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IN-VITRO CONTROL OF FUSARIUM OXYSPORUM BY ASPERGILLUS SP AND TRICHODERMA SP ISOLATED FROM VERMICOMPOST

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ABSTRACT

The effectiveness of agricultural residue derived vermicompost in providing protection against various plant diseases, especially against soil -borne plant pathogens has been studied extensively. In the previous studies effective control of soil-borne plant pathogen infections was observed on application of vermicompost. Most of the research is focused on elucidating the mechanism of soil – borne pathogen suppression and the potential types of interactions between micro-flora of vermicompost and the pathogens. The current study was aimed at assessing the potential for suppression of *Fusarium oxysporum* (causative agent of Fusarial wilt of common vegetable crops) by *Trichoderma* and *Aspergillus* sp. isolated from Vermicompost. Mycelial disc (5 mm diameter) of F.oxysporum was placed at one edge of Petri plate containing PDA and incubated at 270C for four days. Forty eight hours later, mycelial discs (5 mm in diameter) of *Trichoderma* isolate was placed on the opposite side facing *F.oxysporum* in the same Petri plate and incubated. The results showed that *Aspergillus* sp. and *Trichoderma* sp. effectively suppressed *F.oxysporum*. This indicates that the fungal isolates from vermicompost have antagonistic effect against the plant pathogen. This study gives substantial evidence for the suppressive nature of vermicompost, which has the potential to replace the currently used fungicides in agriculture.

Keywords: Antagonism, Vermicompost, *Fusarium oxysporum*, *Aspergillus*, *Trichoderma*, Suppression.

Number of Tables : 2	**Number of Figures :2**	**Number of References : 15**

INTRODUCTION

The excessive use of chemical fertilizers and pesticides causes environmental hazards which greatly affect the human health and environment. The quantum of organic waste generation from domestic and agriculture sectors has increased annually and poses disposal problems (Reinecke *et al*., 1992; Gupta, 2005; Garg *et al*., 2006). On the other side chemicals being used are persistent in environment. All these have led to the need for alternative substances which are not hazardous to surroundings. It is well established that earthworms have beneficial effects on soil and soil fertility (Edwards, 1985). These effects include biological and chemical effects on soil organic matter degradation (Edwards and Bohlen, 1996, and Edwards, 1998). Plant diseases especially soil-borne and seed infecting pathogens are serious issue in both green houses and field production of many horticultural crops. Organic farmers and ecologically minded agricultural scientists have long recognized the importance of using vermicompost to increase soil fertility and many growers today use vermicompost not only to increase nutrients but also to suppress soil-borne plant diseases. 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 tomato bacterial spot disease caused by *Xanthomonas compestris*. 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* (Reddy *et al.*, 2012). Many studies have demonstrated the effectiveness of vermicompost in providing protection against various plant diseases (Chaoui *et al*., 2002; Arancon *et al*., 2002). Various studies have demonstrated the effectiveness of vermicompost in providing protection against various plant diseases. In vermin composting the active component involved in the biodegradation and conversion process during composting is the resident microbial community, among which fungi play a very important role (Sparling *et al.,* 1982; Wiegant 1992). The microbial population present in vermicompost has a major role in decreasing the disease severity.

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 (Reddy *et al.,*, 2012). The protective effect increased in proportion to the rate of application of vermicompost, vermicompost lost its activity after heating, sterilized extract of vermicompost added to potato dextrose agar stimulated the growth of *F.oxysporum.* This result indicated that microbial population that was present in vermicompost played an important role in decreasing the soil borne diseases in plants (Szczech, 1988).

MATERIALS AND METHODS

Vermicompost used for the present study was collected from the vermicompost units of Mount Carmel College, Bangalore, India. The substrate used for composting mainly comprised of assorted leaf litter from college garden. Serial dilution technique followed by spread plate technique was carried out for isolation of fungi from air dried vermicompost. Dry vermicompost (0.1 g) was taken and serially diluted (upto 10^{-6} dilutions) and plated on Potato Dextrose agar. Plates were incubated for 5 days at 27^{o}C.Based on the microscopic observation and conidial structure through wet mount method using lacto phenol cotton blue. The predominant fungal colonies were identified as *Aspergillus* sp. and *Trichoderma sp.*

The above two fungal isolates were sub cultured and maintained in slants. The pathogen (*Fusarium oxysporum*) used in current study was isolated from the infected tomato fruit collected from the field and confirmed by the microscopic observation and conidial structure through wet mount method using lacto phenol cotton blue and it was confirmed by colony color and morphology structure. Pure broth cultures of *F.oxysporum* were prepared for further studies. Whatman no.1 filter paper (5mm diameter) discs were prepared and sterilized. The sterile discs were placed on solidified PDA plates and *F.oxysporum* broth was swabbed uniformly throughout the plate in a sterile condition and incubated it for five days. The pre-grown mycelia disc on Whatman no.1 filter paper was kept ready for the dual cultural technique. The above technique was repeated for *Aspergillus* sp. and *Trichoderma sp.* and the plates were kept ready for dual culture technique.

DUAL CULTURE TECHNIQUE

The antagonism between the fungal isolates from vermicompost (*Aspergillus* sp. and *Trichoderma sp)* against the pathogen (*Fusarium oxysporum*) was studied by dual culture technique. In a sterile condition mycelial disk (5mm diameter) of *F.oxysporum* was placed on right edge of Petri plate containing PDA and mycelial disk (5mm diameter) of *Trichoderma* was placed on left edge of the same Petri plate and the plates were incubated at 27˚C for two weeks. The same technique was applied for *Aspergillus* sp verses *F.oxysporum* sp .The growth of the organisms were monitored over a period of twelve days and measured the growth of both the cultures in terms of centimeters.

RESULTS AND DISCUSSIONS

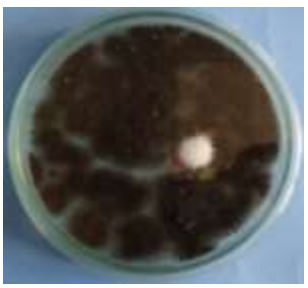

Fig:1

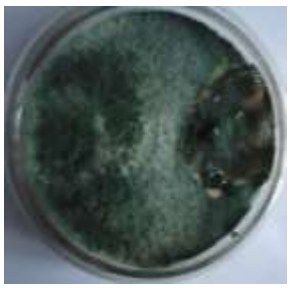

Fig:2

Figure 1 represents the s uppression of *F.oxysporum* by *Aspergillus* sp on 10th day of incubation

Figure 2 represents the suppression of *F.oxysporum* by *Trichoderma* sp on 10th day of incubation.The antagonistic potential of *Trichoderma* and *Aspergillus* spp isolated from vermicompost was assessed against a common

plant pathogen, *F.oxysporum*.

Table 1: *Antagonism between Trichoderma sp and F. oxysporum sp*

Days of Incubation	Species	Plates	Control plates	Inhibition (%)
3rd day	*Trichoderma*	3.2 ± 0.1	3.5 ± 0.1	8.6
3rd day	*F.oxysporum*	2.0 ± 0.1	3.1 ± 0.1	35
4th day	*Trichoderma*	4.2 ± 0.1	4.8 ± 0.1	12.5
4th day	*F.oxysporum*	3.0 ± 0.05	4.2 ± 0.1	28.5
5th day	*Trichoderma*	5.1 ± 0.1	6.0 ± 0.1	15
5th day	*F.oxysporum*	3.2 ± 0.1	4.6 ± 0.1	30.4
6th day	*Trichoderma*	5.6 ± 0.1	7.3 ± 0.2	23
6th day	*F.oxysporum*	3.1	5.4	45

Table 2: *Antagonism between Aspergillus sp and F.oxysporum*

Days of Incubation	Species	Plates	Control plates	Inhibition (%)
3rd day	*Aspergillus*	2.9 ± 0.05	3.0 ± 0.3	23.3
3rd day	*F.oxysporum*	2.0 ± 0.05	3.1 ± 0.1	35.4
4th day	*Aspergillus*	4.0 ± 0.1	4.6 ± 0.1	13.0
4th day	*F.oxysporum*	3.0 ± 0.05	4.2 ± 0.1	28.5
5th day	*Aspergillus*	4.9 ± 0.05	5.8 ± 0.05	15.5
5th day	*F.oxysporum*	3.2 ± 0.05	4.6 ± 0.1	30.4
6th day	*Aspergillus*	5.5 ± 0.05	7.3 ± 0.1	24.6
6th day	*F.oxysporum*	3.1 ± 0.1	5.4 ± 0.1	42.5

It is clear from the results that are summarized in Table: 1 that the plant pathogen was found to be significantly inhibited by *Trichoderma* sp in dual culture technique. With increase in incubation time increase in suppression rate of pathogen was observed, on sixth day 45% suppression of pathogen was recorded. On 10th day of incubation 90% of suppression was noticed. By 12th day *Trichoderma* had grown over *F.oxysporum* leading to complete suppression. The antagonistic potential of *Aspergillus* sp summarized in table- 2 clearly indicates that the plant pathogen was found to be significantly inhibited when it was grown along with *Aspergillus* sp in dual culture technique. As the days of incubation increased the suppression rate also increased, on sixth day plant pathogen showed 42.5% suppression. It was also noticed that *Trichoderma* sp was proved to be a stronger antagonist compared to *Aspergillus* sp as it showed complete suppression on 12th day of incubation. As these organisms were isolated from vermicompost, the study serves as an indication to show the mechanism of pathogen suppression on application of vermicompost to fields. Such a situation of pathogen inhibition on application of vermicompost has been reported earlier (Huber and Schneider 1982; Sparling *et al.*, 1982; Wiegant 1992; Chen and nelson, 2008; Bonanomi *et al.*, 2010). The actual mechanisms associated with suppression of *F.oxysporum* by these two fungal species need further investigations.

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REFERENCES

Arancon, N.Q., Edwards, C.A., and Lee, S. Management of plant parasitic nematode populations by use of vermicomposts. *Proc.Brighton Crop Prot. Conf. – Pests and Diseases*. 8B-2: 705-716, 2002.

Bonanomi, G., Antignani, V., Capodilupo, M. and Scala, F. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. *Soil Biol. Bioch***,** 42(2): 136-144, 2010.

Chaoui, H., Edwards, C. A., Brickner, A., Lee, S., Arancon, N.Q. Suppression of the plant parasitic diseases: *Pythium* (damping off), *Rhizoctonia* (root rot) and *Verticillium* (wilt) by vermicompost. *Proc. Brighton Crop Prot. Conf. – Pests and Diseases*, 8B-3: 711-716, 2002.

Chen, M.H. and Nelson, E. B. Seed-colonizing microbes from municipal biosolids compost suppress *Pythium ultimum* damping-off on different plant species. *Phytopathology*, 98(9):1012-8, 2008.

Edwards, C. A. and Bohlen, P. J. *Biology and ecology of earthworm*. (3rd edn.), Chapman and Hall, London. 426 pp, 1996.

Edwards, C.A. The Commercial and Environmental Potential of Vermicomposting**.** *Waste Handling Equipment***,** June 1998. Section A, 16-18, 1998**.**

Edwards, C.A. The use of earthworms for management of organic wastes. In: International Symposium on Earthworms, Bologna-Carpi, Italy, 31 March–5 April 1985, 1985.

Garg, P., Gupta, A., Satya, S. Vermicomposting of different types of waste using *Eisenia foetida*: A comparative study. *Bioresource Tech*., 97, 391-395, 2006.

Gupta, P.K. Vermicomposting for sustainable agriculture. Bharat Printing Press, Jodhpur, pp: 11-14, 2005.

Huber, D. M. and Schneider, R. W. The description and occurrence of suppressive soils. Suppressive Soils and Plant Disease. R. W. Schneider. St. Paul, M.N., *The American Phytopathological Society***:** 1-9, 1982.

Reinecke, A.J., S.A. Viljioen and R.J. Saayman, The suitability of *Eudrilus eugeniae, Perionyx excavaus & E. foetida* (Oligochaeta) for vermicomposting in southern Africa in term of their temperature requirements. *Soil Biol. Biochem*, 24: 1295-1307, 1992.

Shobha Ananda Reddy, D. J. Bagyaraj and Radha D. Kale., Management of tomato bacterial spot caused by *Xanthomonas campestris* using vermicompost, *J Biopest.*, 5(1): 10-13, 2012.

Sparling, G.P., T.R. Fermor and D.A. Wood. Measurement of the microbial biomass in composted wheat straw and the possible contribution of the biomass to the nutrition of *Agaricus bisporus. Soil Biol. Biochem.*, 14: 609-611, 1982.

Szczech, M. M. Suppressiveness of Vermicompost against *Fusarium* Wilt of Tomato, *J Phytopath.*, 147 (3):155-161, 1988,

Wiegant, W.M. A simple method to estimate the biomass of thermophilic fungi in composts. *Biotech Tech*, 5: 421–426, 1992.