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INFLUENCE OF GREENHOUSE SHADING AND DIFFERENT NUTRIENT MANAGEMENT PRACTICES ON ROOT COLONIZATION BY AMF AND PLANT ROOT ARCHITECTURE OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

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Abstract

The experiment was conducted in a greenhouse during 2017 and 2018 growing seasons to evaluate the impact of the shading and various nutrition programs on percentage root colonization by AM fungus and root growth characteristics of tomato plant (Newton-F1). Split-plot within Randomized Complete Block Design (RCBD) with three replications was conducted in this study. Shading factor was allocated in the main plots and the nutrition programs distributed randomly in the subplots. Results indicate that shading resulted in the decrease of daytime temperature by 5.7°C and increase minimum relative humidity by 11.2% as an average for both seasons, thus a significant increasing was found in main root length and root dry weight. Among the plant nutrition programs, the integrated nutrient management (INM) including the application of organic substances, bio inoculum of AMF and 50% of the recommended dose of chemical fertilizers, lead to the enhancement of root colonization by AMF as well as all root growth characteristics. Generally, combination of both shading and INM showed positive effects and recorded the maximum values of main root length and diameter, secondery root length and root dry weight in 2017, as well as percentage root colonization and secondery root diameter in 2018 growing season.

Key words: root characteristics, biostimulants, amino acids, humic substances, mycorrhizae.

Introduction

Plant survival and fitness pretty much rely on root system architecture. Root characteristics, such as deep root systems, root density in subsoil, secondary root length and diameters as well as root surface area may contribute to enhance water and nutrient uptake. So, modification of root architecture could contribute to improvements of desirable agronomic traits such as vegetative and flowering growth and thereby the plants yield in terms of quantiti and quality, in addition to plants enhancement of resistance to abiotic stresses (Siddique *et al.*, 2015). Furthermore, Root system traits are important in view of current challenges such as sustainable crop production with reduced fertilizer input or in resource-limited environments (Leitner *et al.*, 2014).

The plant root system architecture seems to be strongly regulated by external conditions (such as high temperature), plant nutrition *and* genetic background (Morte and Varma, 2014).

Heat accumulation inside the greenhouses in late spring and summer seasons due to high and long duration of solar radiation leads to expose cultivated plants to high temperature stress. Many literatures indicated that high temperature stress causes various negative effects on the plants growth and development, including root growth characteristics (Wang *et al.*, 2016). Several techniques are used to mitigate the effects of heat stress such as the decrease of light intensity by shading, which is one of the simplest, non-chemical, inexpensive and sustainable approaches to modify the greenhouses environmental conditions in hot seasons.

Also, proper plant nutrient management is an important tool to improve the plant roots architecture. Poultry manure has a significant role on soil fertility and structure through acting on chemical, physical and

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biological properties of soils, thereafter, these improves root architecture and increase nutrient uptake by plants (Ewulo *et al.*, 2008).

Previous studies reported that a variety of biostimulant substances (*i.e.*, humic and fulvic acids, hdrolysed proteins and amino acids containing products) and microbial inoculants (*i.e.*, mycorrhizal fungi) have been introduced as efficient, safe and sustainable tools to optimize root system, boosting crop performance, improving nutrient use efficiency as well as enhancing tolerance to heat stress (Bulgari *et al.*, 2019; Canellas *et al.*, 2015; Duc *et al.*, 2018).

Bio-fertilizer of arbuscular mycorrhizal fungi (AMF) is one of the most important groups of beneficial soil biota, which are establishing mutualistic symbioses with the root systems of approximately 80% of plants, including the most important agricultural crops (Berruti *et al.*, 2015; Smith and Read, 2008). Recently, using the AMF has gained more attention since studies revealed their beneficial effects and ecological sounds especially in sustainable and organic agriculture (Avio *et al.*, 2017; Giovannetti *et al.*, 2012). In addition, AMF plays a significant role in plant performance and nutrition due to its capacity to improve plant minerals uptake (Smith and Read, 2008) by absorbing and translocating mineral nutrients beyond the depletion zones of plant rhizosphere (Rouphael *et al.*, 2015). Moreover, AMF interfere with the phytohormone balance of the plant, thereby it is influencing plants root development and alleviating the effects of biotic and abiotic stresses, which ultimately leads to enhance the biomass accumulation, yield and various quality characteristics (Antunes *et al.*, 2012).

Considering the above-mentioned facts, it is possible to improve the root growth characteristics by applying chemical fertilizers in combination with organic substances and biofertilizers. Therefore, the aims of this study were to examine the influence of shading and different nutrition programs including chemical, organic substances and bio fertilizer of AMF as a biostimulants, alone or in combinations on roots colonization by AM fungus, improving root growth arcitecture of tomato plants under uncontrolled greenhouse conditions.

Materials and Methods

Experimental site and soil analysis

The experiment was carried out during 2017 and 2018 growing seasons at the research farm belongs to the department of Horticulture, College of Agricultural

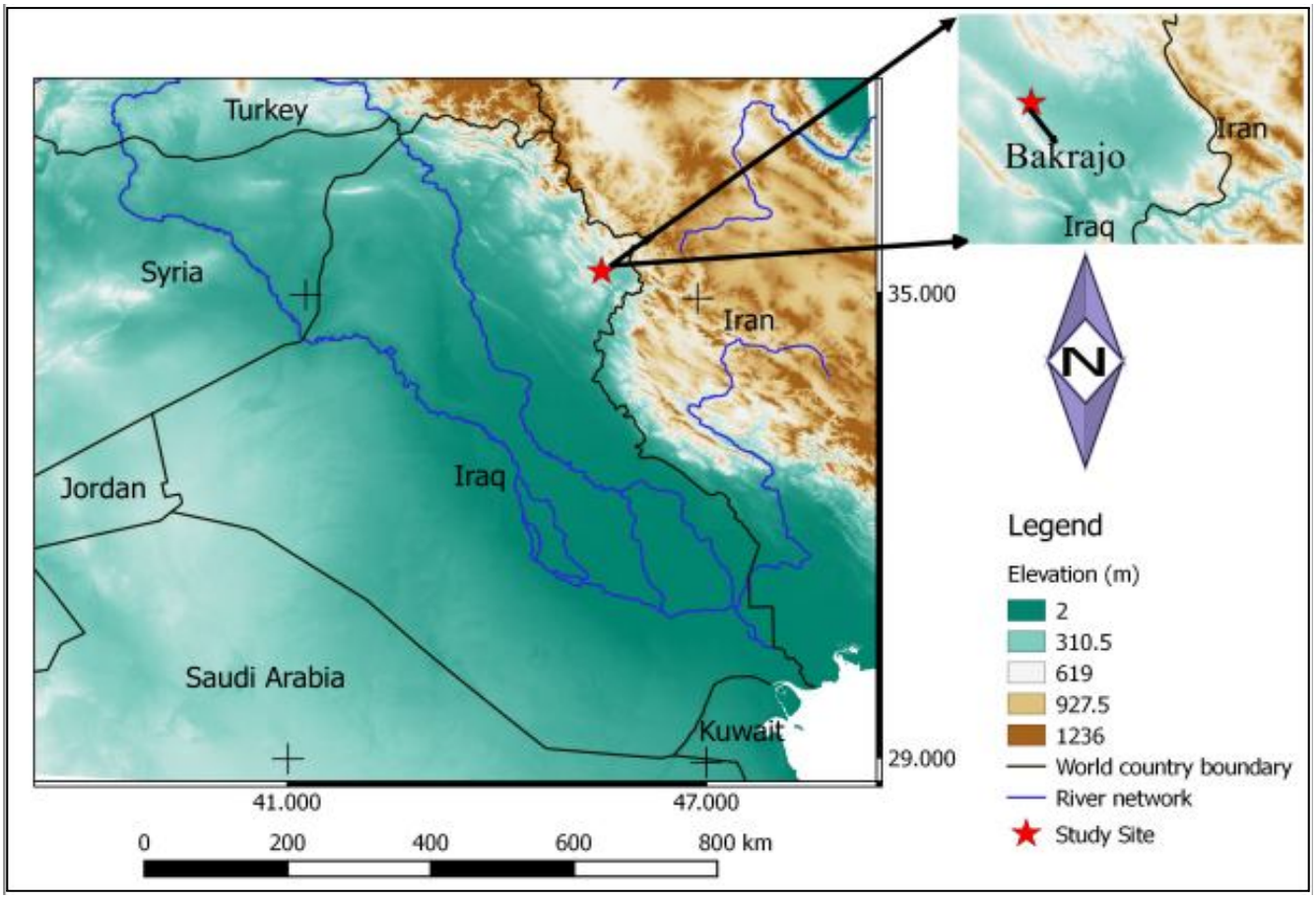

Fig. 1: Regional location of the study site.

Table 1. Some physical and chemical properties of the experiment soil.

Texture	Sand	Silt	Clay	pH	EC	$CaCO_3$	O.M	Total N	Soluble K	Available P	Available Fe
	g kg-1				dS m^{-1}	g kg^{-1}				mg kg^{-1}	
Silty Clay	97.9	439.5	462.6	7.97	1.04	267	10.9	13.7	56.4	6.6	2.91

Engineering Sciences, Sulaimani University, Bakrajo, Sulaimani, Iraq (35° 32' 9.6" N, 45° 21' 54" E) with an altitude 741 masl, in a greenhouse (40 m length, 11 m width, 3.9 m height) covered with 200µm thick polyethylene plastic film. The climate of the study area is classified as arid and semi-arid region which is hot and dry in summer and cold in winter (Najmaddin *et al.*, 2017). GIS software was used to create the study site as shown in (Fig. 1). Soil samples were taken at (0-30 cm) depth in order to determine the baseline soil properties. Samples were air dried and passed *via* a 2mm sieve prior to analysis. Results of some chemical and physical properties of the soil are shown in Table 1.

Plant materials, seedling production and transplanting

An indeterminate F1-hybrid tomato cultivar (Newton-F1) produced by Syngenta® was used in this study. Seeds were sown on 15th February 2017 and 2018 in 54-well seedling trays, which filled with sterilized peat-moss (TS 1, Klasmann-Deilmann GmbH). The seeds were sown under glasshouse conditions and maintained at 23/18 ± 2°C day/night temperature, 14/10 h. light/dark photoperiod and a relative humidity of 65 ± 10%. After reaching at 4-5 true-leaf stage, the seedlings were transplanted to the experimental units. Seedlings were planted in black polyethylene bags (18.5 cm width, 45 cm height and 12 kg of soil capacity) to facilitate getting the whole roots system easily and safely. In order to improve drainage, a layer of 5 cm of gravel was placed in the bottom of the plastic bags.

Experimental design and treatments detail

Split-plot within Randomized Complete Block Design (RCBD) with three replications was conducted in this study. Shading factor was allocated in the main plots and the nutrition programs distributed randomly in the subplots.

The greenhouse was divided into two longitudinal halves that one half was covered with the shade net above the plastic cover to reduce the light intensity by relatively 40%. While the other half was free of the shade net covering. The shading process was implemented in the middle of May, when the weather temperature started to warm up.

Regarding the nutrition treatments, eight nutrition programs were arranged randomly as a sub plots within each replicate in the main plots as the following: *T1*: Absolute control, *T2:* Full recommended dose of chemical fertilizer (100% RDCF). The application included macro and micro nutrients and applied in two methods: soil and foliar application (Table 2). This treatment was

Table 2: Applied full recommended dose of chemical fertilizers (100% RDCF).

Weeks after transplanting	Chemical fertilizers types [1] (Soil application)	Dosages (g $plant^{-1}$)	Chemical fertilizers types (Foliar application)	Dosages (g L^{-1} or ml L^{-1})
5th	NPK15-30-15	1.5	NPK[2] 12-48-8 + CALMAX[3]	2
6th		2.5	NPK 12-48-8 + CALMAX	2
7th		3	NPK 12-48-8 + CALMAX	2
8th	NPK20-20-20	3	NPK 20-20-20 + CALMAX	2
9th	Calmag+ZnN-P-K-CaO-MgO-Zn 13-0-0-16-6-0.2	3	NPK 20-20-20 + CALMAX	2
10th	NPK15-30-15+20-20-20 (1:1)	3	NPK 20-20-20 + CALMAX	2
11th		3	NPK 9-15-30 + CALMAX	2.5
12th	Calmag+ZnN-P-K-CaO-MgO-Zn 13-0-0-16-6-0.2	3	NPK 20-20-20 + CALMAX	2.5
13th	NPK20-20-20+12-8-40 (1:1)	3	NPK 9-15-30 + CALMAX	2.5
14th		3	NPK 20-20-20 + CALMAX	2.5
15th	NPK12-8-40	3.5	NPK 9-15-30 + CALMAX	2.5
16th		3.5	NPK 9-15-30 + CALMAX	2.5
17th		3.5	NPK 9-15-30 + CALMAX	2.5

[1] SANGRAL™ fertilizers (SQM Iberian SA, Barcelona, Spain) were used for soil application.
[2] NUTRI-LEAF® fertilizers (NPK) manufactured by (Miller chemical & fertilizer, LLC, Hanover) were used for foliar application.
[3] The chemical composition of the foliar liquid fertilizer CALMAX (Omex, UK) in (w/v) units is as follows: Total Nitrogen (N) 15%, Calcium (CaO) 22.5%, Magnesium (MgO) 3%, Manganese (Mn EDTA) 0.15%, Iron (Fe EDTA) 0.75%, Boron (B) 0.75%, Copper (Cu EDTA) 0.06%, Zinc (Zn EDTA) 0.03%.

Table 3: Monthly maximum, minimum and average air temperature (°C) inside the greenhouse compartments during both growing seasons 2017 and 2018.

Months	2017						2018					
	Without shading			With shading			Without shading			With shading		
	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
May	37.2	14.9	29.5	32.2	14.6	25.1	34.1	15.2	27.3	28.1	16.3	26.3
June	42.4	17.4	32.8	37.2	17.1	29.4	40.4	18.1	30.8	34.8	17.9	28.2
July	46.1	22.5	36.1	39.8	22.3	32.2	43.9	21.4	32.8	38.2	20.8	30.6
August	49.3	22.8	37.2	42.1	20.7	33.7	46.4	20.8	33.9	39.9	20.3	31.2
September	43.6	20.3	33.8	38.9	19.4	30.9	44.1	20.1	31.7	39.2	19.1	30.3

implemented after four weeks of transplanting, *T3:* Organic nutrition program (ONP), the locally produced poultry manure (SHAMAL) was added at a rate of (5 t ha^{-1}). The following two liquid organic fertilizers as a biostimulants were also added: (i) HUMATE, which contains 25% humic and fulvic acids, 4% N, 4% K and 1% Fe, was applied (2L ha^{-1}) six times during the growing seasons, the first application was conducted after four weeks of transplanting and the others at 10 days intervals. (ii) VEGEAMINO, which contains 24.8% w/v free amino acids was added by foliar spraying (1ml L^{-1}), which applied once every three weeks from transplanting for 4 times. *T4:* the microbial biostimulant of arbuscular mycorrhizal fungi (AMF), *Glomus mosseae*, was conducted by applying 25g of the inoculum per plant during the transplanting time in case which most of the seedling roots were attached the inoculum. Each gram of the inoculum contains approximately 47 spores of the fungus. The inoculum obtained from the Al-Zaefaraniya Agricultural Research Center, Ministry of Sciences and Technology, Baghdad. *T5:* ONP+AMF, *T6*: ONP+50% RDCF, *T7:* AMF+50% RDCF, *T8:* Integrated Nutrient Management (INM), which included (ONP+AMF+50% RDCF).

Growth conditions

During the experimental period, the air temperatures and relative humidity inside the greenhouse compartments were measured by using data logger device (Model: Perfect-Prime TH0160) with fifteen-minute intervals. One device was placed in the center of each compartment at 1.5m above the seedlings. Maximum, minimum and average of air temperatures and relative humidity during the growing seasons inside the greenhouse were summarized in Tables 3 and 4.

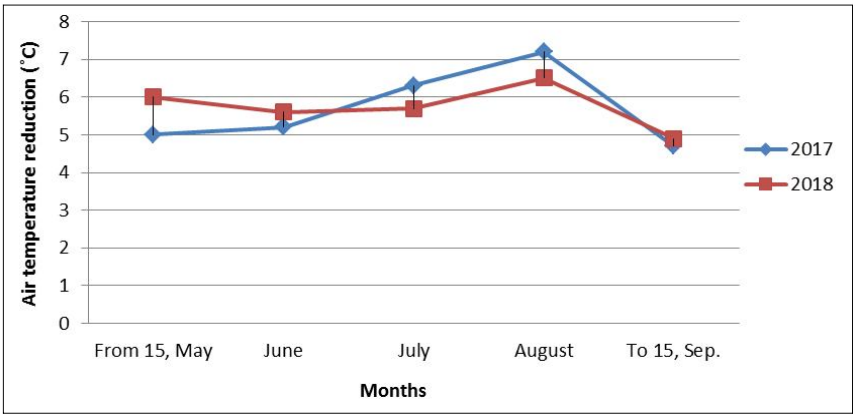

Fig. 2: Effect of greenhouse shading on the air daytime temperature reduction during 2017 and 2018 growing seasons.

Statistical data analysis

Data were submitted to the analysis of variance (Two-way ANOVA) using JMP 7.0.1 statistical analysis software. Least Significant Difference (LSD) test at $P \leq 0.05$ was used to compare the means.

Measurements

At the end of the seasons, the root system was extracted manually for two representative plants. The roots were carefully cleaned from the dirt using tap water as described by (Allawi, 2013; Liang *et al.*, 2010) and the following parameters were recorded: Root colonization (RC) by AM fungus, Main root length (MRL), Main root diameter (MRD), Secondary root length (SRL), Secondary root diameter (SRD), Root surface area (RSA) and Root dry weight (RDW).

For determination of root colonization (RC) by AM fungus, root samples were rinsed carefully with tap water and then stored in a weak formalin, acetic acid and alcohol (FAA) solutions (50, 50 and 900ml respectively) at room temperature until ready for clearing and staining process. The roots were chopped into approximately 10mm segments and then the segment samples were cleared with 10% KOH at 90°C for 20 minutes. The root segments were washed with distilled water and placed in 10% hydrogen peroxide (H_2O_2) at the room temperature for 60 minutes and washed again with distilled water. Lastly, the samples were soaked in 1% of HCl for three minutes. After finishing clearing, the samples were stained with 0.01% (w/v) acid fuchsin in lactoglycerol (14:1:1

Table 4: Monthly maximum, minimum and average relative humidity (%) inside the greenhouse compartments during both growing seasons 2017 and 2018.

Months	2017						2018					
	Without shading			With shading			Without shading			With shading		
	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
May	68.0	27.2	42.2	69.5	36.8	48.2	70.4	45.6	61.2	72.8	51.4	66.8
June	54.6	22.9	34.8	61.6	33.8	43.2	63.1	32.5	41.3	69.8	43.1	49.7
July	44.6	21.6	29.5	55.3	34.1	40.1	60.0	25.9	41.8	61.5	36.2	44.7
August	47.5	19.6	30.6	62.0	35.3	44.3	57.6	22.5	36.3	65.7	38.1	50.4
September	49.0	16.3	28.9	57.7	28.1	39.5	60.8	30.6	40.6	69.1	39.7	52.6

lactic acid, glycerol and water), according to (Kormanik *et al*., 1980). Following that, percentage of root colonization calculated by manifesting 10 root segments under an optical microscope at 40X magnifications (Giovannetti and Mosse, 1980) and then the root colonization percentage was calculated as the following equation:

$$\text{Root colonization (\%)} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments examined}} \times 100$$

RSA was measured after extraction the roots from the soil by photographing technique using a digital camera. The Digimizer software version 4.5 was used to analyze the root images to calculate the surface area of the roots (Allawi, 2013). RDW was measured by placing the root samples in the forced-air oven (Model: LOD-250N, LabTech®, Korea) at 70°C for about 72h., until the weight was stable and then the dried samples were weighted by a digital scale.

Results

Effects of greenhouse shading on air temperature reduction and increasing the relative humidity

Shading had significant impact on reducing daytime air temperature inside the greenhouse from the middle of the May to the middle of the September for two consecutive growing seasons 2017 and 2018. The greenhouse shading decreased the average of the maximum air temperature by 5, 5.2, 6.3, 7.2 and 4.7°C in 2017 and 6, 5.6, 5.7, 6.5 and 4.9°C in 2018 for the months of May, June, July, August and September, respectively. The overall reduction of the air temperature in daytime for the both seasons was 5.7°C (Table 3 and Fig. 2).

Furthermore, the greenhouse shading increased the average percentage of the minimum relative humidity by 9.6, 10.9, 12.5, 15.7 and 11.8°C in 2017 and 5.8, 10.6, 10.3, 15.6 and 9.1 in 2018 for the months of May, June, July, August and September, respectively. The overall increasing of the relative humidity in daytime for the both seasons was 11.2% (Table 4 and Fig. 3).

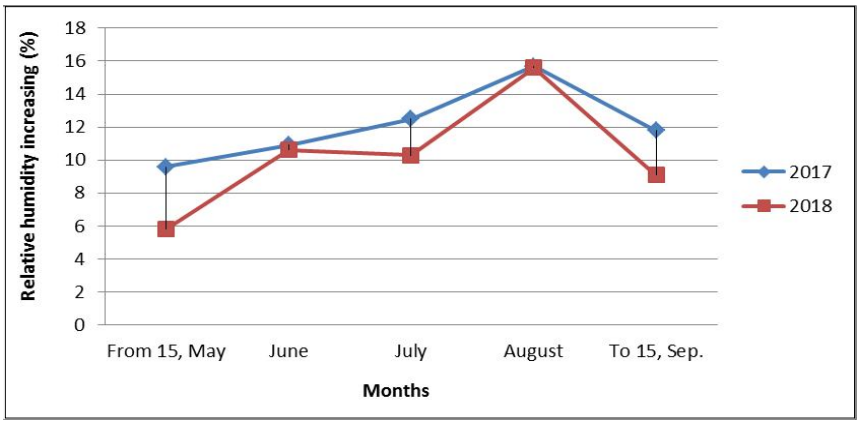

Fig. 3: Effect of greenhouse shading on the air daytime temperature reduction during 2017 and 2018 growing seasons.

Effects of shading on plant root colonization by AMF and root architecture

The impacts of greenhouse shading on tomato root colonization by AM fungus and root architecture characteristics are shown in Table 5. Based on the outcomes, if compared to plants in non-shade circumstances, the shading factor results in a substantial rise in the main root length and root dry weight in both seasons. It was recorded (57.38 and 56.15 cm) for the main root length and (47.73 and 49.99 g) for the root dry weight in seasons 2017 and 2018, respectively. While there were no substantial variations in the percentage root colonization, main root diameter, secondary root length, secondary root diameter and root surface area during both seasons between plants growing under shade and non-shade conditions.

Effects of different nutrition programs on plant root colonization by AMF and root architecture

Tomato root colonization by AM fungus and root architecture characteristics were affected significantly by different nutrition programs in both seasons (Tables 6 and 7). In the first growing season (2017), the nutrition program (ONP+AMF) registered the highest percentage of roots colonization (74.17%) which was not different

Table 5: Effects of greenhouse shading on plant root colonization by AMF and root architecture in 2017 and 2018 growing seasons.

Effect of shading	RC (%)	MRL (cm)	MRD (mm)	SRL (cm)	SRD (mm)	RSA (cm^2)	RDW (g)
First season (2017)							
Without shading	36.46 a	49.90 b	3.31 a	22.47 a	1.06 a	114.90 a	40.47 b
With shading	37.71 a	57.38 a	2.79 a	24.38 a	1.08 a	130.79 a	47.73 a
LSD $P \leq 0.05$	n.s	6.890	n.s	n.s	n.s	n.s	5.396
Second season (2018)							
Without shading	37.29 a	51.08 b	4.23 a	25.56 a	1.03 a	122.96 a	42.63 b
With shading	41.04 a	56.15 a	4.02 a	26.45 a	1.06 a	141.54 a	49.99 a
LSD $P \leq 0.05$	n.s	4.169	n.s	n.s	n.s	n.s	4.160

substantially with the (INM) which registered (67.50%). Furthermore, the integrated nutrient management (INM) registered the highest values of main root length (64.00cm) and diameter (4.18mm), secondary root length (30.08cm) and root dry weight (59.13g) which it was significantly superior to the control treatment and majority of the other nutrition programs. In addition, plants that colonized with AMF gave the highest value of secondary root diameter (1.20mm) while this treatment was not different significantly with the other nutrition programs in this character, but it was only overcome the control treatment. Concerning the average of the root surface area, the nutrition program (ONP+50%RDCF) measured the highest value (146.34 cm^2) which is statistically distinct only with control and AMF treatments (Table 6).

Similar results were observed in the second growing season (2018). No significant differences were found between the nutrition programs (ONP+AMF and INM) in the percentage roots colonization. They registered the highest percentage (75.83 and 74.17%) respectively. Whereas, the lowest percentage (2.50 and 6.67%) was recorded by plants that treated with (100% RDCF) and control treatments respectively. Concerning to the root characteristics, the nutrition program (ONP+50% RDCF) registered the longest main root (61.25cm), which differed significantly from the control treatment and the nutrition programs (100% RDCF, ONP and AMF). The maximum value of the main root diameter (4.81mm) was obtained from (ONP+AMF) and it was significantly overcome the control and AMF treatments. There were no significant differences in secondary root length among nutrition programs, while all of them were considerably superior over the control. The highest value of secondary root diameter was recorded by the nutrition programs (INM and ONP+AMF) (1.13mm) which they were not statistically different from the other treatments except the control. Treatments of (INM and ONP+AMF) significantly improved the average root surface area, by supplying 164.24 and 158.03 cm^2 respectively, compared to most other treatments, including control. Finally, the nutrition program (ONP+AMF) clearly influenced the root dry weight that was given (52.16g), while this treatment did not showed significant differences with the treatments of (ONP+50% RDCF, AMF+50% RDCF and INM) in this trait.

Notably, the minimum values of all parameters that related to the tomato root architecture were observed in the control treatment in the both growing seasons (Table 6 and 7).

Combination effects between greenhouse shading and different nutrition programs on plant root colonization by AMF and root architecture

Tables 8 and 9, demonstrate the combined impacts between greenhouse shading and different nutrition programs on the tomato root colonization and root

Table 6: Effects of different nutrition programs on plant root colonization by AMF and root architecture in 2017 growing season.

Nutrition programs	RC (%)	MRL (cm)	MRD (mm)	SRL (cm)	SRD (mm)	RSA (cm^2)	RDW (g)
Control	2.50 d	39.58 e	1.79 d	18.25 d	0.91 b	86.76 c	27.50 e
100% RDCF	1.67 d	52.25 cd	2.91 bc	21.25 bcd	1.03 ab	123.83 ab	38.98 d
ONP	17.50 c	60.75 ab	2.89 bc	22.25 bcd	1.05 ab	120.53 ab	44.73 bcd
AMF	61.67 b	48.00 d	2.50 cd	19.67 cd	1.20 a	99.92 cb	42.03 cd
ONP + AMF	74.17 a	62.42 ab	3.46 ab	24.97 abc	1.05 ab	140.39 a	48.58 b
ONP + 50% RDCF	11.67 c	56.08 b	3.28 b	26.25 ab	1.10 a	146.34 a	45.98 bc
AMF + 50% RDCF	60.00 b	46.00 de	3.41 b	24.67 abc	1.07 ab	131.27 a	45.85 bc
INM	67.50 ab	64.00 a	4.18 a	30.08 a	1.14 a	133.74 a	59.13 a
LSD $P \leq 0.05$	8.770	7.287	0.734	5.502	0.187	27.214	5.910

Table 7: Effects of different nutrition programs on plant root colonization by AMF and root architecture in 2018 growing season.

Nutrition programs	RC (%)	MRL (cm)	MRD (mm)	SRL (cm)	SRD (mm)	RSA (cm^2)	RDW (g)
Control	6.67 d	46.25 e	3.16 c	19.83 b	0.87 b	97.39 d	29.88 d
100% RDCF	2.50 d	52.83 bcd	4.27 ab	25.14 a	1.00 ab	129.28 bc	45.01 c
ONP	18.33 c	49.92 cde	4.34 ab	28.14 a	1.06 a	125.71 c	46.11 bc
AMF	59.17 b	47.58 de	3.74 bc	26.09 a	1.01 ab	98.81 d	46.04 bc
ONP + AMF	75.83 a	59.25 ab	4.81 a	28.51 a	1.13 a	158.03 a	52.16 a
ONP + 50% RDCF	26.67 c	61.25 a	4.06 abc	28.40 a	1.12 a	148.21 ab	48.70 abc
AMF + 50% RDCF	50.00 b	54.75 abc	4.23 ab	24.87 a	1.05 a	136.35 bc	51.06 ab
INM	74.17 a	57.08 ab	4.38 ab	27.05 a	1.13 a	164.24 a	51.52 ab
LSD P ≤ 0.05	11.072	6.510	0.951	4.421	0.142	21.636	5.913

architecture during both seasons. At the first growing season (2017), the nutrition program (ONP+AMF) without shade conditions (Non-shade × ONP + AMF) recorded the maximum percentage of the roots colonization (75.00%) which was significantly different with the majority of the other combinations except (Non-shade × INM), (Shade × INM) and (Shade × ONP + AMF). The root system in the plants that fertilized with full recommended dose of chemical fertilizers (100% RDCF) was not colonized by AMF. The nutrition program (INM) under shade conditions (Shade × INM) recorded the maximum values of the main root length (74.00cm), main root diameter (4.22mm), secondary root length (31.83cm) and roots dry weight (64.13g). This treatment showed extremely important distinctions with all the other treatment combinations in roots dry weight and most other treatment combinations in the main root length and diameter as well as secondary roots length, are overcome. Tomato plants which colonized with AMF and grown in non-shade compartment (Non-shade × AMF) gave the maximum value of secondary roots diameter (1.23mm) which was significantly superior only over control plants whether grown under shade or non-shade circumstances. As for the average surface area of the roots, plants cultivated in shade compartment and fertilized with ONP + 50% RDCF (Shade × ONP + 50% RDCF) registered the largest value (157.96cm^2) although it did not differ with some other treatments including (Shade × INM) (Table 8).

In the second season (2018). the highest percentage of the roots colonization (80.00%) was stated from the combination of (Shade × INM). This treatment showed extremely important distinctions with the majority of the other treatment combinations, with exception of (Shade × ONP + AMF), (Non-shade × INM) and (Non-shade × ONP + AMF). The minimum percentage of the roots

Table 8: Combination ffects between greenhouse shading and different nutrition programs on plant root colonization by AMF and root architecture in 2017 growing season.

Effect of shading	Nutrition programs	RC (%)	MRL (cm)	MRD (mm)	SRL (cm)	SRD (mm)	RSA (cm^2)	RDW (g)
Without shading	Control	3.33 efg	39.83 f	1.96 gh	17.83 e	0.89 c	80.13 f	23.17 h
	100% RDCF	0.00 g	56.67 bcde	2.78 efg	20.17 de	0.97 abc	116.53 bcdef	36.47 fg
	ONP	15.00 de	59.00 bcd	3.18 bcdef	20.67 cde	1.07 abc	124.27 abcde	40.87 def
	AMF	61.67 bc	40.50 f	2.76 efg	18.33 de	1.23 a	90.41 ef	38.43 efg
	ONP+AMF	75.00 a	60.67 bcd	3.79 abcde	24.77 abcde	1.01 abc	127.36 abcde	45.90 bcde
	ONP+50% RDCF	10.00 defg	47.67 ef	4.03 abc	23.83 bcde	1.18 ab	134.71 abc	40.93 def
	AMF+50% RDCF	61.67 bc	40.83 f	3.87 abcd	25.83 abcd	1.02 abc	130.72 abcd	43.87 cdef
	INM	65.00 abc	54.00 cde	4.15 ab	28.33 abc	1.10 abc	115.09 bcdef	54.13 b
With shading	Control	1.67 fg	39.33 f	1.63 h	18.67 de	0.92 bc	93.38 def	31.83 g
	100% RDCF	3.33 efg	47.83 ef	3.05 cdef	22.33 bcde	1.08 abc	131.13 abcd	41.50 def
	ONP	20.00 d	62.50 bc	2.59 fgh	23.83 bcde	1.04 abc	116.78 bcdef	48.60 bcd
	AMF	61.67 bc	55.50 bcde	2.24 fgh	21.00 bcde	1.16 ab	109.43 cdef	45.63 cde
	ONP+AMF	73.33 ab	64.17 abc	3.12 bcdef	25.17 abcde	1.09 abc	153.42 ab	51.27 bc
	ONP+50% RDCF	13.33 def	64.50 ab	2.52 fgh	28.67 ab	1.02 abc	157.96 a	51.03 bc
	AMF+50% RDCF	58.33 c	51.17 de	2.94 defg	23.50 bcde	1.13 abc	131.81 abcd	47.83 bcd
	INM	70.00 abc	74.00 a	4.22 a	31.83 a	1.19 a	152.39 ab	64.13 a
	LSD P ≤ 0.05	12.402	10.305	1.038	7.782	0.265	38.486	8.359

Table 9: Combination ffects between greenhouse shading and different nutrition programs on plant root colonization by AMF and root architecture in 2018 growing season.

Effect of shading	Nutrition programs	RC (%)	MRL (cm)	MRD (mm)	SRL (cm)	SRD (mm)	RSA (cm^2)	RDW (g)
Without shading	Control	6.67 gh	47.83 f	3.29 de	18.73 d	0.85 d	88.70 g	27.79 f
	100% RDCF	1.67 h	52.17 cdef	4.20 abcde	24.70 abcd	0.98 bcd	126.03 cde	39.66 de
	ONP	16.67 fgh	48.83 ef	4.40 abcd	26.12 abc	1.06 abc	130.96 cde	47.61 cd
	AMF	63.33 bc	47.33 f	4.08 abcde	26.52 abc	1.01 abcd	95.11 fg	45.73 cd
	ONP+AMF	75.00 ab	57.83 bcde	4.85 a	29.69 ab	1.14 ab	135.80 bcd	44.76 cd
	ONP+50% RDCF	23.33 f	53.67 bcdef	3.47 bcde	27.38 ab	1.10 ab	130.39 cde	47.21 cd
	AMF+50% RDCF	43.33 de	49.33 ef	4.74 abc	26.15 abc	1.04 abcd	128.64 cde	44.43 cd
	INM	68.33 abc	51.67 cdef	4.82 a	25.17 abc	1.08 abc	148.04 bc	43.88 cd
With shading	Control	6.67 gh	44.67 f	3.03 e	20.93 cd	0.90 cd	106.08 defg	31.96 ef
	100% RDCF	3.33 h	53.50 bcdef	4.34 abcde	25.59 abc	1.02 abcd	132.53 cde	50.36 bc
	ONP	20.00 fg	51.00 def	4.27 abcde	30.17 a	1.06 abc	120.46 cdef	44.62 cd
	AMF	55.00 cd	47.83 f	3.40 cde	25.66 abc	1.02 abcd	102.51 efg	46.36 cd
	ONP+AMF	76.67 ab	60.67 abc	4.76 ab	27.32 ab	1.11 ab	180.26 a	59.55 a
	ONP+50% RDCF	30.00 ef	68.83 a	4.65 abc	29.41 ab	1.14 ab	166.02 ab	50.18 bc
	AMF+50% RDCF	56.67 cd	60.17 abcd	3.73 abcde	23.58 bcd	1.07 abc	144.05 bc	57.70 ab
	INM	80.00 a	62.50 ab	3.93 abcde	28.92 ab	1.19 a	180.44 a	59.16 a
	LSD P ≤ 0.05	15.658	9.206	1.345	6.252	0.201	30.598	8.362

colonization (1.67%) recorded by the combination of (Non-shade × 100% RDCF). Tomato plants that grown under shade conditions and fertilized with ONP + 50% RDCF (Shade × ONP + 50% RDCF) reached the maximum length of the main roots (68.83cm), but it was not different significantly than some of the other combinations including (Shade × INM), (Shade × ONP + AMF) and (Shade × AMF + 50% RDCF). The findings indicate that the treatment combinations (Non-shade × ONP + AMF) and (Non-shade × INM) offered the highest value of the main root diameter, which they were recorded 4.85 and 4.82 mm respectively. Some variations were noted between the treatment combinations in the average length and diameter of the secondary roots, the combination of (Shade × ONP) recorded the largest value of the secondary root length (30.17 cm) but the maximum value of the secondary root diameter (1,19mm) was recorded in the combination of (Shade × INM). Greenhouse shading that combined with the nutrition programs (ONP + AMF and INM) showed highly significant effects on roots surface area and dry weight. They were recorded (180.26 and 180.44cm^2 respectively for root surface area) and (59.55 and 59.16 g respectively for root dry weight), which they were superior to most of the other combinations (Table 9).

Discussion

Under our research conditions the shading treatment creates more suitable circumstances for tomato plants growth through decreasing the maximum air temperature by 5.7°C and increasing minimum relative humidity by 11.2% under shading area of the greenhouse (Tables 3 and 4, Figs. 2 and 3). These changes in the climatic conditions improve the photosynthesis efficiency, nutrients uptake and vegetative growth of the plants (data not shown), which consequently reflected positively on the root growth. In addition, decreasing light intensity by shading treatment may reduce the photo-oxidation of the phytohormones, particularly auxins, in the plants which are considered significant hormones that stimulate root growth and development (Banerjee and Roychoudhury, 2016; Iwasa and Roughgarden, 1984). Therefore, the root architecture improved under shaded condition compared to non-shaded ones. Although, among the studied root measurements only the main root length and root dry weight statistically reached the significant level in both seasons (Table 5). Also, our results showed no significant effect of shading treatment on the AMF roots colonization percentage (Table 5). This may be due to that shading does not alter the soil environment to the extent that causes AMF growth.

Proper plant nutrient management is an important tool to improve root colonization by AMF fungus and the plant roots architecture. Effect of organic matter is very clear in our results for root colonization by AMF, for instance the treatment of (ONP + AMF) resulted significantly in the increase of root colonization compared to the (AMF) alone. Although no significant differences were found between (ONP + AMF) and (INM) (Tables 6 and 7). These results are in agreement with (Bilalis *et al*., 2015; Hammer *et al*., 2011) who reported that both

organic and chemical fertilizers have effects on enhancing plant root colonization with mycorrhizae, but the effect of organic was more obvious.

Generally, our study revealed that the plants that treated individually with each of chemical, organic substances and bio-fertilizer of AMF caused increasing in the tomato root growth characteristics compared to control in both seasons. The effects of the fertilizers combination which constitute of (INM) and (ONP+AMF) on the root growth characteristics could be the reason for their superiority to the control and some of the other nutrition programs in both seasons (Tables 6 and 7).

Such results are in agreement with previous studies that reported about the effect of each of chemical fertilizers (Hajabbasi and Schumacher, 1994; Sainju *et al.*, 2003), poultry manure (Baldi and Toselli, 2013) a variety of biostimulant substances such as humic substances (Jindo *et al.*, 2012), free amino acids (Fischer *et al.*, 1998) and mycorrhizal fungi symbiosis (Gamalero *et al.*, (2004) on improving plants root architecture. Also, our results are in consistence with previous studies which stated that the plants root architecture improved by using integrated organic and bio-inoculums with reducing chemical fertilizers to 50%, for example (Allawi, 2013) on sweet pepper and (Al-shaibany, 2005) on tomato.

The concentration of soluble nutrients in the soil determines root morphology such as root diameter, root length, lateral root formation *and* root surface area (Hajabbasi and Schumacher, 1994). Inorganic nutrients especially phosphorous helps to initiate root growth of tomato and therefore aids in early establishment of the plant immediately after transplanting. Starter solution containing high concentration of phosphorous is normally applied to tomato plants within few days after transplanting for early root development and establishment in the soil. The vigorous root growth stimulated by phosphorous helps in better utilization of water and other nutrients in the soil and promotes a sturdy growth of stem and healthy foliage (Sainju *et al.*, 2003).

Studies have demonstrated that the soil application of poultry manure and humic substances (HS) have a significant role on soil fertility and structure through acting on chemical, physical and biological properties of soils, thereafter, these improve root architecture and increase nutrient uptake by plants (Du Jardin, 2015; Kumar Sootahar *et al.*, 2019; Trevisan *et al.*, 2010).

Poultry manure is rich with nutrient compounds and organic matters which encourages the activity of the different soil microorganisms and increases the availability of various nutrients. Besides, the secretions of growth regulators such as auxins, gibberellins and cytokinins as well as organic acids by microorganisms can lead to increase root lengths and thickness as well as nutrient accumulation, which are reflected in the accumulation of dry matter in the roots (Allawi, 2013). The application of organic matter to the soil may affect root growth by increasing inorganic ions and HS that induce a proliferation of lateral roots and root hairs and cause a higher differentiation rate of root cells (Baldi and Toselli, 2013).

HS in the rhizosphere may release auxin-like compounds that promote root growth (Calvo *et al.*, 2014). Auxins induce plasma membrane (PM) H+ -ATPase activities in cell roots, which couple adenosine triphosphate (ATP) hydrolysis to H+ transport across cell membranes. Consequently, the apoplast is further acidified, the cell walls are loosened and cells eventually elongate, thereby favoring an increase in root growth (Jindo *et al.*, 2012).

Zandonadi *et al.*, (2010) demonstrated that HS is capable of inducing nitric oxide (NO), which is a bioactive molecule that is involved in numerous plant physiological processes including root development. The application of HS on roots of cucumber plants caused a primary increase in NO accumulation and it was associated with the expression of the following morphological root changes: increase in the number of secondary roots, increase in root thickness *and* increase in root fresh weight (Mora *et al.*, 2012).

Concerning the biostimulant action of the amino acids on plants root growth, Fischer *et al.*, (1998) mentioned that amino acids are the precursors of phytohormones and other growth substances in plant tissues which are responsible for the rate of stem and root elongation.

Microbial biostimulant of AMF often induces modifications in the root architecture of plants, in particular root length, density, diameter *and* number of lateral roots. Better root system architecture in mycorrhizal plants allowed the extraradical hyphae to extend beyond depletion zones of plant rhizosphere making the uptake of water and various nutrients especially low mobile nutrients (Rouphael *et al.*, 2015). Gamalero *et al.*, (2004) investigated the effect of AMF (*G. mosseae*) on root architecture, plant growth and P acquisition of the tomato plant. They reported that AMF inoculation improved significantly plant root architecture, such as total root surface area, total root volume, number of tips and degree of root branching, increased shoot fresh weight and P content of inoculated plants compared to untreated plants.

According to our results, more suitable circumstances in shade compartment and the (INM) among the nutrition

programs led to improve the plants root architecture. For this reason plants that fertilized with (INM) and grew under shading conditions (Shade × INM) enhanced majority of the root system growth characteristics (Tables 8 and 9).

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