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RESEARCH ARTICLE

BIOLOGICAL SUPPRESSION OF *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* (SACC.) SYNDER AND HANS USING ANTAGONISTIC FUNGUS *TRICHODERMA HARZIANUM* (RIFAI) IN TOMATO (*LYCOPERSICON ESCULENTUM* (MILLER))

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ABSTRACT

Trichoderma harzianum (Rifai) isolate was collected from the Rhizosphere soil of Tomato field and tested for their antagonistic activity against *Fusarium oxysporum f.* sp. *lycopersici* ((SACC.) Synder and Hans) causing *Fusarium* wilt of Tomato. The antagonistic fungus was very effective in inhibiting the mycelial growth of the pathogen in Dual culture. The antagonistic fungus produced lytic enzyme (Chitinase) which was found to inhibit the growth of the pathogen *in vitro*. In green house experiments, the antagonist *Trichoderma harzianum* was effective in the suppression of the pathogen *Fusarium* causing wilt in Tomato plants. Seed treatment with *Trichoderma harzianum* broth recorded an increase in the seed vigour index by 69.02 % over the control also promoting the Plant Growth (PGPF).

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum f.* sp. *lycopersici* in Tomato which is both soil borne and seed borne is a major factor which has lead to considerable yield loss. The most common symptom noticed due to Tomato *Fusarium* wilt is the yellowing and drooping of the lower (or) older leaves. This symptom often occurs on one side of the plants (or) on one shoot. Subsequently successive leaves become yellow and the plant wilts and dies often before it reaches maturity. If the main stem is cut dark brown streaks may be seen running lengthwise through the stem. As the disease progresses growth is stunted and little (or) no fruits develop. The most common method of managing the disease is seed treatment with Fungicide

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Trichodema harzianum has been found as effective Bio control agents against *Fusarium* wilt (Rattink, 1993; Hartman and Fletcher 1991; Lemanceau an Alabouvette, 1993). Chitinase as a lytic enzyme produced by *Trichoderma harzianum* may also be involved in the complete degradation of mycelial walls of phytopathogenic fungi (Cherif and Benhamou, 1990). *Trichoderma harzianum* inhibits the growth of the pathogen *Fusarium oxysporum f. sp. lycopersici* (Santander *et al.,* 2003). The present study reports the efficacy of the antagonistic fungi *Trichoderma harzianum* in the biological suppression of *Fusarium* wilt of tomato as well as the fact that the antagonist is the good plant growth promoting fungus.

MATERIALS AND METHODS

Isolation of Organism

The pathogen *Fusarium oxysporum f.*sp *lycopersici* was isolated from infected tomato plant (PKM-I variety). The

antagonistic fungus *Trichoderma harzianum* was isolated in the soil collected from the tomato field and the isolates were maintained on Potato Dextrose Agar (PDA) slants at 4°c.

Colony forming units in different media

Mycoparasitic fungi *Trichoderma harzianum* was inoculated in sterilized Potato Dextrose broth, Rose Bengal broth and sabourauds dextrose broth and incubated for 72 h. Then it was plated using *Trichoderma* specific medium (Elad and Chet, 1980).

Dual culture Technique

The efficacy of the antagonist was tested by Dual culture technique (Dennis, Webster, 1971) using PDA medium. Cork borer (Five mm diameter) was used to place the mycelial discs of pathogen and fungal antagonist in the opposite sides at the periphery of the petriplate. The plates were incubated at 28 ± 2ºC. The linear growth of the antagonist and pathogen was measured at 24, 48, 72 and 96 h incubation.

Effect of Antagonist on the growth and vigour of Tomato seedlings

Roll towel method (ISTA, 1976) was used to study the effect of the antagonist on growth and vigour of Tomato seedlings. *Trichoderma harzianum* broth containing more than 3 x 10^6 CFU/ ml was used to treat the seeds of PKM-I Tomato seeds (15 seeds per towel). There were 2 treatments including control with three replication per treatment. Roll towels with treated seeds were incubated at 25 ± 2ºC relative humidity in the germination room. The germinated seeds were observed after 14 days. Observations were recorded on shoot length, Root lengths and Germination percentage. Vigour index of tomato seeds were calculated following by the procedure suggested by Abdul-Baki and Andersen (1973).

Induction of Extracellular chitinolytic enzymes in *Trichoderma* sp.

To study the induction pattern of chitinase by *Trichoderma harzianum*, glucose in the enzyme production medium (Harman *et al*., 1993) was subcultured with the cell walls of the test pathogen prepared form Sabourauds Dextrose broth, as the carbon source (2 g/ lit.). two mycelial discs (9 mm diameters) from 4 days old culture of *Trichoderma harzianum* were inoculated into enzyme production medium and incubated at 28 ± 2º C for five days. Then the culture was pooled and filtered through whatman no 1 filter paper and the filterate was precipitated with 80% ammonium sulphate for overnight incubation at 4°c. This was centrifuged at 5000 rpm for 30 min. The pellet was resuspended in minimal amount of phosphate buffer (pH 7.0). The dialysate was lyophilized to concentrate the protein and was used for SDS-PAGE.

Antifungal assay of purified chitinase

The assay was carried out in 9cm diameter petriplates containing 15ml of PDA medium. For assay, three sterile filter papers discs of 0.6mm diameter were placed radically in the petridish (3cm away from the center). A mycelial disc of *Fusarium oxysporum* f. sp. *lycopersici* was placed at the center. The plates were incubated at room temperature until the mycelial growth from the central disc had reached nearer to the filter paper disc. Purified chitinase at different concentrations was added on to the paper discs. A suitable control with buffer alone was also maintained. This was done to determine the lowest inhibitory concentrations. Inhibition zone was visualized after two days.

Efficacy of *Trichoderma harzianum* against Fusarium wilt of Tomato under green house conditions

Potting soil (red soil: sand: decomposed cow dung at 1:1:1 by w:w:w. Available NPK, Calcium, Magnesium of red soil where 160, 18, 280, 0.28 and 0.19kg/ hectare respectively and pH and 7.1) was autoclave sterilized for 1 hour on 2 consecutive days and was placed in earthen pots. Antagonist treated seeds of Tomato were sown thickly. Carbendazim as seed treatment at 2gm/kg of seeds and after transplanting 2gm pot as soil application was included for comparison (T_5) 30 day old treated seedling were transplanted (3 seedlings per pots) in earthen pots (Size 0.35 meter diameter, 3.5. meter height) filled with potting soil (Volume of Soil 0.04m^3). Four other treatments were carried out 10 days after transplanting soil application with 10ml of *Trichoderma harzianum* suspension was done. One day after the soil application of the antagonist, 50ml of conidial suspension 10^3 microconidia per ml was inoculated per pot.

Treatments are as follows

T1-Pathogen +*Trichoderma harzianum* Seed treatment +*Trichoderma harzianum* Soil Application
T_2- Pathogen + *Trichoderma harzianum* Seed treatment + *Trichoderma harzianum* Soil application+Foliar spray after 20 days. (DAT- Date after Transplanting)
T_3 – Pathogen + *Trichoderma harzianum* seed treatment + *Trichoderma harzianum* Soil Application+Foliar spray after 40 days (DAT)
T_4 – Pathogen + untreated Seedlings (Control)
T_5- Pathogen + Bavistin Seed treatment + Bavistin Soil drenching (Comparison)
Wilt incidence was recorded 50 days after DAT using the formula.

$$\text{Percent disease incidence (PDI)} = \frac{\text{No. of infected plant}}{\text{Total No. of Plant}} \times 100$$

There were three replications for each treatment. Pots were arranged in randomized manner.

RESULTS AND DISCUSSION

Trichoderma harizanum was isolated in the soil obtained from the tomato field at 10^{-6} dilution. Conidiophores were found to erect and produce side branches bearing whorls of short phialides. Phialides were densely clustered. Branches bore terminal conidial heads. Individual conidia were globose whose size range from 25-70 µm in diameter. Colonies grew quickly producing white, mycelia which turned into green cushions of sporulating filaments. After the incubation period of five days,

Fusarium oxysparum f. sp. *lycopersici* colonies were observed as pinkish white coloured and round shaped in the individual plates and successive colonial growth of the fungus was also in evidence. The pathogen was observed under the microscope and was found to be sickle shaped macroconidia and ovoid microconidia. The maximum colony forming units of *Trichoderma harzianum* was found to be Potato Dextrose followed by Sabourauds Dextrose and the least CFU'S were observed in Rose Bengal broths. Results of Dual plate technique showed that the maximum growth of six cm of antimycotic fungus *Trichoderma harzianum* was observed after three days of incubation in the petriplate and the minimum growth of three cm of the disease causing pathogen *Fusarium oxysperum* f.sp. *lycopersici* was observed. Further it could be seen from the result that the *Fusarium* growth was vehemently arrested by the beneficial fungus *Trichoderma harzianum* there by inhibiting the growth of *Fusarium oxysporum* f.sp. *lycopersici* by 64.70 % on the sixth day. On the seventh day *Trichoderma harzianum* over grew the pathogen. Hence Biological Suppression of root wilt disease in Tomato is feasible by *Trichoderma harzianum* as Biocontrol agent. From this it is obvious that the growth of the fungus was very much suppressed by the saprophytic fungus *Trichoderma harzianum* which play a vital role to contain the infection in the root zone of the tomato plant. Seeds of Tomato (PKM-1 variety) treated with *Trichoderma harzianum* at concentration 3x106 CFU/ml has shown an increase in seed vigour index of 69.02% over the untreated seeds.

The extracellular chitinolytic enzymes were separated by using SDS – PAGE. Results showed that the chitinase enzyme produced by the *Trichoderma harzianum* has the molecular weight of 42KDa. The bands were separated in the gel and its molecular weight was estimated by using marker loaded in the Lane – I. The marker carbonic anhydrase has the molecular weight of upto 96kda. The chitinase enzyme produced by *Trichoderma harzianum* is responsible for the control of most disease causing pathogens. Purified chitinase of *Trichoderma harzianum* is effective enough to control the radial growth of the fungus *Fusarium oxysporum f.sp. lycopersici* at concentration of 40 µl. Since inhibition zone is more at A than B, where the concentration was 20 µl. It can also be seen that there is over growth of the pathogen over control were buffer alone was used. From the results of the pot culture studies conducted at the premises of BCL, PKKVK, Puducherry on the Biological Suppression of *Fusarium* wilt in tomato by the antimycotic fungus *Trichoderma harzianum* it is observed that the treatment T5 is significantly superior to all other treatment as it has recorded the minimum PDI at 18.63. Treatment T2 is significantly superior to treatment T1, T3 and T4 with PDI of 20. 83. T3 is significantly by superior to T1 with a PDI of 22.8. Though T2 is not superior as T5 which is chemical control, Continuous use of the Biopesticide *Trichoderma harzianum* against *Fusarium* wilt of tomato it will be longer lasting and stable than T5 because though chemical control may be spectacular due to immediate control of the pathogen it also results in, Resistance of pathogen to the chemical, Residual effect of the chemical in the fruit, Pollution of soil and environment, Chemical may also kill beneficial organism.

T2 is superior to T1 and T3 because *Trichoderma harzianum* not only controls spread of the disease through soil and seed but foliar spray of *Trichoderma harzianum* also induces systemic resistance to the tomato plant at the earliest. This implies that biopesticides should be used as seed treatment, main field application and also foliar spray at the earliest on the crop.

Conclusion

Above the findings, it is clear that Mycofungicide formulation using *Trichoderma harzianum* as the biocontrol agent is found to the effective against *Fusarium oxysporum f.sp.lycopersici* in Tomato

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