Full Length Research Paper

The Potential of Microbial enrichment Compost to Enhance the Growth of Green Bean (*(Phaseolus vulgaris* L.)) under Organic and Global GAP systems

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Organic fertilizers such as biofertilizer and compost play a significant role in the environment and food safety. Some microbial enriched compost i.e. compost+*Trichoderma virdie*, compost+*Rhizobium tropici* and compost+*T. virdie*+*R. tropici* are used to enhance the growth of green bean (*Phaseolus vulgaris* L.) cv. Poulista. The treatment of compost+*R. tropici*+*T. virdie* gave the best increasing in both of plant height, number of branches, fresh weight, dry weight, nodule leghaemoglobin level, nitrogen and protein in pods of green been plants. While, the treatment of compost+*R. tropici* gave the best increasing in leaves number and nodules number on green been roots at 50% N-fertilizer level.

**Keywords:** Green bean, Compost, Trichoderma, Rhizobium, biofertilizer

INTRODUCTION

Green bean (*Phaseolus vulgaris* L.) is the world’s most important legume grown for human nutrition (Peix *et al.*, 2011). Egypt is the leading Mediterranean top producer of snap bean, which is one of our important first cash crops and the second exportable vegetable crop after potato to the European Union. Besides, this crop comprises an important nutrient source in the Egyptian diet. La Rue and Paterson, (1981), Wolff *et al.*, (1993) and Hardarson, (1993) declared that bean plants are considered a poor fixer of atmospheric N compared with other legume crops and generally responds poorly to Rhizobium inoculation in the field.

Biofertilizers are the micro-organisms and their products which are utilized to increase soil fertility. The N*₂*-fixing micro-organisms are *Anabaena*, *Nostoc*, *Aulosoria*, *Rhizobia*, *Tolypothrix* etc. Biofertilizers are products containing living cells of different types of microorganisms which when applied to seeds, plant surface or soil, colonize the rhizosphere or the exterior of the plant and promotes growth by converting nutritionally important elements (nitrogen and phosphorus) from unviable to viable form through biological process such as nitrogen fixation and solubilization of rock phosphate (Rokhzadi *et al.*, 2008). El-Yazid *et al.*, (2007) found that beneficial microorganisms in...
biofertilizers accelerate and improve plant growth and protect plants from pests and diseases. The property of symbiotically fixing nitrogen within nodules of vascular plants is found in two major groups of bacteria one of them, rhizobia that associate essentially with leguminous,(Sprent,2001). Another important group of nitrogen fixing bacteria is that of the cyanobacteria, found in association with a large variety of higher and lower plants, fungi and algae.( Meeks and Elhai, 2002). Franke et al., (2009) reported that leguminous plants can obtain their nitrogen by association with rhizobia via differentiation on their respective host plants of a specialized organ, the root nodule. Bhattacharyya and Pati (2000) found that \textit{Rhizobium} sp., produced high amount (10^7\text{microg/ml}) of IAA in culture from tryptophan supplemented Yeast Extract Mannitol medium. The production was maximum when the bacteria reached its stationary phase of growth. Inorganic fertilizer plays a significant role in environmental pollution. Among the inorganic fertilizers, nitrogen fertilizer increases denitrification, resulting in elevated emission of nitrous oxide (N\textsubscript{2}O) to the atmosphere which contributes to global warming (Smith et al.,2008). It has also been reported that application of nitrogen fertilizers may deplete soil organic carbon in the long run (Khan et al., 2007).Instead, biofertilizers, the products containing living cells of different microorganisms, can prevent the depletion of the soil organic matter (Jeyabal and Kuppuswamy, 2001). It has also been reported that application of biofertilizers increases yield and reduce environmental pollution (Mia and Shamsuddin, 2010). Recently, great attention has been focused on the organic agricultural production in Egypt by using biofertilizers imported from the European countries in order to avoid plant and environmental pollution with different elements and to reduce the usage of chemical or mineral fertilizers especially in the newly reclaimed lands. Furthermore, Sinclair et al., (2001) reported that legumes and their symbiotic root nodule bacteria are extremely sensitive to drought stress as occur in the newly reclaimed areas in Egypt. In addition, Jagnow et al., (1991) noticed that many autotrophic bacteria produce an endogenous phytohormone like auxin, cytokinens and gibberellins which enhance growth of roots, shoots and consequently plant yield. Organic manures are well established to be useful in fertilization of plants due to their beneficial effect on the physical, chemical and biological characteristics of the soil, which in turns, influence growth and increase plant production (Molla et al., 2005 and Meunchang et al., 2006). In recent years, biofertilizers the products containing living cells of different types of microorganisms are also used in the integrated nutrient supply system. Biofertilizers can convert nutritionally important elements from unavailable form through biological processes leading to crop yields (Hegde et al., 1999).

Fungi of the genus \textit{Trichoderma} are biocontrol agents that are successfully used as biopesticides worldwide. Several species of \textit{Trichoderma} are reported to produce secondary metabolites with antibiotic activity (Harman et al., 2004; Reino et al., 2008 and Vinale et al., 2008). It is well documented that the interaction of \textit{Trichoderma} strains with the plant may promote growth, improves crop yield, increase nutrient availability and enhance disease resistance (Harman et al., 2004 and Benitez et al., 2004). In addition, some species of \textit{Trichoderma} are able to colonize root surfaces, interact with the plant, and exchange compounds that can cause substantial changes in plant metabolism (Yedidia et al., 2001). Celar and Valic, (2005) showed that effects of the \textit{Trichoderma} species in promoting seedling establishment, enhancement of plant growth and elicitation of plant defense reaction in some vegetable crops have been examined by a number of researchers. Combined application of poultry refuse and \textit{Trichoderma harzianum} was found to be highly effective to control soil-borne diseases in seedbed nurseries(Anon, 2007). Yehia et al., (1988) proved antagonistic effect of \textit{Trichoderma viridie} against \textit{Fusarium solani} of faba bean and found that seeds coating with \textit{T. virdie} increased fresh and dry weight of shoots, roots and nodules number. Researchers studied the role of organic manures, which was incorporated with biofertilizer as stimulating the plant growth and yield of vegetables (Shaheen et al., 2007). Jensen et al., (2002) evaluated the effect of \textit{Bacillus subtilis} and \textit{T. harzianum} alone or in combination with Capton 400 and Vitavax 200 as biocontrol treatments against the dry bean root rot pathogens. They also recorded that seed application of both biocontrol agents increased plant biomass and decreased disease severity, under greenhouse conditions.

Dave et al., (2013) found that there was quantitative increase in total phenol, total protein and major three fatty acids after treatment. \textit{Trichoderma} also observed to introduce resistance capacity of Indian mustard plants. Some \textit{Trichoderma} rhizosphere competent strains has been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer sufficiency, percentage and rate of seed germination and stimulation of plant stimulation of plant defenses against biotic and abiotic damage,(Shoresh, 2010).

Several studies have also shown that the use of compost in agriculture is beneficial to soil, crops and environment (Rodd et al., 2002 and Rahman et al., 2006). Moreover, compost acts as a long term reserve and slow-release sources of major nutrient like N, P and K (Sullivan et al., 2002).
Hoitink & Fahy, (1986) mentioned that Composts are made from organic waste that has been degraded by thermophilic and mesophilic microorganisms. Pérez Piqueres et al., (2006) showed that the impact of compost on soil characteristics varies according to the nature of the compost and the soil type. For example, spent mushroom composts alter biological parameters in clay and silty clay soil, while green waste compost does not. While, Postma et al., (2003) demonstrated that Composts are used to improve the physical structure of soils. They also mentioned that composts have the potential to provide biological control of various disease pathogens, particularly the soil-borne pathogens through microbial actions. Also, Parr et al., (2002) stated that Liquid bio-products commonly referred to as biofertilizers contain living micro-organisms that influence the soil ecosystems and produce supplementary substances for plant growth. The species of the micro-organisms and their quantity may vary depending on the source of cultures and raw materials used for producing the product. Presently, different types of bio-products are available and their quality differs mainly due to the kinds of raw materials used, forms of utilization, and the sources of microorganisms (Higa and Parr, 1994). Also, Naidu et al., (2010) stated that compost extracts considered as bio-fertilizers have been found to enhance plant growth and to suppress pathogens.

Tricho-compost; a *Trichoderma* based compost fertilizer; was developed by mixing a definite concentration of spore suspension of a *Trichodermaharzianum* strain with measured amounts of processed raw materials, such as cow dung poultry refuse, water hyacinth, vegetable wastes, sawdust, maize bran, and molasses. Tricho-leafate; a liquid by-product of the Tricho-compost; was obtained during decomposition of Tricho-compost materials. These bio products were tested both in the laboratory and in seedbed nurseries to evaluate their effectiveness against soil-borne pathogens for growing cabbage seedlings (Nahar et al., 2012). Application of Tricho-compost and Tricho-leafate reduced the seedling mortalities of cabbage caused by *Sclerotium rolfsii* by about 98%. In laboratory tests, *Trichodermaharzianum*, after re-isolation from Tricho-compost and tricho-leafate, was also found to be highly effective to arrest the growth of *S. rolfsii*. *T. harzianum* destroyed the radial growth of *S. rolfsii* mycelium by 59.7% after five days and effected total destruction of the mycelium in 10 days. In seedbed nurseries, soil applications of Tricho-compost and Tricholeachate significantly increased the seedling germination rate and reduced the incidence of soil-borne diseases and infestation of root-knot nematodes. Field experiment showed that combined application of Tricho-compost and Tricho-leafate reduced the seedling mortalities by 40.9% to 64.5% in Gazipur and 53.3% to 62.1% in Bogart. Application of Tricho-leafate at 500 ml per sq. meter increased plant weight by about 55.6%, and reduced the seedling mortality by about 84.0% in Gazipur. Seedbed nurseries treated with Tricho-compost and Tricho-leafate had only *Pythium* spp as a soil-borne pathogen, whereas the control plot had as many as four soil-borne pathogens: *Pythium, Rhizoctonia, Sclerotium* and *Fusarium* spp. Use of Tricho-compost and Tricho-leafate also reduced the infestation of root-knot nematode by about 80.7% to 91.0%. The results clearly showed that use of Trichocom post and Tricho-leafate is highly effective for production of healthy cabbage seedlings. Obied et al., (2005) cleared that, olive mill wastes are known to contain a number of biologically active substances. The phytotoxic and antimicrobial properties of these residues have been extensively investigated and are associated with the presence of phenolic compounds and free fatty acids.

Della Greca et al., (2001), Fiorentino et al., (2003) and Isidori et al., (2005) pointed out that several investigators have reported on the inhibition of plant and microbial growth by low-molecular-weight phenols present in olive mill wastes. Bisignano et al., (1999) stated that, high-molecular-weight polyphenols such as oleuropein or lignin like polymers have also shown toxic activity. Romero et al., (2002) find out that Hydroxytyrosol has been identified as one of the major natural phenolics present in olive mill wastes. However, many compounds remain unidentified and there is still controversy about the exact type and amounts of phytotoxic components in olive residues. For instance, Gonzalez et al., (1990) found that the antibacterial activity of phenolic acids (tested separately and in mixtures) did not coincide with the inhibitory effect of olive mill waste waters. Furthermore, Capasso et al., (1992) stated that some researchers have found toxicity even after total extraction of phenols, suggesting that other chemical products contribute to the overall toxicity (Martirani et al., 1996) mentioned that Phytotoxic and antimicrobial properties of olive mill wastes have been frequently approached as a negative attribute that limits the beneficial re-use of such materials. Thus, several methods have been developed in the last years to degrade phenols in liquid and solid olive oil residues.

**MATERIALS AND METHODS**

These experiments were conducted at the experimental station farm of Genetic Engineering and Biotechnology Research Institute (GEBRI) in Sadat City University, Egypt in successive season of 2013 to study the response of green bean plants c.v. Paulista to the inoculation with different forms of biofertilizers namely, *Rhizobium tropici* and *Trichoderma viride* along with four levels of mineral-N.
This experiment was prepared in Queisna for agricultural development. The windrow quantity 100 ton (mix compost). Preparation of compost were added. Potassium sulphate (50% K) and Superphosphate (15.5% P) were added. Phosphorus and potassium, where calcium was maintained at 30-35 °C. First animal manure (cow manure) 65 ton and sugarcane mill waste 35 ton were mixed thoroughly and piled. These piles were given time to be composited and to complete digestion process for about 60-70 days. During piling, the mixture was mixed, turned and watered after every three days to maintain moisture content of 50-60 %. A turner was used for turning process to maintain uniformity and thoroughly. The composting process is carried out by a diverse population of predominantly aerobic micro-organisms that decompose organic material in order to grow and reproduce. The activity of these micro-organisms is encouraged through management of the carbon-to-nitrogen (C:N) ratio, oxygen supply, moisture content, temperature, and pH of the compost pile.

Biofertilizers Stocks

*Trichoderma viride* and *Rhizobium tropici* that were used in this study have been provided by the Organic Agriculture Research Unit, Department of Environmental Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City.

Inocula Preparation

*Rhizobium tropici* was initially isolated from common bean. The reference strains of *R. tropici* was grown for 72hrs in Yeast Mannitol Broth (YMB), Vincent, (1970) for physiological enhancement before inoculation in the further experiments. *R. tropici* bacterium was grown on the appropriate medium and incubated at 28°C for 3 days until early log phase. Vermiculite supplemented with 10% black brownish peat plus rock phosphate and feldspar was packed on polyethylene bags (300 g carrier per bag), then sealed and autoclaved at 121°C for 2 hrs. Bacterial culture was injected into sterilized vermiculite to satisfy 60% of the maximal water holding capacity, then the inoculation rate were used as 300gr inoculation per Fadden for each microorganism (100 % for seed inoculation before planting). Before sowing, 300 militaries of *Rhizobium* strain were added to carrier formula each1Kg consisted from of peat moss, rock-phosphate and feldspar media to inoculation with seeds every hill, taken one spoon 5 grams of these strains.

The experimental design was a split plot with four replicates, the four-nitrogen fertilizer levels were arranged within main plot and five treatments of biofertilizers were represented as the subplot. The plot area was 50m² (five rows, which was 10 m length and 1m width). Seeds were sown on the 15th-February in experimental season at both sides of row at 10 cm distance between hills. Two seeds were sown with 5 grams of different strains and covered by sandy soil in a hill under sandy soil condition using drip irrigation system.

RESULTS AND DISCUSSION

Green beans (*Phaseolus vulgaris* L.) cv. Paulista was treated with four treatments (compost, compost+ *Trichoderma viride*, compost+*Rhizobium tropici* and compost+*T. viride*+*R. tropici*) in along with four levels of mineral-N fertilization (0, 50, 75 and 100%) as ammonium nitrate in order to reduce the amount of chemical N fertilizer. Plant height of green bean plants was determined in Figure (1) which cleared that the best result of all the treatments was observed at 50% mineral N fertilization. Application of compost+*T. viride*+*R. tropici* caused the highest increase in plant height followed by the application of compost+*R. tropici*. Also, the same applications gave the same results at the different levels of N-fertilizers.

Figure (2) illustrate the effect of the tested treatments in along with the four levels of mineral N-fertilizer on the number of branches/plant and showed that the best results was conducted at 50% level of mineral N-fertilizer. The treatment of compost+*T. viride*+*R. tropici* caused the highest increase in the number of branches/plant at the four N-fertilizer levels followed by compost+*R. tropici*.

Figure (3) cleared that the best results of leaves number/plant were shown at 50% mineral fertilizer followed by 75%,
Figure 1: Effect of mineral N-fertilization and biofertilizers each alone or in tries combination on plant height.

Figure 2: Effect of mineral N-fertilization and biofertilizers each alone or in tries combination on number of branches/plant.

Figure 3: Effect of mineral-N fertilization and biofertilizers each alone or in tries combination on plant number of leaves.
100% and 0% resp.,. The treatment of compost+ *T. viride*+ *R. tropici* gave the best result (27.3) and followed by the treatment of compost + *R. tropici* at 50% (25.8).

Green bean plant fresh weight (g/plant) was shown in figure 4 which indicate that the treatment of compost+ *R. tropici* gave the best increase in plant fresh weight (78.1 g/plant) at 50% level of mineral N-fertilization followed by the treatment of compost+ *T. viride*+ *R. tropici* (75.65g/plant) which gave the best increase at the other 3 levels of mineral N-fertilization.

Figure (5) showed that there is a great increase in green bean plant dry weight when treated with compost+ *T. viride*+ *R. tropici* at 50% mineral N-fertilization level (17.5 g/plant) and also the best one at 0% and 100% N fertilization levels (13.33 and 14.8 g/plant cons.,) while the treatment of compost+ *R. tropici* gave the best result at 75% mineral N-fertilization level.

Effect of mineral N-fertilization and biofertilizers each alone or in tries combination on protein contents of pods was estimated in figure (6) which cleared that the treatment of
Figure 6: Effect of mineral N- fertilization and biofertilizers each alone or in tries combination on protein contents of pods.

Figure 7: Effect of mineral N- fertilization and biofertilizers each alone or in combinations on Nodule number.

Figure 8: Effect of mineral N- fertilization and biofertilizers each alone or in tries combination on nodule leghaemoglobin (LHb) (mg g⁻¹ nodule).
compost + T. viride + R. tropici gave the best result followed by 
compost + R. tropici, compost + T. viride and compost
conse.

It was noticed that there are great differences between 
ethe effect of compost + R. tropici on the nodule number at 
50% mineral N-level and the other treatments followed by 
ethe treatment of compost + T. viride + R. tropici. There is no 
any nodules on the roots of green bean plants at 100%
mineral N-level.

Figure (8) clear that the treatment of compost + T. 
viride + R. tropici was the most effective one in nodule 
leghaemoglobin (LHB) at the three mineral N biofertilization.

Using of microbial enriched compost as biofertilizer 
enhanced the growth of green bean (Phaseolus vulgaris 
L.) cv. Pouliiesta. At the first, this compost led to reducing 
50% N-fertilizer. This result is in agreement with Jeyabal 
and Kuppuswamy, (2001) who showed that biofertilizers 
can prevent the depletion of the soil organic matter. Also, 
Sinclaire et al., (2001) focused on the organic agricultural 
production in Egypt in order to avoid plant and 
environmental pollution with different elements and to 
reduce the usage. Biofertilizers are products when applied 
to seeds, plant surface or soil, colonize the rhizosphere and 
protomes growth by converting nutritionally important 
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At 50% N-fertilizers The treatment of compost + R. 
tropici + T. viridi gave the best increasing in both of plant height, 
number of branches, fresh weight, dry weight, nodule 
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green bean plants. While, the treatment of 
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number and nodules number on green bean roots. These 
results are in agreement with Bhattacharyya and Pati, 
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produce secondary metabolites with antibiotic activity and 
promote growth, improves crop yield, increase nutrient 
availability and enhance disease resistance, weight of roots 
and roots and nodules number (Harman et al., 2004; Reino 
et al., 2008, Vinale et al., 2008, Benetz et al., 2004 and 
Yehia et al., 1988). Researchers studied the role of 
organic manures, which was incorporated with biofertilizer 
as stimulating the plant growth, total protein, nutrient 
uptake, seed germination and yield of vegetables (Shaheen 
et al., 2007, Dave et al., 2013 and Shoreh, 2010).

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