Using plant growth promoting rhizobacteria for improving tomato growth under saline stress

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Abstract

Salinity is a crucial constraint that slow downs agriculture production in many areas in Egypt. Inoculation with plant growth promoting microorganisms may enhance plant growth under salt stress conditions. The objective is to evaluate the inoculation efficieny of biostimulant strains (*Pseudomonas fluorescence D23*, *Bacillus pumilus D139* and *Azospirillum lipoferum D178*), humic acid and organic manure (compost) on growth and yield of tomato (*Solanum lycopersicum* L.). This experiment was conducted in greenhouse conditions at the Experimental Farm Station of Faculty of Agricuture Moshtohor during 2011. The highest significant increase of dehydrogenase, nitrogenase and phosphatase was observed in tomato inoculated with biostimulant combined with humic acid + compost at one and half dose. The highest records of macronutrients uptake by tomato shoots were observed when tomato amended with biostimulant combined with compost at different doses + humic acid. Application of humic acid combined with compost significantly decreased the proline content in tomato, whereas, the reverse was observed in nitrate reductase. Dual treatment of tomato with biostimulant and compost gave higher records of tomato growth characteristics and yield.

Key words: Compost, nitrate reductase, plant growth-promoting rhizobacteria, PGPR, praline, saline stress, tomato.

Introduction

Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases. Tomato is a major vegetable crop that has achieved tremendous popularity over the last century. Salinity is one of the most critical constraints which hampers agriculture production in many areas around the world, including Egypt. About 9.5 billion ha of the world's soil are saline, except for large areas of secondary salinized soil in cultivated land (Aşik *et al.*, 2009). Out of 14.12 million ha of arable area of Egypt, 4.2 millions are salt affected. Most of these lands are annually lost for cultivation due to salinity. It is hypothesized that the use of plant growth promoting microorganisms as inoculants can enhance plant growth under salt stress conditions (Nadeem *et al.*, 2006). Plant growth promoting rhizobacteria (PGPR) are free living soil-borne bacteria or a symbiotic one which colonize the rhizosphere. These bacteria enhance plant growth either by direct or indirect mechanisms (Pallai, 2005). The aim of this research is to study the inoculation efficiency of tomato with salt-tolerant PGPR combined with compost and/or humic acid on tomato growth performance, productivity and yield quality.

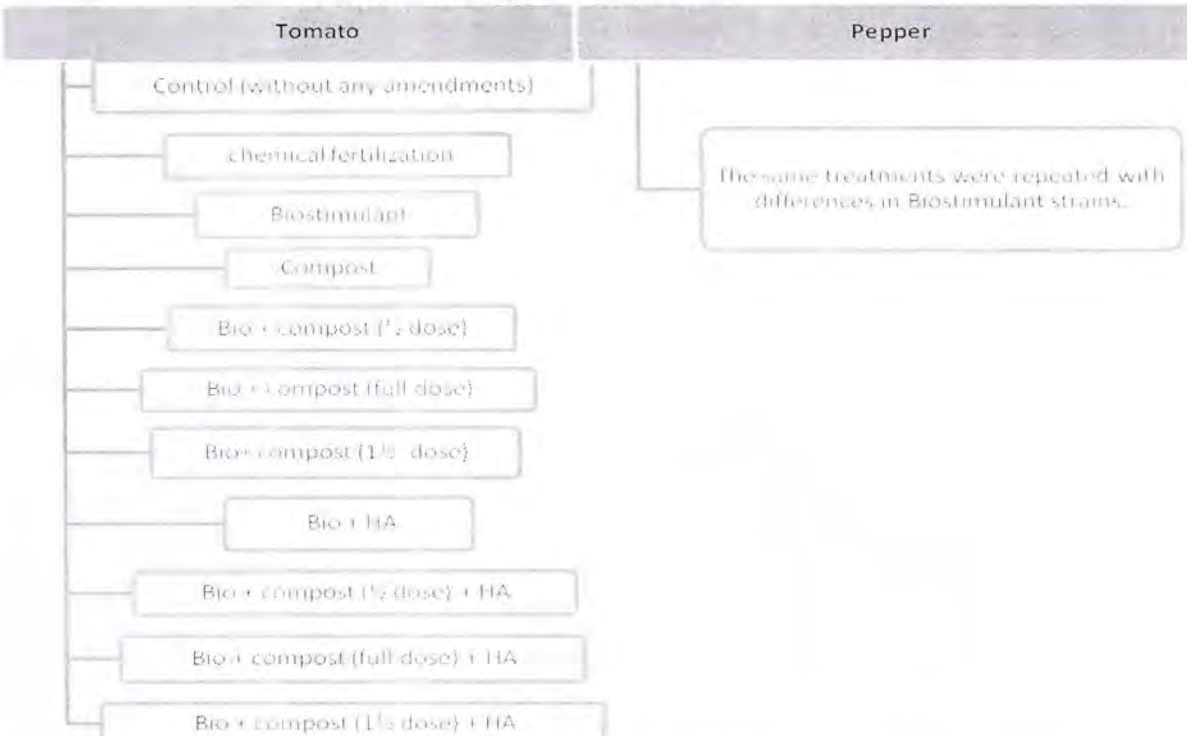
Table 1. The experimental soil texture and chemical analyses.

Parameters		Unit	Values	
Soil texture			Soil before adjustment	Soil after adjustment
Sand		(%)	45.01	50
Silt		(%)	16.32	33
Clay		(%)	38.67	17
Textural class		(%)	Clay	Clayey silt
Chemical analysis				
EC		$ds\ m^{-1}$	24.34	13.92
pH			8.30	8.61
Organic matter		(%)	0.62	1.12
Total nitrogen		(%)	0.010	0.114
Soluble cations	$Na^{+} + K^{+}$	$meq\ L^{-1}$	1.34	8.27
	Ca_2^{+}		14.22	35.0
	Mg^{2+}		27.12	38.0
Soluble anions	$CO_3^{=}$	$meq\ L^{-1}$	Zero	Zero
	HCO_3^{-}		9.93	28.00
	Cl^{-}		186	81.00
	SO_4^{-2}		47.75	30.20

Experimental design

Treatments were distributed in a randomized complete block design with three replicates.

Flow chart of experiment treatments



adhesive agent. The same prepared PGPR inocula were added to grown plants three times throughout the growing season at a rate of 300 mlpot^{-1}. The recommended dose (full dose) of compost was 8-10 ton fed^{-1} for vegetable crops. Whereas, humic acid was added to soil at rate of 3-4 kg fed^{-1}. A half dose of inorganic nitrogen fertilizer (50 kg N fed^{-1}) as ammonium sulphate was supplemented for treatments of biostimulant and biostimulant + humic acid. Also, a full dose of inorganic phosphorus fertilizer (25 kg P_2O_5 fed^{-1}) as calcium super-phosphate and potassium fertilizer (40 kg K_2O fed^{-1}) as potassium sulphate were supplemented for all treatments.

Materials and methods

Soil and soil mixtures

Soil of the experiment was obtained from Port Said Governorate, Sahl El- Hussinia. Experimental soil was subjected to adjustment with soil gypsum and sand, analyses were carried out according to the method described by Page *et al.* (1982).

Determination

Dehydrogenase activity was assayed in soil according to Glathe' and Thalmann (1970). Phosphatase activity was estimated according to Drobnikova (1961). Nitrogenase activity was

Mechanical (particle size distribution) and chemical analyses are presented in Table 1.

Preparation of biostimulant inocula

The biostimulant inocula for tomato (*Pseudomonas fluorescence D23, Bacillus pumilus D139 and Azospirillum lipoferum D178*) were prepared in specific broth media. Cell suspension of *A. lipoferum* contains about (1 x 10^6 cfu ml^{-1}) 7 days-old, *B. pumilus* (90 x 10^6 cfu ml^{-1}) 2 days-old and *Ps. fluorescence* (20 x 10^6 cfu ml^{-1}) 5 days-old.

Cultivation process

Prior to transplanting, tomato seedlings were soaked by dipping the root system in a mixture of PGPR inocula (cell suspension of biostimulant) for 60 minutes; sucrose solution (40 %) was used as an measured by using the acetylene reduction technique given by Diloworth (1970).

Growth and yield traits

Leaves number, flowers number, dry weights of the plants were determined at flowering stage (60 days), plant height was determined after 120 days of transplanting. Number of fruits plant^{-1}, fruits yield and weight of fruit plant^{-1} were estimated.

Macro-element content

Total nitrogen, phosphorus and potassium contents were determined according to the methods described by (A.O.A.C, 1970); A.P.H.A. (1992) and Dewis and Freitas (1970), respectively.

Proline and nitrate reductase

Proline was determined according to the method of Bates *et al.* (1973). Nitrate reductase was determined using the method of Abdel-Samad *et al.* (2004).

Statistical analysis was carried out according to Snedecor and Cochran (1989). The differences between the means value of various treatments were compared by Duncan's multiple range test (Duncan's, 1955).

Results and discussion

Effect of tomato inoculation with biostimulants in presence of compost and/or humic acid on the activity of Dehydrogenase (DHA) is shown in Fig.1. The rhizosphere of tomato cultivated in salt-affected soil with no amendments (control) gave lower DHA values, this may be due to the high salt concentration which decreases the microbial activities. The DHA in various treatments were significantly higher at flowering stage (60 days) than vegetative one. This increase of DHA could be attributed to the beneficial effect of root exudates which increase during flowering stage. Higher activity of DHA at flowering stage is likely to be due to the higher multiplication rate of different soil microorganisms.

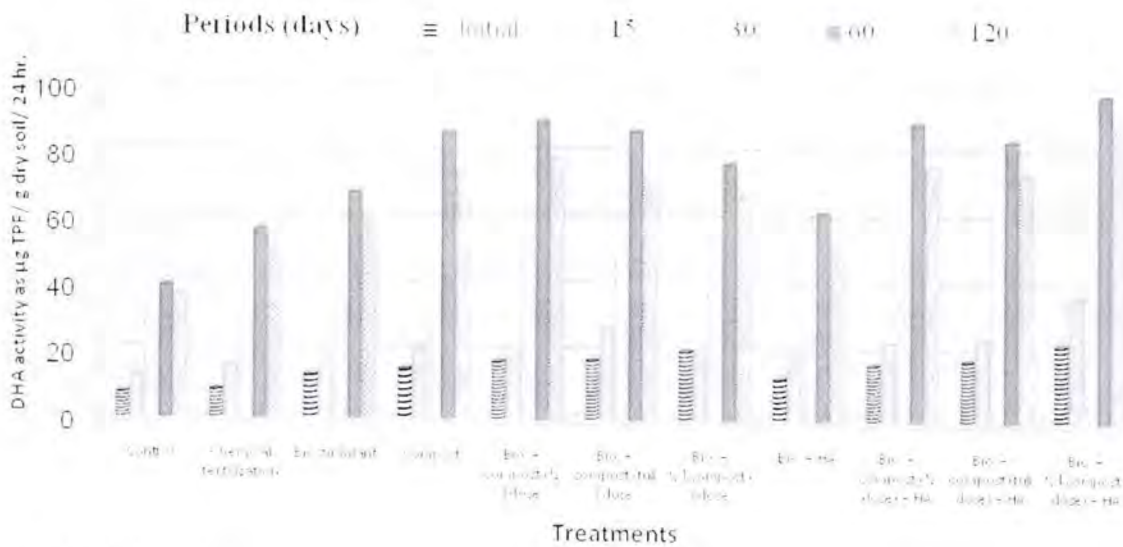


Fig 1. Periodical changes in dehydrogenase activity in soil cultivated with tomato.

Data in Fig. 1 also revealed that the highest significant increase of DHA was observed in case of tomato inoculated with biostimulant combined with humic acid and compost at one and half dose. This result is likely to be due not only to the promotion effect of biostimulant on microbial proliferation but also to the beneficial effect of compost and humic acid. Liu *et al.* (1992) reported that the addition of humic acid to soil enhanced DHA at vegetative and flowering stages. This result could be attributed to the synergistic effect between biostimulant and natural microbial flora occurred in compost which increased the microbial respiration rate. Obtained data showed that relative lower records of DHA were observed in soil amended with chemical fertilization than soil amended with humic acid and/or compost. This result was observed with most experimental periods and was in accordance with Marinara *et al.* (2000) who reported that higher DHA values were observed in soil amended with compost compared to soil fertilized with chemical fertilizers.

Phosphatase activity

Data illustrated in Fig. 2 showed that inoculation of tomato with *Pseudomonas fluorescence D23, Bacillus pumilus D139* and *Azospirillum lipoferum D178* resulted in a significant increase of phosphatase activity compared with either the compost or the chemical fertilization. Obtained results revealed that no significant difference was observed between phosphatase activity in soil treated with biostimulant only and soil treated with chemical fertilizers at 15 and 30 days. Soil inoculated with biostimulant combined with humic aid + compost at one and half dose gave the highest significant values of phosphatase activity.

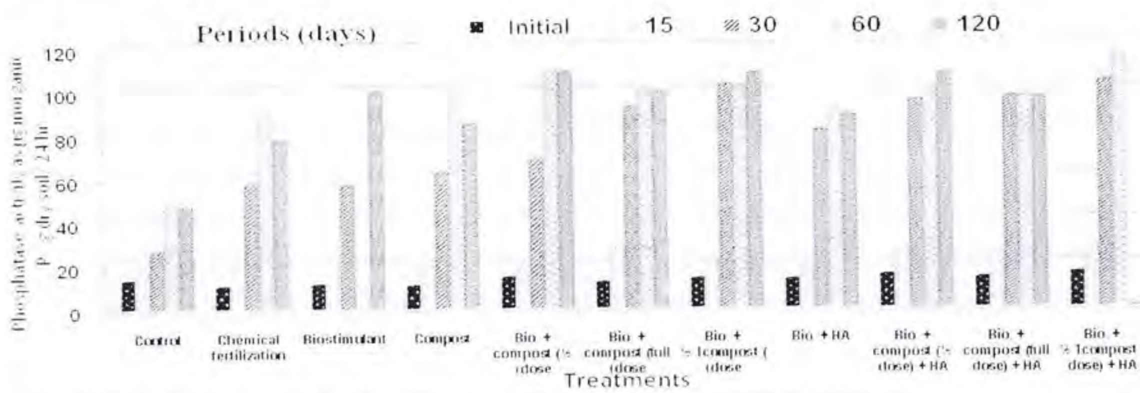


Fig 2. Periodical changes in phosphatase activity in soil cultivated with tomato.

This result may be attributed not only to the effect of inoculation on microbe's number in rhizosphere but also to the beneficial effect of compost on indigenous and introduced biostimulant strains for proliferation and their activities. Balakrishnan *et al.* (2007) found that the application of compost in combination with phosphate solubilizing bacteria significantly increased the soil enzyme activities such as phosphatase. Also, Bama *et al.* (2008) applied humic acid at 20 or 10 kg ha^{-1} with foliar spray and recorded an increase of enzymatic activities such as catalase, dehydrogenase and phosphatase. Data also showed that phosphatase activity was significantly higher at flowering stage than vegetative one. In case of nitrogenase activity (N2-ase) (Fig. 3), it was affected by the investigated treatments. Tomato amended with chemical fertilizers gave the lowest values of N_2-ase activity compared to other treatments. This result is in harmony with that obtained by Anne-Sophie *et al.* (2002) who found that the addition of chemical fertilizers such as ammonium nitrate decreased the nitrogenase activity.

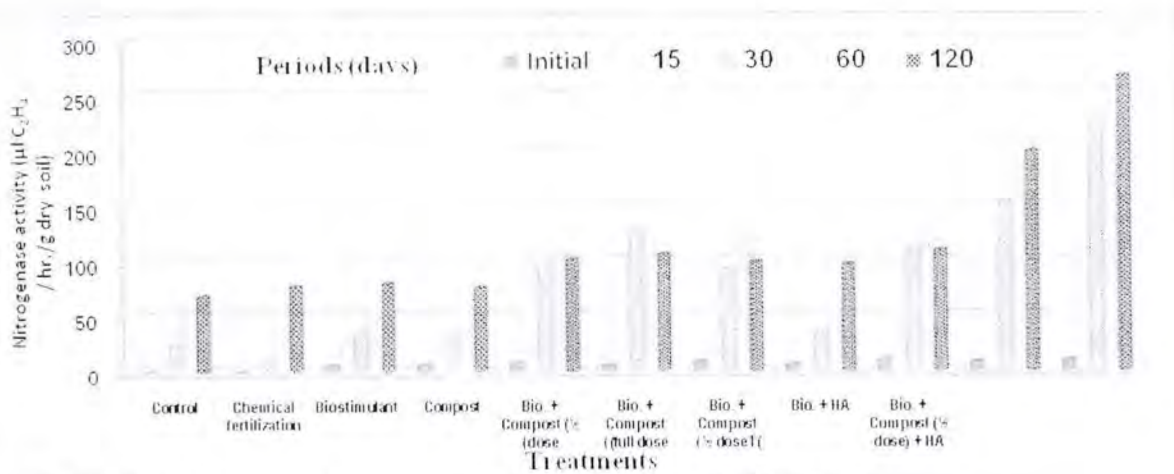


Fig 3. Periodical changes in nitrogenase activity in soil cultivated with tomato.

amended with compost at one and half dose and humic acid in combination with biostimulant. This result may be due to the enhancement of humic acid to the native and introduced microorganisms and also increased the synergistic effect of inocula addition with other microorganisms. These results are in harmony with Meunchang *et al.* (2006) who mentioned that compost promotes plant growth when amended with N_2 - fixing bacteria.

Also, data revealed that soil without any amendments gave significant higher nitrogenase activity than soil treated with NPK fertilizers. It may be due to the activity of native microorganisms. Higher records of N_2-ase activity were observed in soil treated with compost than biostimulant each one singularly. Enhancement of biological activities caused by compost might be due to containing native microorganisms. The highest significant values of N_2-ase activity were observed in soil

Table 2. Growth characteristics of tomato planted in salt-affected soil in response to different soil amendements.

Treatment	Leaf number $plant^{-1}$	Flower number $plant^{-1}$	Shoot length	Root dry weight	Shoot dry weight
	------------no--------		Cm	-----------g------------	
Control[a]	12^{g}	10^{f}	42^{f}	8.9^{a}	15.7^{a}
Chemical fertilization	30^{b}	24^{ab}	86^{a}	7.8^{ab}	14.5^{b}
Biostimulant (Bio)[b]	15^{ef}	13^{ef}	44^{ef}	5.4^{c}	10.9^{d}
Compost	19^{d}	15^{de}	46^{ef}	7.4^{ab}	8.00^{e}
Bio .+ compost (½ dose)	14^{fg}	19^{cd}	51^{de}	8.4^{a}	6.50^{f}
Bio .+ compost (full dose)	17^{de}	19^{cd}	54^{cd}	6.7^{bc}	10.8^{d}
Bio. + compost (1½ dose)	22^{c}	19^{cd}	50^{de}	6.8^{bc}	12.9^{c}
Bio .+ HA[c]	15^{ef}	14^{ef}	54^{cd}	7.2^{ab}	8.20^{e}
Bio. + compost (½ dose) + HA	30^{b}	21^{bc}	58^{c}	7.0^{ab}	14.4^{b}
Bio.+ compost (full dose) + HA	30^{b}	26^{a}	57^{cd}	6.5^{bc}	14.0^{bc}
Bio. + compost (1½ dose) + HA	40^{a}	26^{a}	70^{b}	7.8^{ab}	15.3^{a}

[a]Control:=without any soil amendments.
[b]Biostimulant strains = *Ps. fluorescence D23, B. pumilus D135 and A. lipoferum D178.* [c]HA= Humic acid

Growth characteristics

Data in Table 2 clearly indicated that the lowest records of tomato growth characteristics i.e. leaves number, flowers number and shoot length were observed in plants cultivated in salt-affected soil without any amendments (control). Dual treatment of tomato with biostimulant strains and compost gave higher records of growth characteristics than plants cultivated in soil treated with either biostimulant or compost only. This might be due to the synergistic effect of compost and biostimulant (Table 2).

Significant increase in tomato growth characteristics was observed in soil treated with chemical fertilizers than soil treated with biostimulant only. Respecting the interaction effect between the biostimulant and compost amendment, results revealed that the combination of biostimulant with compost at one and half dose gave high records of tomato shoot length. Similar results were observed by Meunchang *et al.* (2006) who mentioned that the compost promote plant growth when it was amended with N_2-fixing bacteria because the N_2-fixing bacteria colonize roots when compost was used and enhance shoot and root growth. Also, Ranganathan *et al.* (1995) demonstrated that inoculation of tomato seedlings with *Azospirillum* spp. increased growth, flowering and dry matter of plants. It is worthy to mention that leaf number was not affected by the amount of compost, therefore tomato grown in soil amended with compost at half, full and one and half dose gave similar records. Moreover, when soil amended with compost at different doses in presence of humic acid and biostimulant tomato gave higher values of most determined characteristics than other treatments.

Whereas, at 120 days soil amended with biostimulant gave higher values of N_2-ase activity than compost only, this result explained the importance of the poost inocula which added to the experimental soil. Obtained data in Fig. 3 showed that soil treated with compost in combination with biostimulant gave significant higher values of N_2-ase activity in tomato rhizosphere than soil solely treated with each only.

Nitrogen, phosphorus and potassium uptake

Results in Table 3 showed that the lowest values of macronutrients uptake were observed in salt-affected soil without any amendments (control). This may be attributed to the negative effect of salinity on macronutrients absorption. These results are in harmony with Lopez and Satti (1996) who proved that salinity can reduce N_2 accumulation in plants, P concentrations and the uptake of K in plants due to the inhibitive effect of Na on such process. Obtained data revealed that N, P and K uptake were significantly increased in plants grown in soil treated with chemical fertilization than soil amended with compost or biostimulant strains, each one. Moreover, N, P and K uptake by tomato shoots were higher in case of dual application with biostimulant and compost than those recorded in either biostimulant or compost solely with each one. This may be due to the beneficial effect of dual application on macronutrients availability and uptake. The highest records of macronutrients uptake by tomato shoots were observed when soil was amended with biostimulant combined with compost at different doses in presence of humic acid. This might be due to the positive effect of compost on chemical properties of salt-affected soil which might cause release of macronutrients or availability of nutrients. Similar results were observed by Alvarez *et al.* (1995) who reported that the addition of compost to soil cultivated with tomato may affect the release of nutrients to plants directly through the inherent nutrients or indirectly by their effect on the cation-exchange capacity.

Table 3. Uptake of N, P and K by tomato cultivated in salt-affected soil in response to soil amendements.

Treatment	N	P	K
		mg plant^{-1}	
Control[a]	66.58^{c}	8.25^{c}	31.82^{b}
Chemical fertilization	92.84^{b}	13.3^{a}	43.88^{a}
Biostimulant (Bio)[b]	80.16^{bc}	9.25^{b}	40.62^{a}
Compost	84.91^{bc}	9.80^{b}	38.90^{ab}
Bio. + compost (½ dose)	89.67^{bc}	11.1^{ab}	35.26^{ab}
Bio. + compost (full dose)	90.89^{b}	11.2^{ab}	36.68^{ab}
Bio. + compost (1½ dose)	98.98^{b}	11.4^{ab}	38.78^{ab}
Bio. + HA[c]	89.16^{bc}	11.0^{ab}	36.00^{ab}
Bio. + compost (½ dose) + HA	108.9^{ab}	13.4^{a}	33.64^{b}
Bio. + compost (full dose) + HA	118.4^{ab}	13.6^{a}	43.04^{a}
Bio. + compost (1½ dose) + HA	123.9^{a}	14.6^{a}	44.80^{a}

[a]Control:=without any soil amendments.

[b]Biostimulant strains = *Ps. fluorescence D23, B. pumilus D135 and A. lipoferum D178.* [c]HA= Humic acid

In addition, soil treated with humic acid gave higher values of N, P and K uptake by tomato shoots compared with control. This is due to the beneficial effect of humic acid application on the decrease of soil salinity. This result is in agreement with Masciandaro *et al.* (2002) who found that humic substances may enhance the nutrients uptake and reduce the uptake of some toxic elements. Therefore, it could be said that the application of humic substances could improve plant growth under salinity conditions.

Proline accumulation and nitrate reductase activity

Data recorded in Table 4 clearly indicated that salt-affected soil without any amendments (control) gave the highest amounts of proline in tomato plants. It may be due to the response of plants to high concentration of salts. Tomato inoculated with biostimulant combined with compost at one and half dose in presence of humic acid gave the lowest amounts of accumulated proline. This result could be attributed to the beneficial effect of natural microbial flora occurred in compost or biostimulant which have been demonstrated to induce plant tolerance to salinity. This result is in agreement with Mohamed *et al.* (2007) who reported that proline content significantly increased with an increase of NaCl concentration. Also, Martinez *et al.* (1996) found a positive relationship between proline accumulation and NaCl tolerance. In addition, the application of humic acid combined with compost significantly decreased the accumulated proline amounts in tomato plants rather than the absence of humic acid. It may be likely due to the role of humic acid in decreasing the effect of salinity on plants. Humic acid could be used as a growth regulator to regulate hormone level, improve plant growth and enhance stress tolerance (Serenella *et al.*, 2002).

Results clearly indicated that except for the control, chemical fertilization treatment gave the lowest values of nitrate reductase (NR-ase).

Table 4. Proline accumulation and nitrate reductase activity in tomato leaves in salt-affected soil in response to soil amendements.

Treatment	Proline accumulation	Nitrate reductase activity
	mg g^{-1}	μ mol NO_2 g^{-1} hr^{-1}
Control[a]	0.84^{a}	194.7^{a}
Chemical fertilization	0.82^{a}	165.0^{c}
Biostimulant (Bio)[b]	0.72^{b}	177.2^{b}
Compost	0.45^{c}	141.3^{d}
Bio. + compost (½ dose)	0.28^{c}	76.10^{g}
Bio. + compost (full dose)	0.38^{d}	99.00^{f}
Bio. + compost (1½ dose)	0.45^{c}	100.5^{f}
Bio. + HA[c]	0.79^{a}	170.6^{c}
Bio. + compost (½ dose) + HA	0.49^{c}	52.70^{i}
Bio. + compost (full dose) + HA	0.51^{c}	58.90^{h}
Bio. + compost (1½ dose) + HA	0.45^{c}	123.5^{c}

[a]Control:=without any soil amendments.

[b]Biostimulant strains = *Ps. fluorescence D23, B. pumilus D135 and A. lipoferum D178,* [c] HA= Humic acid

Tomato inoculations with biostimulant in combination with compost at different doses significantly increased the nitrate reductase activity compared to either biostimulant or compost each one individually. Also, nitrate reductase increased with the increasing of compost amounts since the addition of compost at one and half dose gave higher records than either half or full dose. The highest values of NR-ase were observed in case of soil amended with compost at one and half dose in presence of humic acid and biostimulant. This result may be due to the beneficial effect of the native microorganisms occurred in compost and their synergistic effect with biostimulant. These results are in agreement with Zhang *et al.* (2008) who stated that the positive effects of humic acid on plant growth could be mainly due to hormone-like activities of the humic acid through their involvement in oxidative phosphorylation, protein synthesis, antioxidant and various enzymatic reactions.

Yield and yield components

Data in Table 5 revealed that the lowest number of fruits/plant was observed in tomato grown in salt-affected soil without any amendments (control), while the treatments that contain biostimulant, compost and/or humic acid gave higher number of fruits plant^{-1}. Similar results were observed by Ullah *et al.* (1994) who reported that tomato fruit production was negatively affected by high salt concentrations. Results also showed that the number of fruits plant^{-1} was similar in case of tomato inoculation with biostimulant combined with compost at different doses in presence of humic acid. This trend of results is in agreement with Zandonadi *et al.* (2007) who reported that humic acid increased growth and yield of various crops including vegetables. Chemical fertilization of tomato gave significant higher number of fruits than tomato inoculation with either biostimulant or compost. The highest weight of tomato fruits was observed when tomato inoculated with biostimulant in combination with compost at one and half dose in presence of humic acid. Obtained data in Table 5 also revealed that tomato yield was significantly increased with the increasing of compost dose, it may be due to the synergistic effect of natural microbial flora occurred in compost on the introduced inocula (biostimulant). Chemical fertilization of tomato gave higher yield of fruits plant^{-1} than tomato inoculated with either biostimulant or compost. The highest significant yield of tomato fruits was observed when tomato was inoculated with biostimulant in combination with compost at one and half dose in presence of humic acid. This result could be due to the beneficial effects of humic acid and compost. These results are in harmony with Zhang *et al.* (2008) who reported that the positive effects of humic acid on productivity, which seem to be concentration-related, could mainly be due to hormone like activities of the humic acid through their involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, antioxidant and various enzymatic reactions.

Table 5. Yield and yield components of tomato grown in salt-affected soil in response to soil amendements.

Treatments	Number of fruits plant^{-1}	Weight of one fruit	Fruits yield plant^{-1}
	no	g	kg
Control[a]	9[b]	45.7[d]	0.411[f]
Chemical fertilization	15[ab]	50.7[bcd]	0.761[cd]
Biostimulant (Bio)[b]	12[ab]	53.8[abc]	0.646[e]
Compost	14[ab]	51.5[bcd]	0.721[de]
Bio. + compost (½ dose)	14[ab]	54.2[abc]	0.759[cd]
Bio. + compost (full dose)	15[ab]	57.8[a]	0.867[ab]
Bio. + Compost (1½ dose)	15[ab]	55.5[ab]	0.833[bc]
Bio. + HA[c]	14[ab]	49.1[cd]	0.687[de]
Bio. + compost (½ dose) + HA	16[a]	59.1[a]	0.946[a]
Bio .+ compost (full dose) + HA	16[a]	58.3[a]	0.934[a]
Bio. + compost (1½ dose) + HA	16[a]	59.7[a]	0.955[a]

[a]**Control:=without any soil amendments.**

[b]**Biostimulant strains = *Ps. fluorescence D23, B. pumilus D135 and A. lipoferum D178,*** [c]**HA= Humic acid**

Also, Ulukan (2008) reported that the improving soil conditions and establishing equilibrium among plant nutrients are also important for soil productivity and plant production. Moreover, humic substances and organics improve the soil characteristics and increase the yield of vegetable crops.

Conclusion

This study recommended that the use of salt tolerant PGPR as biostimulant for tomato grown in salt-affected soils can improve growth performance and productivity under saline stress. Results also indicated that tomato inoculation with PGPR in combination with compost and humic acid enhanced plant resistance to stress through the reduction of proline accumulation and increase of nitrate reductase.

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