Vermicompost as a biocontrol agent in suppression of two soil-borne plant pathogens in the field

Shobha Ananda Reddy[1], Davis Joseph Bagyaraj[2], Radha Dinakar Kale[1]

[1]Department of Environmental Sciences, Mount Carmel College, Bangalore, Karnataka, 560052, India; [2]Centre for Natural Biological Resources and Community Development, #41 RBI Colony, Anand Nagar, Bangalore, Karnataka, 560024, India;
Email: shobhanand64@gmail.com

ABSTRACT

Two serious soil-borne plant pathogens *Fusarium oxysporum* and *Ralstonia solanacearum* are causing considerable damage to vegetable crops in India. These pathogens are difficult to manage by chemical methods. Current interest is to promote their biological management. The present study was carried out to evaluate the effect of seed treatment with 10% aqueous extracts of vermicompost prepared from different organic substrates (agricultural waste, leaves of *Azadiracta indica* (neem), *Parthenium hysterophorous* (Parthenium), *Lantana camara* (Lantana) and application of vermicompost neem to soil. The test crop was tomato (*Lycopersicon esculentum*) for *F. oxysporum* and eggplant (*Solanum melongena*) for *R. solanacearum*. The results showed that the best treatment for suppression of fusarial wilt in tomato and bacterial wilt in eggplant was seed treatment (1 hour) with 10% aqueous extract of vermicompost neem coupled with the application of vermicompost neem to the soil both during sowing as well as on transplantation. This treatment reduced the incidence of both fusarial and bacterial wilt by 100%. The application of vermicompost alone was not enough to protect the plants against the disease but coupling it with aqueous seed treatment is important to achieve complete disease suppression and increase the yields.

Keywords: vermicompost, plant pathogens, disease suppression, eggplant, tomato

INTRODUCTION

Frequent application of chemicals to soil for the management of soil-borne pathogens and the practice of mono-cropping has resulted in development of resistance by most of the pathogens. Indiscriminate use of chemicals that are not targeted only to the pathogens can result in loss of beneficial organisms in soil. From environmental safety aspects and to maintain the necessary ecological balance in the soil community, it is essential to identify alternate methods for the management of pathogens. *Fusarium oxysporum* and *Ralstonia solanacearum* are common soil-borne pathogens that are causing considerable damage to vegetable crops all over the world. These pathogens are difficult to manage by chemical methods and there is a current interest to promote their biological management. The suppression of disease caused by soil-borne pathogens on application of vermicompost has been reported [1-3]. In the earlier *in vitro* studies it was found that certain soil-borne fungal and bacterial plant pathogens are suppressed by earthworm exudates [4]. Improved growth, flowering, yields, pests and pathogen suppression, on application of aqueous solutions of vermicomposts were observed in eggplant and tomato [5-12].

Research Article, Acta Biologica Indica 2012, 1(2):137-142

Lantana and parthenium are common problematic weeds which are found extensively in agricultural fields. Subjecting these materials to vermicomposting can help in weed management. Neem is a common tropical tree and its seeds are known to have fungicidal properties [13]. There will be periodic shedding of leaves and hence they are available in sufficient quantity for farmers to produce vermicompost which can be applied to soil during cultivation of vegetable crops to alleviate the severity of incidence of diseases caused by pathogens. The results of *in vitro* antimicrobial assay and pot culture studies conducted earlier [14] formed the basis for the present study to evaluate the effects of seed treatment with 10% aqueous extracts of vermicompost prepared from different substrates [agricultural wastes, leaves of *Azadiracta indica* (neem), *Parthenium hysterophorous* (parthenium) and *Lantana camara* (lantana)], and soil application of vermicomposted neem. The response of susceptible crops, e.g., tomato (*Lycopersicon esculentum*) and eggplants (*Solanum melongena*) in the infected fields were evaluated for developing effective biological management.

MATERIALS AND METHODS

Preparation of substrate for vermicompost production

Fresh plant material of neem (*Azadirachta indica* A. Juss), lantana (*Lantana camara* L.), parthenium (*Parthenium hysterophorus* L.) were collected and allowed to undergo partial decomposition by adding thin slurry of cow manure with occasional turning in small containers (10 cm × 6 cm). After four weeks of decomposition 10 g earthworms (*Eudrilus eugeniae* Kinb.) were released into the one kg material. Earthworms fed on the organic matter and excreted the undigested matter as castings. The entire material was converted into vermicompost in two weeks time. Later on large scale production of vermicompost were prepared in bins (1 × 1 × 0.45 m) on similar lines [14].

Preparation of vermicompost extracts

Aqueous extracts of vermicompost were prepared by adding 100 ml of sterile distilled water to 10 g of vermicompost (10%) and placed on a magnetic shaker for four hours and then centrifuged at 4000 rpm for 30 min; the supernatant fluid was filtered through membrane filter and stored at 4°C for further studies [1,4,14]. The filtrate was free from any bacterial contamination.

Pathogen cultures

Pure cultures of plant pathogens on solanaceous crops were obtained from Plant Pathology Department, University of Agricultural Sciences, Bangalore, Karnataka, India such as *Ralstonia solanacearum* (Smith) Yabuuchi et al. and *Fusarium oxysporum* Schlechtend ex Fr. f. sp. *lycopersici* (Sacc.) Snyder and Hans. Microbial suspensions were prepared for inoculating into fields. Nutrient broth culture of the bacterial isolate *Ralstonia solanacearum* was prepared and incubated for 48 h at room temperature (27±2°C). The optical density of 48 h nutrient broth culture was adjusted to 0.450 at 610 nm using UV visible spectrophotometer to get the inoculum of 1×10^8 CFU ml^{-1} [15]. The fungal pathogen *Fusarium oxysporum* was inoculated into Czapek Dox broth and incubated at 27±2°C for 10 days. After 10 days the mycelial mat was taken out macerated using a homogeniser and suspended into 10 ml of 0.01M $MgSO_4.7H_2O$. Serial dilution followed by plating was carried out to determine the fungal concentration in CFU ml^{-1}.

Microplot studies

Microplots each of 2 × 1.5 m size in triplicates for each of the treatments were prepared in a randomized way (24 for tomatoes and 15 for eggplants). Each microplot had 50 plants. Pathogen inocula (1×10^8 CFU ml^{-1}) of 60 ml were inoculated into each plot five days before sowing. After four days the levels of pathogens in inoculated soils were determined using serial dilution method. This was to confirm the sickness of the soil. The recorded levels of pathogens were *R. solanacearum* 22×10^3 CFU ml^{-1} for the plots for eggplants and *F. oxysporum* 19×10^3 CFU ml^{-1} for the plots with tomatoes.

Seed treatment and raising of seedlings

The seeds were soaked in 10% aqueous extracts of different vermicompost (as mentioned earlier) for experimental plots and they were soaked in water for control plots for one hour before sowing in the nursery beds [14]. The seedlings were raised in separate nursery beds of size 1m × 1m to which vermicompost was applied at the rate of 5 tons ha^{-1}. From the nursery beds seedlings were transplanted to plots and each plant received 250 g of vermicompost (spot application) in two split doses like 50% at the time of transplantation and 50% after one month. Microplots without any amendment were used as control and the application of farm yard manure served as an additional control. The prepared microplots for both the crops received different vermicompost as mentioned in table 1 and 2. Seed treatments before sowing are also provided in these tables. During crop growth, plants were monitored for survival rates and numbers of plants infected with the pathogen. The fruit yield per plot was recorded for each treatment. Possible treatment differences were identified by the analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) by running MSTAT-C and Microsoft Excel software.

RESULTS AND DISCUSSION

The survival of tomato plants treated with different bioagents is summarised in table 1. The survival was 100% in the treatment T_4 (seeds soaked in aqueous extract of vermicomposted neem leaves + vermicomposted neem leaves added to soil) followed by 96% in the treatments T_5 (seeds soaked in aqueous extract of vermicomposted agricultural waste + vermicomposted neem leaves added to soil) and T_7 (seeds soaked in aqueous extract of vermicomposted parthenium leaves before onset of flowering + vermicomposted neem leaves added to soil). Treatments T_8 (94%), T_6 (92%), T_3 (86%) had comparatively better survival compared to 70% and 58% in control treatments T_2 and T_1 respectively. The yields of tomato fruits per plot from three harvests are shown in table 1. Yields were higher in the second harvest in all the treatments and it decreased during the third harvest. The duration between each harvest was 10 days. Among all treatments, treatment T_4 (seeds soaked in aqueous extract of vermicomposted neem leaves + vermicomposted neem leaves added to soil) produced the highest yield (385 kg $plot^{-1}$) followed by treatments T_7 (361.66 kg $plot^{-1}$), T_6 (360.33 kg $plot^{-1}$), T_5 (333 kg $plot^{-1}$), T_8 (325 kg $plot^{-1}$) and T_3 (136.66 kg $plot^{-1}$). Very low yields were recovered from treatments T_1 and T_2. The yield recovered in treatment T_3, i.e., seeds soaked in water + addition of vermicomposted neem leaves to soil was 136.66 kg $plot^{-1}$ indicating that application of vermicompost alone was not enough to protect the plants against the disease but coupling it with the aqueous seed treatment was important to achieve complete disease suppression and to obtain better yields. Good foliar growth and early flowering and fruiting was seen in treatments T_4, T_5, T_6, T_7 and T_8 where seeds were soaked in aqueous plant or vermicompost extracts combined with vermicompost applications to soil. The fruit size was also larger in these treatments compared to

those treatments in T_1, T_2 and T_3 where seeds were soaked in water with no soil treatment or amended with FYM or vermicompost.

Table 1. Yield recovery of tomato on seed treatment and manurial applications (n=3).

Treatment code	Treatment	Survival rate (%)	Yield (kg) plot^{-1} $\bar{x}$±SE
T_1	Seeds soaked in water + no amendment to soil	58	15.66±1.20^f
T_2	Seeds soaked in water + farm yard manure added to soil	70	58.33±0.88^e
T_3	Seeds soaked in water + VN Soil	80	136.66±4.41^d
T_4	Seeds soaked in aqueous extract of vermicompost neem leaves + VN Soil	100	385± 7.63^a
T_5	Seeds soaked in aqueous extract of vermicompost agricultural waste + VN Soil	96	333±6.24^c
T_6	Seeds soaked in aqueous extract of *lantana* flowers + VN Soil	92	360.33±2.91^b
T_7	Seeds soaked in aqueous extract of *Parthenium* leaves before onset of flowering + VN Soil	96	361.66±6.01^b
T_8	Seeds soaked in aqueous extract of *Parthenium* leaves on onset of flowering + VN Soil	94	325±2.88^c

VN Soil - vermicomposted neem leaves added to soil; Means with same superscript are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level.

Table 2. Eggplant yields in response to aqueous seed treatments and vermicompost applications (n=3).

Treatment code	Treatment	Survival rate (%)	Yield (kg) plot^{-1} $\bar{x}$±SE
T_1	Seeds soaked in water + no amendment	22	4. 38±2.96^e
T_2	Seeds soaked in water + farm yard manure added to soil	60	11.25±3.45^d
T_3	Seeds soaked in water + VN Soil	80	21.65±2.32^c
T_4	Seeds soaked in aqueous extract of vermicomposted neem leaves + VN Soil	98	32.5±1.45^a
T_5	Seeds soaked in aqueous extract of vermicomposted agricultural waste + VN Soil	88	27.5±2.03^b

VN Soil - vermicomposted neem leaves added to soil; Means with same superscript are statistically on par by Duncan's Multiple Range Test (DMRT) at 5% level.

The survival of eggplants treated with different bioagents in microplots is summarised in table 2. The survival was 98% in treatment T_4 (seeds soaked in aqueous extract of vermicomposted neem leaves + vermicomposted neem leaves added to soil). This was followed by T_5 (88%) in which seeds were soaked in aqueous extract of vermicomposted agricultural waste + vermicomposted neem leaves added to soil. The least survival of 22 % was encountered in the control treatment with seeds soaked in only water with no soil amendments. The yield per plot from first harvest is summarized in table 2. Among all the treatments, treatment T_4 (seeds soaked in aqueous extract of vermicomposted neem leaves + vermicomposted neem leaves added to soil) produced the highest yield of 32.5 kg plot^{-1}. This was followed by the treatment T_5 (seeds soaked in aqueous extract of

vermicomposted agricultural waste + vermicomposted neem leaves added to soil) with a yield of 27.5 kg plot^{-1}. The yield in treatment T_3 was 21.65 kg plot^{-1} which was significantly higher than that of the control with no soil amendments (4.38 kg plot^{-1}) indicating that application of vermicompost prepared from neem can help in suppressing the pathogen.

The present study has shown that the incidence of both fusarial wilt in tomatoes and bacterial wilt in eggplants could be totally managed by treating the seed with neem-based vermicompost extract combined with application of the vermicomposted neem to soil. Better biomass and yields were observed with application of neem vermicompost. It is also clear that the vermicompost prepared from specific substrates and their extracts can be effective in bringing about specific pathogen suppression. Several other reports are on pathogen and pest suppression by aqueous extracts of vermicompost [4,16-19].

Augmenting earthworm populations (*L. terrestris*) at the rate of 11 earthworms m^{-2}, was effective in suppressing *Verticillium* wilt of eggplant [20]. Better quality of grapeberries was observed with *in situ* vermiculture when compared to vermicompost application [21]. This suggests that the contents of earthworm gut, body wall contents and coelomic fluid may have an influence on antimicrobial activity. The beneficial soil microbes present in the gut of the earthworms may antagonise the pathogens in soil. Hence using *in situ* vermiculture, better suppression might be obtained when compared to vermicompost application. But this is possible only in case of plantation crops. In plantation crops canopy cover and organic mulch supports the moisture retention which is necessary for survival of earthworms. In dryland conditions there will not be sufficient moisture and organic matter input when the fields are left fallow during dry season. Lack of moisture and sufficient organic matter is the limiting factor for *in situ* vermiculture.

The present study confirms the antimicrobial properties of vermicompost. For dryland situation vermicompost application serves as means to restore the soil health. If this is confirmed vermicompost can be effectively used in agriculture since it is one of the potential bioagents which can serve both as biofertilizer and biopesticide. Use of vermicompost extract as foliar spray can effectively decrease the incidence of pest attacks and diseases [19]. In the current study neem-based vermicompost had the best results in suppressing both wilt (fusarial and bacterial). Hence it can be concluded that soil applications of neem vermicompost, coupled with seed treatment with 10% aqueous extract of neem vermicompost for one hour, can reduce significantly the incidence of wilt disease caused by *F. oxysporum* in tomatoes and *R. solanacearum* in eggplants. These results can be further exploited for weed control and litter management through vermicomposting and formulating the vermicompost extracts for seed treatment for management of diseases of common vegetable crops. More investigations are needed for isolation and characterization of antimicrobial moieties and recommendation for field applications.

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