



INFLUENCE OF MINERAL NITROGEN, COMPOST AND NITROGEN FIXING BACTERIA ON TOMATO PLANTS GROWN IN SANDY SOIL

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Keywords: Tomato, Mineral fertilization, Compost, Nitrogen fixing bacteria, Biofertilization

ABSTRACT

Pot trials were conducted under plastic house condition during two successive seasons of 2013/2014 and 2014/2015, at the experimental site of Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center, Giza, Egypt. The present study aims to determine the partial replacement of mineral nitrogen fertilization of tomato by nitrogen fixing bacteria with or without adding compost in sandy soil. Tomato seedlings (Lora F₁Hybrid) were transplanted during the first week of October into plastic pots (30 cm diameter) filled with 10 kg of sandy soil. Three rates 25, 50 and 75% of the recommended mineral nitrogen in the nutrient solution for tomato with adding compost at 2% and nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) at 20 ml/plant either individually or in combinations were investigated on growth, mineral composition and yield of tomato plants compared to 100% of recommended nitrogen only (control). The plants were irrigated daily by drip irrigation and received 200 ml/plant of nutrient solution twice a weekly. The results showed that using 50 or 75% of N-mineral fertilizer + compost + nitrogen fixing bacteria gave the highest values of growth, mineral composition and yield of tomato. It is recommended that 50% of nitrogen mineral fertilizers for tomato plants could be replaced by nitrogen fixing bacteria in presence of compost, which in turn, reduce environment pollution caused by extensive application of mineral nitrogen fertilizers.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is one of the most popular and widely grown vegetable crops in the world. The total cultivated area in Egypt was 0.52 million feddans (feddan = 0.4 hectare), produced about 8.6 million tons on annual basis with an average of productivity 16.636 tons/feddan (Ministry of Agriculture and Land Reclamation, 2013). The tomato crop is highly responsive to nitrogen fertilizer application, where the nitrogen availability may be limited factor for plant growth in many areas especially in low organic soils. (Taber, 2001).

Mineral nitrogen fertilizers often are easy dissolving and quickly leaching in soils, thus they can pollute soils and groundwater (Dhar, 1962). Excess use of nitrogen fertilizers deteriorates the soil health, increases ground water pollution, encourages nitrate accumulation in fruits and makes plants susceptible to pest and disease incidents (Chatterjee et al 2014). Therefore, management N fertilizer such as rate and type of N fertilizer is very important (De Pascale et al 2006). Thus, integrated nutrient management has become an accepted strategy to bring about improvement in soil fertility and protecting the environment. This strategy utilizes a judicious combination of inorganic, organic and bio fertilizers (Premsekhar and Rajashree, 2009).

In Egypt, the organic matter of cultivated clay soils is between 1.0- 2.5%, while in the calcareous and sandy desert soils, it is usually less than 0.5% under arid and semiarid conditions (Abd El-Ghaffar, 1982); so most of Egypt soils need to add organic amendments to improve their properties and consequently their productivity and natural fertility. The application of compost to these soils

improve characteristics and fertility of soil and consequently increase the growth and development of plant roots, shoots and quantity of yield (Mamo et al 1998; Elashry et al 2008), as well as reduce the using of chemical fertilizers, which have adverse environmental effects (Mahmoud et al 2009).

Biological nitrogen fixation is one way of converting elemental nitrogen into plant usable form (Gothwal et al 2007). Nitrogen fixing bacteria (NFB) that function transform inert atmospheric nitrogen to organic compounds (Bakulin et al 2007). These bacteria provide the plant with fixed nitrogen, hormones, signal molecules, vitamins, iron, etc (Kavadia et al 2007; Mikhailouskaya and Bogdevitch, 2009). Azotobacter and Azospirillum are the two most important non-symbiotic N-fixing bacteria in non-leguminous crops. Under appropriate conditions, Azotobacter and Azospirillum can enhance plant development and promote the yield of several agricultural important crops in different soils and climatic regions (Okon and Labendera-Gonzalez, 1994; Jagadeesha, 2008). Applying Nitrogen fixing bacteria is not only reducing mineral nitrogen requirements by 25%, but also increases the availability of various nutrients, enhances the resistance of plants to root disease and reduces the environmental pollution (Rizk and Shafeek, 2000).

Many studies showed that the combination of biofertilizers with organic or chemical fertilizers further enhanced the growth, yield and quality of plants such as Togun and Akanbi (2003), Toor et al (2006); Fawzy et al (2007); Glala et al (2010); Habibi et al (2011); Glala et al (2012) and Glala et al (2013).

This work aims to the possibility of partial replacement of mineral nitrogen fertilization of tomato by nitrogen fixing bacteria with or without adding compost in sandy soil.

MATERIALS AND METHODS

The experiment was carried out at the experimental site of Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center, under plastic house conditions, during the two successive seasons of 2013/2014 and 2014/2015. Tomato seeds (Lora F₁ Hybrid) were sown in the nursery on 27 August and the seedlings were transplanted to plastic house on 7 October in the both seasons. The seeds were sown in the seedling trays, which were filled with peat moss and vermiculite 1:1 (v:v) for 40 days. Then they were transplanted into plastic pots 1 seedling / pot (30 cm diameter and 25 cm height), the pots were filled with 10 kg of sandy soil. Each treatment included 12 plants in one row, the space within plants and between rows was 50 cm, the space between double rows was 75 cm. The plants were irrigated by drip irrigation (4 L/hr) daily according to water rations program for tomato plants under plastic houses at Giza Governorate (Ministry of Agriculture and Land Reclamation, 1988).

The compost was added to sand pots as soil amendment at rate 2% (200 g / pot) before one week from transplanting except control treatment, as 200 g of additional sand soil was added per pot. The analyses of soil and compost were carried out according Chapman and Pratt (1961) and were presented in Tables (1 and 2) respectively.

Table 1. Physical and chemical analyses of experimental soil

Sand %	Silt %	Clay %	Texture	pH	EC dS/m	Cations meq/l				Anions meq/l			
						Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺	CO ₃ ⁼	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁼
90.76	6.78	2.46	Sandy	7.74	1.13	2.58	1.24	1.82	3.94	1.14	1.92	3.38	3.25

Table 2. Chemical analyses of compost

pH 1:5	EC 1:10 dS/m	O.M (%)	C/N ratio	N%	P%	K%
7.86	4.62	28.58	15.63	1.28	0.93	1.08

Nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*) as pure local

strains were kindly provided by Microbiology Dept. Soil, Water and Environment Research Institute, Agricultural Research Center. Soil application technique was carried out by using liquid culture of Ashby media at a rate of 20 ml/plant (1ml contains 10^8 cell) according to **Mashhoor et al (2002)** after diluted by water without Chlorine at 1 : 20. Nitrogen fixing bacteria were applied to the soil surface

beside plants at twice times, after 1 and 3 weeks from transplanting.

The plants received 200 ml/plant of nutrient solution (**Abou-Hadid et al 1989**) twice a weekly. The electrical conductivity (EC) of the nutrient solution was maintained at 2.5 dS/m; while pH was maintained at 5.5 - 6.5 by using nitric and phosphoric acids (3:1 v/v) as described **Abou-Hadid et al (1989)**. The contains of the nutrient solution were showed in **Table (3)**.

Table 3. Contains of the nutrient solution

pH	EC dS/m	Macronutrients (ppm)						Micronutrients (ppm)					
		N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Mo	Zn
6.5	2.5	260	35	300	160	50	221	5.0	1.0	0.3	0.1	0.1	0.1

Three rates 25, 50 and 75% (65, 130 and 195 ppm respectively) of mineral nitrogen from the recommended dose in nutrient solution (260 ppm) as 1.44 g calcium nitrate (15.5% N) and 0.84 g potassium nitrate (13% N) / L of water with adding compost and nitrogen fixing bacteria individually or in combinations were investigated for production of tomato comparing to 100% of mineral nitrogen from the recommended dose in nutrient solution for tomato according to **Abou-Hadid et al (1989)** as a control.

The experimental treatments

- 100% N mineral fertilization in nutrient solution (260 ppm) as a control.
- 25% N mineral fertilization in nutrient solution (65 ppm) + compost.
- 50% N mineral fertilization in nutrient solution (130 ppm) + compost.
- 75% N mineral fertilization in nutrient solution (195 ppm) + compost.
- 25% N mineral fertilization + Nitrogen fixing bacteria (NFB).
- 50% N mineral fertilization + Nitrogen fixing bacteria (NFB).
- 75% N mineral fertilization + Nitrogen fixing bacteria (NFB).
- 25% N mineral fertilization+ compost + Nitrogen fixing bacteria.
- 50% N mineral fertilization+ compost + Nitrogen fixing bacteria.
- 75% N mineral fertilization + compost +Nitrogen fixing bacteria.

The experimental treatments were arranged in a completely randomized block design, with three replicates for each treatment. Each replicate was included 12 pots. Tomato plants were grown in plastic house on the main branch only with the removal of all side branches. Supported strings were wrapped around the plants weekly.

After 60 days from transplanting, three plants from each replicate were randomly chosen to measure plant height, stem diameter and number of leaves on plant, as well as chlorophyll reading in the fourth upper leaf was measured by using Minolta Chlorophyll Meter Spad 501.

Total nitrogen, phosphorous and potassium percent were determined in the dry matter of fourth upper leaf according to **Cottenie et al (1982)**. Samples were dried at 70°C for 72 hours according to **ADAS/MAFF (1987)**. Then dried leaves were digested in sulphuric acid and hydrogen peroxide according to **FAO (1980)**. Total nitrogen was determined by Kjeldahl method according to the procedure described by **FAO (1980)**. Phosphorus percent was determined using spectrophotometer according to **Watanabe and Olsen (1965)**. Potassium percent was determined spectrometrically using Phillips Unicum Atomic Absorption Spectrometer as described by **Chapman and Pratt (1961)**.

Fresh and dry shoot weight of tomato plants was measured at the end of harvesting stage. Total yield and number of fruits per plant were recorded after each harvesting accumulatively until the end of harvesting season.

Five ripe fruits (fully red color) from the same cluster position and fruit position on cluster per replicate, were selected to measure fruit weight, also total soluble solids (TSS) were measured by using a manual Refractometer.

Data of the two seasons were arranged and statistically analyzed by the analysis of variance using one way ANOVA according to **Snedecor and Cochran (1980)** with SAS software, version 2004. Comparison of treatment means was done using Tukey test at significance level 0.05.

RESULTS AND DISCUSSION

Data in **Tables (4 and 5)** show the effect of applying N-mineral fertilizer, compost and N-fixing bacteria on vegetative growth parameters, such data show clearly that, using 50 or 75% of N-mineral fertilizer + compost + N-fixing bacteria significantly increased all vegetative growth parameters (plant height, stem diameter, leaf number / plant, fresh and dry shoot weight). Also the same

treatments reflected the highest reading of chlorophyll of plant leaf during both seasons of study. However, the lowest value was found in the treatment of 25% N-mineral fertilizer + compost or N-fixing bacteria. The same trend was found in the second season. The increases in plant growth obtained when partial replacement of mineral nitrogen by N-fixing bacteria in presence of compost, might be due to the improvement of physical and chemical properties of soil by adding compost (**Mamo et al 1998**), which improve soil fertility and biological activity in roots rhizosphere (**Glala et al 2010 and 2012**). As well as, N-fixing bacteria provide the plant with fixed nitrogen, hormones, signal molecules, vitamins, iron, etc which enhance root growth of plants (**Kavadia et al 2007; Mikhailouskaya and Bogdevitch, 2009**). All that, play an important role in increasing nutrient availability for uptake which reflected in better root distribution and vegetative growth. These results agreed with those obtained by **Abdalla et al (2001)** on pepper, **Glala et al (2012)**, on squash, **Glala et al (2010)** and **Glala et al (2013)** on tomato plants.

Table 4. Effect of different treatments on plant height, stem diameter and leaves number of tomato plants (60 days after transplanting) during 2013/2014 and 2014/2015 seasons

Treatments	Plant height cm		Stem diameter mm		Leaf No./plant	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
100% NM (control)	193.83 b	200.42 b	19.67 b	20.00 b	29.33 b	30.67 b
25% NM + C	161.67 d	166.29 e	13.33 fg	13.33 f	19.67 e	20.33 ef
50% NM + C	175.00 c	180.24 d	15.00 def	15.67 de	23.33 d	24.00 cd
75% NM + C	183.33 bc	190.86 c	16.67 cd	17.33 cd	24.33 cd	26.00 cd
25% NM + NFB	158.33 d	164.63 e	13.00 g	13.00 f	19.00 e	19.33 f
50% NM + NFB	174.67 c	179.60 d	14.67 efg	15.33 e	22.67 d	23.67 de
75% NM + NFB	181.33 c	187.50 cd	16.33 cde	17.00 cde	23.67 cd	25.33 cd
25% NM + C + NFB	185.28 bc	192.88 bc	17.33 c	18.00 c	26.33 c	27.33 bc
50% NM + C + NFB	211.73 a	216.44 a	22.33 a	22.67 a	33.33 a	34.33 a
75% NM + C + NFB	214.17 a	221.45 a	23.00 a	23.00 a	34.00 a	35.00 a

Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test.

NM = mineral nitrogen fertilizer

C = compost

NFB = nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*)

Table 5. Effect of different treatments on fresh, dry weight and chlorophyll reading of tomato plants during 2013/2014 and 2014/2015 seasons

Treatments	Fresh weight kg/plant		Dry weight g/plant		Chlorophyll reading Spad	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
100% NM (control)	1.35 a	1.43 b	259.67 b	262.00 b	32.00 b	32.82 b
25% NM + C	1.16 f	1.22 e	216.33 f	217.67 f	22.67 e	23.50 e
50% NM + C	1.23 de	1.27 d	224.33 e	226.33 e	25.67 d	27.13 d
75% NM + C	1.27 cd	1.34 c	233.67 d	236.00 d	27.67 cd	30.35 bc
25% NM + NFB	1.16 f	1.21 e	215.67 f	217.67 f	22.33 e	23.16 e
50% NM + NFB	1.21 ef	1.28 d	223.33 e	225.33 e	25.67 d	27.13 d
75% NM + NFB	1.25 cde	1.33 c	233.33 d	236.33 d	27.67 cd	29.69 cd
25% NM + C + NFB	1.29 bc	1.36 c	240.67 c	243.00 c	28.33 c	29.95 cd
50% NM + C + NFB	1.59 a	1.69 a	287.67 a	289.67 a	35.00 a	36.21 a
75% NM + C + NFB	1.61 a	1.70 a	289.00 a	290.33 a	35.67 a	36.30 a

Means followed in same column by similar letters are not statistically different at 0.05 level according to Tukey test.

NM = mineral nitrogen fertilizer C = compost

NFB = nitrogen fixing bacteria (*Azotobacter chroococcum* and *Azospirillum brasilense*)

Data in **Table (6)** revealed that the highest concentrations of N, P and K were obtained by 50% or 75% N-mineral fertilizer + compost + N-fixing bacteria treatments. On the other hand, applying 25% N-mineral fertilizer + compost or N-fixing bacteria gave the lowest concentrations. These results were similar in both seasons. These findings might be due to the beneficial effects of N-fixing bacteria that help in increasing nitrogen fixation and other nutrients in rhizosphere, also enhance the production of phytohormone (**Kavadia et al 2007; Mikhailouskaya and Bogdevitch, 2009**). Moreover, compost has a high cation exchange capacity exceeded the capacity of sandy soil to maintain nutrients are absorbed by plants. Consequently, root system absorbs more nutrients (**Togun and Akanbi, 2003; Toor et al 2006; Ahmad et al 2008; Fiorentino and Fagnano, 2011; Abou-El-Hassan et al 2014**). All that lead to increment minerals content of plants.

The results in **Table (7)** demonstrate clearly that, using N-mineral fertilizer, compost and N-fixing bacteria affected significantly on number and weight of fruit / plant and TSS in tomato fruits. The highest values of yield, fruit number and TSS were found by 50% or 75% N-mineral fertilizer +compost + N-fixing bacteria treatments. On the contrary, the

lowest values were recorded by the treatments of 25% N-mineral fertilizer + compost or N-fixing bacteria individually. Meanwhile, the other treatments were moderated. These results were true in the two seasons. This increment in the yield and quality of tomato fruits, may be resulted to positive effect of compost in sandy soil, which improve the soil characteristics and fertility consequently increase the growth and development of plant roots (**Mamo et al 1998 and Elashry et al 2008**). As well as, N-fixing bacteria have beneficial effects such as increasing nitrogen fixation and other nutrients in rhizosphere, also production of phytohormone that improve root development and increase the rate of water and mineral uptake by roots (**Okon and Labendera-Gonzalez, 1994; Jagadeesha, 2008**). All these factors combined together produce better nutrients absorption of root, which reflects better vegetative growth, photosynthetic activity and dry matter accumulation. which lead to produce good yield and quality of tomato. These results agreed with those obtained by **Abdalla et al (2001)** on pepper, **Habibi et al (2011)**, on pumpkin, **Glala et al (2012)**, on squash, **Glala et al (2010)** and **Glala et al (2013)** on tomato.

CONCLUSION

It is clear from results of this study that 50% of nitrogen requirement for tomato plants as mineral fertilizers could be replaced by nitrogen fixing bacteria of *Azotobacter chroococcum* and *Azospirillum brasilense* in presence of compost by rate 2%, that improved the use efficiency of nitrogen fertilizers and reduced the environment pollution caused by extensive application of mineral fertilizers.

ACKNOWLEDGEMENT

This work has been supported by Central Laboratory for Agricultural Climate (CLAC) and Central Lab of Organic Agriculture (CLOA), Agricultural Research Center, Giza, Egypt.

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