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Research article

INDUCTIONS OF RESISTANCE IN BRINJAL (SOLANUM MELONGENAE L.) BY AQUEOUS EXTRACT OF VERMICOMPOST AGAINST FUSARIUM WILT

Kamal Lochan Barman, Reena Barman Kalita and Dhruva Kumar Jha*

Microbial Ecology Laboratory, Department of Botany, Gauhati University, Guwahati-14
*Corresponding author Email: dkjha_203@yahoo.com

ABSTRACT: Fusarium wilt of brinjal (*Solanum melongena* L.) caused by *Fusarium oxysporum* f. sp. *melongenae* is a highly destructive disease of brinjal. Vermicompost has the unique ability to improve the chemical, physical and biological characteristics of growing media. In this study the effect of aqueous extract of vermicompost on resistance induction in brinjal plants was evaluated both in vitro and pot experiments. Application of aqueous extract of vermicompost even at low concentration (0.1%) significantly inhibited growth and development of the pathogen. The soil amended with vermicompost showed significant improvement in the growth parameters of brinjal plants over the control. It also induced synthesis of phenolic compounds in brinjal. Maximum phenolic compounds were recorded from plants treated with 5% vermicompost extract and minimum from control. The synthesis of phenolic compounds indicated the induction of resistance in treated plants.

Key words: Aqueous extract, vermicompost, resistance, phenloic compound

INTRODUCTION

Vegetables are rich sources of nutrients and vitamins, and play a major role in a balanced diet for human beings. Next to China, India is the second largest producer of vegetables in the world and accounts for about 15% of the world's production of vegetables. Chemical fertilizers are popularly applied in agricultural fields to improve yield of vegetables. But continuous and improper application of chemical fertilizers has caused various adverse affects on soil micro flora as well as has caused ecological imbalance of soil. Vermicompost has been found as agent to minimize the hazardous effects of chemical fertilizers. Vermicomposting is stabilization of organic material involving the joint action of earthworms and microorganisms. Although microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering the biological activity as discussed by Dominguez [9]. Vermicompost is rich in organic matter resources that have the unique ability to improve the chemical, physical, and biological characteristics of soils or growing media. The addition of vermicompost has a positive effect on soil productivity and other characteristics of soil as they increase organic matter and nutrient levels as discussed by Garcia [13]. Furthermore, when used as soil amendments, they may have highly suppressive effects against diseases caused by a variety of soil borne plant pathogens, such as *Pythium* sp, as reported by Pascual [28], *Phytophthora* spp. as discussed elsewhere [17, 42] and *Rhizoctonia* sp reported by Tuiter [39]. Compost and its aqueous extract have been found to induce systemic acquired resistance in cucumber and *Arabidopsis* [44, 45]. An aqueous extract of a composted manure-straw mixture also has the ability to control diseases caused by *Botrytis cinerea* [23]. The application of aqueous compost extracts has been shown to reduce diseases by necrotrophs as well as biotrophs [12, 3] and also aqueous extracts of vermicompost have been shown to suppress soil borne pathogens and pests [24, 27, 29]. Bare patch disease of wheat caused by *R. solani* was effectively controlled by soil application of vermicompost [34]. Suppressive effects of compost-amended soil for *Rhizoctonia solani* as compared to non-composted soil have also been reported by Voland and Epstein [41]. Research has shown that increased population of certain microorganisms may suppress specific plant diseases such as *Pythium* and *Fusarium* as well as nematodes. Disease suppression by vermicompost has been attributed to the activities of antagonistic microorganisms. The biological control of soil borne plant pathogens in vermicompost amended container media has received much attention in the last decade. Among the organic materials used for the preparation of potting media, some were suppressive against Fusarium wilts [31].

Brinjal is one of the most popular and important commercial crops grown throughout the world. It is commonly known as poor man's meat due to its inherent high nutritive value, vitamin content and dietary fibers. In addition, brinjal is of much medicinal value. It can block free radicals; help control cholesterol levels and is also a source of folic acid and potassium. Many diseases and disorders can affect brinjals during the growing seasons. *Fusarium oxysporum* f. sp. *melongenae* is a highly destructive pathogen of brinjals. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield. The available control methods for Fusarium wilt disease are either insufficient or difficult to apply. Though the chemical fertilizers are effective in vitro against *F. oxysporum*, however under field conditions it is not possible to apply a fungicide directly to roots**.** Therefore the main objective of our experiments was to determine the potential of aqueous extract of vermicompost to suppress the incidence of the plant diseases *Fusarium oxysporum* in brinjal.

MATERIAL AND METHODS

Isolation of fungi

Fusarium oxysporum was isolated from infected brinjal plants. A piece of an infected part was cut and disinfected with sodium hypochlorite (1%) for 5 min. The piece was put on *F. oxysporum* specific medium [peptone pentachloronitrobenzene agar (PPA) modified by Nash and Snyder [25]. After 5 days of incubation at 28 ºC, *F. oxysporum* growth around the part was developed. A section of the developed mycelium from the margin was subculture for three times on potato dextrose agar (PDA) by incubating the plates at 25 ºC for 7 days to for confirming the growth of *F. oxysporum* mycelium. A piece of the isolated *F. oxysporum* mycelium was suspended in a flask containing 250 ml of potato dextrose broth and was incubated until the liquid medium was brown-pink in colour. The number of spores was counted by haemocytometer and it was diluted to obtain 3.3 X 10^5 CFU ml^{-1}. The *F. oxysporum* suspension was used to infect the brinjal plants.

Aqueous extract of vermicompost (AVC)

Air-dried vermicompost (1 kg) was taken and dipped in 2 l sterilized distilled water in 5 l conical flasks and shaken thoroughly 6–8 times in a day. After 24 hr, the supernatant of vermicompost was filtered through absorbent cotton pad. It was refiltered twice to remove the dust particles. The filtrate thus obtained was evaporated and finally a slightly brownish material was obtained (5 g) for making the desired concentrations. The recovered extract was used for invitro bioassay against phytopathogenic fungi. Assessment of the efficacy of different concentrations (1000, 2000, 3000, 4000 and 5000 mg/l) of the extract was done in pot against *Fusarium* wilt disease of brinjal.

Invitro antagonistic effect of vermicompost (VC)

Thirty milliliter of sterile water was poured into a Petri plate with 7 day old mycelium of *F. oxysporum* on PDA. The fungus was suspended in water by stirring it with a sterile string. Then 0.1 ml of the suspension was spread on plates with fresh PDA. After 24 hr of incubation at 28º C, samples (0.5g) of vermicompost placed in the centre of each plate. Plates containing autoclaved vermicompost samples served as a control. There were 3 replicates per treatment. The plates were incubated at 25ºC for 20 days and the development of fungus was observed. The suppressiveness of vermicompost was estimated by the presence of a zone of inhibition around the samples.

Assessment of plant growth

Brinjal seedlings were grown in pots (15.0 cm dia.) containing garden soil previously mixed with aqueous extract of vermicompost (1, 2, 3, 4 and 5 %) along with the control (garden soil only). Seeds were allowed to germinate at 25 ± 2 °C at 8/16 h day/night photoperiod. Each treatment was conducted in triplicate. Observations on various growth parameters were recorded periodically. Simple Randomized Block Design (RBD) and Origin statistical package were used for statistical analysis.

Extraction of phenolic acids

The leaves of 21-day-old brinjal plants were used for assessing the resistance-inducing capacity of vermicompost by the estimation of phenolic compounds by the methodology described by Zieslin and Ben-Zaken [46]. Brinjal plants were excised from randomly selected different plants from each treatment. They were pooled together to make one sample of each treatment for extraction of phenolic acids. At least three samples were prepared from each treatment.

Fresh plant samples (1g) were homogenized in 10ml of 80% methanol and agitated for 15minutes in a water bath at 70 ± 3oC. One ml of the methanolic extract was then added to 5 ml of sterilized distilled water and 250 µl of 1N Folin-Ciocalteu reagent and the solution was kept at 25 ± 20C. After 3 minutes one ml saturated solution of sodium carbonate and one ml of distilled water was added and the reaction mixture was incubated for 1hour at 25oC. The absorbance spectra of developed blue colour were measured using UV-visible spectrophotometer at 725 nm. A standard curve was prepared by dissolving different concentrations of catechol. The amount of phenolic content was expressed as phenol equivalents in µgm/g-1 fresh tissue.

Pot experimentation

For pot experiment, we selected *Solanum melongena-Fusarium oxysporum*, the causal organism of wilt disease of brinjal. Experiments were conducted at Department of Botany, Gauhati University, Guwahati, India. Randomized block design was followed for the pot experiment. Each treatment and control was replicated three times. Brinjal seeds were surface sterilized by immersing them first in 95 % ethanol for 30 s and then in 0.2 % solution of $HgCl_2$ for 3 min, following several rinses with sterilized water to remove traces of the disinfectant. Brinjal seedlings were planted in the pots containing 5 Kg of sterilized soil which was inoculated by adding 10 ml of *Fusarium oxysporum* spore suspension (3.3 x 10^{-5} CFU ml^{-1}) per pot two weeks before planting. The pre inoculation treatments of aqueous vermicompost extract were done before the planting of seedlings whiles the post inoculation after the first appearance of wilting. Plant height, number of leaves and diseases index were recorded at 30 days interval. Growth parameters such as root and shoot biomass were also recorded.

Disease index

The disease index was computed by adapting 0-4 scales to cover all broad symtomological criteria for Fusarium [7]. Disease development was assessed using the index: **0**, plant without symptoms; **1**, very slight browning of hypocotyl, no other symptoms; **2**, some wilting of plant, drying of lower leaves, some browning of vascular system; **3**, wilting of entire plant, leaves drying, hypocotyl with external and internal symptoms; **4**, necrosis of stem, plant dying.

Statistical analysis

The experiments were carried out in complete randomized block design. The average values obtained from three replicates were used for analysis. Data were analyzed by Duncan's Multiple Range Test (DMRT) using SPSS Software (version 10) and mean values were compared using least significant difference (LSD at $P \leq 0.05$).

RESULTS

Invitro suppressive effect of vermicompost

Unsterilized vermicompost strongly inhibited the growth of *F. oxysporum* on agar medium, while sterilization of this substrate could not suppress the mycelial growth of the pathogen (Fig: 1). Microscopic observations of these plates showed that hyphae treated with unsterilized vermicompost were completely destroyed and colonized by microbes. The hyphae growing on plates treated with sterilized vermicompost, however, were unaffected.

Efficacy of aqueous extracts in pot experiments

The effect of pre and post inoculation treatments of aqueous extract of vermicompost on the development of wilt in brinjal due to the infection of *F .oxysporum* is shown in Fig 2. All the concentrations of vermicompost extract were significantly effective ($p \leq 0.05$) against the development of wilt disease in brinjal as compared to the control. The disease index decreased gradually with the increasing concentration of the extract. The lowest disease index was obtained when the plants were treated with highest concentration (0.5%) of the extract. While in control, no differences were observed in disease index in both pre-inoculation and post inoculation treatments. In pot experiments, pre inoculation was more effective in restricting the development of wilt disease in brinjal as compared to the post treatment.

Figure 1: Influence of unsterile (A) and sterile (B) vermicompost on growth of *F. oxysporum* mycelium on agar medium.

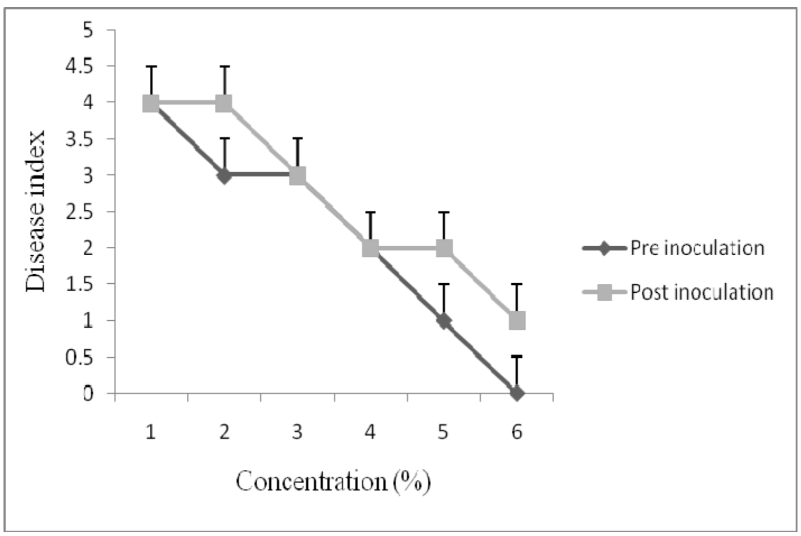

Figure 2: Effect of different concentrations of aqueous extract of vermicompost on disease index in pre and post treated brinjal seedlings. All values are mean of three replicates and bars indicate the standard deviation of the mean.

Growth promoting effect of vermicompost

The soil amended with vermicompost showed significant increase in the various growth parameters of brinjal in comparison to the control. The analysis showed reduction in disease development in vermicompost treated plants in comparison to the control. The various growth parameters of brinjal plants are summarized in Table: 1. Data obtained in the experiment showed that increase in the concentration of vermicompost extract significantly ($p \leq 0.05$) increased the growth and development of brinjal plants. The highest growth and productivity was obtained when treated with highest concentration of vermicompost i.e. 5%. . The analysis of data showed reduction in the severity of the disease symptoms in the vermicompost treated plants. The best response was obtained when the seedlings were inoculated with 5% vermicompost. There were significant differences ($p \leq 0.05$) in number of drooped leaves, yellow leaves and also in total number of leaves. The various parameters of the plants differed significantly ($p \leq 0.05$) in vermicompost treated plants as compared to only *F. oxysporum* inoculated plants.

Table 1: Effect of application of vermicompost on growth of brinjal seedlings (Mean ± SD)

Observation	Vermicompost concentration (%)						LSD
	Control	1	2	3	4	5	
Plant height (cm)	23.3±3.2^{a}	28 ± 3^{ab}	32 ± 1^{bc}	35 ± 4^{cd}	38 ± 3^{d}	40 ± 2^{d}	4.8
Total no. of leaves	17 ± 2^{a}	21 ± 1^{b}	25 ± 2^{c}	29 ± 1^{d}	34 ± 2^{e}	39 ± 1^{f}	2.5
No. of drooped leafs	12 ± 2^{b}	12 ± 3^{b}	11 ± 3^{b}	10 ± 3^{b}	10 ± 1^{b}	5 ± 2^{a}	3.1
Shoot biomass (gm)	6.06±1.9^{a}	7.6±1.8^{ab}	8.2±2.9^{ab}	9.7±0.8^{ab}	10.3±2.2^{b}	10.6±2.4^{b}	2.4
Root biomass(gm)	0.8 ± 0.3^{a}	1.2±0.3^{ab}	1.5±0.2^{bc}	1.7±0.4^{bc}	1.7±0.2^{bc}	1.9 ± 0.4^{c}	5.3
Time of flowering(Days)	52 ± 4^{c}	47 ± 6^{bc}	46± 5^{abc}	43 ± 4^{ab}	40± 2^{ab}	38 ± 3^{a}	7.1
Phenol content (µg/g leaf tissue)	3.3 ± 0.2^{a}	4.6± 0.3^{b}	5.0±0.4^{bc}	5.4±0.3^{cd}	5.8±0.4^{de}	6.2 ± 0.2^{e}	5.2
Chlorophyll. Content in leaf(mg/gm leaf)	1.2 ± 0.4^{a}	1.9 ±0.2^{b}	2.2±0.3^{bc}	2.5±0.4^{cd}	2.7±0.3^{cd}	2.8 ± 1^{d}	5

Data are mean of three replicates.
Mean values followed by different letters in each row differ significantly at $P \leq 0.05$.
LSD refers to the least significant difference at 5% probability level

Phenolic compounds

The phenolic compounds were studied by the methodology as described. Quantitive variation of phenolic compounds have been found in the treatments .Among different concentrations of vermicomposts, 5% concentration observed maximum 6.2 µg/g fresh tissues (Table: 1) which is followed by 4% and 3% concentration that recorded 5.8 and 5.4 µg/g fresh tissues respectively and minimum amount was obtained in control plants (3.3 µg/g fresh tissues).

DISCUSSION

Invitro suppressive effect of vermicompost

Unsterilized vermicompost substituted into sterilized media increased *Fusarium* suppression to a greater extent than sterilized vermicompost, evidence that the resistance was probably due to the high disease suppressive property of the antagonistic microorganisms inhabiting in the vermicompost sample. The ability of vermicompost to suppress the growth of soil- borne fungal plant diseases has been described by only few workers [36-37]. The aqueous extract of vermicompost strongly inhibits the development of powdery mildew of pea and balsam [32]. Hoitink and Fahy [16] and Hoitink et al. [17] also obtained identical results using different composts. In most cases, the ability of compost to suppress *F.oxysporum* was lost once they were heated (sterilized) indicating the biotic nature of the disease suppressive factor of the composts [16]. Unsterilized vermicompost strongly inhibited the growth of F. oxysporum on agar medium, while sterilization of this substrate could not suppress the mycelia growth of the pathogen (Fig: 1) which may be due to loss of antagonistic microorganisms on heating at high temperature.

Efficacy of aqueous extracts in pot experiments

Aqueous extract of vermicompost significantly inhibited the growth and development of several phytopathogenic fungi invitro [32]. In pot experiments, pre inoculation was more effective in restricting the development of wilt disease in brinjal as compared to the post treatment. These results support the fact that use of composts as growth promoting media reduced disease intensity (severity and incidence) in aerial parts of plants attacked by foliar pathogens [2, 22]. Extracts obtained from thermophilic compost proved to be effective against various fungal diseases of leaves and fruits especially when applied prophylactically [30]. Our present findings are in agreement with the suppression of Rhizoctonia disease of cucumber due to application of mixture of agricultural compost as reported by Trillas [38]. The application of vermicompost significantly increased the disease suppressive properties of soil. Cotxarrera [8]; Kavroulakis [19]: Wu [43] also advocated the application of suppressive composts for controlling *Fusarium* wilt.

Growth promoting effect of vermicompost

The analysis of data showed reduction in the severity of the disease symptoms in the vermicompost treated plants. The best response was obtained when the seedlings were inoculated with 5% vermicompost. There were significant differences ($p \leq 0.05$) in number of drooped leaves, yellow leaves and also in total number of leaves. The various parameters of the plants differed significantly ($p \leq 0.05$) in vermicompost treated plants as compared to only *F. oxysporum* inoculated plants. In the present study it was also observed that due to increase in vermicompost concentration plant growth and productivity also increased (Table 1). The application of vermicompost was found to be more effective in improving soil micronutrients content. Vermicompost contribute macronutrients and micronutrients in amount that is required by plants. The higher growth of various plant characteristics in different concentration of vermicompost compared to control was not only because of the presence of greater amounts most of the plant nutrients but also due to the presence of microbial metabolites, the plant-growth promoting hormone-like substances. Nijhawan & Kanwar [26] observed that on application of vermicompost there was an increase in plant height, number of tillers and of leaves in wheat plant than control. Edward & Burrows [10] concluded that emergence of tomato, cabbage and radish seedlings were much better in vermicompost amended soil than in thermophilically composted waste. It is well established that earthworm activity on organic matter can lead to the production of water-extractable plant growth influencing substances in quantities that could significantly influence seed germination, growth, flowering and yield of crops [11]. In our study the growth and yield of brinjal showed great variations in 5% concentration than other concentrations. Our findings also support the results of Suthar [35] that warm cast can act as a best plant growth media. Arancon et al [4] reported that application of vermicompost increased microbial population and activities which were key factor for cycling of plant nutrients, production of plant growth influencing materials and induction of plant resistance or tolerance against disease.

Phenolic compounds

Phenolic acids are the pre-existing biochemical compounds present in low quantity in the plants [1]. They are the most widespread class of plant secondary metabolites, which are found to be bioactive against a number of pathogens. They may function as a part of the structural plant matrix [33] thus acting as constitutive protector against invading organisms [40]. Application of vermicompost in the soil increased the level of phenolic compounds in brinjal plants. Along with the increased in concentration of vermicompost, a significant increase ($p \leq 0.05$) in phenolic compound was recorded. Phenolic compounds can exist as free or bound molecules since they can form complexes with other molecules such as proteins and other cellular components [21]. Cell wall-bound phenolic esters may act directly as defense compounds, or may serve as precursors for the synthesis of lignin, suberin, and other wound induced polyphenolic compounds [14] that provide stronger physical barriers inhibiting the entry of pathogens [6, 20]. Some of the phenolic acids are already known as antifungal and also induce resistance in plants directly or indirectly by the synthesis of phenolic compounds in the hosts [15]. Thus the increase of phenolic compound in the VC-treated plants indicates that the vermicompost can induce resistance in the plants.

In summary the application of aqueous extract of vermicompost not only minimized the disease index in brinjal but its application also enhanced the biological potential of soil and consequently increased seedling growth. Present findings confirm that vermicompost application promotes microbial population that not only promotes plant growth but also induces diseases resistance in plants. It also seen that highly concentrated vermicompost also induce high phenolic compounds which may acts as strong barriers for pathogens.

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