Effect of Compost, NPK and Plant Promoting Rhizobacteria (PGPR) on Growth and Yield of Three Vegetables cultivated on Arenosols

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***Abstract* —** *Three field studies were conducted to determine the effects of compost, plant growth promoting rhizobacteria (PGPR) and NPK on growth and yield of three vegetables. Two PGPR strains (Nitrogen-fixing Bradyrhizobium japonicum and Phosphate-solubilizing Bacillus subtilis) supplemented into compost and bacterial liquid were added into compost during vegetable cultivation, chemical fertilizer (100 N – 80 P_2O_5 – 40 K_2O) and control (non inoculation). The study revealed that compost inoculated with PGPRs can replace 50% chemical fertilizer in three vegetables cultivation, farmers but also saved 50 N – 40 P_2O_5 – 20 K_2O not only minimized environmental pollution.*

Key words - biomass yield, compost, PGPR, trade productivity, vegetable.

I. INTRODUCTION

Soil fertility degradation, caused by erosion and depletion or imbalance of organicmatter/nutrients, is affecting world agricultural productivity [1]. Inorganic fertilizers have played a significant role in increasing crop production since the "green revolution" [2]; however, they are not a sustainable solution for maintenance of crop yields [3]. Long-term overuse of mineral fertilizers may accelerate soil acidication, affecting both the soil biota and biogeochemical processes, thus posing an environmental risk and decreasing crop production [4].

Composting is considered a sustainable and environmental friendly approach for the safe utilization of solid organic wastes such as farm manure and trash of crops [5]. Usually, composts are applied to get equivalent amounts of nutrients but that requires a large amount of application rates. Increase in organic matter and vegetable production are seen in previous research at different levels (22.5, 56, and 112 t ha^{-1}) of compost application [6]. Some reports stated that application of composts improved the soil physical, chemical, and biological as well as yield of crops compared to chemical fertilizers on sustainable basis [7]. Mineralization of compost in the soil results in nutrient release and soil quality enhancement. The useful effects of application of composted organic materials on soils are very extensively recognized all over the world [8]. Total organic matter contents, microbial activity, and nutrient release are also increased with the application of composts [9]. Compost improves the physicochemical properties of soils such as pH cation exchange capacity, bulk density, porosity, and water holding capacity. The major concern associated with the use of organic manures is their rapid rate of decomposition especially under high temperature. Organic matter may be mineralized within a single cropping season and its sustainability is a standing question. Practically, some limitations are associated with the application of composts at higher rates containing some toxic constituents such as heavy metals [10-11]. Excessive applications of composts may release some organic compounds which can contaminate surface waters by runoff and subsurface water when percolates in deep layers [8]. This concern can be addressed with the value addition of composts in terms of higher stability and fertility for sustainable agriculture.

Vegetables are rich source of vitamins, proteins, carbohydrates and minerals, which constitute an important component in human nutrition. Besides the nutritional value of vegetables, increased interest is being bestowed on the functional and therapeutic benefits of vegetables in human health. Agriculture is highly dependent on the use of chemical fertilizers, growth regulators, fungicides and pesticides for obtaining increased yield. This dependence is associated with problems such as environmental pollution, health hazards, interruption of natural ecology, nutrient recycling and destruction of biological communities that otherwise support crop production. The use of bioresources to replace these chemicals is gaining importance. In this context, plant growth promoting rhizobacteria (PGPR) are often considered as novel and potential tool to provide substantial benefits to agriculture. [12].

PGPR are a heterogeneous group of bacteria that can be found in the rhizosphere, which can improve the quality of the plant growth directly and or indirectly [13] as (i) their ability to produce plant growth regulators like indoleacetic acid, gibberellic acid and cytokinins [14], (ii) asymbiotic nitrogen fixation [15], (iii) antagonism against phytopathogenic microorganisms by production of siderophores [16], antibiotics [17] and cyanide [18], (iv) solubilization of mineral phosphates and other nutrients [19] and (v) active removal and bioaccumulation of heavy metals and their capacity to assist the root growth [20].

In addition, PGPR isolates must be rhizospheric competent, able to survive and colonize in the rhizospheric soil [21]. The variability in the performance of PGPR may be due to climate, weather conditions, soil characteristics or the composition or activity of the indigenous microbial flora of the soil that may affect their growth and exert their effect on the plant [22]

Different bacteria that have been reported as PGPR belong to the following genera: *Pseudomonas, Bacillus, Azospirillum, Agrobacterium, Azotobacter, Arthrobacter, Alcaligenes, Serratia, Rhizobium, Enterobacter, Burkholderia, Beijerinckia, Klebsiella, Clostridium, Vario vovax, Xanthomonas, and Phyllobacterium* (23-24). Among these, *Pseudomonas* and *Bacillus* are the most widely reported PGPR.

The aim of this study was to evaluate the effects of composting and PGPR (including nitrogen-fixing bacteria and phosphate-solubilizing bacteria) on three vegetables (leaf-eating vegetable) as sweet cabbage (*Brassica integrifolia* O. B. Schultz), parchoi (*Brassica chinensis* L.), and mustard greens (*Brassica juncea* L.)

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Soil experiment

Soil experiment is arenosols [sandy soil] [25] with pH and physical and chemical characteristics of arenosols (Table 1). Soil experiment has neutral pH but soil fertility is low.

TABLE 1
pH AND PHYSICAL & CHEMICAL CHARACTERISTICS OF ARENOSOLS (SOIL EXPERIMENT)

Characteristics	
pH	7.53
CEC (meq/100g)	14.50
Organic matter (%)	1.13
Available N (mg/kg)	26.55
Available P (mg/kg)	168.37
Exchangeable K (mg/kg)	62,14
N total (%)	0.15
P total (%)	0.05

Origin: Analysed at Advanced Lad., Can Tho University, Vietnam, 2016

2.1.2 Composting procedure

Compost was prepared from rice straw (*Oryza sativa)*. The compost added with 0.02% *Trichoderma* spore (Dept. of Plant Protection, College of Agriculture, Can Tho University), incubated by covering plastic membrane; the compost was inverted and watering fortnightly. After 6 weeks, the volume of compost was reduced 50%, added liquid of PGPR (including nitrogen-fixing bacteria and phosphate-solubilizing bacteria) into compost to keep moisture at 50 – 60%, compost was incubated 4 weeks and compost from the bucket, air dried and later sieved to remove the shaft, shredded and bagged. Compost used in this study with pH and physical and chemical characteristics presented in Table 2.

2.1.3 PGPR production

Nitrogen-fixing bacteria [NFB] (*Bradyrhizobium japonicum* strain CJ02)[26] and Phosphate-solubilizing bacteria [PSB] (*Bacillus subtilis* strain SDN2c)[27] were provided by Biotechnology R&D Institute, CanTho University, Vietnam which proliferated by incubation in container 120-L containing 100 litres water with 10% sugar in 10 days for NFB and 7 days for PSB. PGPR liquid reached to 10^7 cells/ml and they already used to experiment.

TABLE 2
pH AND PHYSICAL AND CHEMICAL CHARACTERISTICS OF COMPOST SUPPLEMENTED WITH PGPR

Characteristics	Result	Comment
pH	6.68	Neutral
Available N (mg/kg)	134.17	High
Available P (mg/kg)	950.01	High
Exchangeable K (mg/kg)	5951.77	High
N total (%)	2.37	Normal
P total (%)	0.29	Normal

Origin: Analysed at Advanced Lab. Can Tho University, Vietnam, 2016

2.2 Experimental procedures

Three field experiments were done for three vegetables, the land for the field experiment was prepared manually. There were three blocks for each experiment, with each block consisting of five beds, making a total number of fifteen beds, with each bed measuring 1 x 3 m and 0.5 m in between beds, and block size of 17.5 x 4.5 m. The total land area used for each experiment was 78.75 m^2 (Figure 1). The seedlings were prepared in the plastic glasses (Figure 2) which were planted with one glass per hole at spacing of 0.4 x 0.30 m. The experimental design was a randomized complete block design. There were 5 treatments: NT1 (control, without fertilizer, compost, PGPR), NT2 (100 N- 80 P_2O_5 – 40 K_2O/ha), NT3 (2 kg compost/m^2), NT4 (3 kg compost/m^2), NT5 (4 kg compost/m^2). However, the treatments: NT3, NT4 and NT5 were supplemented into watering with time 1 (6 day after planting [DAP] with 50 ml/m^2, time 2 (9 DAP with 100 ml/m^2, time 3 (12 DAP) with 150 ml/m^2, time 4 (15 DAP) with 200 ml/m^2, time 5 (18 DAP) with 250 ml/m^2 and time 6 (21 DAP) with 300 ml/m^2 PGPR liquid.

FIGURE 1. Experimental plot as a bed, land were prepared by manually

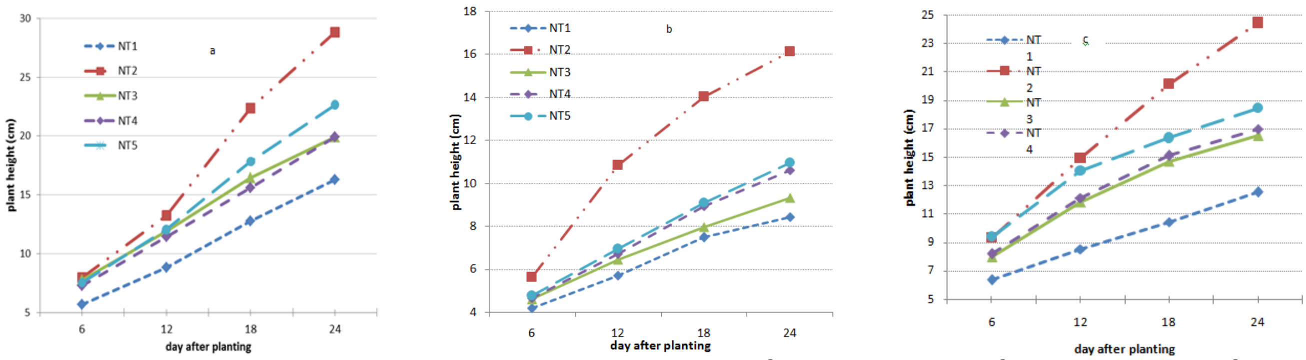
FIGURE 2. Seedlings were prepared to put in a hole

Insecticides did not used in the experiment, weed control by hand and eating-leaf plants were harvested at 24 days-old to measure plant height, leaf length, leaf number/plant, weight of a plant, biomass yield, Available ratio.

III. RESULTS AND DISCUSSION

3.1 Effects of compost on plant height and yield component of vegetables

Application of chemical fertilizer increased plant height of three vegetables and plant height was the lowest in the control treatment, using compost also increased plant height of three vegetables (Figure 3a, 3b and 3c).



NT1=control; NT2=100 N - 80 P_2O_5 – 40 K_2O/ha, NT3=2 kg compost/m^2, NT4=3 kg compost/m^2, NT5=4 kg compost/m^2

FIGURE 3. Effects of compost and PGPR strains on plant height of sweet cabbage (a)[left], pakchoi (b) [between] and mustard green (c) [right].

Similarly, the leaf length and leaf number/plant of chemical fertilizer treatment (NT2) were the higher than others significantly however leaf number/plant of mustard green in the NT3, NT4 and NT5 treatments did not differ from NT2 treatment (Table 3).

TABLE 3
EFFECTS OF COMPOST AND PGPR STRAINS ON LEAF LENGTH (CM) AND LEAF NUMBER/PLANT OF THREE VEGETABLES CULTIVATED ON ARENOSOLS.

Treatment	Leaf length (cm)			Leaf number/plant		
	sweet cabbage	pakchoi	mustard green	sweet cabbage	pakchoi	mustard green
Control	15.81 c	7.49 c	11.67 c	9.92 c	7.03 d	8.70 b
100 N - 80 P_2O_5 – 40 K_2O/ha	27.92 a	13.75 a	23.13 a	15.54 a	11.05 a	10.15 a
2 kg compost/m^2	18.24 bc	8.11 bc	15.62 b	11.30 bc	7.81 cd	9.59 ab
3 kg compost/m^2	19.12 bc	9.35 bc	16.12 b	11.57 b	8.44 bc	9.64 ab
4 kg compost/m^2	21.96 b	9.76 b	17.56 b	12.70 b	8.98 b	10.10 a
Calculated F	17.57**	17.33**	27.31**	23.05**	33.67**	4.48*
C.V (%)	**9.30**	**10.50**	**8.18**	**6.25**	**5.24**	**9.16**

**The numbers followed by the same letter do not differ at 1% level significantly*

3.2 Effects of compost on weight of a plant and biomass yield of vegetables

Application of chemical fertilizer for vegetable cultivation supported weight of a plant and biomass yield and using compost plus PGPRs also increased weight of a plant and biomass yield, application 4 kg compost/m^2 had the highest biomass yield but biomass yield also reached to 50% biomass yield of chemical fertilizer treatment, this showed that chemical fertilizer improved biomass of vegetable in short time in comparison to compost (24 days).

TABLE 4
EFFECTS OF COMPOST, NPK AND PGPR STRAINS ON WEIGHT OF A PLANT (G/PLANT) AND BIOMASS YIELD (KG/M2) OF THREE VEGETABLES CULTIVATED ON ARENOSOLS

Treatment	Weight of a plant (g/plant)			Biomass yield (kg/m^2)		
	sweet cabbage	pakchoi	mustard green	sweet cabbage	pakchoi	mustard green
Control	5.06 c	3.99 c	8.33 c	0.38 c	0.31 d	4.00 c
100 N - 80 P_2O_5 – 40 K_2O/ha	30.92 a	23.40 a	47.43 a	2.18 a	1.74 a	22.80 a
2 kg compost/m^2	12.53 b	4.33 c	19.00 b	0.85 b	0.40 cd	9.20 b
3 kg compost/m^2	11.65 b	7.48 bc	21.40 b	0.75 b	0.62 c	10.27 b
4 kg compost/m^2	16.71 b	11.24 b	26.77 b	0.99 b	0.92 b	12.80 b
Calculated F	262.29**	34.14**	27.27**	86.06**	57.69**	25.33**
C.V (%)	**5.96**	**23.50**	**19.46**	**12.93**	**16.36**	**20.18**

***The numbers followed by the same letter do not differ at 1% level significantly**

Available ratio (%) [trade productivity/biomass yield] depended on kind of vegetable as sweet cabbage had high available ratio (%) in control treatment but chemical fertilizer treatment had the lowest available ratio. On the contrary, on pakchoi, control treatment had the lowest available ratio and chemical fertilizer treatment had the highest available ratio. However, in all vegetables, compost treatment had stable available ratio (Table 5).

TABLE 5
EFFECTS OF COMPOST, NPK AND PGPR STRAINS ON BIOMASS YIELD (TON/HA) AND TRADE PRODUCTIVITY (TON/HA) OF THREE VEGETABLES CULTIVATED ON ARENOSOLS

Treatment	sweet cabbage			pakchoi			mustard green		
	biomass yield	trade product	Avai. ratio	biomass yield	trade product	Avai. ratio	biomass yield	trade product	Avai. ratio
	(ton/ha)		(%)	(ton/ha)		(%)	(ton/ha)		(%)
NT1	0.38 c	0.37 c	97.3 a	0.31 d	0.20 d	63.8 c	4.00 c	3.30 c	88.2 b
NT2	2.18 a	2.01 a	92.2 c	1.74 a	1.45 a	83.1 a	22.80 a	21.07 a	92.4 a
NT3	0.85 b	0.81 b	95.3 b	0.40 cd	0.29 cd	72.5 b	9.20 b	8.40 b	91.3 a
NT4	0.75 b	0.71 b	94.6 b	0.62 c	0.47 bc	74.6 b	10.27 b	9.20 b	89.6 ab
NT5	0.99 b	0.94 b	94.5 b	0.92 b	0.68 b	73.9 b	12.80 b	11.33 b	88.5 ab
Calcula. F	86.06**	79.63**	10.11*	57.69**	42.46**	9.62*	25.33**	24.35**	10.21*
C.V (%)	**12.93**	**13.24**	**6.11**	**16.36**	**21.73**	**5.21**	**20.18**	**21.60**	**5.48**

NT1=control; NT2=100 N - 80 P_2O_5 – 40 K_2O/ha, NT3=2 kg compost/m^2, NT4=3 kg compost/m^2, NT5=4 kg compost/m^2 trade productivity = trade product; Available ratio = Avai. Ratio = trade productivity/biomass yield (%); cal. F = calculated F

***The numbers followed by the same letter do not differ at 1% level significantly**

Application 4 kg compost/m^2 plus PGPRs for three vegetables cultivation had trade productivity more or less 50% in comparison to chemical fertilizer treatment (100 N – 80 P_2O_5 – 40 K_2O) (Figue 4) therefore the equivalent of 4 kg compost/m^2 or 40 tons/ha was calculated as 50 N – 40 P_2O_5 – 20 K_2O/ha or farmers not only saved 100 kg urea, 265 kg super phosphate 15% P_2O_5 and 33 kg KCl 60% K_2O but also kept safe vegetable products, consequently they contributed environmental protection.

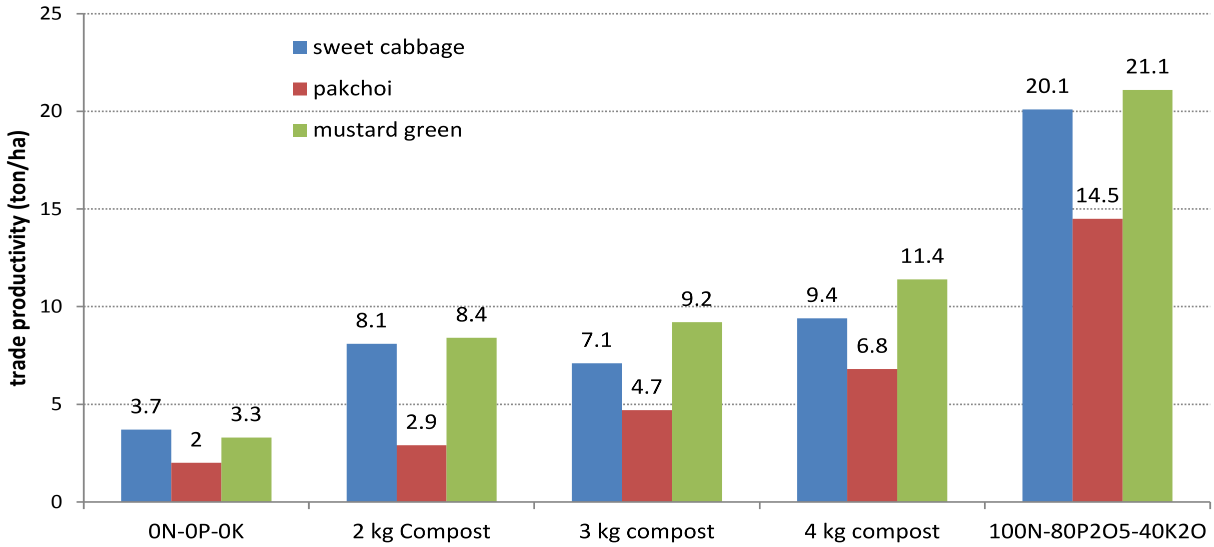


FIGURE 4. Effects of compost, NPK and PGPRs on trade productivity (ton/ha) of three vegetables cultivated on arenosols

Composts used as a soil amendment or in container media may protect plants from diseases caused by soilborne root pathogens [28]. Several organisms antagonistic to soilborne root pathogens have been isolated from suppressive composts [29]. These findings suggest that suppressive organisms may be at least partly responsible for the decreased disease incidence observed on plants grown in compost substrates. Compost generally increased growth rate, leaf area and dry matter accumulation of the two okra cultivars compared to control under varying light intensities. Compost at 15 t/ha performed better and increased fruit number by 66% on the field. The application of compost at 15 t/ha is therefore recommended for optimum yield of okra under low light intensity [30]. The use of compost with half fertilizer was better in increasing grain yield, especially with higher BC proportion in the compost than FM [31].

Plant rhizosphere is known to be the preferred ecological niche for various types of PGPR (*Rhizobium*, *Azotobacter* and *Azospirillium*) due to rich nutrient availability. The three main intrinsic characteristics of PGPR must be ability to: (i) colonize roots, (ii) survive and multiply in microhabitats associated with the root surface, in competition with other microbiota, at least for the time needed to express their plant promotion/protection activities, and (iii) promote plant growth [32-33].

The impact of rhizobacteria generally on plant growth and health may be classified as neutral, deleterious or beneficial (34). However, PGPR specifically are beneficial and the beneficial effects have been utilized in many areas including biofertilizer, disease control, microbe-rhizoremediation, biopesticide, in forestry as well as probiotics (35). Plant growth-promoting rhizobacteria (PGPR) benefit plants through different mechanisms of action, including, for example, (i) the production of secondary metabolites such as antibiotics, cyanide, and hormonelike substances; (ii) the production of siderophores; (iii) antagonism to soilborne root pathogens; (iv) phosphate solubilization; and (v) dinitrogen fixation [36]. The establishment in the rhizosphere of organisms possessing one or more of these characteristics is interesting since it may influence plant growth. The effect of PGPR in crop productivity varies under laboratory, greenhouse and field trials. Because, soil is an unpredictable environment and an intended result is sometimes difficult to achieve. Plant growth promoting traits do not work independently of each other but additively as it was suggested in the ''additive hypothesis,'' that multiple mechanisms, such as phosphate solubilization, dinitrogen fixation, ACC deaminase and antifungal activity, IAA and siderophore biosynthesis etc. are responsible for the plant growth promotion and increased yield [37]. Chabot *et al.* [38] used phosphate-solubilizing *Rhizobium leguminosarum* biovar phaseoli on lettuce and Antoun *et al.* [39] also used *Rhizobium* and *Bradyrhizobium* species on ridishes *(Raphanus sativus* L.) and they noticed positively from these rhizobia species.

Kalita *et al.* [40] showed that the mixture of PGPRs increased the shoot height, number of leaves, and total biomass content of plants as tomato, chili, cauliflower, brinja after treatment. Kumar *et al.* [41] recognized that bitter gourd with plant growth promoting rhizobactreia (PGPR) enhanced its growth, yield and quality attributes, especially with *Bacillus subtilis.* In the experiment in Long An province, using 15 ton/ha compost + 35 N – 24 P_2O_5 – 12 K_2O had biomass yield of mustard green was equivalent with 70 N – 48 P_2O_5 – 24 K_2O kg/ha without compost but compost treatment only had 4.78 mg/kg nitrate in biomass while chemical treatment had 286 mg/kg nitrate [42]. The results of effects of compost plus PGPRs on mustard green cultivated on alluvial soil of Can Tho city showed that biomass yield of treatment compost (1 ton/ha plus 50 N – 40 P_2O_5 – 20 K_2O kg/ha) in did not differ with biomass yield of chemical fertilizer treatment (100 N – 80 P_2O_5 – 40 K_2O kg/ha) without compost but nitrate content in leaves of mustard green was low [43]. Our result showed that the effectiveness of compost plus PGPRs (nitrogen-fixing rhizobia and phosphate-solubilizing bacilium) on three vegetables cultivated on arenosols only reached to 50% biomass yield of chemical fertilizer treatment.

IV. CONCLUSION

Compost was produced from rice straw and *Trichoderma* spore, supplemented with PGPRs (Nitrogen-fixing *Bradyrhizobium japonicum* and Phosphate-solubilizing *Bacillus subtilis*) and its effect on three vegetables cultivated on arenosols reached to 50% amount of chemical fertilizers both biomass yield and trade productivity.

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