CLEAN
Soil Air Water

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Research Article

Recycling of Organic Waste through Four Different Composts for Disease Suppression and Growth Enhancement in Mung Beans

Compost is beneficial for agriculture fields in many ways such as soil conditioner, fertilizer, and natural pesticide and above all it helps to manage organic wastes and adds vital humic acids to soil. Four indigenous composts prepared from readily available organic wastes viz. vermicompost, banana, NADEP, and *Calotropis* were used in the present investigation for growth and disease suppression in mung beans. The composts were amended with *Trichoderma viride* in the concentration of 0.1 and 0.2% to determine their influence on length and weight of roots and shoots, disease incidence, soil moisture, and soil microflora in plants. The best results were observed in the treatment with *T. viride* (0.2%), followed by *T. viride* (0.1%) in vermicompost, while the treatment *T. viride* (0.1%) with *Calotropis* compost showed little growth and suppression of disease. All composts enhanced the soil moisture content and microbial populations in amended soil resulting in the reduction of disease incidence. Among *T. viride* enriched composts, the counts of fungi, bacteria, and actinomycetes were higher in the vermicompost and banana compost-amended soils. Thus, preparing these composts from readily available organic wastes and amending soil with *T. viride* enriched composts hold a great promise for improving soil fertility and suppressing the soil-borne plant pathogens for sustainable agriculture.

Keywords: Compost; *Macrophomina phaseolina*; Microbial population; *Trichoderma viride*

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1 Introduction

Mung bean (*Vigna radiata* L. Wilczek) is one of the key pulse crops gaining importance all over the world. It is rich in proteins and contains amino acids in higher quantities than any other cereals and pulses. It is affected by a number of diseases caused by fungi, bacteria, and viruses but the root rot caused by *Macrophomina phaseolina* (Tassi.) Goid. is the most serious disease due to its seed and soil borne nature. Though chemical fungicides are available, but their use is discouraged for several reasons like high cost, potential environmental pollution, health hazards, adverse effect on non-target fungi, phytotoxicity, and the growing demand for organic foods. Under these circumstances, employment of composts for the control of disease forms a realistic, safe, and economically viable alternative to fungicides.

Different types of composts have been used with varying levels of success for the suppression of many soil borne plant pathogens and induced diseases. They have been in vogue in agriculture with beneficial effects for years in China, Japan, and other parts of the world [1, 2]. Compost-amended sandy soil held more water than non-amended soil, which in turn reduced *M. phaseolina* population and its infection on the host plant through enhanced antagonism and/or competition for sites [3]. Organic amendments are excellent sources of nutrition which favor native antagonist to proliferate and suppress the soil borne diseases [4]. Various researchers have reported farm yard manure (FYM) to be the most suitable carrier for *Trichoderma* multiplication [5, 6]. In recent years, there has been growing interest in vermicompost for soil enrichment and its suppressive effect on certain soil borne phytopathogens. However, the suppressive effect of vermicompost against *M. phaseolina* has not been studied in detail. Besides, there are many organic wastes which can be used to prepare composts. This is an area which needs specific attention in isolation as well as comparative evaluation of composts prepared from plant residues. *Calotropis* is grown wildly in tropical areas and the litter is available in abundance in this region which can be effectively recycled for the preparation of composts. Banana peels have been considered for preparation of composts with a view to utilize this waste from vegetable markets, hotels, and hostels. Besides, there is ample availability of cow dung in India and vermicompost is undoubtedly a useful amendment for organic crops. The present communication deals with the use of four different indigenous composts prepared from organic wastes found abundantly in India along with *T. viride* as soil amendment to observe their influence on mung bean plant growth, disease incidence, soil moisture, and microbial population.

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Abbreviations: **cfu**, colony forming unit; **FYM**, farm yard manure

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2 Materials and methods

2.1 Biological materials

Seeds of mung bean (*Vigna radiata* L. Wilczek cv. RMG-492) commonly grown in Rajasthan, India, were procured from Krishi Vigyan Kendra (KVK), Banasthali University. The test pathogen, *M. phaseolina* was isolated from root rot infected plants of mung bean cv. RMG-492 grown in the District Tonk, Rajasthan, and multiplied on maize meal/sand medium (9:1 w/w) for 15 days at 28°C. To prepare *M. phaseolina* infested soil, the inoculum was added to each pot at the rate of 20 g kg^{-1} soil, 15 days before sowing. In the laboratory, it was maintained on potato dextrose agar slants. The biocontrol agent, *T. viride* P-127-1 was obtained from the Agriculture Research Station, Durgapura, Jaipur.

2.2 Soil characteristics

The soil of the experimental site was loamy sand formed from calcareous rocks. It contained 41% coarse sand, 37.6% fine sand, 14.3% clay, 7.1% silt, 0.26% organic carbon, 4.4 kg ha^{-1} available phosphorus, and 300 kg ha^{-1} available potassium. It had a pH value of 8.2 and an electrical conductivity 0.42 dS m^{-1} (KVK, Banasthali, unpublished report).

2.3 Preparation of composts

2.3.1 Banana and calotropis composts

Fully dried residues of banana (*Musa accuminata*) and *Calotropis* (*C. procera*) were used for the preparation of the composts. The process of composting was initiated under partially anaerobic conditions in separate plastic containers (35.6 cm diameter × 35.6 cm height) according to the principles of the Indore method [7]. A total of 2 kg of residue enriched with 1% gypsum and 2% urea was filled layer by layer in each plastic container. Each layer was provided with sufficient moisture and covered with a mixture of 800 g cow dung and 1200 g field soil. Three such layers of residues with soil/dung mixture were placed in each container. These containers were finally covered with a plastic lid and kept for 90 days. The content was mixed at seven to eight days intervals from top to bottom.

2.3.2 Vermicompost

Fresh cow dung was collected and water was sprinkled on it for eight to ten days for cooling and removal of methane gas. Mixing was done once or twice. The prepared cow dung was then spread uniformly on the shaded bed (1200 × 300 × 200 cm). Approximately, 3000 worms (*Pheretima posthuma*) were spread evenly on the above-prepared bed. After spraying water, the bed was covered with jute cloth. The first inversion was done after 25 days of bed filling; water was sprinkled and the bed was covered again with jute cloth. The second inversion was done after 25 days of the first inversion. After 70 days, the desired vermicompost was available for use.

2.3.3 NADEP compost

Fully dried residue of farm waste in sufficient quantity was used for the preparation of NADEP compost (20:80). The process of composting was initiated in pits (457.2 × 182.9 × 91.5 cm). Firstly, farm waste layer was spread over the base of the pit and then covered with cow dung, repeating the layering five times. The pit was finally covered with the mixture of cow dung and black soil and thereafter water was sprinkled. It took around 90 days for NADEP compost to mature.

2.4 Experimental design

Trichoderma viride was added to all four composts in two different concentrations *i.e.*, 0.1 and 0.2%, with a total of eight treatments and it was left to multiply for seven to eight days. The composts were mixed with soil at a rate of 25 g kg^{-1} and were filled to a depth of 12 cm by a spade in separate clay pots (22.9 cm diameter × 30.5 cm height) containing *M. phaseolina* (1.60×10^3 colony forming unit (cfu) g^{-1}) infested soil, two days before sowing. Pots with *M. phaseolina* and soil served as a control. Four replications were used for each treatment. Mung bean seeds (cv. RMG-492) were sown at ten seeds per pot. The length and weight of roots and shoots and disease incidence were recorded after 60 days of sowing. The experiment was conducted from 24 July 2009 to 25 September 2009 and subsequently repeated from 28 July 2010 to 29 September 2010. The percent disease incidence was determined using the following formula:

$$\%\text{Disease incidence} = \frac{\text{No. of rotted plants}}{\text{Total no. of plants observed}} \times 100 \tag{1}$$

To determine the moisture content, the fresh weight of soil samples with and without enrichment of *T. viride* was measured when harvested. The soil was then oven dried at 80°C for 3 h and weighed. Thereafter, the percent soil moisture was calculated.

2.5 Microbial assay

For biological assays, soil samples were collected from each pot after five to six days of mixing composts and at the time of harvesting. The soil samples were mixed to form a composite sample from each replication (four pots for two consecutive years) of each treatment. This sample was processed for the determination of microbial populations. cfu were estimated by the standard dilution plate method on potato dextrose agar for fungi and Thornton's Agar (1 g mannitol, 1.5 g asparagine, 1 g K_2HPO_4, 500 mg KNO_3, 200 mg $MgSO_4$, 100 mg $CaCl_2$, 100 mg NaCl, 2 mg $FeCl_3$, 15 g agar, 1 L distilled water, pH 8.1) was used for bacteria and actinomycetes.

2.6 Statistical analysis

Statistical analysis was carried out using SAS software version 9.2 (2010). The data were subjected to *F*-test and one way ANOVA followed by Duncan's multiple range test. The values of $p < 0.05$ were considered as statistically significant.

3 Results

3.1 Effect of compost on length and weight of root and shoot with and without addition of *T. viride*

Table 1 shows that the *T. viride* (0.2%)-amended banana compost resulted in the maximum length of roots in comparison to the other treatments. Regarding shoot length, the treatment with *T. viride* (0.1%) in NADEP and *Calotropis* composts and *T. viride* (0.2%) in

Table 1. Effect of composts on length and weight of root and shoot of mungbeans with and without addition of *T. viride* after 60 days of sowing

Treatment	Root length (cm)	Shoot length (cm)	Root weight (g)	Shoot weight (g)
T. viride (0.1%)				
Vermicompost	19.70^{ab}	19.90^{b}	0.324^{b}	3.002^{b}
Banana compost	14.00^{f}	14.60^{e}	0.147^{i}	1.490^{k}
NADEP compost	18.09^{cd}	18.30^{c}	0.204^{e}	2.091^{g}
Calotropis compost	18.00^{cd}	18.70^{c}	0.201^{ef}	2.448^{f}
T. viride (0.2%)				
Vermicompost	18.33^{c}	18.90^{c}	0.226^{d}	2.512^{e}
Banana compost	20.63^{a}	20.53^{a}	0.444^{a}	3.579^{a}
NADEP compost	16.00^{e}	17.45^{d}	0.164^{h}	1.794^{j}
Calotropis compost	16.93^{de}	17.44^{d}	0.186^{g}	1.988^{i}
Without *T. viride*				
Vermicompost	15.86^{e}	17.55^{d}	0.189^{fg}	2.015^{h}
Banana compost	18.90^{bc}	19.51^{b}	0.233^{d}	2.897^{c}
NADEP compost	19.20^{bc}	19.73^{b}	0.311^{b}	2.858^{d}
Calotropis compost	13.90^{f}	14.44^{e}	0.142^{i}	1.456^{l}
CD at 5%	1.22	0.61	0.022	0.012

Mean followed by same letters are not significantly different at $p < 0.05$.
CD, critical difference.

vermicompost showed no significant differences. The maximum shoot length was found in the treatment with 0.2% *T. viride* in banana compost, which was significantly better than the other compost treatments with and without *T. viride*. This treatment was followed by vermicompost enriched with 0.1% *T. viride*, which was statistically at par to the banana and NADEP composts without *T. viride*. A maximum root and shoot weight was observed in the treatment with 0.2% *T. viride* in banana compost which was significantly better than the other compost treatments with and without addition of *T. viride*.

3.2 Effect of compost on disease incidence and population density of *M. phaseolina* with and without the addition of *T. viride*

For Kharif 2009, a maximum disease suppression was observed after the addition of *T. viride* (0.2%) in vermicompost which was significantly better than any other compost treatment in the presence or absence of *T. viride* (Fig. 1). This was followed by the treatment with 0.1% *T. viride* in banana and vermicompost, *T. viride* (0.2%) in banana and NADEP compost and the treatment without *T. viride* in vermicompost. During 2010, the treatments with both concentrations of *T. viride* in vermicompost and the higher concentration in banana compost were found promising to suppress the dry root rot.

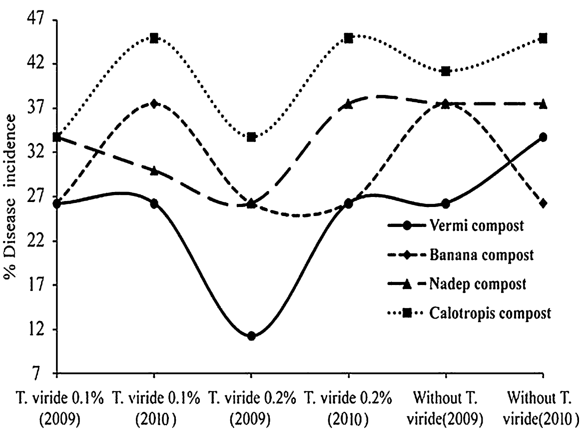

Figure 1. Composition of % disease incidence between two years (2009 and 2010).

The reduction in the population density of *M. phaseolina* in the agriculture soil was observed for all amendments. It showed a decrease from 1.60×10^3 in control to 1.10×10^3 pathogen g^{-1} after addition of vermicompost and *T. viride* (0.2%) (Table 2). When comparing the treatments with and without *T. viride*, a maximum reduction in the population of pathogen was found in *T. viride* (0.2%) in the vermicompost treatment, followed by *T. viride* (0.1%) in the same compost and both concentrations of *T. viride* in banana compost. Banana and vermicompost were found equally effective in the reduction of the pathogen population in treatments without *T. viride*. The highest population of pathogen was recorded in 0.1% *T. viride*-amended *Calotropis* compost.

Table 2. Effect of composts on population density of *M. phaseolina* with and without addition of *T. viride* after 60 days of sowing

Treatment	Mean population density ($\times 10^3$ pathogen g^{-1} soil)
T. viride (0.1%)	
Vermicompost	1.16^{H}
Banana compost	1.26^{G}
Nadep compost	1.40^{D}
Calotropis compost	1.50^{B}
T. viride (0.2%)	
Vermi compost	1.10^{H}
Banana compost	1.26^{G}
Nadep compost	1.30^{E}
Calotropis compost	1.48^{D}
Without *T. viride*	
Vermi compost	1.26^{F}
Banana compost	1.26^{F}
Nadep compost	1.40^{D}
Calotropis compost	1.48^{C}
Control	1.60^{A}

"A" signifies the highest density of *M. phaseolina* and ascending letters show reduction in population density.

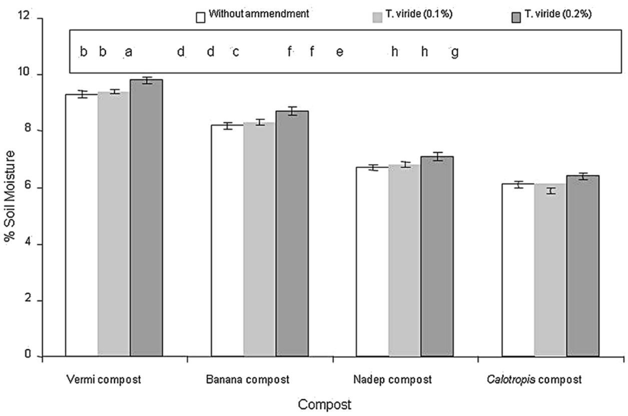

Figure 2. Effect of different composts and amendments on soil moisture (same letters indicate that there is no significant difference at 5% level between different composts with and without addition of *T. viride* after 60 days of sowing). Bars denote standard error (SE).

3.3 Effect of composts with and without *T. viride*-amended soil on soil moisture

The available percent soil moisture always remained higher in soil amended with vermicompost as compared to all other compost-amended treatments; banana compost gave the second best results (Fig. 2). The treatment, *T. viride* (0.2%) + vermicompost + soil, could retain maximum moisture content during the experiment. Soil enrichment with banana compost and *T. viride* (0.1 and 0.2%) also retained more moisture than NADEP and *Calotropis* composts. Non-amended soil irrespective of inoculum could retain least amount of available soil moisture at the time of harvest.

3.4 Microbial population

It is clear from Table 3 that amongst all four composts enriched with *T. viride*, the population of total fungi was significantly higher in the vermicompost compared with the other *T. viride* enriched composts before addition to soil. After incorporation of different composts enriched with *T. viride* (0.1 and 0.2%) in pot soil, the maximum population of fungi was recorded in the treatment with higher concentration of *T. viride* in the vermicompost at the initial stage and was found to be significantly better than all other compost treatments. In contrast, at the harvest stage, this treatment was significantly equal to the treatment with *T. viride* (0.1%) in vermicompost. Similarly, *T. viride* enriched vermicomposts were found superior in bacterial population also before the addition of the composts to soil, which were significantly better than the other *T. viride* enriched compost treatments. These treatments were also found better in the bacterial population even after addition of the composts to field soil followed by the treatments with *T. viride* (0.1 and 0.2%) in banana and NADEP compost at initial and harvesting stages, respectively. None of the *T. viride* enriched compost-amended soils showed significant improvement in actinomycetes population.

4 Discussion

The studies on *T. viride*-amended compost revealed that the treatments with *T. viride* (0.2%) in vermicompost and banana compost showed the highest suppression of dry root rot along with plant growth enhancement. A lower concentration (0.1%) of *T. viride* in these composts was also effective. Variations observed in the growth parameters of mung bean in different composts can be attributed to differences in physical, chemical, and biological properties of the composts as also observed earlier by different researchers [8, 9]. In general, better plant growth of mung bean in *T. viride* enriched vermicompost-amended soil could be due to an increased availability of soil moisture, enhanced population of beneficial microbes and more nutrients. A significant increase was noticed by Lodha et al. in the yield of cluster beans through pearl millet, cluster bean, and cauliflower leaves composts, both in normal and low rainfall conditions [10]. In contrast, a lower fresh

Table 3. Microbial population in composts enriched with *T. viride* (0.1 and 0.2%) and in soil amended with compost at initial (five to six days) and harvesting stages of mung bean plants

	Fungi ($\times 10^3$ cfu g^{-1}compost)			Bacteria ($\times 10^5$ cfu g^{-1}compost)			Actinomycetes ($\times 10^5$ cfu g^{-1}compost)		
		Compost-amended soil			Compost-amended soil			Compost-amended soil	
Treatment	Compost	At initial stage	At harvest stage	Compost	At initial stage	At harvest stage	Compost	At initial stage	At harvest stage
T. viride (0.1%)									
Vermi compost	10.33^{a}	7.00^{d}	8.83^{b}	29.33^{a}	20.00^{gh}	20.50^{fg}	1.50^{ab}	1.00^{bcde}	1.00^{bcde}
Banana compost	7.17^{d}	5.83^{fg}	6.83^{de}	23.17^{d}	17.50^{j}	18.83^{i}	1.17^{bcd}	0.67^{de}	0.83^{cde}
NADEP compost	7.00^{d}	5.17^{hi}	6.00^{fg}	21.17^{f}	17.50^{j}	19.17^{hi}	1.00^{bcde}	0.67^{de}	0.83^{cde}
Calotropis compost	5.50^{ghi}	4.50^{j}	5.00^{ij}	20.00^{gh}	13.00^{m}	17.00^{j}	0.83^{cde}	0.50^{e}	0.67^{de}
T. viride (0.2%)									
Vermi compost	10.50^{a}	8.00^{c}	8.50^{bc}	29.50^{a}	22.17^{e}	23.33^{d}	1.83^{a}	1.50^{ab}	1.33^{abc}
Banana compost	7.17^{d}	6.00^{fg}	7.33^{d}	27.00^{b}	17.50^{j}	18.50^{i}	1.33^{abc}	1.17^{bcd}	1.00^{bcde}
NADEP compost	8.00^{c}	5.83^{fg}	7.00^{d}	24.67^{c}	19.17^{hi}	20.33^{fg}	1.33^{abc}	0.67^{de}	1.17^{bcd}
Calotropis compost	6.33^{ef}	5.00^{ij}	5.67^{gh}	16.50^{jk}	14.00^{l}	14.33^{l}	1.00^{bcde}	0.67^{de}	0.67^{de}
CD at 5%		0.624			0.943			0.562	

Mean followed by same letters are not significantly different at $p < 0.05$.
CD, critical difference.

weight of lettuce plants grown in compost-amended soil was attributed to the release of phytotoxic compounds from decomposing composts during the first week of crop growth [11]. In the present experiment, amendments were applied before planting mung bean seeds, therefore, phytotoxic volatiles, if at all, might have released mostly before planting without any observable effect on the plants.

The addition of compost was responsible for the disease suppression during the experiment. The management of dry root rot caused by *M. phaseolina* in various crops by using organic amendments had been reported by many workers [3, 12–14]. Ratnoo and Bhatnagar reported a reduction in the incidence of *M. phaseolina* in cowpea by wheat straw amendment [12], whereas Sharma et al. recorded the same by mustard cake and cauliflower residues [13]. The application of oil cakes of neem, castor, groundnut, and mahua at the rate of 0.2 and 1% (w/w) was reported to reduce the population of recoverable propagules of *M. phaseolina* in cotton [14]. Organic amendments in soil reduced the number of fungal propagules through germination and stimulation, followed by lysis [15]. Mathur et al. observed that vermicompost was significantly superior in reducing the incidence of *Fusarium oxysporum* causing wilt of fenugreek under field conditions [16]. Similar results have been reported by Sun and Huang with S-H mixture in *Fusarium* wilt of watermelon, where it supported higher length of vines [17]. Organic amendments were found to be an excellent source of nutrition, which favor native antagonists to proliferate and suppress the pathogen by antibiotic production or competition [4]. Pre-incubation of *Trichoderma* in different composts improved the degree of protection by pre-establishing and multiplying of antagonists in a large number in composts.

In arid and semi-arid regions, soil moisture is the most critical factor that affects plant growth, incidence of dry root rot and seed yield of crops that are grown under rain-fed conditions. Limited soil moisture can be efficiently utilized for the crop growth by soil amendment with composts [10]. Earlier, Lodha reported that conserved soil moisture affected the increase in native bacterial population with a corresponding decrease in total fungi including *M. phaseolina* [18]. Besides, the antagonistic role of soil bacteria in reducing the sclerotial population of *M. phaseolina* in the presence of adequate soil moisture was documented by Dhingra and Sinclair [19]. Unlike soil bacteria, the antagonistic activity of actinomycetes was not always hampered at low soil moisture. Bumbieris and Lloyd detected lysis of fungal hyphae in soil too dry for bacterial activity and concluded that actinomycetes were responsible for the lysis in the dry soils [20].

The microbial count was found to be maximum in the *T. viride* enriched vermincompost followed by *T. viride* enriched banana compost-amended soil. It can be inferred from the results that addition of composts to soil, in general, and especially of vermicomposts and banana composts enriched with *T. viride* increased the soil microflora by supporting higher survival and proliferation of fungi, bacteria and actinomycetes as well as causing a reduction in the population of *M. phaseolina* in soil. Low build-up of pathogen can be attributed to the antagonists/saprophytes present as natural microbial flora in soil which compete with the soil borne pathogens for the nutrients and thereby, affect the multiplication of mung bean root pathogens in soil adversely. The high microbial activity and biomass caused by the “general soil microflora” in compost-amended soil prevented germination of pathogenic propagules and infection of the host, presumably through microbiostasis [21]. Majumdar et al. reported a decrease in soil organic carbon under chemical fertilization [22]. Similarly, Prasanna et al. reported an increase in soil organic carbon levels with increased quantity of organic residues added to soils [23]. Organic manure resulted in better growth, enhanced yield and reduced disease incidence in mustard crops [24]. Shaktawat and Shekhawat observed that the application of farmyard manure to Kharif crops significantly improved the available N, P, and K levels of soil after harvesting and a decline was recorded in the nutrient status of soils in the absence of farmyard manure [25]. This could be due to the increased microbial population with addition of manure causing greater mineralization of added leaf fall, root biomass, incorporated trash, native nutrients, and also due to high enzyme activity [26, 27]. Soil microbial population (actinomycetes, bacteria, fungi, and blue green algae) and enzymatic activity were enhanced due to the application of organic inoculants as compared to control and recommended fertilizer application [28, 29].

The present findings are in close agreement with the earlier reports and confirm the possibility of controlling root rot of mung bean by using composts with antagonistic fungi like *T. viride*. A higher dosage of *T. viride* in vermicompost and banana compost was most effective in managing the pathogen, thereby resulting in better plant health. Many factors may be involved in reducing the incidence of dry root rot and enhanced root and shoot length and biomass due to soil amendment with different composts with *T. viride*: i) the composts improved the physical soil structure thus improving the aeration of the roots. A better root growth of mung bean plants in *T. viride* enriched compost-amended soil is an indication for this phenomenon; ii) the composts supported higher levels of the total microbial population including antagonists. An increased microbial population in *T. viride* enriched compost-amended soil in our study evidently confirmed this enhancement in soil. In general, *M. phaseolina* has a poor competitive saprophytic ability. An increase in the population of other microbes, particularly bacteria and actinomycetes might have suppressed its pathogenic activity. The propagules of this pathogen might have been prevented from infecting the roots because of the presence of an increased population of antagonists; iii) the composts improved the moisture holding capacity of the soil, which in turn reduced the pathogenic propagules and disease; and iv) biological factors in compost-amended soil also might have contributed to an increased nitrogenase activity resulting in healthier plants that may resist the attack of the pathogen.

5 Conclusion

Our results conclusively demonstrated that various organic wastes such as banana, cow dung, NADEP, and *Calotropis* can be effectively recycled for their use in compost preparation, because they are easily decomposable materials. *T. viride*-amended composts should be made available to farmers, gardeners, industries and in markets to make use of the same for promoting crop development/agricultural/horticultural activities. Amended soil with composts enriched with *T. viride* holds a great promise in both rain-fed and irrigated agriculture for improving soil fertility. There exists enormous demand of utilizing composts with *T. viride* for suppression of soil-borne plant pathogens for sustainable agriculture.

The authors have declared no conflict of interest.

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