STUDY OF THE EFFECTS OF MYCORRHIZA, FULVIC ACID, SEAWEED EXTRACT AND UREA ON PHYSIOLOGICAL TRAITS AND LEAF YIELD OF TOBACCO (BURLEY 21)

SHABNAM MORADI, BABAK PASARI*, and REZA TALEBI

Department of Agronomy and Plant Breeding, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran
* Corresponding author: bpasary@yahoo.com

ABSTRACT

To study the effects of mycorrhiza, fulvic acid, seaweed extract and urea on the physiological traits and leaf yield of tobacco, an experiment was carried out in split plots in a randomized complete block design with three replicates at Marivan during 2015. In this study, the main factor was two levels of inoculation with mycorrhiza including: control: no inoculation and inoculation with mycorrhiza (*Glomus interaradices*) and subplots treated with growth stimulants at 5 kinds: control-distilled water, fulvic acid, seaweed extract, urea, fulvic acid + seaweed extract + urea. The results indicate that the number of leaves and dry weight of the middle leaves of plants inoculated with mycorrhiza were increased significantly. In addition, the foliar application of growth stimulants significantly affected the relative water content, fresh and dry weight of the lower and middle leaves and also the greenness and fresh weight of the upper leaves of tobacco.

Keywords: canopy; dry weight; fertilizer; growth promotion; symbiosis

Introduction

Although the application of chemical fertilizers has resulted in a huge increase in world food production (Banerjee et al. 2011; Garai et al. 2014), it has contributed significantly to the pollution of water, air and soil (Ignacimuthu and Vendan 2007). Phosphorus has been applied regularly for many years and the level of P is now relatively high at some locations (Magarey et al. 2005). Tobacco is a cash crop and the best quality tobacco comes from crops cultivated in regions in Iran where it is humid and the soil is fertile. However, it is important that tobacco does not contain toxic or heavy metals. To achieve this it is vital to reduce the use of chemical fertilizers that contain toxic and heavy elements, such as cadmium, because these fertilizers increase the risk of Cd entering the food chain, which has an adverse effect on the kidneys, lungs, cardiovascular and musculoskeletal systems of humans (Roberts 2014). In order to prevent or decrease the incidence of such problems it is necessary to produce healthy crops by using environmentally friendly fertilizers such as organic fertilizers or biofertilizers instead of chemical fertilizers. Arbuscular mycorrhiza is known to assist plants in their uptake of phosphorus and certain trace elements such as zinc and copper. The mycorrhiza invades the root system of its host and utilizes carbon from the plant and in return effectively increasing the soil volume from which the plant may draw nutrients. This enables plants to produce high yields even when growing in soil with low levels of P (Magarey et al. 2005). Increasing concentrations of N, P, K and S in the rhizosphere and dry weight of onion bulbs and maize shoots by inoculating these plants with mycorrhizae is reported (Amal et al. 2014). Munda et al. (2016) report better productivity of soybean when grown in plots treated with biofertilizers and that the microbes also improve P availability for the next crop.

Fulvic acid is an organic fertilizer with a non-toxic mineral chelating additive and water binder that maximizes its uptake through leaves and stimulates plant productivity (Malan 2015). It attracts water molecules, which helps in keeping soil moist and aids the movement of nutrients into plant roots. Fulvic acid easily binds or chelates minerals such as iron, calcium, copper, zinc and magnesium, and can deliver these elements directly to plants (Yamauchi et al. 1984). Various studies report that it increases the fluorescence of chlorophyll a, inhibits ROS and enhances the antioxidant enzymes that destroy ROS (Lotfi et al. 2015), decreases water stress or stress due to hot and dry winds during ear development, increasing grain yield by 7.3–18.0%, enhances root activity, increases ion uptake and the rate of transport of phosphorus to the grains (Xudan 1987), increases the number and length of root hairs in Arabidopsis plants (Schmidt et al. 2005), ameliorates the growth of rice and radish resulting in taller plants (Khang 2011), limits the development of some pathogens, e.g. Fusarium spp (Yigit and Dikilitaş 2008) and the availability of phosphorus (Yang et al. 2013). Seaweed extract, another organic fertilizer, contains micro and macro nutrients and growth promoters (Prasad et al. 2010). It also contains plant growth hormones that enhance yield (Latique et al. 2013). Application of seaweed extract stimulates growth and yield and increases the tolerance of plants to environmental stress (Pramanick et al. 2013), improves germination of maize seeds by 10–19%, promotes shoot and root growth by 30–68% (Matysiak et al. 2011), increases the resistance of herbaceous plants to drought (Zhang and Ervin 2004), promotes vegetative growth, yield and oil content of peanuts (31.69%, 14.27%) and sunflower (31.69%, 14.27%),

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respectively (Karthikeyan and Shanmugam 2015). In addition, spraying plants with seaweed extract improves growth, yield and tuber quality of potatoes in terms of their nitrogen, total soluble solids and protein contents (Haider et al. 2012), increases root growth, nutrient absorption, stem thickness and growth (Jensen 2004), ameliorates effect of salt stress on bean seeds because of the presence of growth hormones, nutrients and other important phytochemical compounds (Latique et al. 2014).

Therefore, this study compares the effectiveness of organic and chemical fertilizers in terms of the increase in yield of leaf tobacco.

Material and Methods

This experiment was carried out in split-plots based on a randomized complete block design with three replicates in a farmer's tobacco field at Marivan in the northwest of Iran (situated at 35°N and 46°E and at 1324 m above mean sea level), where the annual precipitation during the 2015 growing season was 750 mm. In this study, the main factor was the inoculation of the mycorrhizal fungus (*G. intaradices*) and a control (not inoculated) and the sub-factor application of growth compounds of 6 kinds: distilled water (control), fulvic acid, seaweed extract, urea, fulvic acid + seaweed extract + urea). The fulvic acid was prepared by Fanavari Sabz Shargh Co, Tehran, Iran. The seaweed extract (Acadian^{TM}) was made from *Ascophyllum nodosum* obtained from Nova Scotia, Canada. Sterilized and coated seeds of tobacco (*Nicotiana tabacum* cv. Burley 21) were obtained from the Iranian Tobacco Administration. These seeds were germinated in a float tray in a greenhouse two months before planting in a field. At the end of May, seedlings were transplanted to another field and planted 40 cm apart in rows that were 1 meter apart. For the inoculation of mycorrhiza, mycorrhiza powder (3.33 g/p) was placed in rows and then covered with 1 cm of soil prior to transplanting the seedlings. The growth compound were sprayed (2/1000 w/v concentration) on the plants one month before the lower leaves were harvested. The watering of the plants was achieved using furrows. At various stages of the growth of the tobacco plants they were watered and weeds were removed. In this study, the following characters were recorded after spraying: the greenness, temperature and relative water content of the leaves. The greenness was based on the readings from 10 leaves (lower, middle, upper) using a Chlorophyll Meter SPAD-502 and the temperature of the same leaves using an Infra-red Thermo meter CEMDT-8810. Relative water content was measured using Baslam and Goicoechea (2012) method.

$$RWC = 100 \times (FW - DM) / (TM - DM)$$

In this formula, FW is fresh weight, DM dry weight and TW turgid weight.

Fresh weight is that of ten 2 cm diameter pieces of leaves that were previously kept in an ice box. In order to calculate TM, the leaf pieces were placed in distilled water for 24 h, wiped to remove excess water and then weighed. DM was obtained by drying the leaf pieces in an oven at 75 °C for 48 h before weighing them. At harvest, the lower, middle and upper leaves were collected from two plants in each plot together with a record of the number and dry and fresh weight of the leaves. Finally, data were analyzed according to the experimental design in $SAS_{9.1}$ software package and comparison of the trait means using Duncan's multiple range test at 0.05 probability.

Results and discussion

Leaf temperature

Leaf temperature was not affected significantly by the mycorrhiza inoculations (Table 1), but as shown in Table 2, they resulted in a decrease in the temperature of the middle and upper leaves by improving the water balance of the plants, possibly by increasing the uptake of water from the soil. Probably, if the plants had been subject to water stress then a greater effect of mycorrhiza would have been recorded as Bakr et al. (2018) report that inoculation of mycorrhiza decreases canopy temperature significantly only in water-limited conditions. Also Chen et al. (2017) conclude that mycorrhiza enhances the gas exchange capacity of plants by improving "stomatal opening, reducing stomatal resistances and increasing transpiration fluxes". The effects of mycorrhiza in decreasing the temperature of leaves is due to their increasing water absorption, improving water use efficiency and effect on stomatal conductance (Chen et al. 2017; Bakr et al. 2018). Probably the high rainfall and air humidity in the area studied accounts for the non-significant effect of mycorrhiza on leaf temperature.

Also, the effects of foliar application of growth compounds and interaction between inoculation with mycorrhiza and foliar application were not significantly different. As shown in Table 2, despite the no significant difference between treatments, the foliar application of growth compounds decreased canopy temperature compared with the control. The minimum temperature of the middle leaves was recorded when fulvic acid was applied. Xudan (1987) report that foliar application of fulvic acid improves resistance to drought in wheat via partial closure of the stomata, which reduces transpiration and raises the water potential during flowering; it also enhances root activity. In this study, a significant effect of growth compound was not recorded as the field was irrigated and the rainfall and humidity were high.

Greenness

This character was not affected significantly by inoculation with mycorrhiza as it increased slightly in the lower and upper but not in middle leaves. It is concluded that the increase in availability of nutrients, especially nitrogen, due to inoculation with mycorrhiza (cf. Amal et al. 2014) can affect greenness, as nitrogen is needed for producing chlorophyll. Chen et al. (2017) also reports that the inoculation with mycorrhiza increases the contents of some nutrients such as nitrogen and therefore of chlorophyll. Bakr et al. (2018) also report that inoculation with mycorrhiza enhances the relative chlorophyll index only under drought conditions with no increase when there is an optimum supply of water.

In this study, the foliar application of growth compounds had a significantly different effect on the greenness of the upper leaves (Table 1). As shown in Table 2 it had a positive effect on the greenness of the lower, middle and upper leaves compared with the control. However, for the upper leaves the maximum greenness was recorded for those treated with seaweed extract, fulvic acid and urea. It is possible that the nutrients in the soil, especially nitrogen, are sufficient to meet the needs of the plants until the middle of the vegetative season, but after that when there was a reduced level of nutrients in the soil the application of growth compounds resulted in an increase in chlorophyll in the upper leaves. This result may also be attributed to the location and exposure of the upper leaves to sunlight along with the translocation of nitrogen to the upper parts of the plant, especially the upper leaves.

These results are in accordance with the findings of other researchers. Xudan (1987) reports that spraying with fulvic acid resulted in a higher level of chlorophyll in the leaves and a greater uptake by the roots in wheat and Lotfi et al. (2015) report that the application of fulvic acid improved the maximum quantum efficiency of PSII (Fv/Fm) and the performance index (PI) of plants under both well-watered and drought conditions. They also report that application of FA significantly increases the fluorescence of chlorophyll a, inhibits ROS production and enhances antioxidant enzymes activity that destroys ROS.

Relative water content

Relative water content was not affected significantly by inoculation with mycorrhiza, but there was an increase in relative water content of all the leaves (Table 2). It is likely that mycorrhiza could increase relative water content via increasing root growth and the uptake of phosphorus from soil (Magarey et al. 2005), resulting in greater water uptake and increase in the RWC of the leaves. As it was mentioned in the results of leaf temperature, if this experiment was conducted under limited irrigation or rainfed conditions, increasing water absorption and consequently, enhancing relative water content of the leaves likely to be done by mycorrhiza.

In this study, the foliar application of growth compounds on the RWC in the lower leaves differed signif-

Table 1 Results of the analysis of variation of the effects of mycorrhiza, fulvic acid, seaweed extract and urea on the physiological traits of tobacco.

SOV	df	Leaf temperature			Greenness			RWC		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Block	2	0.99^{ns}	10.59^{ns}	12.85^{ns}	12.42^{ns}	36.75^{ns}	7.46^{ns}	52.93^{ns}	442.75^{**}	19.89^{ns}
Mycorrhiza	1	1.79^{ns}	2.91^{ns}	7.29^{ns}	3.52^{ns}	24.24^{ns}	10.87^{ns}	102.52^{ns}	15.19^{ns}	145.99^{ns}
Ea	2	5.58	5.99	10.89	5.45	44.60	1.32	259.94	6.40	378.86
Growth compounds	4	1.87^{ns}	4.04^{ns}	0.88^{ns}	3.68^{ns}	17.09^{ns}	16.66^{*}	145.33^{*}	34.37^{ns}	58.52^{ns}
M × G	4	1.10^{ns}	2.73^{ns}	1.33^{ns}	2.90^{ns}	11.58^{ns}	1.47^{ns}	32.98^{ns}	80.08^{ns}	80.01^{ns}
Eb	16	2.06	5.53	2.77	2.60	9.38	7.24	50.24	61.82	23.93
CV (%)		5.27	8.56	5.64	4.48	9.48	7.30	10.83	10.66	16.06

**, * Significant at 1% and 5% level, respectively, ^{ns} non-significant.

Table 2 Comparison of the average effects of mycorrhiza, fulvic acid, seaweed extract and urea on the physiological traits of tobacco.

Treatment		Leaf temperature (°C)			Greenness			RWC (%)		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Mycorrhiza	Control	26.984^{a}	27.781^{a}	30.027^{a}	35.958^{a}	33.189^{a}	36.224^{a}	63.562^{a}	72.987^{a}	67.015^{a}
	Inoculation	27.473^{a}	27.158^{a}	29.041^{a}	36.280^{a}	31.391^{a}	37.482^{a}	67.259^{a}	74.410^{a}	71.521^{a}
Growth compounds	Control	28.320^{a}	28.565^{a}	29.886^{a}	35.228^{a}	30.875^{a}	34.010^{b}	63.182^{b}	71.280^{a}	70.342^{a}
	Seaweed	27.850^{a}	27.192^{a}	29.206^{a}	36.251^{a}	30.975^{a}	37.815^{a}	64.000^{b}	73.090^{a}	67.897^{a}
	Fulvic	27.665^{a}	21.318^{a}	30.003^{a}	37.186^{a}	31.678^{a}	37.742^{a}	65.147^{ab}	72.088^{a}	67.698^{a}
	Urea	26.686^{a}	27.770^{a}	29.193^{a}	35.551^{a}	33.035^{a}	37.970^{a}	60.950^{b}	74.722^{a}	66.277^{a}
	Seaweed+fulvic+urea	26.621^{a}	27.503^{a}	29.381^{a}	35.525^{a}	34.885^{a}	36.593^{ab}	73.775^{a}	77.312^{a}	74.225^{a}

Averages with the same letter in each column are not significantly different at a 0.05 probability level using Duncan's multiple range test.

icantly (Table 1). The maximum RWCs were recorded in the lower leaves when fulvic acid + seaweed extract + urea was applied. It also had a positive effect on the RWCs of the middle and upper leaves, but it was not significant. It is concluded that the higher level of RWC in lower leaves may be due to their proximity to the roots and receiving a larger amount of growth compound due to their larger size when the growth compounds were applied. This is supported by the decrease in the RWC of the middle and a greater decrease in that of the upper leaves of the plant (Table 2). On the other hand, the middle and upper leaves are more exposed to sunlight and consequently transpire more water, therefore did not show a significant response to this compound.

Xudan (1987) reports that the foliar application of fulvic acid improves the drought tolerance of wheat by inducing a partial closure of the stomata, which reduces transpiration and increases the water potential during flowering and enhances root activity, resulting in an increase in ion uptake and high rate of transport of phosphorus to the grains.

Numbers of leaves

Inoculation with mycorrhiza significantly affects the number of middle leaves on tobacco plants (Table 3). Comparison of the average number of middle leaves indicates they are more numerous on plants inoculated with mycorrhiza. Number of leaves is very important, in term of leaf yield and consequently farmer income. All tobacco plants produce about 20–50 leaves in a growing season. In addition, most of the highest quality leaves are middle leaves, which have lower nicotine content.

As it takes time for the colonization and growth of fungal mycelium and the onset of symbiosis and as a consequence the positive effect of this symbiosis is only likely to affect the middle and upper leaves and then increases in air and soil temperature reduce its effect. As shown by Augé et al. (2015) mycorrhiza promotes an increase of about 10% when air temperature is less than 27 °C but negative increases at higher temperatures. In another study, it is reported that an increase in air temperatures of 3 °C reduces colonization by mycorrhiza (Wilson et al. 2016). The decline in the activity of mycorrhiza at high temperatures is also reported by Mohan et al. (2014).

However, increased leaf numbers, leaf area, shoot and root biomass and higher chlorophyll a, b in plants inoculated with mycorrhiza is also reported (Lu and Wu 2017).

There were no significant effects of foliar applications of growth compounds on the number of leaves (Table 3). Comparison of the average numbers of leaves reveal that the highest number of both lower and middle leaves developed on plants sprayed with seaweed extract and fulvic acid + seaweed extract + urea. Latique et al. (2013)

Table 3 Results of the analysis of variance of the effects of mycorrhiza, fulvic acid, seaweed extract and urea on the leaf traits of tobacco.

SOV	df	Numbers of leaves			Leaf fresh weight			Leaf dry weight		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Block	2	0.433^{ns}	7.05**	2.508^{ns}	0.0001^{ns}	0.003^{ns}	0.0004^{ns}	9.33^{ns}	0.00006^{ns}	0.000008^{ns}
Mycorrhiza	1	0.133^{ns}	35.20**	0.008^{ns}	0.0003^{ns}	0.530^{ns}	0.00002^{ns}	8.53^{ns}	0.0002*	0.00004^{ns}
Ea	2	1.033	0.108	1.108	0.0010	0.378	0.0004	0.00002	0.000009	0.00003
Growth compounds	4	0.395^{ns}	1.80^{ns}	0.32^{ns}	0.0010**	0.063**	0.0068**	0.00003*	0.0008**	0.00007^{ns}
M × G	4	0.112^{ns}	0.18^{ns}	0.195^{ns}	0.0002^{ns}	0.009^{ns}	0.0005^{ns}	0.00001^{ns}	0.00005^{ns}	0.00006^{ns}
Eb	16	0.191	5.55	1.277	0.0002	0.012	0.0013	0.00001	0.0001	0.00008
CV (%)		10.100	14.09	19.880	11.21	15.82	14.35	16.7	7.94	19.11

**, * Significant at 1% and 5% levels, respectively, ^{ns} non-significant.

Table 4 Comparison of the average effects of mycorrhiza, fulvic acid, seaweed extract and urea on the leaf traits of tobacco.

Treatment		Numbers of leaves			Leaf fresh weight (g/p)			Leaf dry weight (g/p)		
		Lower	Middle	Upper	Lower	Middle	Upper	Lower	Middle	Upper
Mycorrhiza	Control	4.266^{a}	15.630^{b}	5.700^{a}	0.148^{a}	0.677^{a}	0.225^{a}	0.020^{a}	0.127^{b}	0.048^{a}
	Inoculation	4.400^{a}	17.800^{a}	5.660^{a}	0.154^{a}	0.752^{a}	0.253^{a}	0.021^{a}	0.133^{a}	0.046^{a}
Growth compounds	Control	4.000^{a}	16.583^{a}	5.916^{a}	0.130^{b}	0.598^{c}	0.252^{abc}	0.019^{ab}	0.143^{a}	0.052^{a}
	Seaweed	4.583^{a}	17.250^{a}	5.500^{a}	0.166^{a}	0.834^{a}	0.241^{bc}	0.023^{a}	0.130^{ab}	0.049^{a}
	Fulvic	4.166^{a}	16.917^{a}	5.916^{a}	0.158^{ab}	0.762^{a}	0.290^{a}	0.022^{a}	0.140^{a}	0.047^{a}
	Urea	4.333^{a}	15.833^{a}	5.666^{a}	0.132^{b}	0.609^{bc}	0.205^{c}	0.017^{b}	0.114^{c}	0.045^{a}
	Seaweed+fulvic+urea	4.583^{a}	17.000^{a}	5.416^{a}	0.163^{a}	0.745^{ab}	0.281^{ab}	0.022^{a}	0.124^{bc}	0.043^{a}

Averages with the same letter in each column are not significantly different at a 0.05 probability level using Duncan's multiple range test.

report that seaweed contains growth hormones that enhance yield. The same results are reported for potato (Sarhan 2011; Haider et al. 2012), peanut and sunflower (Karthikeyan and Shanmungam 2015). Conversely, when the initial soil organic matter and NO3-N levels are high seaweed extract has no effect on yield (Wickham and Davis 2015).

Since, in this experiment the leaves developed before spraying, the fact they did not increase in number is reasonable, but the size or weight of the leaves were expected to increase.

Leaf fresh weight

Leaf fresh weight was not affected significantly by inoculation with mycorrhiza (Table 4). Like the effects of mycorrhiza on RWC, this is to be expected, because mycorrhiza helps plants to obtain water, however, the effect was not significantly different.

Bakr et al. (2018) report that fresh weight of moderately stressed plants inoculated with mycorrhiza was increased by 73% but for those well supplied with water it was only 8%.

The effect of foliar applications of growth compounds on leaf fresh weight were significantly different for all leaves. The maximum leaf fresh weight was recorded for the lower leaves of plants sprayed with seaweed extract and fulvic acid + seaweed extract + urea and for the middle leaves sprayed with seaweed extract and fulvic acid and for upper leaves with fulvic acid. It seems that, the difference in the effect of growth compound is due to the variation in the needs of tobacco leaves in different positions, due to their age, size, exposure to solar radiation and temperature.

Esringu et al. (2015) show that humic and fulvic acids promote plant growth in terms of the number of buds, plant height, number of main shoots, number of side shoots, plant diameter, root length, fresh root weight and fresh shoot weight.

Leaf dry weight

Inoculation with mycorrhiza had a significant effect on the dry weight of the middle leaves (Table 4). Probably this is due to an increase in the absorption of macro and micronutrients from the soil as is reported by Amal et al. (2014) and Munda et al. (2016).

Chen et al. (2017) report that the net photosynthetic rate and stomatal conductance is increased significantly in plants inoculated with mycorrhiza by 58.76 and 95.65%, respectively. However, for other species of mycorrhiza, the increase in these traits is reported to be 35.79 and 21.26%. They conclude that the enhancement of photosynthesis in these treatments is associated with an increase in ribulose-bisphosphate carboxylase/oxygenase (RuBisCO) and Calvin cycle enzymes. Finally, they state that plant height, dry weight of shoot and root, root to shoot ratio, root activity, chlorophyll content, photosynthetic characteristics and nutrient uptake, including N, P, K, Ca, Cu, Fe, Mn, Mg, Zn, and S of inoculated plants are all significantly higher. In another study, the improvement in the growth of plants inoculated with mycorrhiza is due to improvements in water use efficiency, stomatal conductance and photosynthetic efficiency (Bakr et al. 2018).

Mycorrhizal symbiosis can greatly improve yield when the level of P in the soil is low (Magarey et al. 2005), hence we conclude that if pre-sowing application of chemical fertilizers had not occurred in this experiment, it is likely the yield increase would have been higher. The results of Munda et al. (2016) indicate that the application of biofertilizers can improve the availability of P for the next crop. However, Hendrix (1993) indicates that the symbiosis between mycorrhiza and tobacco reduces the yield of commercial tobacco and is the cause of a poor growth condition called "tobacco stunt".

In this study, the foliar application of growth compounds resulted in significantly different dry weights of the lower and middle leaves. The maximum weight of lower leaves was recorded for the plants sprayed with seaweed extract, fulvic acid and fulvic acid + seaweed extract + urea. For the middle leaves it was for plants sprayed with distilled water and fulvic acid.

The lack of an effect of the growth compounds in increasing the dry weight of the middle and upper leaves can be attributed to two factors. Initially, these growth stimulants may have increased leaf quality, such as their colour, size, shape and soluble sugar content, which were not measured in this experiment. Secondly, since the inflorescence is cut off when the upper leaf of tobacco ceases growing (known as: Topping), thus removing a strong drain on photosynthetic products, which enables these products to be transferred to the growing leaves and therefore obscuring any effects the growth compounds may have had. The increase in the photosynthesis of the leaves may also be similarly determined.

Wickham and Davis (2015) also report that the application of seaweed extract does result in an increase in yield when the soil initially contains high levels of organic matter and NO3-N. The positive results of growth compounds are also reported by others such as: grain yield increase of 7.3–18.0% with application of fulvic acid (Xudan 1987), promoting effects of fulvic acid on dry weights of roots and shoots (Esringu et al. 2015), enhancing of antioxidant enzymes by fulvic acid (Lotfi et al. 2015), significant increase in plant height, number branches and the percentage dry matter in shoots of pepper after application of seaweed extracts (Marhoon and Abbas 2015) and increase in root growth, nutrient element absorption and stem thickness and growth after application of seaweed extracts (Jensen 2004). Sutharsan et al. (2014) also state that the foliar application of seaweed extract increases shoot dry weight (80.92%), root dry weight (81.57%), fruit number (57.87%), fruit yield per hectare (58.70%), Total Soluble Solids (25.71%) and Total acidity (76.95%) content of fruit over that in the control.

Conclusion

Tobacco is a cash crop grown in the northwest of Iran-Marivan and in order to increase the yield of leaves it is planted in fertile soil and treated with commercial fertilizers. This is likely to result in the entry of chemical and heavy elements in the human food chain and in damaging the environment. The application of bio-growth promoters was introduced as an environmentally friendly alternative to chemical fertilizers. In order to increase the yield of tobacco leaves, in this experiment a mycorrhizal fungus (*G. intaradices*) and the bio-stimulants fulvic acid, seaweed extract and chemical stimulant urea were used.

The results of this study indicate that the inoculation of mycorrhiza had positive effects on some of the characters studied, such as an increase in the number and dry weight of the middle leaves of tobacco. Inoculation with mycorrhiza increased the number and dry weight of the leaves by 13.88 and 4.72%, respectively, in comparison with the control. Since the number and weight of leaves determines the final yield and income of the farmers, increasing these components is very desirable. There were positive effects of mycorrhiza on other traits but they were not significant. It is concluded that due to irrigation and high rainfall in the area studied, the positive effects of mycorrhiza was less apparent than expected. In addition, soil fertility and the application of chemical fertilizers before planting may have reduced the positive effects of mycorrhiza. Lower colonization of plants by mycorrhiza is reported when the levels of potassium and magnesium in the soil are high (Zare-Maivan et al. 2017).

On the other hand, mycorrhiza has a more positive effect on plants subject to drought and high salinity. Fernández-Lizarazo and Moreno-Fonseca (2016) conclude that mycorrhiza can enhance the water potential, stomatal conductance and nutritional content of plants and finally result in an increase in plant growth during periods of drought. Also a higher dry mass and leaf area and enhancement of chlorophyll content, light energy absorption, gas exchange and Rubisco activity of plants inoculated with mycorrhiza growing under salinity stress is reported by Xu et al. (2018).

Corrêa et al. (2006) report that the response of plants to mycorrhiza depends on "their age (stage of development, leaf area), their initial nutritional status and the amount of nitrogen supplied". They conclude that colonization by mycorrhiza only increases the net photosynthesis rate in young plants and the application of high levels nitrogen inhibits the formation of mycorrhizal symbioses.

In this experiment, probably the effects of the mycorrhizal fungi was less that of the native strains in the soil, because the soil in the pots was sterilized, which is not possible to duplicate under field condition. In other research, higher dry and fresh weights and chlorophyll contents are reported in plants with mycorrhiza than in those grown in sterilized soil (Zare-Maivan et al. 2017).

Gazey et al. (2004) report that indigenous mycorrhizal fungi colonized a higher proportion of roots in two agriculture soils than introduced species. Hepper et al. (1998) compared three species of mycorrhizal fungus and confirm the superiority of indigenous over introduced species.

Williams et al. (2012), based on studies on six species of mycorrhiza, confirms the superiority of indigenous strains and state that it is better to use indigenous than commercial strains, both in terms of increased plant growth and cost, when regenerating forest.

Also based on the results of this study, the highest numbers of leaves were produced by seaweed and fulvic acid + seaweed extract + urea in the lower leaves side. In lower leaves, the maximum leaf dry weight was obtained from seaweed, fulvic acid and fulvic acid + seaweed extract + urea.

Fulvic acid and seaweed extract contain different macro and micronutrients that can enhance the growth and development of plants resulting in a better quality and greater yield. Matysiak et al. (2011) report that, the spraying of plants with seaweed extract and fulvic acid increased chlorophyll levels by 27–30% and shoot and root weight by 30–68%.

As most of the leaves used to produce tobacco are middle leaves, inoculating tobacco plants with mycorrhiza and the foliar application of growth compounds, especially seaweed extract, can be used to significantly increase the quantity and quality of these leaves.

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