Plant Archives Vol. 20, Supplement 2, 2020 pp. 292-296 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

RESPONSE OF TOMATO *LYCOPERSICON ESCUIENTUM MILL.* AND SOIL MICROBIAL ACTIVITY TO *GLOMUS MOSSEAE*, *TRICHODERMA HARZIANUM* AND CHEMICAL FERTILIZATION

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Abstract

Mycorrhizal fungi, Trichoderma and chemical fertilizer have a desirable affect on tomato yield. However, the concern to eliminate the chemical fertilization and depends on microbes inoculations is still clouded. A field experiment was carried out into greenhouses in the Al-Seyahi area, Babil Governorate to find out the role of bio- fertilization and chemical fertilizers on increasing microbial activity and tomato productivity. The split-plot design was used with three replicates, where the main plot included chemical fertilization 0%, 50% and 100% of the recommended doses. The subplot was included four types of bio-fertilization, control (C), Mycorrhizal (M), Trichoderma (T) and the mixture between Mycorrhizal fungi and Trichoderma (MT). Results showed that there was a significant increase in the microbial activity, bacterial and fungal cell numbers, nitrogen and phosphorus content and the total yield at the 100% and 50% of the recommended doses. Moreover, M led to a significant increase in the number of bacteria (41.7 x 10^{-6} colony-forming unit CFU/ g dry soil), and Mycorrhizal infection rate (80.02%). In addition, in MT treatment, the percentage of organic carbon increased by 0.39%, and the number of fungal cells were significantly higher than others (22.4 x 10^{-3} CFU/ g dry soil). The highest total yield was at MT (121.667 t.ha^{-3}). In conclusion, the combination of Mycorrhizal fungi with Trichoderma at 50% of the recommended doses increased the total yield (151.67 ton. ha^{-1}) and consequences on the reduction of soil contamination.

Keywords: tomato *Lycopersicon escuientum* Mill, Mycorrhizal (*Glomus mosseae*), Trichoderma (*Trichoderma harzianum*), microbial activity, nutrients.

Introduction

The *Lycopersicon escuientum* Mill. (Tomato) belongs to the Solanaceae family. It is a widespread crop that is consumed in large quantities and is important because of the higher nutritional value, carbohydrates, proteins, organic acids and minerals such as potassium, phosphorus, and iron as well as antioxidants (Gol, 2006). However, Fertilization with its various chemical types plays an important role in improving plant growth and increasing productivity, but the high prices of chemical fertilizers and its toxic influence have become a real burden. Furthermore, Iraqis soils are suffered from the low organic matter and higher salinity (AL-Maliki *et al.*, 2018; Al-maliki 2016; AL-maliki *et al.*, 2019; AL-maliki *et al.*, 2014). Therefore, the urgent need to use alternatives that contribute to reducing the use of chemical fertilizers and enhance soil organic matter is an ultimate priority. Biofertilizers were among the most important alternatives tool, including fungus and bacterial inoculations, which play an important role in increasing organic matter decomposition, absorption of nutrients, as well as, hormones production (Kumar *et al.*, 2013; Mathur, 2018). In contrast, an excessive use of chemical fertilizers may cause environmental damages particularly to groundwater. Therefore, the use of biofertilizers is one of the most successful solutions to coping with these pollutants. Mycorrhizal Fungus can contribute to the growth and productivity of tomato by improving the roots density, increasing the nutrient absorption and plant resistance to disease infections (Kumar *et al.*, 2013). Mycorrhizal Fungus can enhance aggregation, infection rate, microbe's numbers as well as potato yield (AL-Maliki and AL-masswdy 2018; AL-Maliki and AL- Zabee 2019) Moreover, Trichoderma is quite beneficial for plant growth (Zhang *et al.*, 2016). The proposed hypothesis is that Mycorrhizal Fungus and Trichoderma might be used as an alternative choice for the chemical fertilizer. Therefore, the study aims was to find out the effect of both Mycorrhizal fungi and Trichoderma fungi on the microbial activity, productivity of tomato and also attempting to reduce the fertilizer recommendation of tomato.

Materials and Methods

A field experiment was carried out in the Al-Seyahi area - Al-Husain Village - Babil Governorate (32 39 '33.54 "N and E 44 55' 09. 62") in 2019. The experiment was designed according to the Randomized complete block design R.C.B.D. The arrangement of split-plot with three replicates was designed (Sahuki and Waheeb, 1990). The main plot included chemical fertilization at three levels (zero, 50% and 100%) of the chemical fertilizer recommendation of tomato. While for the subplot; it was included four types of bio fertilization, (comparison without the addition (C), Mycorrhizal (M), Trichoderma (T) and the mixture between Mycorrhizal and Trichoderma (MT). Soil preparations were conducted included plowing and leveling. The study area was divided into three blocks; each block contained 12 plots. The area of experimental unit was 4 m^2 (2 * 2) in a dimensions. Two meter distance was left between the blocks and 1 m distance was also left between the experimental units to prevent the leaking of the soil chemical fertilizers to the non-aimed samples. Furthermore, the experiment was fertilized according to the approved recommendations at a rate of 200 kg nitrogen (N46%), 60 kg of concentrated calcium superphosphate (p 20%) and 120 kg of potassium sulfate (41.5% k) (Faraj, 2009). Field soil samples were taken from a depth of (0-30) cm in order to analyze particular soil properties as shown in Table 1. The irrigation process was carried out using the drip irrigation system, as the water flow was 75 ml per minute. The following parameters were studied; including the microbial activity according to (Anderson, 1982; AL-Maliki *et al.*, 2017), bacteria and fungi numbers was estimated according to (Stotzky *et al.*, 1993). In addition, the percentage of total organic carbon was measured based on (page *et al.*, 1982) method, infection percentage according to the (Phillips and Hyman, 1970) method, shoot and root (g), nitrogen and total phosphorus in the soil according to (labetowicz 1988).

Table 1 : Particular properties of the field soil before planting

Property		Unit	Value	Reference
Soil PH		-	8.2	(Richards,1954)
The organic matter in the soil		g/kg soil	7.2	(Page, *et al.*, 1982)
Major element	Nitrogen	PPm	0.19	(Labetowicz,1988)
	Phosphorus		502.3	
	Potassium		2505.5	
Texture	the sand	g/kg loam	42	(Page, *et al.*, 1982)
	Silt		35	
	Clay		23	
Vital traits	Bacteria	(CFU*10^{-7} g^{-1} dry soil)	20*10^{-7}	(Stotzky,1993)
	Fungi	(CFU*10^{-3} g^{-1} dry soil)	21*10^{-3}	

Results and Discussion

The results of Table 2 showed a significant effect of chemical fertilization on soil microbial activity. The 100% dose of the recommended fertilizer recorded the highest value of microbial activity (40.60 mg CO_2 g^{-1}-dry soil) compared to the comparison treatment (38.02 mg CO_2 g^{-1}.dry soil). It's possibly that the highest dose of chemical fertilizer adjusted soil pH and stimulated microbial activity as this considers as an energy source for microbes. Additionally, the period of 10 days highly increased microbial activity (61.14 mg CO_2 gm^{-1}-dry soil) compared to other days, suggesting that the organic matter decomposition increased steadily, resulting in releasing the CO_2 from the soil at day 10 but lowered thereafter due to an accessibility of organic maters leading to carbon exhausting. Interestingly, a significant interaction was found between chemical fertilizer and biofertilizer factors confirming the importance of both factors in improving microbial activity. In soil where 100% of the fertilizer recommended dose was combined with MT treatment noticed predominant increases in microbial activity (70.03 mg CO_2 g^{-1}.dry soil). Additionally, M treatment at 100% of the recommended dose recorded higher value of microbial activity (49.57 mg CO_2 gm^{-1}-dry soil) compared to 50% (31.10 mg CO_2 g^{-1}-dry soil). Similarly, the T treatments and 2 days incubation increased microbial activity significantly (62.94 mg CO_2 g^{-1} dry soil) compared to MT treatment. It seems that the chemical fertilizers increased the activity of Mycorrhizal fungi and Trichoderma, which led to an increase in the decomposition of the organic matter and releasing CO_2 from the soil. Mycorrhizal fungi mycelium also secretes molecular weight, sugars, and enzymes that increase the activity of bacteria in the rhizosphere (Toljander *et al.*, 2008). The strategic role of nitrogen, phosphorus, and potassium might contribute to an increase in the activity of Mycorrhizal fungi and secretions of Glomalin (Wetterauer and Killorn, 1996). Trichoderma also plays a role in promoting organic decomposition and releasing CO_2 from the soil (Harman, 2000), by stimulating plant growth regulators and root growth (Martian and Hayes, 2017).

Table 2 : Effect of bio and chemical fertilizers and incubation on the microbial activity mg (CO_2. g^{-1} dry soil)

Bio	Fer	Incubation					Bio.x Fer.	Average Bio
		2	5	10	20	40		
C	0	43.26	50.23	60.50	7.70	15.76	35.49	34.92
	50	41.43	48.76	54.63	5.50	19.80	34.02	
	100	44.73	47.56	57.57	8.80	17.60	35.25	
M	0	40.33	46.56	67.10	8.80	23.10	37.18	39.28
	50	38.13	40.70	53.90	7.36	15.40	31.10	
	100	46.56	50.60	63.06	68.56	19.06	49.57	
T	0	46.20	49.13	63.43	21.63	39.23	43.92	37.27
	50	38.50	42.53	53.90	11.73	16.13	32.56	
	100	38.50	48.03	62.33	9.90	17.96	35.34	
MT	0	39.23	44.36	55.00	8.80	30.06	35.49	39.57
	50	43.26	45.10	56.10	9.16	67.46	44.22	
	100	42.53	47.30	70.03	16.50	18.70	39.01	
LSD		LSD : Bio. X Fer .x. Inc=16.461*					average Bio.xFer 35.09*	LSD: Bio.=4.2501n.s
							LSD Bio.xFer.15.216*	
Bio.x lnc.=		2	5	10	20	40	LSD	
C		43.14	17.72	28.24	59.88	45.58	LSD Bio. X lnc.= 12.145*	
M		48.85	41.67	19.18	14.42	60.37		
T		62.94	45.95	41.06	24.44	11.48		
MT		7.33	61.35	46.56	41.67	38.74		

Fer.x lnc.=	2	5	10	20	40	Average Fer.
0	42.25	47.57	61.50	11.73	27.04	38.02
50	40.33	44.27	54.63	8.44	29.70	35.47
100	43.08	48.37	67.28	25.94	18.33	40.60
LSD	LSD Fer.x lnc.= 10.502					LSD Fer.=3.6807*
Average Inc.	41.89	46.74	61.14	15.37	25.02	LSD lnc.=4.7518*

(C) Control without addition, (M) Mycorrhiza, (T) Trichoderma, (MT) A mixture between Mycorrhiza and Trichoderma. (0) without adding chemical fertilizer (50) Adding half the amount of fertilizer recommendation (100) Adding the full amount of fertilizer recommendation.

Table 3 showed a significant effect of chemical fertilizer on the above-mentioned characteristics. Chemical fertilizer significantly reduced infection rate and organic carbon as compared to control treatment. The reason for why there was lower roots infection rate as chemical fertilizer doses increased could be attributed to the presence of high dependency of Mycorrhiza on chemical fertilizer to meet its carbon need, which might have reduced the chances of creating a symbiotic mycorrhizal relationships leading to lower infection rate of the roots. Another reason for such decrease is that the application of phosphate fertilizers, especially triple superphosphate might affect the cellular membrane permeability of the roots due to the increasing of phospholipids concentrations, which might reduce the secretion of carbon compounds by the roots in the rhizosphere causing a reduction in the infection percentage (Same *et al.*, 1983). These results are consistent with Martin *et al.* (2011) who found that the chemical fertilization decreased the percentage of organic carbon. Moreover, the dose of fertilizer at 50% significantly maximized soil total nitrogen and the total yield. At 100%, bacterial and fungal cells were higher significantly than other doses (25.4 x 10^{-6} and 19.1 x 10^{-3} CFU/ g-dry soil). It's quite known that the chemical fertilizer is an important source of energy for microbes resulting in more cells reproduction. Furthermore, chemical fertilizer can adjust soil pH , which encouraged the activity of the microorganisms and nutrients absorption (NPK) as well as soil aggregates formation (Liu *et al.*, 2019). These results were consistent with Suhail *et al.* (2010) who noticed an increase in nutrients content (NPK) and their role in improving plant growth and the total yield. The increases in total yield of tomato at 50 % chemical recommended dose was due to the higher content of nitrogen in soil and probably due to the higher roots secretions or diminishing influence of chemical fertilizer on plant growth where a tiny amount of chemical fertilizer was applied.

Table 3 : The effect of chemical fertilizers on infection percentage, carbon percentage, bacterial and fungal cell numbers, nitrogen and total phosphorous in the soil and total yield

Fertilization	Parameters						
	Infection percentage %	**Carbon percentage (%)**	**Bacterial cell numbers (CFU*10^{-6} g^{-1} dry soil)**	**Fungal cell numbers (CFU*10^{-3} g^{-1} dry soil)**	**Total nitrogen in the soil ($mg.kg^{-1}$ soil)**	**Total phosphorous in the soil ($mg.kg^{-1}$ soil)**	**Total yield ($ton.ha^{-1}$)**
0	40.00	0.32	6.2	13.5	0.21	327.86	76.04
50	30.00	0.29	7.4	14.3	0.25	331.74	112.91
100	25.09	0.25	25.4	19.1	0.24	461.46	96.95
$LSD_{0.05}$	*0.143	0.0037*	*8.518	*4.181	*0.0174	*122.19	10.37*

Table 4 showed that the bio fertilization significantly affected infection rate, organic carbon, bacterial and fungal community, soil total phosphorus and total yield. M treatments increased the infection rate (80.02%), and the organic carbon (0.39%), as well as the bacterial cell numbers (41.7 x 10^{-6} CFU/ g dry soil), compared to the comparison treatment Furthermore, the MT treatment increased the fungal cell numbers (22.4 x 10^{-3} CFU/ g-dry soil) and the total yield (121.667 ton ha^{-1}) as compared with the comparison treatment . The increase in organic carbon and bacterial cells might be attributed to the role of Mycorrhiza in supporting the amount of organic carbon in the soil by providing the glomalin and chitin (AL-Maliki and AL-Maswodi *et al.*, 2018; Rooney *et al.*, 2009). Mycorrhiza fungi might participate in increasing the activity of microorganisms by excretion the phosphates enzymes leading to an increased phosphorous content (Al Maamouri, 2016). Mycorrhiza might produce organic acids with low molecular weight, including oxalic acid, which affect the phosphorus content in soil (Heil, 2011). This is consistent with Nirmalnath, (2010) and Dubey *et al.* (2011) who confirmed improvements in nutrients content after inoculation of mycorrhiza. The increase in the total yield of tomato is occurred because of the cooperative mission between Mycorrhizal fungi and Trichoderma which increased the infection rate, secretions, plant growth regulators and nutrients uptakes causing a developments in plant growth and total yield. Mycorrhizal fungi and Trichoderma have a role in the decomposition process and enzyme production (Al-Shaibani, 2005; Sirvastava, 2002; Wang *et al.*, 2002) in which more effect on total yield could be happened.

Table 4 : The effect of biofertilizers on infection percentage, carbon percentage, bacterial and fungal cell numbers, nitrogen and total phosphorus in the soil and total yield

Bio.	Studied traits						
	Infection percentage %	Carbon percentage (%)	Bacterial cell numbers (CFU*10^{-6} g^{-1} dry soil)	Fungal cell numbers (CFU*$10^{-3}$$g^{-1}$ dry soil)	Total nitrogen in the soil (mg.kg^{-1} soil)	Total phosphorous in the soil (mg.kg^{-1} soil)	Total yield (ton.ha^{-1})
C	0.00	0.18	1.4	10.7	0.23	292.41	46.944
M	80.02	0.39	41.7	14.1	0.23	531.34	110.111
T	0.00	0.31	3.7	15.5	0.25	307.73	102.500
MT	46.78	0.28	5.1	22.4	0.23	363.27	121.667
$LSD_{0.05}$	*0.165	*0.0043	*9.836	4.828 *	$^{n.s}$0.0201	*141.09	11.98*

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