

The Response of Okra (*Abelmoschus esculentus* L. Moench) to Inoculation with the Mycorrhizae and Spray with FeSO₄ and Anti-transpirant

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Abstract: The present experiment was conducted at one field of the research station (B) belongs to Horticulture Department/College of Agriculture/ Baghdad University during the summer season of 2017 in order to study the effect of the inoculation with the mycorrhizae and spray with FeSO₄ and Anti-transpirant (Armurox) on some growth characters and yield of okra *Abelmoschus esculentus* L. Moench. The experiment was lay out as a factorial experiment (2x3x2) in randomized complete block design (RCBD). The three factors used in this experiment included; the inoculation with mycorrhizae (M) (0 and 10 g. plant⁻¹), spray with FeSO₄ (F) (0, 0.5, and 1g. L⁻¹), and the Anti-transpirant 'Armurox' (A) (0 and 5 ml.L⁻¹). The results showed that the inoculation with mycorrhizae had positive effect on chlorophyll content, dry weight of shoot, total number of flowers and total yield with values of 51.15 mg.100 g⁻¹, 28.73%, 116.12 flower. plant⁻¹, 21.92 ton. hectare⁻¹, respectively. The M1F0A0 combination treatment was superior in total number of flowers per plant (132.90), while the M1F2A1 combination treatment was superior in chlorophyll content, dry weight and total yield with values of 68.23 mg. 100 g⁻¹, 31.82%, and 25.29 ton. Hectare⁻¹, respectively.

Keyword: Okra, mycorrhizae, FeSO₄, Anti-transpirant, dry weight, total yield.

I. INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench), which belongs to Malvaceae family, is considered as an important summer vegetable crop cultivated in most of tropical and subtropical regions of the world [1] ,[2],[3]. It is known as a good source of some vitamins such as B1, B2, B3, C, E, and K in addition to some mineral nutrition and protein, carbohydrate and unsaturated fats. Each 100g of pod contains 88.6 g water, 8.20 g carbohydrate, 2.10 g protein, 0.02 g fat, 1.70 g fiber, 90 mg phosphorus, 84 mg calcium, 1.2 mg iron, 0.60 mg nickel, 185 mg β-carotene, 0.02 mg B2 vitamin, 0.04 mg B3 vitamin, 47 mg ascorbic acid (vitamin C) in addition to 36 Kcal. Moreover, it was mentioned that mature okra seeds contain some of unsaturated fats [4].

Bio fertilizer refers to a substance that contains groups of living microorganisms participate in activating series of bio-reactions at which the supply or availability of some mineral nutrients is increased [5]. Siddiqui et al. [6] mentioned that the fungi as a component of bio fertilizers are characterized by producing secondary compounds which promote plant growth and yield. They contain some phytohormones such as IAA, cytokinins and GA₃ that excrete to the rhizosphere and translate to plant tissues via the symbiosis with the mycorrhizae. Also, fungi belong to this group is characterized by their ability to produce a protein called globine that acts to hold soil particles that increases the capability of soils to retain water for extended period of time [7]. Mycorrhizae is considered as the most important fungi for plants that may compensate for 50% of the chemical fertilizers added.

Gong et al.[8]studied the effect of three kinds of mycorrhizae on potato growth and yield and they found that the kind known *Glomus mosseae* caused an increase in plant height, fresh and dry weight of shoot, number of tubers in addition to increase in rate of phosphorus and nitrogen absorption. Segun et al.[9] found that the inoculation of okra with *G. mosseae* resulted in higher number and weight of pods per plant.

It was found that foliar application is suitable mean of fertilizing plants because of the less amount used especially the macronutrients [10] in addition to other features related to the stage of plant growth [11]. Iron is a micronutrient that play an important role in oxidation-reduction process in addition to its importance in carbon assimilation, biosynthesis of cytochromes and ferredoxin and as a result in increasing the growth rate [12]. Iron can make complicated compounds with the available organic matter to form the chelated compounds, which participate in supplying iron to plants as known siderophores [13]. It was found that spraying okra cv, phule utkarsha with FeSO₄ at 0.05% caused significant increase in yield components of plant [14].

Stress conditions that plants exposed to in Iraq such as high temperature, lack of irrigation in summer, increase of respiration, loss of water by the transpiration, all can cause adverse physiological changes that affect plant growth and development. In order to reduce those adverse effects, there is a need to use the Anti-transpirant compounds by spraying them on plants to help in increasing plant water efficiency use and reduce transpiration which considered as the main source of water loss at the later stage of plant growth [15]. Moussa [16], found that spray with the Anti-transpirant

(Folicote) on potato plants caused a gradual increase in tubers yield as the concentration of the Anti-transpirant increased from 0 to 15%.

Therefore, and due to the economic importance of okra, we use the inoculation with mycorrhizae and spray with FeSO₄ and Anti-transpirant (Armurox) in order to investigate their role in increasing plant growth and yield per unit area.

II. Materials and Methods

The present experiment was carried out at one field of the research station belongs to Horticulture Department/College of Agriculture/Baghdad University/Al-Jadria, during 2017 summer growing season. Seeds were sown at 1/3/2017 in seedling beds containing a mixture of peat moss and the mycorrhizae. The mycorrhizae inoculum contains an infected roots and spores and hypha of the fungi which was obtained from the Agricultural Research Office in Al-Zafarana District/ Baghdad/ Ministry of Science and Technology. The density of the inoculum was 51 spore. g-1 soil. A 100 gram of sterilized peat moss was placed in the transplant bed for mycorrhizae inoculation treatment, and 10 g of the inoculum was added in a way that the inoculum be in touch with the plant roots. The experiment field was divided into three blocks, each block consists of 12 treatments with a distance of 40 cm between plants and 70 cm between rows. Chemical and physical properties of soil of the field were analyzed at the graduate student laboratory /College of Agriculture/Baghdad University (table 1). Plants were irrigated using drip irrigation system. FeSO₄ was prepared as a solution at three concentrations; 0, 0.5 and 1g. L-1 and spray with these concentrations was done twice; first spray was after 45 days of planting and the second one after two weeks of the first spray. The Anti-transpirant was used at two concentrations; 0 and 5 ml. L-1 and the spray was done once after one week of the spray with the FeSO₄. Harvesting of pods was done for 35 times during the period from 9/5/2017 until 29/8/2017. At the end of the growing season, the following parameters were measured:

1. Chlorophyll content (mg.100g-1 fresh weight). A sample of 0.25 g fresh leaves was taken and extracted twice with acetone (80%). Then, the extraction was filtered and the absorption of the solution was read at 663 and 645 nm wave length using spectrophotometer. Total chlorophyll was calculated according to the following formula:
 Total chlorophyll (mg. 100g-1 FW) = 20.2 D (654 nm) + 8.02 D (663 nm) [17].
2. Dry weight percentage of shoot. Five plants were chosen randomly, weight, dried in the shade for several days, then dried in electrical oven at 70c for 72 days. After that, the dry weight was taken as a percent dry weight [18].
3. Total number of flowers was counted for five plants randomly chosen from the field.

Table 1: Physical and chemical properties of soil of the experiment

Analysis		Unit	Value	g.kg-1			
EC		ds.m -1	2.10	Sand	Silt	Clay	Texture
pH			7.23	892	60	48	Loamy sand
Dissolved Cations	Ca+2	mg.L-1	10.22				
	Mg+2		7.27				
	Na+		4.45				
Anion	HCO3-		Nil				
	CO3-2		Nil				
	Cl-		17.13				
	SO4-2		4.83				
NPK Available	N	%	0.004				
	P	mg.Kg-1 soil	10.13				
	K		104.3				
		%	0.73				
Bacterial and fungi number		Bacteria	25X10 -6				
		Fungus	24X10 -3				

4. Fruit set. It was calculated as follow:

$$\text{Fruit set (\%)} = (\text{total number of pods per plant} / \text{total number of flowers per plant}) \times 100 \text{ [19].}$$

5. Pod weight (g). It was calculated by dividing the weight of total pods in each experimental unit on the total number of pods of that experimental unit [20].
6. Total yield (ton. Hectare-1). It was calculated by dividing pod yield of the experimental unit on the area of that experimental unit multiply by 10000.

Treatments were arranged in randomized complete block design (RCBD) as a factorial experiment (2x3x2) with three replications. Treatments means were compared using LSD at 0.05 level.

III. Results and Discussion

1. Chlorophyll content in leaves (mg.100g-1 FW)

Results of table 2 showed that the inoculation with the mycorrhizae caused significant increase in chlorophyll content. Leaves of inoculated plants contain 51.15 mg.100g-1 FW in compare to 41.85 mg.100g-1 FW for the control. Also, spray with FeSO₄ caused a pronounced increase in chlorophyll content which reached an amount of 52.12 mg.100g-1 FW at F1 (0.5 mg.L-1) treatment while the control treatment recorded an amount of 39.29 mg.100g-1 FW. For the Anti-transpirant, it was clear from the same table that the spray with Armurox at 5 ml. L-1 (A1 treatment) was superior over control in chlorophyll content (50.37 mg. 100g-1 FW for A1 vs 42.62 mg.100g-1 FW for control). With regard to the interaction between the inoculation with mycorrhizae and the spray with FeSO₄, M1F2 combination treatment resulted in higher chlorophyll content (58.42 mg. 100g-1 FW) than that for the control (31.94 mg. 100g-1 FW) although it did not differ significantly from M0F1 combination treatment. Also, the M1A1 combination treatment (inoculation with the mycorrhizae along with the spray with the Anti-transpirant) resulted in an increase in chlorophyll content (54.68 mg. 100g-1 FW) over the control (37.63 mg. 100g-1 FW). In addition, combination between FeSO₄ and Anti-transpirant had also significant effect on chlorophyll content. At the F1A0 treatment, the chlorophyll content was 54.62 mg. 100g-1 FW in compare to 29.45 mg. 100g-1 FW for the control.

Table 2: Effect of the inoculation with mycorrhizae (M) and spray with FeSO₄ (F) and Anti-transpirant (A) and their interaction on chlorophyll content (mg. 100 g-1 FW) of leaves of okra cv. Petra.

A	F	M0	M1	A×F	Average
A0	F0	22.49	36.41	29.45	42.62
	F1	51.41	57.84	54.62	
	F2	38.98	48.61	43.79	
A1	F0	41.39	56.88	49.13	50.37
	F1	60.33	38.92	49.63	
	F2	36.50	68.23	52.37	
LSD		6.354		4.493	2.594
A x M					
A0		37.63	47.62	LSD= 3.669	
A1		46.07	54.68		
F x M					
F0		31.94	46.64	39.29	
F1		55.87	48.38	52.12	
F2		37.74	58.42	48.08	
LSD		4.493		3.177	
Average		41.85	51.15		
LSD		2.594			

For the interaction among the three factors, it was noted the M1F2A1 combination treatment recorded the highest chlorophyll content (68.23 mg. 100g-1 FW) while the lowest amount of chlorophyll was found at the control..

2. Percent of shoot dry weight

It was clear that the dry weight percent was affected by the three factors and their interaction. Dry weight percentage were 28.735%, 27.95% and 26.05% when plants treated with the mycorrhizae, FeSO₄ at 1g. L-1, and the Anti-transpirant at 5 ml. L-1, respectively in compare to 22.32%, 23.33%, and 25.01%, respectively, for the control.

Table 3: Effect of the inoculation with mycorrhizae (M) and spray with FeSO₄ (F) and Anti-transpirant (A) and their interaction on percent of shoot dry weight of okra cv. Petra.

A	F	M0	M1	A×F	Average
A0	F0	18.83	27.05	22.94	25.01
	F1	21.79	27.30	24.54	
	F2	24.45	30.62	27.53	
A1	F0	20.57	26.87	23.72	26.05
	F1	23.39	28.74	26.66	
	F2	24.92	31.82	28.37	
LSD		0.538		0.381	0.219
A x M					
A0		21.69	28.32	LSD= 0.311	
A1		22.96	29.14		
F x M					
F0		19.70	26.96	23.33	
F1		22.59	28.02	25.30	
F2		24.69	31.22	27.95	
LSD		0.381		0.269	
Average		22.32	28.73		
LSD		0.219			

For the bi-interaction, the M1F2 combination treatment was superior in giving higher percent of dry weight (31.22%) while the control gave the least percent (19.70%). In the same way, the M1A1 recorded the higher percent of dry weight amounted of 29.14% compare to 21.69% for M0A0. Also, F2A1 combination treatment resulted in higher percent of dry weight amounted of 28.37% compare to 22.94% for the control. It was clear from the same table that using the inoculation with the mycorrhizae along with the FeSO₄ spray at 1 mg. L⁻¹ and the Anti-transpirant (M1F2A1 treatment) recorded the higher percent of dry weight among other treatments.

The noticed increase in chlorophyll content and percent of dry weight of plant was due to the positive effects of the three factors used in this study. Wang and Song[21], have found that the inoculation with the mycorrhizae during the early stage of plant growth caused an increase in some vegetative parameters after transfer of plants to the field. Also, [22] have stated that the inoculation with VAM increased the rate of photosynthesis and increase the absorption of phosphorus. Same results were mentioned by [23]. In addition, the increase in chlorophyll content may be due to the important role of iron in synthesizing some compounds such as cytochromes and ferredoxin and their involvement in photosynthesis and increase growth rate. Also, it was noted that chlorophyll increased as the FeSO₄ levels increased and this was reflected in increasing photosynthesis and accumulating of dry matter [24],[12]. In addition, iron may increase the biological processes in plant due to its role in assimilating of nucleic acids and plastids and then increase the chlorophyll content and the efficiency of photosynthesis [25]. This result comes in agreement with results of [26], who found that iron enhanced the photosynthesis and plant growth of okra.

Anti-transpirant contains some amino acids and silicon. Some investigators showed that the increase in chlorophyll content may be due to the role of silicon in increasing plant tolerance in open field and increasing the water use efficiency under water stress in addition to increase absorption of calcium and potassium and increasing the antioxidants which all be reflected in increasing plant performance. The current result agreed with the results of [27],[28].

3. Number of total flowers

Table 4 showed that the inoculation with mycorrhizae increased significantly number of total flowers per plant. Inoculated plants had higher number of flowers (153.3) than the non-inoculated ones (105.9). Spray with FeSO₄ at 1 g. L⁻¹ recorded the highest number of flowers amounted of 140.5 while the control gave the lowest number of flowers (123.5). Use of the anti-respiration compound on plants had no effect.

For the interaction, the M1F0 treatment (interaction between mycorrhizae and FeSO₄) was superior in giving the highest number of flowers (164.3) which did not differ significantly from M1F1 treatment, while the control treatment

resulted in the least number of flowers (82.6). Also, interaction between the mycorrhizae and the anti-respiration had significant effect. The M1A1 combination treatment recorded the highest number of flowers amounted of 159.8 flower. Plant-1. Also, Interaction between the FeSO₄ and the anti-respiration had significant effect. The F2A1 combination treatment recorded the highest number of flowers amounted of 142.7 flower. Plant-1 Moreover, interaction among the three factors significantly affects the number of flowers. The highest number of flowers per plant was obtained at M1F2A1 combination treatment while the lowest number was at the control.

Table 4. Effect of the inoculation with mycorrhizae (M) and spray with FeSO₄ (F) and Anti-transpirant (A) and their interaction on total number of flowers (flower. plant-1) of okra cv. Petra

A	F	M0	M1	A×F	Average
A0	F0	78.7	167.0	118.0	127.0
	F1	96.7	161.1	126.3	
	F2	100.8	146.9	138.2	
A1	F0	100.0	141.1	128.9	131.7
	F1	100.7	120.8	123.3	
	F2	108.2	177.3	142.7	
LSD		10.53		7.40	N.S
A x M					
A0		108.4	146.7	LSD= 6.08	
A1		103.4	109.8		
F x M					
F0		82.7	164.3	123.0	
F1		100.7	144.0	124.8	
F2		129.4	101.0	140.0	
LSD		7.40		0.27	
Average		105.9	153.3		
LSD		4.30			

4.Fruit set

It was noted that neither the use of the mycorrhizae nor the Anti-transpirant had any effect on fruit set, while the spray with FeSO₄ at 1g.L-1 recorded the highest percent of fruit set (table 5) .

Table 5. Effect of the inoculation with mycorrhizae (M) and spray with FeSO₄ (F) and Anti-transpirant (A) and their interaction on percent of fruit set of okra cv. Petra.

A	F	M0	M1	A×F	Average
A0	F0	93.24	96.76	95.00	95.67
	F1	95.77	96.17	95.97	
	F2	96.90	95.16	96.03	
A1	F0	95.77	95.64	95.70	95.90
	F1	95.47	95.46	95.47	
	F2	95.91	97.14	96.52	
LSD		1.437		1.016	N.S
A x M					

A0	95.30	96.03	LSD= 0.830NS
A1	95.72	96.08	
F x M			
F0	94.50	96.20	95.35
F1	95.62	95.82	95.72
F2	96.40	96.15	96.28
LSD	1.016		0.719
Average	95.51	96.05	
LSD	N.S		

The same table showed that the bi-interaction had significant effect on fruit set. The M0F2 combination treatment gave the highest value of fruit set amounted of 96.40% while the control treatment gave the least value (94.50%). Interaction between the mycorrhizae and the spray with the anti-respiration had no significant effect on fruit set. For the interaction between the spray with FeSO₄ and the Anti-transpirant, the F2A1 combination treatment gave the highest percent of fruit set (96.52%). Interaction among the three factors caused significant increase in fruit set. The fruit set was 97.14%, 96.90%, 96.76 and 96.17% at the combination treatments M0F2A0, M1F0A0, M1F0A0 and M1F0A0, respectively, in compare to 93.24% for the control.

5. Pod weight (g. pod-1)

Inoculation with the mycorrhizae caused significant decrease in pods weight (6.20 g. pod-1) compare to control (6.43 g. pod-1) while spray with the Anti-transpirant cause significant increase in pod weight which was 6.47 g. pod-1 compare to 6.17 g. pod-1 for the control. Spray with the FeSO₄ at both concentrations had no effect (table 6).

Table 6. Effect of the inoculation with mycorrhizae (M) and spray with FeSO₄ (F) and Anti-transpirant (A) and their interaction on pod weight (g. pod-1) of okra cv. Petra.

A	F	M0	M1	A×F	Average
A0	F0	6.18	6.09	6.13	6.17
	F1	6.77	6.13	6.45	
	F2	6.15	5.70	5.92	
A1	F0	6.53	6.22	6.38	6.47
	F1	6.66	6.24	6.45	
	F2	6.32	6.80	6.56	
LSD		0.540		0.382	0.220
A x M					
A0		6.36	5.97	LSD= N.S	
A1		6.50	6.42		
F x M					
F0		6.35	6.16	6.25	
F1		6.72	6.19	6.45	
F2		6.23	6.25	6.24	
LSD		0.382		N.S	
Average		6.43	6.20		
LSD		0.220			

Interaction between the inoculation with the mycorrhizae and FeSO₄ at 0.5 g.L-1 recorded the highest pod weight which was 6.72 g. pod-1 while the control treatment gave the lowest weight (6.35 g. pod-1). Also, interaction between the

mycorrhizae and the spray with FeSO₄ caused significant effect with the M0A1 combination treatment recorded the highest pod weight of 6.50 g. pod-1 in compare to 6.36 g.pod-1 for the control. Combination between spray with FeSO₄ and Anti-transpirant had significant effect also. The F2A1 treatment recorded the highest value which did not differ from F1A1, F1A0 and F0A1 treatments. In addition, combination among the three factors caused significant increase in pod weight. The highest weight was obtained at M1F2A1 combination treatment but it did not differ significantly from M0F1A0, M0F1A1, M0F0A1 and M0F2A1 treatments, while the control gave the lowest weight (6.18 g. pod-1).

6.Total yield (ton. Hectare-1)

Results of table 7 indicated a significant increase in total yield due to the inoculation with the mycorrhizae. Total yield reached 21.92 ton. Hectare-1 when plants inoculated with the mycorrhizae (F1) while the yield was 16.54 ton. Hectare-1 for the non-inoculated plants. Also, plants sprayed with FeSO₄ at 1 g. L-1 gave an average yield of 20.24 ton. Hectare-1 compare to 18.11 ton. Hectare-1 for the control. In the same way, plants sprayed with the Arumox get an average yield of 19.65 ton. Hectare -1 while the yield was 18.81 ton. Hectare-1 for the control.

Table 7. Effect of the inoculation with mycorrhizae (M) and spray with FeSO₄ (F) and Anti-transpirant (A) and their interaction on total yield (ton. hectare-1) of okra cv. Petra

A	F	M0	M1	A×F	Average
A0	F0	12.77	24.66	18.71	18.81
	F1	18.38	21.31	19.85	
	F2	19.18	16.58	17.88	
A1	F0	14.01	20.99	17.50	19.65
	F1	18.53	22.72	20.62	
	F2	16.37	25.29	20.83	
LSD		1.702		N.S	0.695
A x M					
A0		16.77	20.85	LSD= 0.983	
A1		16.30	23.00		
F x M					
F0		13.39	22.82	18.11	
F1		18.46	22.02	20.24	
F2		17.77	20.93	19.36.	
LSD		1.204		0.851	
Average		16.54	21.92		
LSD		0.695			

The same table showed that the bi-interaction had significant effect. The M1F1 combination treatment gave an average yield of 20.93 ton. Hectare-1 which did not differ from the M1F1 combination treatment while the control treatment recorded an average yield of 13.39 ton. Hectare-1. For the interaction between the mycorrhizae and the spray with the anti-respiration, the M1A1 combination treatment resulted in an average yield of 23.00 ton. Hectare-1 in compare to 16.77 ton. hectare-1 for the control. Interaction between the FeSO₄ and the Anti-transpirant had no effect on total yield. Interaction among the three factors had also significant effect on total yield. The highest yield was achieved at the M1F2A1 combination treatment with an average of 25.29 ton. Hectare-1 while the control treatment (M0F0A0) gave the least amount of total yield (12.77 ton. hectare-1).

The increase in values of the parameters measured in this study came as result of the inoculation with the mycorrhizae and the spray with the FeSO₄ and the Anti-transpirant "Armurox". It is known that the inoculation with the mycorrhizae promotes the plant growth via the strategic systems by which these microorganism work especially the availability of mineral nutrients and increasing plant tolerance to adverse biotic and a biotic environmental stresses in addition to the production of some phytohormones [29]. Also, the increase in total yield of inoculated plants with the mycorrhizae may be due to the role of the microorganism in improving root growth of plants through the formation of vigor root system. These results come in accordance with the results of [30] on pepper and [31] on cotton and pepper and [32],[33] on tomato and pepper. Moreover, iron is believed to activate the oxidation-reduction processes in addition to its

role in chlorophyll biosynthesis and in turn promoting plant growth [24],[34]. Also, the increase in plant yield due to the use of the anti-respiration 'Armurox' was attributed to the promoting effect of amino acids and silicon as components of the Armurox [35].

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