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USE OF COMPOST WITH MICROBIAL INOCULATION IN CONTAINER MEDIA FOR MUNGBEAN (*Vigna radiata* L. Wilckzek*)* AND PECHAY (*Brassica napus* L.)

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ABSTRACT

Space-intensive agricultural production such as those using containerized low-cost growing media could provide income and food to those urban poor who have limited opportunities for livelihood. A container set-up with soil was used to determine the effects of compost with microbial inoculation on the growth of mungbean and pechay. Compost prepared from coconut coir dust-chicken manure mixture was inoculated with nitrogen-fixing bacteria (*Azotobacter* sp.) and/or cellulolytic fungi (*Trichoderma harzianum*), incubated for 7 days, and applied in potted mungbean plants at 5 g pot^{-1} and grown for 30 days. Mungbean (*Vigna radiata* L. Wilckzek*)* supplied with uninoculated compost had the lowest fresh biomass and the lowest number of nodules. Compost with combined inoculation of *Azotobacter* sp. and *T. harzianum* (each added into compost at 0.5%, w/w) significantly effected the highest plant fresh biomass and number of nodules. The same treatments gave similar results for plant growth and yield levels in pechay (*Brassica napus* L.). The inoculated compost in this study could thus help reduce the use of chemical fertilizers and promote container growing for urban agriculture.

Key words: coconut coir dust, nitrogen-fixing bacteria, cellulolytic fungi, chemical fertilizers, *Azotobacter* sp., *Trichoderma harzianum*.

INTRODUCTION

Compost can be produced from a variety of organic wastes through suitable processing. If more organic wastes could be reduced to compost showing enhanced commercial value, then both the problems of unavailability of fertilizer inputs in agricultural production and environmental pollution can be alleviated. Compost when properly prepared and used can help promote low-input agricultural system to become more sustainable and productive (Golabi et al. 2003). Matured composts, even without microbial inoculation, are already valuable. Continuing research, however, shows that options that employ microbial inoculation in compost tends to further improve its productivity (Kostov and Lynch, 1998).

Different kinds of growing media can be used in container-growing or even in soil-less media for seedlings (Robbins and Evans, 2009). These materials may harbor different kinds and number of microorganisms. Certain media like peat and inorganic constituents such as perlite and mineral wool, for example, will contain low initial population of microorganisms. Certain microbial inoculants may be introduced into these low-microbe-containing substrates. Some *Trichoderma* inoculants could be added to control certain pathogens and enhance growth of plants in the media. Select rhizobacteria may also be added for the same purpose. Other additives may be used such as certain types of tree bark that may reduce pathogen activity in the media. They suppress other microbes. Thus, microbial inoculants and certain additives in either soil or soil-less media may offer considerable benefits to growers (Carlile and Wilson, 1991).

The use of organic inputs as external nutrient sources is a logical alternative to expensive fertilizers in a developing country like the Philippines. Alternative and low-cost sources of nitrogen must be sought in countries where nitrogen fertilizers are imported and the technology to manufacture them is limited or too expensive. Biological materials may offer a solution to soil fertility problems and help in increasing food production. The use of farm-derived sources such as crop residues, manure, household wastes, and compost, has commonly been adopted in the management of soil fertility (Alexander and Wagner, 2006). Animal manure and compost can increase the water holding capacity and cation exchange capacity of the soil (Nkongolo et al., 2001). The promotion of biological nitrogen fixation (BNF) has been used in farming systems to minimize expenses on fertilizers. The use of an effective and persistent *Rhizobium* strain provides several benefits such as non-repeated application of nitrogen fertilizers and higher pod yield due to increased nodulation. Rates of nitrogen fixation of 1 to 2 kg N per ha per day is possible in most legumes in tropical cropping systems (Otieno et al. 2009).

There had been numerous local experiences in the preparation of microbial inoculants for straight application into seeds or seedlings (Espiritu et al. 1993a; Espiritu et al. 1993b). Likewise, the benefits of microbial inoculation in composts for various field crops had also been demonstrated (Espiritu et al. 1993c; Espiritu et al. 2008). Therefore, improved nitrogen management, in which microbial inoculants and composts could play important roles, is needed to optimize economic returns to farmers and minimize environmental concerns associated with nitrogen use.

This study was conducted to determine the effects of prepared compost supplied with inoculants of nitrogen-fixing bacteria (*Azotobacter* sp.) and/or cellulolytic fungi (*Trichoderma harzianum*) on the growth of mungbean (*Vigna radiata* L. Wilckzek) and pechay (*Brassica napus* L.) in container set-ups.

MATERIALS AND METHODS

Compost Preparation

Compost was prepared in heaps (2m x 6m x 0.5m) from coconut coir dust-chicken manure mixture (50/50, w/w), composted for 30 days after initial wetting (50%, w/w), with weekly turning over of the materials. Samples coming from the central portion of the compost heap were collected in plastic bags, sieved through 1/8-inch screen, mixed 4 times, and set up as stock for subsequent testing. The test soil (Table 1) and the resulting compost were analyzed for selected properties using recommended procedures (Table 2). Four large plastic bags were set aside (each subsequently subdivided into 4 small plastic bags to contain 200 g of uniform compost, adjusted to 30% moisture content, after appropriate treatment and incubation). The bagged composts were given the following treatments in 4 replications: T1, control (compost alone, without microbial inoculants); T2, inoculated compost (with *T. harzianum.* at 1%, v/w); T3, inoculated compost (with *Azotobacter* sp. at 1%, v/w); and T4, inoculated compost (with *Azotobacter* sp. at 0.5 %, v/w, and *T. harzianum* at 0.5%, v/w). Compost inoculation was done to allow a 7-day incubation period prior to use.

Inocula Preparation

Stock cultures of *Azotobacter* sp. HIBFA4b were purified in plates and a single colony was multiplied in 4-ml Burk medium in tubes with shaking at 30^0C for 4 days. Two-ml cultures were inoculated into composts in bags corresponding to T3. Stock cultures of cellulolytic fungus *Trichoderma* harzianum SS33 were also retrieved, purified in PDA plates and a single colony was multiplied in 4-ml PDA broth in tubes with shaking at 30^{0}C for 4 days. Two-ml cultures were inoculated into composts in bags to correspond to T2. One-ml cultures each of *Azotobacter* sp. and *T. harzianum* were mixed in bagged composts to correspond to T4. All bagged composts, whether uninoculated or inoculated, were incubated at 30^{0}C for 7 days prior to use in test plants.

Germination and Treatment of Test Plants

Inoculated compost effects on container-grown mungbean

Seeds of mungbean (*Vigna radiata* L. Wilczek cv. Pag-asa 1) were germinated in 3 days on top of 3-kg soil Lipa clay loam (Table 1) in black plastic pots wetted to field capacity with distilled water. Treated composts were applied according to the following treatments: T1, control (compost alone, without microbial inoculants); T2, + 5 g inoculated compost (with *Trichoderma harzianum* at 1%, v/w); T3, +5 g inoculated compost (with *Azotobacter* sp. at 1%, v/w); and T4, +5 g inoculated compost (with *Azotobacter* sp. at 0.5 %, v/w, and *T. harzianum* at 0.5%, v/w). Each treatment was replicated 4 times. Composts corresponding to these designated treatments were applied 3-cm deep into the potted soil, then covered with soil. Plants were watered regularly with 10-ml distilled water every other day. The test plants were maintained at the BIOTECH screenhouse for 30 days. The experiment was set up in a randomized complete block design.

Table 1. Lipa clay loam soil characteristics.

Parameter	Mean Value
pH	6.0
Total N, %	0.07
Organic matter, %	1.11

Another experiment consisting of a set of potted soil (Lipa clay loam, Table 1) and compost (with nutrient analysis as in Table 2) were prepared. Treatments were imposed according to the following: T1, + Chemical fertilizer as control (1g urea + 2 g complete fertilizer per pot); T2, Inoculated compost, 20 g pot^{-1}; T3, Inoculated compost, 7 g pot $^{-1}$. The plants were maintained as in the container-grown mungbean experiment. Here, the inoculated compost used was that treated with *Azotobacter* sp. at 0.5 %, v/w, and *T. harzianum* at 0.5%, v/w. Treatments were replicated 4 times.

Effects of chemical fertilizer and inoculated compost on container-grown pechay (Brassica napus* L.*)

Pechay plants were tested using potted 3-kg soil Lipa clay loam (Table 1) and compost (with nutrient analysis) (Table 2). Treatments were imposed according to the following: T1, control (with uninoculated compost at 20 g pot^{-1}); T2, treated with chemical fertilizer consisting of 1g urea + 2 g complete fertilizer pot^{-1}, N-P-K of 14-14-14; T3, inoculated compost, 20 g pot^{-1}; T4, treated with combination of inoculated compost, 20 g pot $^{-1}$ and chemical fertilizer consisting of 1g urea + 2 g complete fertilizer pot^{-1}, N-P-K of 14-14-14. The plants were maintained as in the container-grown mungbean experiment. Here, the inoculated compost used was that treated with *Azotobacter* sp. at 0.5 %, v/w, and *T. harzianum* at 0.5%, v/w. Treatments were replicated 4 times.

Table 2. Chemical analysis of compost used in the experiment.

Raw material	pH	Total C (%)	Total N (%)	C/N
Coconut coir dust-chicken manure blend (60/40, w/w)	6.7	50.64	1.51	31.45

Data Gathering and Analysis

Mungbean plants were harvested after the 30-day growing period by removing the whole plant with the roots, with a trowel, from the pot. Whole plants were weighed from each pot and the shoots were separated from the roots. Fresh root weights and nodule number were taken from each plant. The pechay plants were maintained at the BIOTECH screenhouse for 45 days, plant heights were measured, after which the plants were harvested and shoot fresh and dry weights were determined. All data in the plant experiments data were subjected to analysis of variance and when the F-test was significant, the treatment means were compared using LSD at 5% level of significance.

RESULTS AND DISCUSSION

For mungbean, all treatments were supplied with compost at 5 g pot^{-1}, the only difference being the control having compost without microbial inoculants (Table 3). Both the control and T2 (supplied with compost inoculated with 1% *T. harzianum,* v/w) had similar levels of fresh biomass weights (0.765 and 0.910 g pot^{-1}, respectively). The *Azotobacter*-inoculated compost gave increased mean weight (1.12 g pot^{-1}) but was not significantly different over the control. The highly significant increase in biomass (1.43 g pot^{-1}) was produced by compost with combined inoculation of *Azotobacter* and *T. harzianum.* Biomass increase was also one of the key parameters improved by the use of inoculated compost in chickpea (Shazad et al., 2008).

Table 3. Effect of inoculated compost on fresh biomass yield of mungbean.

Treatment no.	Treatment description	Fresh biomass (g pot^{-1})	% Increase
T1	Control (+ uninoculated compost)	0.77 b	-
T2	Compost inoculated with 1% *Azotobacter* sp. (v/w)	1.12 b	45.4
T3	Compost inoculated with 1% *T. harzianum* . (v/w)	0.91 b	18.2
T4	Compost inoculated with 0.5% *Azotobacte*r sp. + 0.5% *T. harzianum*. (v/w)	1.43 a	85.7

Means followed by the same letter within a column are not significantly different at 5 % level by LSD.

The tallest plants (6.26 cm plant^{-1}) were produced by compost application with combined inoculants of *Azotobacter* and *T. harzianum* (Table 4). Increase in height was also attained by compost with *Azotobacter* (5.83 cm plant^{-1}). Plants treated with *Trichoderma*-inoculated compost (5.46 cm plant^{-1}) were only as tall as the control plants (4.87 cm plant^{-1}). Plant height was also one of the readily observable traits affected by inoculated compost in herbs as reported by Abdelaziz et al., (2007).

Plants which received only compost treated with combined inoculation produced significantly more roots (17.5 mg pot^{-1}) (Table 4). The other two treatments did not provide beneficial effects on root growth over the control. In contrast, overall growth in tomato was promoted by fungal inoculation alone in soilless compost (Sivapalan *et al.,*1994).

Single inoculation with *Azotobacter* or *T. harzianum* in compost did not result in significant nodule formation in mungbean as compared to the control (Table 4). However, when each inoculant was combined in compost, the effects were highly significant, producing 17.5 nodules plant^{-1}, which is about three times the number of nodules than the control.

Table 4. Effect of inoculated compost on fresh root weight and plant height of mungbean.

Treatment no.	Treatment description	Number of nodules per plant^{-}	Fresh root weight (g pot^{-1})	Plant height (cm plant^{-1})
T1	Control (+ uninoculated compost)	5.0 c	6.0 c	4.87 b
T2	Compost inoculated with 1% *Azotobacter* sp. (v/w)	7.0 b	8.2 b	5.83 a
T3	Compost inoculated with 1% *T. harzianum*. (v/w)	6.0 c	7 c	5.46 b
T4	Compost inoculated with 0.5% *Azotobacter* sp. + 0.5% *T. harzianum* (v/w)	17.5 a	17.5 a	6.26 a

Means followed by the same letter within a column are not significantly different at 5 % level by LSD.

Potted mungbean plants were treated with chemical fertilizer or inoculated compost and the biggest plants were those supplied with inoculated compost at 20 g pot $^{-1}$ (Table 5). The lower rate of compost application (at 7 g pot $^{-1}$) and treatment with chemical fertilizer both gave plants of relatively lower biomass and smaller stand. These results confirmed that compost inoculated with beneficial microorganisms at suitable application rate can improve significantly the growth of mungbean in containerized media.

Table 5. Effect of inoculated compost on the growth of mungbean in the second pot experiment.

Treatment no.	Treatment description	Mungbean growth			
		Plant height (cm)	Apparent foliage span (cm)	No. of leaves per pot	Apparent surface area of largest leaf (cm^2)
T1	Chemical fertilizer (1 g urea + 2 g complete fertilizer pot $^{-1}$)	12.0 b	14.5 b	8 b	10.5 b
T2	Inoculated compost, 20 g pot $^{-1}$	17.5 a	21.0 a	11 a	28 a
T3	Inoculated compost, 7g pot $^{-1}$	15.0 b	13.0 b	10 b	9 b

Means followed by the same letter in a column are not significantly different by LSD at 5% level of significance.
Inoculated compost refers to compost to which inoculants of 0.5% *Azotobacter* sp. + 0.5% *Trichoderma harzianum* (v/w) were added.

The results of the two mungbean experiments would show that compost when inoculated with desired beneficial organisms, in this case other than rhizobia, could lead to the growth promotion. Mungbean, a legume, and the organism of direct benefit to mungbean is the rhizobia which could exhibit specific symbiotic interaction with the legume host. The soil used in the study was not sterile, and should harbor native rhizobia, as shown by the presence of nodules in the control plants. The control plants were supplied with uninoculated compost and showed mean biomass yield level of 0.77 g $plant^{-1}$. The other treatments supplied with variously inoculated composts showed increasing mean biomass yields over the control (0.91 to 1.43 g $plant^{-1}$). The biomass yield of plants supplied with compost with *T. harzianum* alone (0.91 g $plant^{-1}$) was not significantly different from that of the control, and so were those of plants supplied with compost loaded with *Azotobacter* alone (1.12 g $plant^{-1}$). However, when *Azotobacter* and *T. harzianum* were loaded together into compost, this combination treatment produced a highly significant yield (1.43 g $plant^{-1}$) over the control. Other workers have reported a similar trend as with *Azospirillum,* for example, which is also considered to be a "helper" strain stimulating nodulation, nodule activity, and plant metabolism. All of these stimulate many plant growth variables and plant resistance to unfavorable conditions (Itzigsohn et al. 1993). A helper strain is one that improves some plant-microbe interaction and this occurs in its presence than without it. The helper strain may be producing IAA or cellulase that helps the members in achieving more positive effects in their interaction and the mode of action involved is actually biochemical (Egamberdieva *et al*., 2010).

Another successful combination that had been tried included *Azotobacter* mixed with *Streptomyces* (Elshanshoury, 1995), and *Azospirillum* with the fungal biocontrol agent, *Phialophora radicola* (Flouri et al., 1995). It is said that co-inoculation of microorganisms frequently increased growth and yield, compared to single inoculation, and provided the plants with more balanced nutrition, and improved absorption of nitrogen, phosphorus, and mineral nutrients (Bashan, 1998).

Some beneficial effects of the combined inoculation of compost with nitrogen-fixing bacteria and cellulolytic fungi might explain the expressed biomass increases in the target plants, which were not clearly detected in those treated with composts inoculated with *Azotobacter* or *T. harzianum* alone.

Experiments involving pechay showed plant height was highest in plants treated with microbial inoculants (Table 6). Fresh and dry weights of pechay were likewise significantly increased by combined inoculation of *Azotobacter* sp. and *T. harzianum*. The combination of low chemical fertilizer rate and inoculants also significantly increased growth and yield of the test plants. These results would show the potential of microbial inoculants as key components of low-cost management systems for vegetables. The microbial inoculants significantly improved plant height, plant fresh weight, and plant dry weight of pechay in containerized medium. The strains used stimulated plant growth and resulted into significant yield increase over the control. Inoculation with the microbial strains apparently improved nutrient uptake, and could have the potential of improving plant yield under organic growing conditions. Using other kinds of microbial inoculants, Yildirim et al. (2009) obtained similar positive effects on *Brassica olearacea* under field conditions.

Given that the significant enhancement in growth of mungbean and pechay took place mainly with compost (cellulose-rich material) when added with combined inoculants of *Trichoderma harzianum* and *Azotobacter* sp., the mechanism for this beneficial effect could be due to the cooperative degradation of cellulose in compost in which the fungus *Trichoderma harzianum* provided the cellulase function and the aerobic bacterium, *Azotobacter* sp., providing the nitrogenase function. These combined inoculants apparently utilized the cellulose in the coconut coir dust-chicken manure compost as the carbon source for N_2 fixation, resulting in more dinitrogen fixation and available nutrients benefitting the test plants, as compared with the inoculant of fungus or bacterium alone. The combined inoculants could have developed in apparently aerobic niches in the compost with both aerobes presumably providing respiratory protection to the nitrogen-fixation function. This mechanism of cooperative cellulase and dinitrogen fixation functions was substantiated by Veal and Lynch (1984) in their experiments with T*richoderma harzianum* and *Clostridium*. In what appeared to be the same mechanism, Darmwal and Gaur (1988) obtained the maximum benefit in grain yield and N uptake in wheat grown in soil amended with rice straw with combined inoculation of cellulolytic *Aspergillus awamori* and nitrogen-fixing *Azospirillum lipoferum*.

Table 6. Mean effects of coir dust-chicken manure treated compost on plant height, fresh and dry weights of pechay.

Treatment no.	Treatment	Yield		
		Plant height (cm)	**Fresh weight (g plant^{-1})**	**Dry weight (g plant^{-1})**
T1	Control (with untreated compost)	17.86 c	17.18 c	1.60 c
T2	Chemical fertilizer (1g urea + 2 g complete fertilizer pot $^{-1}$)	19.60 bc	31.43 c	1.07 c
T3	*Azotobacter* and *T. harzianum* inoculant (20 g compost inoculant with *Azotobacter* and *T. harzianum*)	24.70 a	48.20 ab	3.55 ab
T4	Inoculant and chemical fertilizer (20 g compost inoculant with *Azotobacter* and *T. harzianu*m and 1 g urea + 2 g complete fertilizer pot $^{-1}$)	18.40 bc	36.30 b	2.20 b

Means followed by a common letter(s) in a column are not significantly different at 5% level by LSD.

SUMMARY AND CONCLUSIONS

Composts were prepared in potted media and given different treatments aimed at determining the effect of inoculated compost in container media on mungbean and pechay growth and yield. The highly significant increase in biomass was produced by compost with combined inoculation of *Azotobacter* and *T. harzianum.* Single inoculation with *Azotobacter* or *Trichoderma* in compost did not effect significant nodule formation in mungbean. However, when each inoculant was combined in compost, increase in number of nodules and biomass yield were observed. The combined use of inoculants of *Azotobacter* sp. and *Trichoderma harzianum* added into the coconut coir dust-chicken manure compost supplied into the test plants could have significantly promoted growth with improved degradation of compost enhancing nitrogen fixation and the supply of available nutrients.

These results suggest that inoculated compost could be an important option for containerized plant-growing and this could contribute to the urban scenario with limited space for food production.

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