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Enrichment of recycled organic waste with N fertilizer and PGPR containing ACC-deaminase for improving growth and yield of tomato

M. Tahir, M. Arshad, M. Naveed, Z.A. Zahir, B. Shaharoona and R. Ahmad*
Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad, Pakistan

Abstract

Recycling of organic wastes enriched with nitrogen and inoculated with plant growth promoting rhizobacteria could convert organic waste material into a value added organic/biofertilizer, which may be effective even when added in substantially small amounts (kg ha^{-1}). In this study, organic waste of fruit and vegetables was collected and composted in a locally-fabricated unit. The composted material was enriched with 25% of full dose (114 kg ha^{-1}) of N fertilizer (95 g kg^{-1} compost) and inoculated with plant growth promoting rhizobacteria (PGPR) to convert it into a biofertilizer. Organic/biofetilizer was applied @ 300 kg ha^{-1} (2 mg pot^{-1}) to the pots fertilized with NPK (114-62-57 kg ha^{-1}), respectively. The P and K fertilizers were applied as basal treatment in all pots, while N was applied in two splits. Two separate pot trials were conducted to evaluate the effectiveness of organic/biofertilizers in the presence of 50% chemical N fertilizer. Seeds of tomato cultivars (Money maker and Nagina) were sown. Results of pot trials revealed that organic/biofertilizer supplemented with 57 kg ha^{-1} (50% of full dose of N fertilizer) was more effective in improving the growth and yield of tomato as compared to full dose of N fertilizers. It increased the root length, dry root/shoot weight, number of fruit pot^{-1} and fruit weight pot^{-1} up to 91, 98/62, 71 and 46%, respectively, over control, in 1st trial, while in 2nd trial, an increase up to 63, 115/70, 81and 68%, respectively, over control, was recorded. Similarly, N concentration in dry fruit and straw of tomato plants were significantly improved (35 and 43% over control, respectively) in case of biofertilizer plus 50% N fertilizer in both the trials. These findings may imply that organic waste could be recycled into value added soil amendment (biofertilizer) for improving growth, yield and nutrient uptake of tomato on sustainable basis. Supplementary role of biofertilizer can improve the yield up to a level that otherwise cannot be achieved by the use of chemical fertilizers alone.

Key words: Recycled organic waste, organic/bio-fertilizer, NPK-fertilizers, tomato

Introduction

Organic matter is known to improve soil health and availability of plant nutrients. Although some of the organic wastes are utilized to some extent in agriculture but most of them are either burnt or remained unutilized, specially in developing countries. Both of the latter practices not only pose serious threat to the environment, but also result in the loss of useful nutrient pool which otherwise can be made available to plant. Organic waste materials are available in huge amounts in the form of farm waste, city waste (sewage sludge), poultry litter and industrial wastes (food, sugar, cotton and rice industry). The continuous accumulation of these wastes is becoming a potential source of land, water and air pollution. These organic wastes could be used as soil amendments; however, direct application of waste materials in raw form is usually not suitable for soil health. As per the conventional practice, organic wastes (either composted or non-composted) are being used in t ha^{-1} for the improvement of crop productivity (Nevens and Reheul, 2003; Wolkowski, 2003; Terrance *et al*., 2004). Thus, the availability of organic materials/wastes in bulk volumes to be applied at several t ha^{-1} could be a limiting factor in its extensive use and may also not be cost effective.

Composting is one of the major recycling processes in which organic materials are biologically/biochemically converted into an amorphous humus like substance (under conditions of optimum temperature, moisture and aeration) that can be handled, stored and applied to land without environmental impacts (Gallardo- Larva and Nogades, 1987). Over the couple of decades, compost production has got dramatic attention in agricultural, industrial and municipal sectors. This is mainly due to increased solid waste management costs, and heightened public enthusiasm for organic

* E-mail: bio@fsd.comsats.net.pk

waste recycling. Composting provides a way to manage high volume of organic waste in environmentally sound manners (Hoitink and Fahy, 1989; Lasaridi and Stentiford, 1999). In general, composted materials are highly regarded for their ability to improve soil health and plant growth. Moreover, compost has also been found to aid in suppression of pathogens and plant diseases.

Beneficial rhizobacteria often referred to as plant growth-promoting rhizobacteria or PGPR (Kloepper *et al*., 1989), affect plant growth either directly or indirectly through various mechanisms of action (Glick *et al*., 1998; Mantelin and Touraine, 2004; Khalid *et al*., 2004, 2006). Glick *et al*. (1998) have reported that some PGPR function as a sink for 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene in higher plants, by hydrolyzing it into α-ketobutyrate and ammonia, and in this way promote root growth by lowering endogenous ethylene levels in plant. PGPR with ACC-deaminase trait usually give very consistent results in improving plant growth and yield and thus, are good candidate for bio-fertilizer formulation (Shaharoona *et al*., 2006a, b).

Very recently, interest has been renewed in compost technology as a solid waste management strategy. A wise manipulation of composted material not only can reduce application rates of compost but could also help in achieving the product of desired characteristics. A novel approach to convert composted material into value-added product may be the enrichment/blending of the compost with nutrients (organic fertilizer) and/or plant growth promoting rhizobacteria (bio-fertilizer).

The present study is focused on developing an effective soil amendment (organic- or bio-fertilizer) from fruit and vegetable wastes by using both composting and enrichment with nitrogen and PGPR containing ACC-deaminase to increase per unit yield of tomato on sustainable basis. This approach could help to obtain high yield potential on one hand and reduce dependence on chemical fertilizers on the other hand.

Materials and methods

Two pot experiments were conducted to test the effectiveness of composted organic wastes enriched with nitrogen (organic fertilizer) and inoculated with PGPR containing ACC-deaminase (bio-fertilizer) for improving growth, yield and nutrient uptake of tomato.

Preparation of N-enriched compost (organic fertilizer)

Fruit and vegetable wastes collected from various locations (local fruit and vegetable market and juice shops etc.) of Faisalabad city were composted in a locally fabricated unit consisting of drier, crusher/grinder and processor. First organic wastes were air-dried for couple of days to remove excessive moisture and sorted out to remove unwanted materials (e.g. pieces of metals, glass, polythene bags etc.). The sorted organic material was oven-dried at 70^{o}C for 24 h and ground into finer particles (<2.0 mm) with the help of an electric grinder. The crushed material was transferred to a vessel (500 kg capacity) for composting under controlled temperature and aeration (shaking at 50 rev min^{-1}). A moisture level of 40% (v/w) of the compost was maintained during the composting process. Temperature rose up from 30 to 70^{o}C in the composting unit during 2nd and 3rd day of composting process and then reduced gradually to 30^{o}C after 4th day process. Composting was done for 5 days and composted organic material was analyzed for macro- and micro-nutrients (Table 1).

Table 1: Analysis of raw and recycled organic waste

Parameter	Raw organic waste (Before composting)	Recycled organic waste (After composting)
Carbon (%)	31.7	23.15
Nitrogen (%)	1.35	2.27
Phosphorous (%)	0.19	0.31
Potassium (%)	1.16	1.59
Iron (mg kg^{-1})	375.00	597.00
Copper (mg kg^{-1})	1.11	1.31
Zn (mg kg^{-1})	39.00	46.11
Manganese (mg kg^{-1})	34.21	45.27

The finished compost was enriched by mixing it with N-fertilizer @ 95 g kg^{-1} compost (28.5 kg N = 25% of 114 kg ha^{-1} N) to enhance the quality and nutritional value of the organic product. Thus, 300 kg batch of compost received 28.5 kg N for tomato crop.

Preparation of biofertilizer

The bio-fertilizer was prepared by inoculating organic fertilizer (N-enriched compost) with the selected strains of PGPR containing ACC-deaminase activity (ACC4 and ACC14). For this purpose, rhizobacteria were isolated from tomato rhizosphere by dilution plate technique using DF salt minimal medium containing ACC as a sole N source (Glick *et al*., 1994). The rhizobacteria were screened for plant growth promoting activity under axenic conditions (data not shown). The inoculum was prepared by growing the selected PGPR carrying ACC-deaminase activity in 250 mL flasks containing DF minimal salt medium. The flasks were incubated at 28 ± 1 ºC for 48 h under shaking (100 rev min^{-1}). The suspension of selected rhizobacterium [10^8 colony forming unit (cfu) mL^{-1}] was mixed with the N-enriched compost (10 mL $kg^{-}1$ compost) and kept for 24 h at room temperature.

Pot trials

Two pot experiments during the same season were carried out in the wire house using sandy clay loam soil. The air-dried and sieved (2 mm) soil was analyzed before filling into pots. The analysis of composite soil sample revealed a pH of 7.6; electrical conductivity, 2.6 dS m^{-1}; organic matter, 0.68%; total N, 0.05%; available P, 7.8 mg kg^{-1} and extractable K, 129 mg kg^{-1} soil.

Each pot was filled with sieved soil (12 kg soil pot^{-1}) mixed with P and K fertilizers (applied at 57 and 62 kg ha^{-1} as single super phosphate and sulphate of potash, respectively). The P and K fertilizers were applied in all the treatments as a basal dose. Nitrogen as urea was applied at 114 kg ha^{-1} in two splits. The organic- and bio-fertilizers (on air dry weight basis) were applied @ 300 kg ha^{-1} (2 mg pot^{-1}) supplemented with 50% i.e. 57 kg ha^{-1} N fertilizer (428 mg pot^{-1}). The details of treatments are given in Tables 2-5. Soil was thoroughly mixed with the amendments before filling of pots.

Nursery of tomato varieties (Mony maker and Nagina) was sown separately. After one week, uniform-sized seedlings were selected and transplanted in the pots (two plants pot^{-1}) in two separate trials; one for each variety. The pots were arranged randomly in wire house with four replications at ambient light and temperature. The pots were kept moist near field capacity (60% WHC) by using good quality canal water (electrical conductivity = 0.03 dS m^{-1}, sodium adsorption ratio = 0.26 (mmol $L^{-1})^{1/2}$, residual sodium carbonate = 0) meeting the irrigation quality criteria for crops (Ayers and Westcot, 1985). Data regarding various growth parameters like number of fruits pot^{-1}, total fruit weight pot^{-1}, root depth, dry root weight and dry shoot weight was recorded at maturity. Fruit and straw samples were collected for the analyses of NPK contents. The data from both the trials were subjected to statistical analysis using the completely randomized design as described by Steel and Torrie (1980). Means were compared by Duncan' multiple-range test (Duncans, 1955).

Results

First Trial

The results of first trial (Table 2) revealed that application of bio-fertilizer (organic fertilizer inoculated with ACC4) supplemented with 57 kg ha^{-1} N fertilizer (50% of 114 kg ha^{-1} N) significantly increased the root length up to 91% over control. Next to it, the highest root length was recorded with bio-fertilizer (organic fertilizer inoculated with ACC14) in the presence of 57 kg ha^{-1} N fertilizer that was 84% more than control. Root length obtained by N-enriched compost supplemented with 57 kg ha^{-1} N fertilizers differed non-significantly from full dose (114 kg ha^{-1}) of N fertilizer. The maximum root dry weight (98% increase over control) was observed with bio-fertilizer (ACC14) plus 57 kg N ha^{-1} that was at par with bio-fertilizer fertilizer (ACC4) plus 57 kg N ha^{-1} followed by N-enriched compost in the presence of 57 kg ha^{-1} N fertilizer and full dose of N fertilizer, respectively. Regarding shoot dry weight, bio-fertilizer (ACC14) gave maximum dry weight (62% over control) in the presence of 57 kg N ha^{-1} that was statistically similar with bio-fertilizer (ACC4). The shoot dry weight recorded with full dose of N fertilizer differed non significantly from the N-enriched compost supplemented with 57 kg ha^{-1} N fertilizers. Data regarding number of fruit and fruit weight pot^{-1} revealed that bio-fertilizer (organic fertilizer inoculated with ACC4) plus 57 kg ha^{-1} N fertilizer produced maximum number and weight of tomato fruit (42 and 71%, respectively over control) that

Table 2. Effect of organic-/bio-fertilizers supplemented with chemical-fertilizers on root length, dry root weight, dry shoot weight, number of fruits pot^{-1} and fruit weight pot^{-1} of tomato [Trial-1 (Money maker); the data are average of three replications]

Treatments[a]	Root length (cm)	Root dry weight (g)	Shoot dry weight (g)	Number of fruits pot^{-1}	Fresh fruit weight pot^{-1} (g)
Control (P and K only)	23.00 d[e]	6.39 c	63.00 c	26.00 c	480.00 c
Urea fertilizer (114 kg ha^{-1})	37.67 bc	9.60 b	99.93 ab	33.33 b	765.33 b
EC[b]+ 57 kg ha^{-1} N	36.67 c	9.93 b	89.33 b	33.00 b	751.67 b
Biofertilizer[c] + 57 kg ha^{-1} N	44.00 a	11.83 a	101.67 a	38.00 a	822.00 a
Biofertilizer[d] + 57 kg ha^{-1} N	42.33 ab	12.67 a	102.00 a	37.33 a	805.46 a

[a]The P and K fertilizers were applied @ 62 and 57 kg ha^{-1}, respectively in all the treatments
[b]Enriched compost (95 g N kg^{-1} compost). The composition of compost is given in Table 1
[c]Enriched compost inoculated with selected PGPR strain (ACC4)
[d]Enriched compost inoculated with selected PGPR strain (ACC14)
[e]Values sharing similar letter(s) in a column do not differ significantly at $P < 0.05$, according to Duncan's multiple range test

was at par with bio-fertilizer (organic fertilizer inoculated with ACC14) plus 57 kg ha^{-1} N fertilizer. Similarly, enriched compost supplemented with 57 kg ha^{-1} N and full dose of N fertilizers remained at par in number and weight of fruits pot^{-1}. Maximum N content in dry fruit was observed with bio-fertilizer (ACC4) in the presence of 57 kg ha^{-1} N (26% more than control) (Table 4). However, it differed significantly from all other treatments. Next to it, the highest N content (24.5% over control) was recorded with bio-fertilizer (ACC14) and 57 kg ha^{-1} N fertilizer followed by full dose of N and enriched compost in the presence of 57 kg ha^{-1} N fertilizer. Statistically similar N contents in dry shoot were observed with bio-fertilizers (organic fertilizer inoculated with ACC4 and ACC14) supplemented with 57 kg ha^{-1} N and full dose of N fertilizer.

Second Trial

The data regarding the effect of organic-/bio-fertilizer (inoculated with ACC4 and ACC14) in the presence of 57 kg ha^{-1} N fertilizer on root length, root/shoot dry weight, fruit number/weight pot^{-1} and N contents in dry fruit/shoot are summarized in Table 3 and 4. The maximum root length (up to 63% over control) was recorded with bio-fertilizers (ACC14 and ACC4) supplemented with 57 kg ha^{-1} N fertilizer. Root length recorded by N-enriched compost in the presence of 57 kg ha^{-1} N fertilizer differed non significantly from full dose of N fertilizer. Regarding root dry weight, bio-fertilizer (organic fertilizer inoculated with ACC14) and 57 kg ha^{-1} N gave maximum root dry weight i.e. 115% over control that differed significantly from all other treatments. Bio-fertilizer (ACC4) supplemented with 57 kg ha^{-1} N and full dose of N fertilizer remained at par in root dry weight. Maximum shoot dry weight was observed (70% over control) with bio-fertilizer (ACC14) and 57 kg ha^{-1} N fertilizer that differed non significantly from full dose of N fertilizer. Shoot dry weight recorded with bio-fertilizer (ACC4) plus 57 kg ha^{-1} N and N-enriched compost in the presence of 57 kg ha^{-1} N fertilizer were statistically at par (i.e. 52.5 and 46% increase, respectively over control). Data regarding number of fruits pot^{-1} revealed that bio-fertilizer (ACC14) plus 57 kg ha^{-1} N fertilizer gave maximum number of fruits pot^{-1} (68% more than control) that was significantly different from all other treatments. Maximum fruit weight pot^{-1} (81% over control) was produced with bio-fertilizer (ACC14) supplemented with 57 kg ha^{-1} N fertilizer that was at par with bio-fertilizer (ACC4) plus 57 kg ha^{-1} N fertilizer. Fruit weight obtained by full dose of N fertilizer differed non significantly from N-enriched compost in the presence of 57 kg ha^{-1} N fertilizer. The maximum N content in dry fruit (up to 35% over control) was recorded with bio-fertilizers (ACC14 and ACC4) in the presence of 57 kg ha^{-1} N fertilizer that was significantly different from all other treatments. Regarding N content in dry shoot, bio-fertilizer (ACC14) plus 57 kg ha^{-1} N fertilizer gave maximum N content (40% over control) that was statistically at par with bio-fertilizer (ACC4) plus 57 kg ha^{-1} N fertilizer. Nitrogen content in dry shoot recorded by N-enriched compost in the presence of 57 kg ha^{-1} N differed non significantly from full dose of N fertilizer.

Table 3. Effect of organic-/bio-fertilizers supplemented with chemical-fertilizers on root length, dry root weight, dry shoot weight, number of fruits pot^{-1} and fruit weight pot^{-1} of tomato [Trial-2 (Nagina); the data are average of three replications]

Treatments[a]	Root length (cm)	Root dry weight (g)	Shoot dry weight (g)	Number of fruits pot^{-1}	Fresh fruit weight pot^{-1} (g)
Control (P and K only)	25.33 c[e]	6.23 d	40.00 c	15.67 d	442.00 d
Urea fertilizer (114 kg ha^{-1})	33.00 b	8.68 b	62.00 ab	22.33 bc	680.47 bc
EC[b]+ 57 kg ha^{-1} N	33.33 b	8.23 c	58.33 b	22.00 c	643.33 c
Biofertilizer[c] + 57 kg ha^{-1} N	39.00 a	10.20 b	61.00 b	24.00 b	745.00 ab
Biofertilizer[d] + 57 kg ha^{-1} N	41.33 a	13.42 a	68.00 a	26.33 a	800.77 a

[a]The P and K fertilizers were applied @ 62 and 57 kg ha^{-1}, respectively in all the treatments
[b]Enriched compost (95 g N kg^{-1} compost). The composition of compost is given in Table 1
[c]Enriched compost inoculated with selected PGPR strain (ACC4)
[d]Enriched compost inoculated with selected PGPR strain (ACC14)
[e]Values sharing similar letter(s) in a column do not differ significantly at $P< 0.05$, according to Duncan's multiple range test

Table 4. Effect of organic-/bio-fertilizers supplemented with chemical-fertilizers on % N contents in dry fruits and shoots of tomato (Trial-1 and Trial-2); the data are average of three replications)

Treatments[a]	Trial-1		Trial-2	
	N contents in dry fruits (%)	N contents in dry shoots (%)	N contents in dry fruits (%)	N content in dry shoots (%)
Control (P and K only)	1.59 d[e]	0.500 c	1.58 d	0.510 c
Urea fertilizer (114 kg ha^{-1})	1.97 bc	0.697 ab	1.99 b	0.673 b
EC[b]+ 57 kg ha^{-1} N	1.96 c	0.680 b	1.93 c	0.670 b
Biofertilizer[c] + 57 kg ha^{-1} N	2.00 a	0.767 a	2.12 a	0.687 ab
Biofertilizer[d] + 57 kg ha^{-1} N	1.98 b	0.710 a	1.14 a	0.700 a

[a]The P and K fertilizers were applied @ 62 and 57 kg ha^{-1}, respectively in all the treatments
[b]Enriched compost (95 g N kg^{-1} compost). The composition of compost is given in Table 1 [c]Enriched compost inoculated with selected PGPR strain (ACC4)
[d]Enriched compost inoculated with selected PGPR strain (ACC14)
[e]Values sharing similar letter(s) in a column do not differ significantly at $P< 0.05$, according to Duncan's multiple

Discussion

Results regarding the growth and yield of tomato revealed that N-enriched compost (organic fertilizer) supplemented with 57 kg ha^{-1} N (urea) was comparable in effectiveness to full dose (114 kg ha^{-1}) of N fertilizer (urea) in both trials. Over all, there was ~25% saving of N-economy with the application of 300 kg ha^{-1} (2 mg pot^{-1}) organic fertilizer. Our findings support the work of other scientists who reported that application of compost (an organic material) could save ~20-35% mineral N fertilizer (Bajpai *et al*., 2002; Pooran *et al*., 2002; Nevens and Reheul, 2003).

The increase in growth and yield of tomato could be attributed to the enhanced nutrient use efficiency in the presence of organic fertilizer being source of macro and micro nutrients (Table 1). This premise is further supported by the fact that total N uptake in tomato were significantly increased (see Table 4) in response to combined application of organic and chemical fertilizers. Similar findings have been reported by other scientists that the use of composted material enhanced the fertilizer use efficiency (Kademani *et al*., 2003; Nevens and Reheul, 2003; Rubapathi *et al*., 2002).

Application of bio-fertilizer (inoculated organic fertilizer) supplemented with 57 kg ha^{-1} N significantly increased the growth and yield of tomato in both the trials compared to full dose of N-fertilizer and showed superiority over organic fertilizer. It implies that inoculation with PGPR strains (ACC4 and ACC14) further improved the effectiveness of organic fertilizer. It increased the root length, dry root/shoot weight, number of fruit pot^{-1} and fruit weight pot^{-1} up to 91, 115/70, 81 and 68%, respectively, over control in both the trials.

Figure 1: Effect of bio-fertilizer supplemented with 57 kg ha^{-1} N (50% of 114 kg N) on root growth of tomato in a pot trial [bio-fertilizer was prepared by inoculating organic fertilizer (enriched compost) with PGPR strain ACC4]

Figure 2: Effect of bio-fertilizer supplemented with 57 kg ha^{-1} N (50% of 114 kg N) on root growth of tomato in a pot trial [bio-fertilizer was prepared by inoculating organic fertilizer (enriched compost) with PGPR strain ACC14]

Similarly, N concentration in dry fruit and straw of tomato plants were improved (35 and 43% over control, respectively) in case of biofertilizers and 50% N fertilizer in both the trials. Bio-fertilizer application enhanced the nutrient uptake, growth and yield of tomato most likely by promoting root growth (Figure 1 and 2) because of ACC-deaminase activity, in addition to the positive attributes of organic fertilizer. Very recently, it has been reported that inoculation with PGPR containing ACC-deaminase significantly increased maize yield under field conditions (Shaharoona *et al*., 2006b). This may imply that inoculation of organic fertilizer with such traits of PGPR could convert organic product into an effective bio-fertilizer and make it superior over organic fertilizer.

The novelty of the approach being used in our study was the application of biofertilizer just at the rate of 300 kg ha^{-1} (2 mg pot^{-1}), as previously researchers reported saving of N fertilizer and increase in crop yield by applying raw/compost organic material in tons ha^{-1} (Bajpai *et al*., 2002; Pooran *et al*., 2002; Cheuk *et al*., 2003; Nevens and Reheul, 2003; Guar and Geeta, 1993). The technology is, therefore cost effective as it reduces dependence on chemical fertilizers and helps in nutrients and water conservation. Moreover, it is economical and safe disposal of organic wastes, so the reduction of huge piling of organic waste is an extra benefit. Integrated use of value-added organic/bio-fertilizer and chemical fertilizers to get higher yields is, therefore better than sole application of either huge amount of low quality raw organic material or adequate amount of chemical fertilizer.

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