

RELATIVE EFFICACY OF *PSEUDOMONAS* SPP., CONTAINING ACC-DEAMINASE FOR IMPROVING GROWTH AND YIELD OF MAIZE (*ZEA MAYS* L.) IN THE PRESENCE OF ORGANIC FERTILIZER

M. NAVEED¹, M. KHALID^{1*}, D.L. JONES², R. AHMAD³ AND Z.A. ZAHIR¹

¹*Institute of Soil & Environmental Sciences, University of Agriculture Faisalabad, Pakistan*

²*School of the Environment and Natural Resources, Environment Centre Wales, Bangor University, Gwynedd LL57 2UW, UK*

³*Land Resources Research Program, National Agriculture Research Centre, Islamabad-45500, Pakistan.*

Abstract

This study was designed to assess the performance of plant growth promoting rhizobacteria (PGPR) containing ACC-deaminase for improving growth and yield of maize in the presence of organic fertilizer. Organic fertilizer was prepared by composting fruit and vegetable wastes in a locally-fabricated unit and enriched with N fertilizer applied @ 147 g kg⁻¹ compost. This 'organic fertilizer' was used to formulate bio-fertilizers by using three PGPR strains containing ACC-deaminase, *Pseudomonas putida* biotype A (Q₇), *Pseudomonas fluorescens* (Q₁₄) and *Pseudomonas fluorescens* biotype G (N₃), separately. The organic- and/or bio-fertilizers were applied to maize @ 300 kg ha⁻¹ without/with 88 kg ha⁻¹ urea-N in the field trials. A basal dose of P and K (100 and 50 kg ha⁻¹, respectively) was applied to all plots and also tested in the field trials in the absence of organic-/bio-fertilizer. Results of field study revealed that the organic fertilizer supplemented with 88 kg ha⁻¹ N was equally effective to full dose of N-fertilizer (175 kg ha⁻¹) in improving cob weight, fresh biomass and grain yield of maize. However, bio-fertilizer supplemented with 88 ha⁻¹ N fertilizer significantly increased the growth and yield of maize over full dose of N-fertilizer and exhibited superiority over organic fertilizer. Organic-/bio-fertilizer application also significantly enhanced N, P and K uptakes. The *Pseudomonas fluorescens* biotype G (N₃) containing biofertilizer was found best to increase grain yield and nutrient uptake both in the presence or absence of 88 kg N ha⁻¹. Results may imply that organic waste could be composted into value-added soil amendment by enriching/blending it with N and PGPR containing ACC-deaminase activity. This approach is based on using organic- or bio-fertilizers (N-enriched and inoculated compost) at lower rates (just 300 kg ha⁻¹) instead of tons ha⁻¹ of non-enriched composts. Moreover, this strategy could also be useful to protect our environments against threat posed by organic wastes.

Introduction

Usually, composted or non-composted organic wastes are being used in large amounts (t ha⁻¹) for crop production (Wolkowski, 2003; Loecke *et al.*, 2004). Availability of organic wastes in huge bulk, and their transport and application cost are becoming limiting factors now-a-days. This has forced the farming community to rely on chemical fertilizers only as ready nutrient source.

Composting organic wastes and their enrichment with suitable amount of chemical fertilizer could enhance fertilizer use efficiency and recycle organic waste materials/ organic matter into soil, restoring soil health and improving crop yield on sustainable basis (Ahmad *et al.*, 2008 a, b). Composting is a biological process which converts heterogeneous organic wastes into amorphous and stable humus like substances by microbial decomposition under controlled conditions (Ahmad *et al.*, 2007a). This compost product can be handled, stored and applied to land without environmental impacts (Millner *et al.*, 1998; Eghball *et al.*, 2004).

*Corresponding author: E-mail: drkhaliduaf@yahoo.com

However, exclusive use of chemical fertilizers (cause decline in organic matter status) and/or compost (decrease crop yield) which is not acceptable under the present agriculture system. Integration of both of these nutrient and /or organic matter sources could enhance crop yield and improve soil health on sustainable basis. A novel approach is to convert composted material into value-added product through the enrichment/blending of the compost with nutrients (organic fertilizer) and/or plant growth promoting rhizobacteria (bio-fertilizer) (Ahmad *et al.*, 2006; Ahmad *et al.*, 2008a). This wise manipulation of composted material, not only reduced application rates of compost but also helped in achieving the product of desired characteristics (Ahmad *et al.*, 2007a; Zahir *et al.*, 2007).

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 & 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.*, 2006b, 2007).

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 maize on sustainable basis. This approach could help to obtain high yield potential and also reduce dependence on chemical fertilizers without compromising per unit yield of maize. Contrary to conventional application of organic wastes in $t\ ha^{-1}$, this approach is based on using the value-added product at just $300\ kg\ ha^{-1}$. This environmental friendly strategy can also help in tackling the pollution problem created due to the piling up of huge volumes of organic wastes.

Materials and Methods

Two field 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) compared with chemical fertilizer for improving growth, yield and nutrient uptake of maize.

Preparation of organic fertilizer: Waste fruit and vegetables collected from various locations (local fruit and vegetable market and juice shops etc.) of Faisalabad city (longitude $72^{\circ} 0'$ and $73^{\circ} 45'$ east and $30^{\circ} 30'$ and $32^{\circ} 0'$ north), Pakistan were composted in a locally fabricated unit consisting of drier, crusher/grinder and processor. The waste was air-dried for couple of days to remove excess moisture and unwanted materials (e.g. pieces of metals, glass, polythene bags etc.) were sorted out manually. The sorted organic material was oven-dried at $70^{\circ}C$ for 24 h and ground into finer particles ($<2.0\ mm$) with the help of an electric grinder. The ground 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 $70^{\circ}C$ in the composting unit during 2nd and 3rd day of composting process and then reduced gradually to $30^{\circ}C$ after 4th day

process. Composting was done for 5 d and composted organic material was analyzed on dry weight basis for carbon contents (Nelson & Sommers, 1996), and macro- and micro-nutrients (Ryan *et al.*, 2001). The C/N, C/P and C/K ratios were determined (Figs. 1 & 2).

The enrichment of compost was carried out by mixing it with N-fertilizer (urea, Engro Fertilizer Company Ltd., Pakistan) @ 44 kg N 300 kg⁻¹ compost (44 kg N = 25% of 175 kg ha⁻¹ N) during composting to enhance the quality and nutritional value of the organic product.

Preparation of bio-fertilizers: Bio-fertilizers were prepared by inoculating organic fertilizer (N-enriched compost) with selected strains of PGPR containing ACC-deaminase activity. For this purpose, rhizobacteria were isolated from maize 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 and characterized as described previously (Shaharoon *et al.*, 2006a). Three selected strains of PGPR, *Pseudomonas putida* biotype A (Q7), *Pseudomonas fluorescens* (Q14) and *Pseudomonas fluorescens* biotype G (N₃) containing ACC-deaminase activity exhibiting the highest growth promoting activity (Shaharoon *et al.*, 2006b) were used for bio-fertilizer formulation. The inoculum of each strain was prepared by growing them in 500 ml flasks containing DF minimal salt medium. The flasks were incubated at 30°C for 48 h under shaking (100 rev min⁻¹). The suspension of selected rhizobacteria [10⁸ colony forming unit (cfu) ml⁻¹] was mixed with three different batches of N enriched compost (10 ml kg⁻¹ compost) and kept for 24 h at room temperature. The bio-fertilizers were named as Biofert_{Q7}, Biofert_{Q14} and Biofert_{N₃}, respectively. Each bio-fertilizer had 10⁹-10¹⁰ bacterial cells g⁻¹ compost at the time of application to soil.

Field trials: Two parallel field trials on maize were conducted at the Agronomy Field Station of Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. Composite soil samples were prepared by mixing individual samples collected before the experiment and analyzed for various physical and chemical characteristics. The soil was sandy clay loam having pH, 7.6; ECe, 1.85 dS m⁻¹; organic matter, 0.72%; total N, 0.047%; available P, 8.50 mg kg⁻¹ and extractable K, 130 mg kg⁻¹ soil.

The hybrid variety (Corn-786) of maize was sown in the fields keeping row to row distance 75 cm and plant to plant distance 25 cm with a plot size of 12 m². The experiments were laid out in randomized complete block design with four replications. Whole dose of P and K fertilizers was applied at the time of seed bed preparation as a basal dose in all blocks, while N was applied according to the treatments in two split doses. First dose of N (urea) was applied after germination with a drill along with organic-/bio-fertilizer while second dose of N was applied before tasseling along the plant rows. The organic- and bio-fertilizers were applied @ 300 kg ha⁻¹ as a band placement with a drill and supplemented with either no N or with 88 kg N. The details of treatments are given in Tables 1-4. Good quality canal water [electrical conductivity, 0.05 dS m⁻¹; sodium adsorption ratio, 0.1 (mmol l⁻¹)^{1/2} and residual sodium carbonates, 0.02 me l⁻¹] meeting the irrigation quality criteria for crops in the area (Ayers & Westcot, 1985) was used for irrigation in both trials. The data regarding plant height, cob weight, total biomass, grain yield and 1000-grain was recorded at maturity. Grain and shoot samples of maize plants were analyzed for N, P and K concentrations (Ryan *et al.*, 2001) and their total uptake in maize plants were determined.

Table 1. Effect of organic-/bio-fertilizer on plant height, cob weight, total biomass, grain yield and 1000-grain weight of maize (Average of four repeats).

Treatments [†]	Plant height (cm)	Cob weight (g)	Total biomass (t ha ⁻¹)	Grain yield (t ha ⁻¹)	1000-grain weight (g)
Control (P & K only)	175.0 d [§]	130.4 c	14.0 c	4.2 d	228 d
Organic fertilizer [§]	180.2 b	141.0 b	15.6 bc	4.8 c	244 c
Biofert _{Q7} [*]	182.5 b	153.4 a	17.0 b	5.1 b	260 b
Biofert _{Q14} ^{**}	184.8 b	155.2 a	18.1 ab	5.3 ab	268 a
Biofert _{N3} ^{***}	191.0 a	160.5 a	19.5 a	5.5 a	272 a

[†]The P and K fertilizers were applied @ 100 and 50 kg ha⁻¹, respectively in all the treatments

[§]Means sharing similar letter(s) do not differ significantly at p=0.05

[§]Enriched compost (147 g N kg⁻¹ compost). The composition of compost is given in Figure 1&2.

^{*}Enriched compost inoculated with selected PGPR strain, *Pseudomonas putida* biotype A (Q7)

^{**}Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* (Q14)

^{***}Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* biotype G (N₃)

Table 2. Effect of organic-/bio-fertilizer on nitrogen, phosphorus and potassium uptake of maize (Average of four repeats).

Treatments [†]	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)
Control	48 d [§]	20 e	65 d
Organic fertilizer [§]	63 c	25 d	80 b
Biofert _{Q7} [*]	75 b	28 cd	91 a
Biofert _{Q14} ^{***}	84 ab	31 bc	96 a
Biofert _{N3} ^{***}	92 a	34 a	100 a

[†]The P and K fertilizers were applied @ 100 and 50 kg ha⁻¹, respectively in all the treatments

[§]Means sharing similar letter(s) do not differ significantly at p=0.05

[§]Enriched compost (147 g N kg⁻¹ compost). The composition of compost is given in Figure 1&2.

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^{**}Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* (Q14)

^{***}Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* biotype G (N₃)

Table 3. Effect of organic-/bio-fertilizer supplemented with chemical N fertilize on plant height, cob weight, total biomass, grain yield and 1000-grain weight of maize (Average of four repeats).

Treatments [†]	Plant height (cm)	Cob weight (g)	Total biomass (t ha ⁻¹)	Grain yield (t ha ⁻¹)	1000-grain weight (g)
Urea fertilizer (175 kg ha ⁻¹ N)	202.0 b [§]	218.4 b	22.3 b	6.9 c	284 b
Organic fertilizer [§] + 88 kg ha ⁻¹ N fertilizer	201.6 b	211.3 b	21.8 b	6.8 c	278 b
Biofert _{Q7} [*] + 88 kg ha ⁻¹ N fertilizer	202.4 b	241.0 a	23.8 ab	7.2 bc	297 ab
Biofert _{Q14} ^{**} + 88 kg ha ⁻¹ N fertilizer	207.3 a	246.8 a	24.7 a	7.5 ab	310 a
Biofert _{N3} ^{***} + 88 kg ha ⁻¹ N fertilizer	205.1 ab	250.2 a	25.6 a	7.8 a	315 a

[†]The P and K fertilizers were applied @ 100 and 50 kg ha⁻¹ respectively in all the treatments

[§]Means sharing similar letter(s) do not differ significantly at p=0.05

[§]Enriched compost (147 g N kg⁻¹ compost). The composition of compost is given in Figure 1&2.

^{*}Enriched compost inoculated with selected PGPR strain, *Pseudomonas putida* biotype A (Q7)

^{**}Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* (Q14)

^{***}Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* biotype G (N₃)

Table 4. Effect of organic-/bio-fertilizer supplemented with chemical N fertilize on nitrogen, phosphorus and potassium uptake of maize (Average of four repeats).

Treatments [†]	N (kg ha ⁻¹)	P (kg ha ⁻¹)	K (kg ha ⁻¹)
Urea fertilizer (175 kg ha ⁻¹ N)	122 c [§]	41 b	132 b
[§] Organic fertilizer + 88 kg ha ⁻¹ N fertilizer	118 c	42 b	129 b
Biofert _{Q7} * + 88 kg ha ⁻¹ N fertilizer	130 b	43 b	135 b
Biofert _{Q14} *** + 88 kg ha ⁻¹ N fertilizer	135 ab	45 ab	138 b
Biofert _{N3} *** + 88 kg ha ⁻¹ N fertilizer	141 a	48 a	146 a

[†]The P and K fertilizers were applied @ 100 and 50 kg ha⁻¹, respectively in all the treatments

[§]Means sharing similar letter(s) do not differ significantly at p=0.05

[§]Enriched compost (147 g N kg⁻¹ compost). The composition of compost is given in Figure 1&2.

*Enriched compost inoculated with selected PGPR strain, *Pseudomonas putida* biotype A (Q7)

**Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* (Q14)

***Enriched compost inoculated with selected PGPR strain, *Pseudomonas fluorescens* biotype G (N₃)

The data were analyzed statistically (Steel *et al.*, 1997). Means were compared by Duncan's multiple range test (Duncan, 1955).

Results

First trial: Results of the first field trial revealed that application of organic and bio-fertilizer significantly improved the growth and yield of maize (Table 1). The plant height and cob weight was increased up to 9 and 23% over control in response to organic-/bio-fertilizer. Similarly, total biomass was enhanced by 11.4 and 39.3% by organic- and bio-fertilizer over control respectively, showing a supremacy of biofertilizer over organic fertilizer.

The increase obtained in grain yield and 1000 grain weight was 14.2 and 7.0% over control by organic fertilizer which doubled in case of biofertilizer. Results revealed that biofertilizer effect was significantly higher than organic fertilizer in improving yield and yield attributes of maize.

Biofertilizer application also enhanced nutrient uptakes over organic fertilizer and control (Table 2). Maximum increase in N, P and K uptake (91.6, 70 and 53.8% over control, respectively) was obtained by biofertilizer. Organic fertilizer also differed significantly (31.2, 25 and 23% increase) from control in N, P and K uptake, respectively. Comparison of biofertilizers showed that Biofert N₃ performed best, followed by Biofert Q₁₄ and Biofert Q₇.

Second trial: Biofertilizer in combination with N fertilizer increased yield and yield attributes of maize (Table 3). By comparison with full dose of N fertilizer, organic fertilizer plus 88 kg ha⁻¹ N was no different producing similar plant height, cob weight and biomass. Biofertilizer plus 88 kg ha⁻¹ N, however, differed significantly producing 14.5 and 15.2% more cob weight and biomass than full dose of urea fertilizer (175 kg ha⁻¹ N).

Grain yield, main parameter of concern and 1000 grain weight were also affected significantly by the integrated use of biofertilizer and 88 kg N. Maximum grain yield (13% more than full dose of N fertilizer) and 1000 grain yield (315 g) were obtained by Biofert N₃ plus 88 kg N, followed by Biofert Q₁₄ and Biofert Q₇. Organic fertilizer in combination with 88 kg N and full N fertilizer gave similar results.

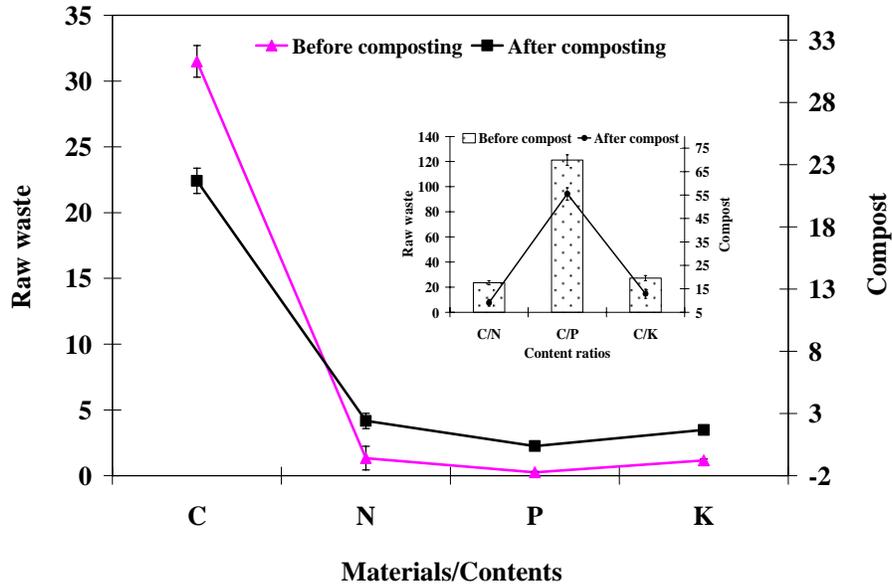


Fig. 1. Macronutrient analysis and their ratios of raw and composted fruit and vegetable wastes. Average of four repeats \pm Standard error.

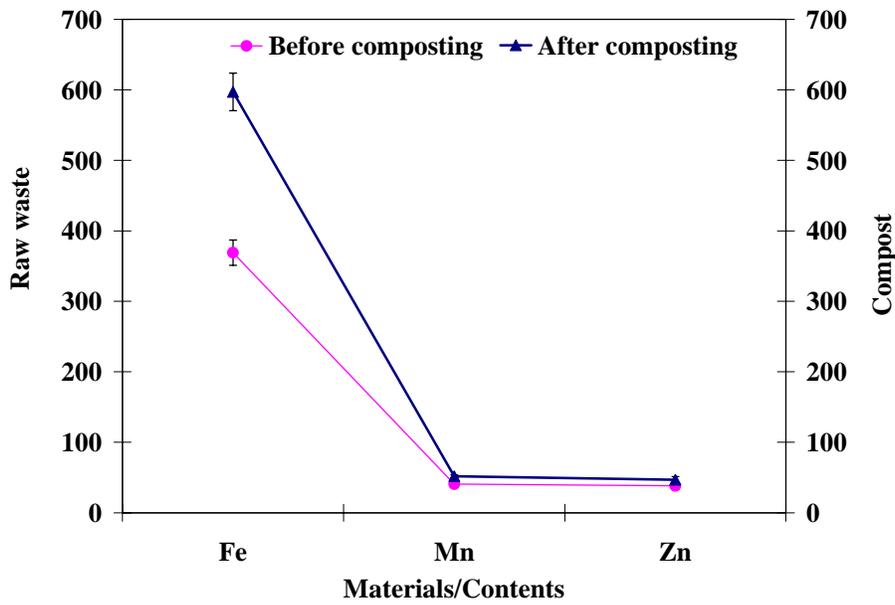


Fig. 2. Micronutrient analysis of raw and composted fruit and vegetable wastes. Average of four repeats \pm Standard error.

Nutrient (N, P and K) uptakes followed almost the same pattern that was observed in agronomic data (Table 4). Among biofertilizers however, only Biofert N₃ in the presence of 88 kg N differed significantly from full dose of N and caused 10-15% increase in N, P and K uptakes. Rest of the treatments showed non significant difference when compared with each others.

Discussion

This study demonstrated the effectiveness of organic-/bio-fertilizer for improving growth, yield and nutrients (N, P and K) uptake of maize both in the presence or absence of N fertilizer. Organic fertilizer applied at 300 kg ha⁻¹ and supplemented with 88 kg ha⁻¹ N (urea) was comparable in effectiveness to full dose (175 kg N ha⁻¹) of N-fertilizer (urea). Over all, there was ~25% saving of N-economy with the application of 300 kg ha⁻¹ organic fertilizer. These findings support our previous work that application of 300 kg ha⁻¹ organic fertilizer (N-enriched compost) saved ~25% mineral N fertilizer when supplemented with 50% of standard rates of cereals (Ahmad *et al.*, 2006; Zahir *et al.*, 2007; Ahmad *et al.*, 2007b). The increase in growth and yield of maize could be attributed to the enhanced nutrient use efficiency in the presence of organic fertilizer. Studies have shown that the composted organic materials release nutrients slowly and may reduce the leaching losses, particularly N (Paul & Clark, 1996; Muneshwar *et al.*, 2001; Nevens & Reheul, 2003) and thus enhance nutrient use efficiency. This premise is further supported by the fact that total N and P uptakes in maize were significantly increased (Table 2 & 4) in response to combined application of organic and chemical fertilizers.

Formulation of bio-fertilizer (by inoculating organic fertilizer) and its application at 300 kg ha⁻¹ for improving growth and yield of maize was novelty of this study. Biofertilizer supplemented with/without 88 kg N ha⁻¹ significantly increased the growth and yield of maize compared to full dose of N-fertilizer and showed superiority over organic fertilizer. It implies that inoculation with PGPR strains *Pseudomonas fluorescens* biotype G (N₃) further improved the effectiveness of organic fertilizer. It is highly likely that greater effectiveness of strain N₃ formulated biofertilizer might be related to its high root colonization ability and chitinase activity in addition to ACC-deaminase activity, which made this strain more competitive than other strains (Shaharoono *et al.*, 2006b). In addition to the positive attributes of organic fertilizer, biofertilizer (ACC-deaminase containing PGPR) application enhanced nutrient uptake, growth and yield of maize is most likely due to promotion of root growth by the decreased ethylene levels attributed to ACC-deaminase activity (Shaharoono *et al.*, 2006a; Ahmad *et al.*, 2008a). The rhizobacteria-containing ACC-deaminase are well known for improving root growth of plants as a consequence of decreased ethylene synthesis through ACC hydrolysis into NH₃ and α -ketobutyrate in the inoculated roots (Glick, 1995; Penrose & Glick, 2001; Shaharoono *et al.*, 2007). Very recently, we have reported that inoculation with PGPR containing ACC-deaminase significantly increased wheat and maize yield, under field conditions (Shaharoono *et al.*, 2006b, 2007; Ahmad *et al.*, 2008a). This implies that inoculation of organic fertilizer with such traits of PGPR could convert organic product into an effective biofertilizer and make it superior over organic fertilizer. Further, comparison of bio-fertilizers regarding yield and yield attributes and nutrient uptake, *Pseudomonas fluorescens* biotype G (N₃) containing biofertilizer (Biofert N₃) was found best for formulation of bio-fertilizer than *Pseudomonas fluorescens* (Q₁₄) *Pseudomonas putida* biotype A (Q₇).

The economic analysis of enriched compost indicated that this technology is cost effective if organic material is collected and transported by the Government. In conclusion, organic waste materials can be converted into value-added organic-/bio-fertilizer by the addition of lower doses of chemical nitrogen fertilizer and inoculation with PGPR. It is possible to get higher yield levels with the complimentary use of PGPR (microbial) inoculation, composted organic waste and inorganic (chemical) fertilizers than the application of organic or chemical fertilizers alone. The improvement in soil health and reduction in piling of organic wastes could be extra benefit.

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