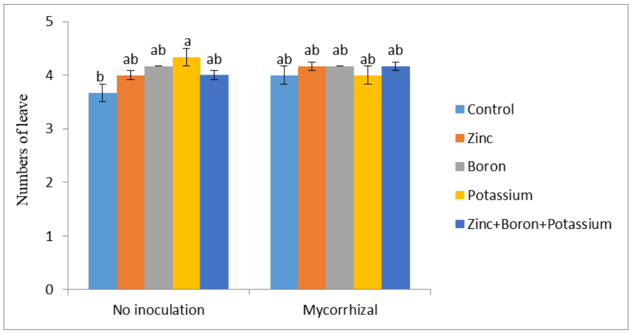


**Fig. 3. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the number of lower leaves of tobacco.**

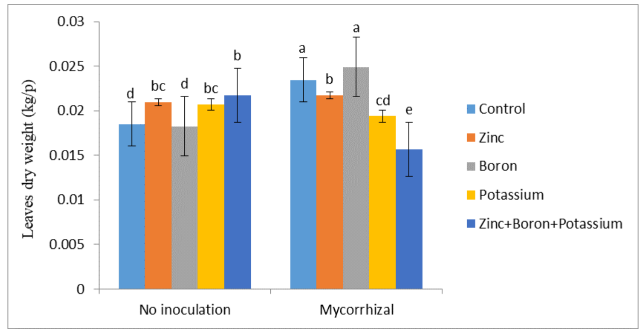


**Fig. 6. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the dry weight of the lower leaves of tobacco.**

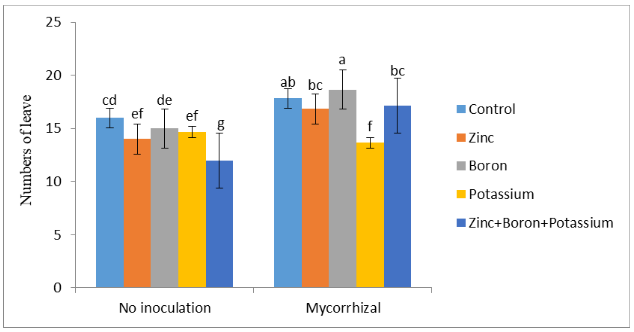


**Fig. 4. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the number of middle leaves of tobacco.**

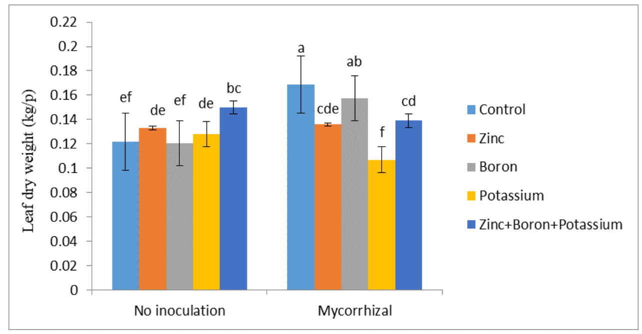


**Fig. 7. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the dry weight of the middle leaves of tobacco.**

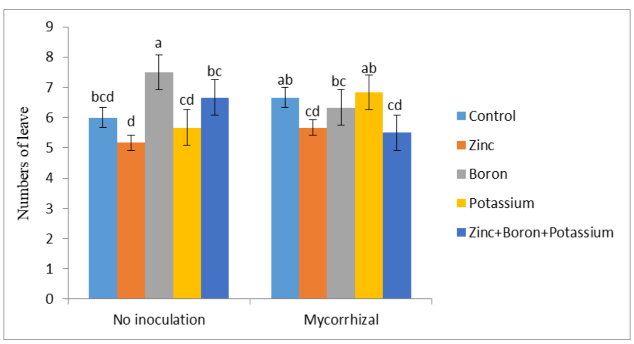


**Fig. 5. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the number of upper leaves of tobacco.**

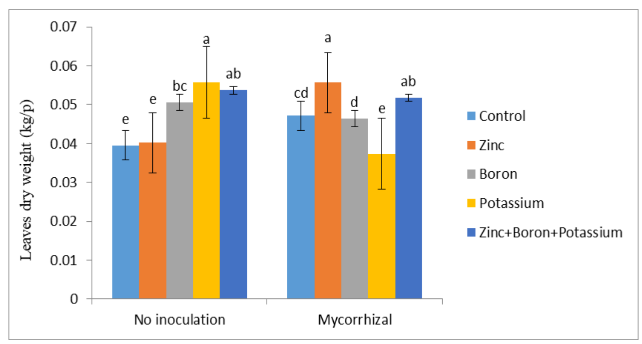


**Fig. 8. Interaction of mycorrhizal inoculation and micronutrient and macronutrient application on the dry weight of the upper leaves of tobacco.**

tobacco were evident. The highest numbers of leaves were obtained as a result of foliar application of boron in the middle leaves (9.73% more than the control) and by application of zinc in the upper leaves (27.72% more than the control). Other nutrients also had a positive effect but lower on the number of upper leaves. Also, the highest dry weight was obtained as a result of application of potassium in the upper leaves.

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