Management of tomato bacterial spot caused by *Xanthomonas campestris* using vermicompost

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ABSTRACT

The *in vitro* efficacy of different aqueous extracts of vermicompost prepared from leaves of *Azardirachta indica, Lantana camera, Parthenium hysterophorous* were tested for the management of the pathogen. Vermicompost was prepared by mixing the respective substrates with cow dung slurry (9:1 ratio w/w) independently. Among the three aqueous extracts, vermicomposted neem was found to be superior to that of vermicomposted *Lantana* and *Parthenium* in suppression of growth of *X.campestris.* Based on the results of the *in vitro* studies field studies were taken up as a combination of seed treatment with vermicompost extract and soil application of vermicompost. The seeds of tomato were soaked for one hrs in different aqueous extracts of vermicomposts before sowing. The seedlings were raised in separate nursery beds of size 1m x 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 250g of vermicompost (spot application) in two split doses. Since the aqueous extract of vermicomposted neem showed better suppression of the pathogen in *in vitro* studies, the same vermicompost was used for soil application. The results showed that the best treatment for suppression of bacterial spot in tomato was seed treatment (1 h) with 10% aqueous extract of vermicomposted neem coupled with application of vermicomposted neem to the soil both during sowing as well as on transplantation. This treatment reduced the incidence of bacterial spot by 98% and resulted in maximum yield of 15.4 t ha^{-1}. Bioagent like aqueous extract of vermicomposted neem along with the soil amendment with the same vermicompost is essential for the management of the pathogen in affected soils. Use of such alternate materials which are non-polluting, cost effective, non hazardous and do not disturb ecological balance can reduce the use of copper based bactericides, leading to elimination of copper entry into the soil system.

Key words: Seed treatment, soil-borne plant pathogen, soil amendment, vermicompost

INTRODUCTION

Bacterial spot is one of the most devastating diseases of tomato grown in warm, moist environments. It is highly destructive both in greenhouses and field grown tomatoes causing 10 – 50 % yield loss in many tomato production areas (Kallo, 1991; Mao *et al.,* 1998). In India losses ranging from 10 – 80 % have been reported. On an average, annual loss due to this disease is 10-20 %, which may go up to 80 % in rare cases (Sharma and Sharma, 2005). The recurrent and indiscriminate use of bactericides has posed a serious threat to human health. Some of them have already been proved to be mutagenic, teratogenic and carcinogenic. Keeping in view the harmful effects and drawbacks of the chemical methods of plant pathogen management, use of bioagents for pathogen management is gaining importance. The present study was carried out to evaluate the efficiency of vermicomposts prepared from leaves of *Azardirachta indica*, *Lantana camera* and *Parthenium hysterophorous* for the management of bacterial spot in tomato at field level in microplots.

MATERIALS AND METHODS

Vermicompost production and preparation of vermicompost extracts

Vernicompost was produced by subjecting the

fresh plant material (leaves of *Azadirachta indica* A. Juss (neem), *Lantana camara* Linn., *Parthenium hysterophorus* Linn. and assorted leaf litter), independently for primary decomposition for three weeks after mixing with thin slurry of cow manure with occasional turning in small containers (10 x 6 x 4 cm). After three weeks of decomposition the earthworms [*Eudrilus eugeniae* (Kinb)] were released into the material. Vermicomposts from individual containers were collected separately for preparation of 10% aqueous extracts with sterile distilled water. Fresh vermicompost (10g) was taken in a conical flask and mixed with 100 mL of sterile distilled water. The flask was kept in orbital incubator shaker for 4 hrs and allowed to settle for one hrs. The supernatant was filtered using membrane filter of size 0.4 µ and stored at 4^oC for future use (Shobha and Kale, 2009).

Pathogen culture

Pure culture of *Xanthomonas campestris pv vesicatoria* (Diodge) Dye, was obtained from Plant Pathology Department, University of Agricultural Sciences, Bangalore. An inoculum of size 10^8 CFU/mL of each of the isolates was prepared according to the method of Bauer *et al.* (1966). Nutrient broth cultures of the bacterial isolates were prepared and incubated for 48 hrs at room temperature (27 ± 2^0C). The optical density of 48 hrs nutrient broth culture was adjusted to 0.450 at 610 nm using UV-visible spectrophotometer to get the inoculums of 1×10^8 CFU/mL.

***In-vitro* antibacterial studies**

Antibacterial activity of the above mentioned extracts was determined, using the agar well diffusion assay method (Holder and Boyce, 1994).The bacterial culture was swabbed onto the Nutrient agar plates. Wells of uniform size were bored in the medium and 1mL of the respective extracts was added into the wells. The antimicrobial activity was recorded on the basis of diameter of zone of inhibition, which was measured after 24 hrs of incubation at 37^0C. Sterile distilled water was used as control. Plates were maintained in triplicates.

Seed treatment and raising of seedlings

Tomato seeds (S-22) were soaked in 10% aqueous extracts of different vermicomposts (T1-Seeds soaked in water+no amendment to soil; T2-seeds soaked in water+FYM amendment to soil; T3-seeds soaked in water+soil amendment with vermicomposted neem leaves; T4- seeds soaked in aqueous extract of vermicomposted neem leaves; T5-seeds soaked in aqueous extract of vermicomposted assorted leaf litter + soil amendment with vermicomposted neem leaves; T6- seeds soaked in aqueous extract of lantana flowers+ soil amendment with vermicomposted neem leaves and T7- seeds soaked in aqueous extract of Parthenium leaves+ soil amendment with vermicomposted neem leaves) for experimental plots and they were soaked in water for control plots for one hrs before sowing in the nursery beds. The seedlings were raised in separate nursery beds of size 1 x 1 m to which vermicompost was applied @ rate of 5 tons ha^{-1}.

Microplot studies

Microplots each of size 2 x 1.5 m were prepared in triplicates using completely randomized block design. Pathogen inoculum (60 mL) was inoculated into each plot at the rate of 1×10^8 CFU mL-1 five days before sowing the seeds. The presence of pathogen was confirmed in the field on fourth day (*X. campestris* –34 x 10^3 CFU/g). From the nursery beds seedlings were transplanted to plots and each plant received 250 g of vermicompost (spot application) in two split doses - 50% at the time of transplantation and 50% after one month. Micro plots without any amendment was used as control and the application of farm yard manure served as an additional control. Each microplot had a density of 50 plants. During crop growth, plants were monitored for survival rate and number of infected plants. The fruit yield per plot was recorded for each treatment.

Statistical analysis

Possible treatment differences were explored by the analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) by running MSTAT-C and Microsoft Excel software.

RESULTS AND DISCUSSION

Among the different bioagents tested the aqueous extract of vermicomposted neem showed maximum inhibitory effect ($P<0.05$) on *X. campestris* (25.33 ± 1.27) than that of vermicomposted assorted leaf litter (21.66 ± 0.48), vermicomposted *Lantana* (10.50 ± 1.32) and vermicomposted *Parthenium* (9.50 ± 2.15). The vermicomposted neem which was very effective in inhibiting the pathogen in the *in vitro* studies was used for soil application in the field. The survival of tomato plants treated with different bioagents is shown in Figure 1. The survival was 99% in the treatment T_4 followed by 95 % in the treatments T_5 and T_7. Treatments T_6 and T_3 had comparatively better survival as against 70 % and 58 in control treatments T_2 and T_1 respectively.

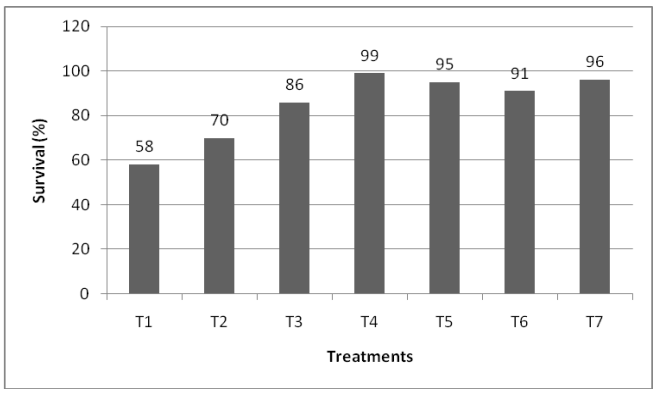

Figure 1. Influence of bioagents on the survival of tomato plants raised in *Xanthomonas infected* soil

The yield of tomato fruits per plot from three harvests was recorded. Yield was higher in second harvest in all the treatments and it reduced during the third harvest. The duration between each harvest was 10 days. Among all the treatments, treatment T_4 (15.4±7.63 t ha^{-1}) registered the highest yield followed by treatments T_7 (14.46±6.01 t ha^{-1}), T_6 (14.41±2.91 t ha^{-1}), T_5 (13.32±6.24 t ha^{-1}) and T_3 (5.46±4.41 t ha^{-1}). Treatments T_1 (0.62±1.20 t ha^{-1}) and T_2 (2.33±0.88 t ha^{-1}) recovered very low yield. The yield in treatment T_3 where seeds were soaked in water before transplanting to plots amended with vermicomposted neem leaves was only 136.66 kg/plot indicating that application of vermicompost alone is not enough to protect the plants against the disease but coupling it with seed treatment is important to achieve complete disease suppression and to get better yield.

In the present study neem based vermicompost has shown best results in suppressing the bacterial spot. It is evident from the several reports that vermicompost extracts are effective antimicrobial agents against soil-borne pathogens (Szczech *et al*., 1993; Orlikowski, 1999; Rodriguez *et al*., 2000; Szczech and Smolinska, 2001; Edwards and Arancon, 2004; Zaller, 2006) and do not produce any residual effects. These bioagents are non-polluting, cost effective, non-hazardous and can be prepared with available materials in the field. It does not disturb soil ecological balance. Vermicomposting of materials such as *Lantana* (Kanwar 2008; Deeksha *et al*., 2009) and *Parthenium* (Biradar and Patil, 2001; Channappagoudar *et al.*, 2007; Vijayakumari and Yadav, 2008) for pathogen management can also help in better weed management. Antimicrobial property of neem and its extracts has been reported by Bhatnagar and McCormick (1988), Agbenin and Marley (2006) and Perumal *et al.* (2008).

It can be concluded that soil application of vermicompost coupled with seed treatment with 10% aqueous extract of vermicomposted neem for one hrs can significantly reduce the incidence of bacterial spot caused by *X. campestris* in tomato. The results also show that the organic matter composition used in the preparation of the vermicompost has a role to play in assessing the performance of the vermicompost in acting against the prevalent pathogens at the site of application.

REFERENCES

Agbenin, O. N. and Marley, P. S. 2006. *In-vitro* assay of some plant extracts against *Fusarium oxysporum f. sp. lycopersici* causal agent of tomato wilt. *Journal of Plant Protection Research*, **46**(3): 215-220.

Bauer, A.W, Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, **45**:493-496.

Bhatnaagar, D. and Mc Cormick, S. P. 1988. The inhibitory effect of neem (*Azadirachta indica*) leaf extracts on aflatoxin synthesis in

Aspergillus parasiticus. *Journal of the American Oil and Chemical Society*, **65**: 1166-1168.

Biradar, A.P. and Patil, M.B. 2001. Studies on utilization of prominent weeds for vermiculturing. *Indian journal of Weed Science*, **33**(3/4): 229-230.

Channappagoudar, B. B., Biradar, N. R., Patil, J. B. and Gasimani, C. A. A. 2007. Utilization of weed biomass as an organic source in sorghum. *Karnataka Journal of Agricultural Sciences*, **20**(2): 245-248.

Deeksha Joshi, Hooda, K. S., Bhatt, J. C., Mina, B. L. and Gupta, H. S. 2009. Suppressive effects of composts on soil-borne and foliar diseases of French bean in the field in the western Indian Himalayas. *Crop Protection*, **28**: 608-615.

Edwards, C. A. and Arancon, N. 2004. Vermicompost supress plant pests and disease Attacks. In: *REDNOVA NEWS*: http://www.rednova.com/display/?id =55938.

Holder, I. A. and Boyce, S. T. 1994. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. *Burns*, **20**: 426–429.

Kallo, G. 1991. Genetic Improvement of Tomato, Springer Verlag Berlin Heidelberg, Germany, 99 **P**.

Kanwar, K. 2008. Recycling of organic wastes by vermicompositing and its utilization in agriculture in Himachal pradesh**.** *ENVIS Bulletin*. **10**(2): Himalayan Ecology.

Mao W., Lewis, J. A., Lumsden, R. D. and Hebbar, K. P. 1998. Biocontrol of selected soil borne diseases of tomato and pepper plants. *Crop Protection*, **17**(6): 535-542.

Orlikowski, L. B. 1999. Vermicompost extract in the control of some soil borne pathogens. In: *International Symposium on Crop Protection.* **64**: 405-410.

Perumal, G., Saravanan, G., Ragupathi, T. and Muthusami, S. 2008. Antimicrobial activity of selected plant extracts against plant pathogens. *Asian Journal of Bio Science*, **3**(1): 130-132.

Rodríguez, J. A., Zavaleta, E., Sanchez, P. and Gonzalez, H. 2000. The effect of vermin-compost on plant nutrition, yield and incidence of root and crown rot of *Gerbera* (*Gerbera jamesonii* H Bolus). *Fitopathologia*, **35**: 66-79.

Sharma, R. C. and Sharma, J. N. 2005. Challenging problems in horticulture and Forest pathology, Indus publishing Company, New Delhi.

Szczech, M. and Smolinska, U. 2001. Comparison of suppressiveness of vermicomposts produced from animal manures sewage sludge against *Phytophthora* var. *nicotianae. Phytopath*ology, **149**(2): 77-82.

Szczech, M., Rondomanski, W., Brzeski, M.W., Smolinska, U. and Kotowski, J. F. 1993. Suppressive effect of commercial earthworm compost on some root infecting pathogens of cabbage and tomato. *Biological Agriculture and Horticulture*, **10**(1): 47-52

Vijayakumari, B. and Yadav, R. H. 2008. Comparison of fresh, composted, vermicomposted *Parthenium hysterophorus* and poultry manure. In: *Biodiversity conservation* (Binoj Kumar, M. S. and Gopalakrishnan, P. K. eds.), Scientific publishers, Jodhpur, India, 123-127 **PP**.

Zaller, J. G. 2006. Foliar spraying of vermicompost extracts: Effects on fruit quality and indications of late-blight suppression of field-grown tomatoes. *Biological Agriculture and Horticulture*, **24:** 165-180.

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