

Inhibition of Pectinolytic and Cellulolytic Enzymes of *Fusarium oxysporum* by *Trichoderma* spp and *Pseudomonas fluorescens* on *Arachishypogaea*.L

P. Rajeswari

Department of Biochemistry and Molecular Biology,
Pondicherry University, Puducherry -605014, India
Email: aksharaasmitha@gmail.com

Abstract – The present study examined the effects of culture filtrates of *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* on *Fusarium oxysporum* infecting *Arachis hypogaea*. *Fusarium* wilt diseases caused by the fungus *Fusarium oxysporum* lead to significant yield losses of crops. Experiments were conducted on the effect of culture filtrates of *T.viride* (1%), *T.harzianum* (1.5%), and *P. fluorescens* (2%) on Pectinolytic and Cellulolytic enzymes produced by *Fusariumoxysporum* on *Arachishypogaea*. The activity of Poly Methyl Esterase (PME), endo polymethylgalacturonase (PMG), and exo polymethylgalacturonase (PMG), produced by *Fusarium oxysporum* in the leaves of infected *Arachis hypogaea* was higher, when compared to control plants. Maximum inhibition of above pectinolytic enzymes (PME, endo and, exo PMG) in the plants sprayed with *T. viride* was followed by *T.harzianum*-sprayed plants and *P.fluorescens*- sprayed plants. The cellulolytic enzyme activity 1,4 β endo, -glucanase, 1,4 β exo-glucanase, and the cellobiase enzyme activity in the leaves of infected *Arachis hypogaea* was higher compared to that of control plants. Activity of cellulolytic enzymes (1,4 β endo, -glucanase, 1,4 β exo-glucanase, cellobiase) was inhibited significantly by *T.viride*-sprayed plants followed by *T.harzianum*-sprayed plants and *P.fluorescens*-sprayed plants. Of all the treatments, *T. viride* treatment showed higher rate of inhibition of Pectinolytic and Cellulolytic enzymes followed by that of *T.harzianum* and *P.fluorescens*. This present study indicates that culture filtrate of *T.viride*(1%) is the best biocontrol agent in the inhibition of *Fusarium oxysporum* causing *Fusarium* wilt of *Arachis hypogaea* .L

Keywords – *Fusarium oxysporum*, Polymethylgalacturonase, 1,4- β -Glucanase, Cellobiase, Poly Methyl Esterase, *Arachis hypogaea*. L.

I. INTRODUCTION

Arachis hypogaea L. (Groundnut) is one of the important crops all over the world. Especially in India, it is one of the major oil seed crops. *Fusarium oxysporum*, the soil borne pathogen causes vascular wilt diseases in a wide variety of economically important crops [1]. *Fusarium oxysporum* produces several enzymes that act upon the pectic and cellulose components of cell walls of host plant [2]. Pectinases catalyse the degradation of pectic polysaccharides, the main component of the middle lamella, i.e., the intercellular cement that holds in place the cells of plant tissues [3]. Pectic enzymes consist primarily of pectin methylesterase, polygalacturonase and pectate lyase [4], [5]. The capacity to produce pectin methylesterase (PME) is a general feature among the

vascular wilt. *Fusarium* species [6]. Polygalacturonases (PGs) are important pectinolytic enzymes produced by phytopathogenic fungi during the process of infection and colonization of host plants [7]. PG is a highly polymorphic enzyme and exhibits either an endo- or exo-mode of action [8]. The role of endo- PGs in pathogenesis is tissue maceration and cell death [9] and the generation of oligogalacturonides that could act as elicitors of plant defence responses. Exo- PGs are responsible for the release of soluble low molecular weight oligogalacturonides from highly polymeric substrates which can enter into the cell where they are catabolised and act as inducers of other pectic enzymes [11]. Cellulase enzymes, designed C1, C2 and Cx are required to degrade cellulose. The C1 and C2 enzymes act upon native, insoluble cellulose to produce linear chains that are attacked by the Cx enzyme to produce cellobiose and glucose [12]. Najar, Anwar, Masoodi, Lubna. and Khar studied the antagonistic activity of *Trichoderma viride*, *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Fusarium solani* f.sp. *melongenae* causing wilt of Brinjal [13]. It has been inferred from the earlier studies that *Trichoderma* spp. inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis and competition [14], [15].

The strains of *Trichoderma* spp. produce many types of secondary metabolites [16] including antibiotics [17] and cell wall degrading enzymes [18], [19] the role of which has been clearly established in biocontrol activity [20]. Mohandas studied the effect of precolonization of banana roots with *Pseudomonas fluorescens* on infection with *Fusarium oxysporum* f. sp. *cubense*. The biocontrol agents *Trichoderma harzianum* and *Pseudomonas fluorescens* are important rhizosphere microorganisms and are efficient in suppressing root and foliar diseases of several plant species [21]. Thus, the present study was carried out to determine the inhibition of pectinolytic and cellulolytic enzymes *F. oxysporum* on *Arachis hypogaea* plants with the application of *Trichoderma* spp and *Pseudomonas fluorescens*.

II. MATERIALS AND METHODS

Trichoderma viride, *Trichoderma harzianum* and *Pseudomonas fluorescens* were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India and were used for the present study. The pathogen *Fusarium oxysporum* was obtained from the infected leaves of

Arachis hypogaea and was obtained from the infected leaves of *Arachis hypogaea* and was purified by single conidium isolation method. The purified culture was stored in the slants of PSA.

Fusarium oxysporum was grown on PSA for 30 days and further grown in Czapek's medium for 7 days and filtrate was taken. *Trichoderma viride* and *Trichoderma harzianum* were grown on Malt Extract agar and *Pseudomonas fluorescens* on ABM Medium and further grown on Czapek's medium in conical flask. It was further centrifuged and culture filtrate was taken.

Four plants of *A. hypogaea* raised from the seeds (JLR – variety) were grown in each of six earthen pots (25cm diameter) upto 75 DAS (Day After Sowing) and grouped into three sets. Control-first set of two pots was sprayed with distilled water on 30 DAS and left without any treatment. Infected- second set of two pots was sprayed with culture of pathogen, *Fusarium oxysporum* on 30 DAS and left without any treatment. Infected-treated - third set was sprayed with pathogen on 30 DAS. These infected plants were sprayed with OIC (Optimum Inhibitory Concentration) of culture filtrates of antagonistic microorganisms, *Trichoderma viride* (1%), *Trichoderma harzianum* (1.5%) and *Pseudomonas fluorescens* (2%) on 40 DAS. On 50 DAS, the leaves of control, infected and infected treated plants were collected for the estimation of Pectinolytic and Cellulytic enzymes produced by *F. oxysporum* on *Arachis hypogaea*.

For pectinolytic enzyme production the pathogens were grown in Czapek's broth, supplemented with pectin as carbon source replacing sucrose. Similarly for cellulolytic enzymes microcrystalline cellulose and carboxy methyl cellulose were used. To 50ml sterilized Czapek's liquid media in a 250ml Erlenmeyer conical flask, the culture filtrate of *T. viride*, *T. harzianum* and *P. fluorescens* in their OIC were amended to the media separately. The two discs of 9 mm were cut from the growing tip of the 7 days old culture of *F. oxysporum* with the help of a cork borer. They were inoculated in each flask and incubated in the BOD incubator at $28^{\circ} \pm 0.2^{\circ}C$ for 7 days. The control and treated flasks were all maintained in triplicates. After incubation, the fungal mat and the liquid media were separated by double layered Whatman No. 1 filter paper placed in Buchner funnel under suction by vacuum pump. The filtrates were further centrifuged in a high speed, cooling centrifuge at 5000 rpm for 10 min and the supernatant was used as the enzyme source

A. Polymethyl esterase (PME)

The PME was assayed by the titration method of Muse, Couch, Moore and Muse [22] with modification.

1.5 g of pectin dissolved in 100 ml of 0.2 M NaCl was blended with the help of the polytron homogenizer, then passed through two layers of cheese cloth and pH was adjusted to 7.

To 3 ml of enzyme, 10 ml of 1.5% pectin substrate was added and pH of this reaction mixture was immediately adjusted to 7. After 24 h of incubation at $28^{\circ} \pm 0.2^{\circ}C$, pH of the reaction mixture was measured in control dynamics pH meter and the solution was titrated back to pH 7 with 0.02 N NaOH. Control was maintained with boiled enzyme as

enzyme source. The activity was expressed as specific activity units (SAU). One unit = μ ml of 0.02 N NaOH required to maintain pH 7/h

B. Polymethyl galacturonase (PMG)

The activity of the Endo-PMG was assayed by measuring the reduction in the viscosity of the substrate caused by the enzyme. The activity of exo-PMG was assayed by measuring the mono galacturonic units and the activity was expressed as SAU [23].

1g of pectin was dissolved in 100 ml of acetate buffer, pH 5.2, heated to $50-60^{\circ}C$ in a water bath and mixed with the help of a polytron homogeniser (blender) and then passed through the two layered cheese-cloth. The pH was adjusted to 5.2 using 1N HCl or 1N NaOH. Few drops of toluene was added to the substrate and stored at $4^{\circ}C$.

To 4 ml of the substrate, 1 ml of the buffer and 2 ml of the enzyme was pipetted out into the Ostwald Viscometer-150. Suction was applied through the large arm of the viscometer to mix the contents and the suction was also applied to the small arm and to determine the viscosity of the mixture (i.e. zero time). The efflux time of the reaction mixture was measured at every 30 min intervals for 3 h and the loss in viscosity was calculated by the formula.

$$V = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

Where,

V = percent loss in viscosity

C. Exo-PMG

From the three hour incubated reaction mixture, 2.0 ml aliquots were pipetted out. To this 2 ml of DNS reagent was added and heated in boiling water bath for 10 minutes. Then cooled and diluted with 10 ml of distilled water. The orange red colour was read at 575 nm. Control was maintained with boiled enzyme reaction mixture.

The enzyme activity was expressed as specific activity units. One unit represents μ g of maltose released/h.

D. Measurement of 1,4 β -Exo-Glucanase (C₁)

To 1 ml of enzyme source, 1 ml of buffer and 0.5 ml of substrate were added in a test tube and incubated at room temperature for 2 h. The reaction mixture was mixed well with vortex mixer at regular interval of 30 minutes. At the end of the reaction, the volume of the reaction mixture was adjusted to 5 ml with distilled water. The tubes were centrifuged for 15 min at 2000 g to deposit the residual avicel cellulose. Soluble sugar in the supernatant was measured with the orcinol reagent. Two ml of the above supernatant, 3 ml of orcinol reagent was taken in the test tubes and 10 ml of anthrone reagent was added on ice. The tubes were mixed well with the help of vortex mixture and heated in a water bath at $80^{\circ}C$ exactly for 20 minutes and immediately cooled under running tap water. The colour developed was read at 485 nm in Systronics Spectrophotometer. A blank was prepared with 2% H₂SO₄ instead of orcinol. Control was maintained with boiled enzyme reaction mixture and with zero time reaction mixture.

E. Measurement of 1,4 β -Endo-Glucanase (C_x)

(C_x) activity was determined by measuring the viscosity loss in reaction mixture [24] and by estimating

the reducing sugars released by the enzyme sources in the same reaction mixture[25].

0.5 g of CMC (carboxyl methyl cellulose) was dissolved in 100 ml of sodium acetate-acetic acid buffer with pH 5.2 and kept in water bath at 50-60°C for 5-10 min. then the mixture is blended with the help of polytron homogenizer. The substrate was filtered in two layered cheese cloth and this was stored at 4°C with a layer of toluene

F. Viscosity measurement

Ostwald viscometer 150 size was used to determine the viscosity loss of cellulose substrate.

4 ml of carboxyl methyl cellulose, 1 ml of the buffer and 2 ml of enzyme was pipetted out into the viscometer. The contents were mixed by drawing air gently through the large arm of the viscometer. Suction was applied to the small arm and the efflux time of the mixture was determined at every 30 min interval for 3 h incubation. The percentage loss in viscosity was calculated by employing the formula of the viscosity assay of Endo-PMG.

G. Estimation of Cellobiase

The amount of reducing sugars released from cellobiose by cellobiase was used to assay the enzyme.

1.5 ml of the buffer, 2.5 ml of 5 mM cellobiose and 1 ml of the enzyme was taken in a test tube and incubated at 30°C for 2 h. The reaction was terminated by placing the test tube in a boiling water bath for 10 min. The amount of glucose liberated by the enzyme using DNS (dinitro salicylic acid) reagent was measured at 575 nm in Systronic Spectrophotometer. Glucose was used as standard.

III. RESULTS AND DISCUSSION

The least activity of PME was recorded in the control (6.98 SAU). The highest activity was recorded in the enzyme source obtained from infected leaves (90.60 SAU). The lowest activity was recorded in the enzyme source obtained from the leaves sprayed with *T.viride*

(10.17 SAU) followed by the enzyme sources treated with *T. harzianum* (15.52 SAU) and *P. fluorescens* (22.65 SAU)(Table1).

Table 1: Effect of culture filtrates of *T.viride*, *T.harzianum* and *P. fluorescens* on the activity of polymethyl esterase in the leaves of *Arachis hypogaea* infected with *Fusarium oxysporum* in vivo (expressed in SAU=Specific activity units)

Control-(Healthy) plants	6.98 ±0.27
Plants infected with <i>Fusarium oxysporum</i>	90.60±0.51 ^a
Infected plants treated with <i>T.viride</i>	10.17±0.30 ^a
Infected plants treated with <i>T.harzianum</i>	15.52 ± 0.45 ^a
Infected plants treated with <i>P.fluorescens</i>	22.65±0.75 ^a

SAU= μ ml of 0.02 N NaOH required to maintain pH 7/h^ap< 0.001 as compared to control

The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=3)

The least reduction of substrate viscosity was recorded in the control (6.52%). The highest activity of endo-PMG was observed in enzyme obtained from the *F. oxysporum*-infected leaves (80% loss of viscosity of the substrate at 180min. The least reduction of substrate viscosity was recorded in the enzyme source obtained from the leaves sprayed with *T.viride* (8.50%), followed by those of *T.harzianum* (10%) and *P.fluorescens* (15%)(Fig1).

The amount of reducing sugars released by the activity of exo-PMG was expressed in SAU. The lowest enzyme activity was recorded in the control (50.66 SAU). Highest activity of exo-PMG was observed in enzyme source obtained from the leaves infected with *F.oxysporum* (888.33 SAU). The lowest activity was recorded in the enzyme source obtained from the leaves sprayed with *T.viride* (191.87 SAU) followed by the enzyme sources treated with *T.harzianum* (207.85 SAU) and *P.fluorescens* (217.49 SAU)(Table2).

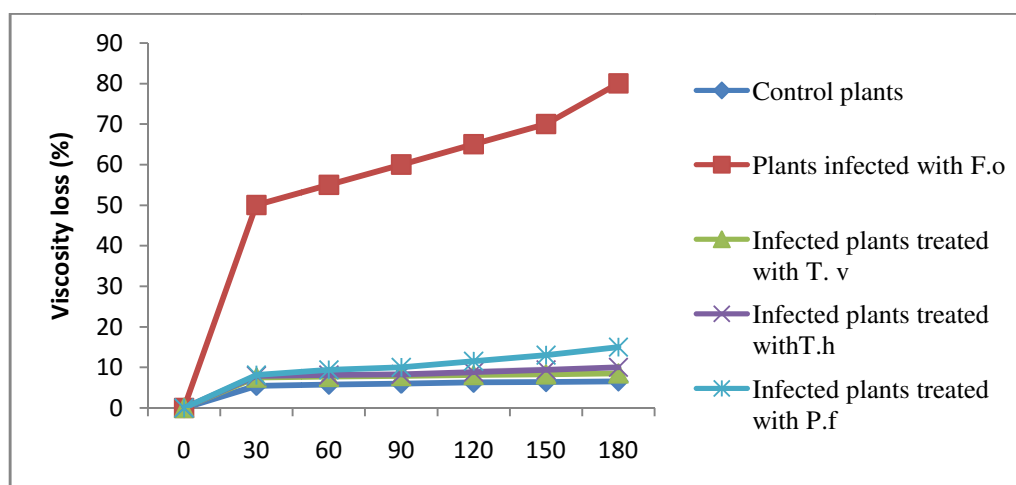


Fig.1. Effect of culture filtrates of *T.viride*, *T.harzianum* and *P. fluorescens* on the activity of endo polymethylgalacturonase in the leaves of *Arachis hypogaea* infected with *Fusarium oxysporum* in vivo

Table2. Effect of culture filtrates of *T. viride*, *T. harzianum* and *P. fluorescens* on the activity of *exopolymethylgalacturonase* in the leaves of *Arachis hypogaea* infected with *Fusarium oxysporum* in vivo (expressed in SAU=Specific activity units)

Control-(Healthy) plants	50.66 ±0.57
Plants infected with <i>Fusarium oxysporum</i>	888.33±0.72 ^a
Infected plants treated with <i>T.viride</i>	191.87±0.62 ^a
Infected plants treated with <i>T.harzianum</i>	207.85±0.71 ^a
Infected plants treated with <i>P.fluorescens</i>	217.49±0.83 ^a

^ap< 0.001 as compared to control
 SAU= change in the absorbance at 547nmof 0.001/h

The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=3)

The least reduction of substrate viscosity of was recorded in the control (7.89%) The highest activity of endo-C_x was observed in enzyme obtained from the *F. oxysporum*-infected leaves (75% loss of viscosity of the substrate at180 min). The least reduction of substrate viscosity was recorded in the enzyme source obtained from the leaves sprayed with *T.viride* (10%) followed by those of *T.harzianum* (15.78%) and *P. fluorescens* (30%)(Fig2).

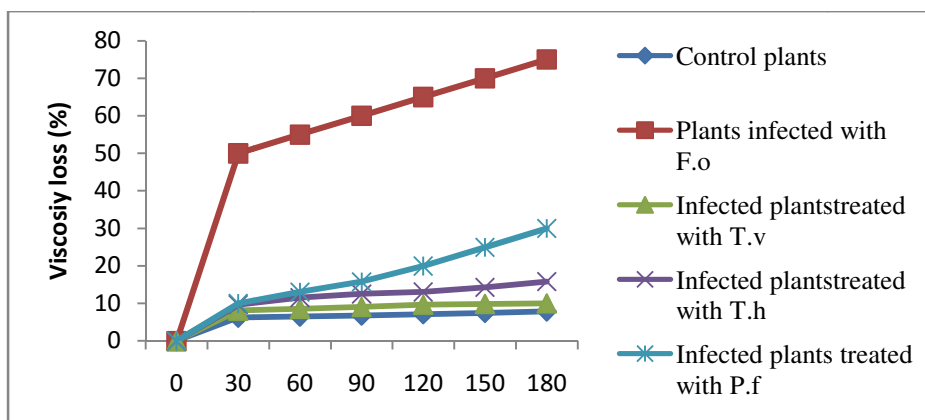


Fig.2. Effect of culture filtrates of *T.viride*, *T.harzianum* and *P. fluorescens* on the activity of *1, 4- β- endo glucanase* in the leaves of *Arachis hypogaea* infected with *Fusarium oxysporum* in vivo

Highest activity of exo-C₁ was observed in enzyme source obtained from the leaves infected by *F.oxysporum* (138.33 SAU). The lowest activity was recorded in the enzyme source obtained from the leaves sprayed with *T. viride* (44.16 SAU) followed by that of *T. harzianum* (49.76 SAU) and *P. fluorescens* (57.25 SAU). The least enzyme activity was recorded in the control (20.66 SAU) (Table3)

Table 3: Effect of culture filtrates of *T. viride*, *T.harzianum* and *P. fluorescens* on the activity of *1,4- β exo glucanase* in the leaves of *Arachis hypogaea* infected with *Fusarium oxysporum* in vivo (expressed in SAU=Specific activity units)

Control-(Healthy) plants	20.66 ±0.57
Plants infected with <i>Fusarium oxysporum</i>	138.33±0.72 ^a
Infected plants treated with <i>T.viride</i>	44.16±0.35 ^a
Infected plants treated with <i>T.harzianum</i>	49.76±0.41 ^a
Infected plants treated with <i>P.fluorescens</i>	57.25±0.51 ^a

^ap< 0.001 as compared to control
 SAU= micro g of maltose equivalent liberated /h

The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=3)

The lowest activity of cellobiase was recorded in the control (10.66 SAU). Highest activity was recorded in the enzyme source obtained from infected leaves (277.94

SAU). The lowest activity was recorded in the enzyme source obtained from the leaves sprayed with *T.viride* (21.77 SAU) followed by the enzyme sources treated with *T.harzianum* (24.35 SAU) and *P. fluorescens* (25.55 SAU) (Table4)

Table 4: Effect of culture filtrates of *T.viride*, *T. harzianum* and *P. fluorescens* on the activity of cellobiase in the leaves of *Arachis hypogaea* infected with *Fusarium oxysporum* in vivo (expressed in SAU=Specific activity units)

Control-(Healthy) plants	10.66 ±0.57s
Plants infected with <i>Fusarium oxysporum</i>	277.94±0.88 ^a
Infected plants treated with <i>T.viride</i>	21.77±0.38 ^a
Infected plants treated with <i>T.harzianum</i>	24.35±0.44 ^a
Infected plants treated with <i>P.fluorescens</i>	25.55±0.47 ^a

^ap< 0.001 as compared to control
 SAU= micro g of maltose equivalent liberated /h

The values within a column followed by different letters are significantly different according to Tukey's HSD multiple range test (TMRT) at 5% level of significance (n=3).

The fungal plant pathogens produce different types of pectin and cellulose lytic enzymes, which act as the main agents for disease development. Chakrabarti and Basuchaudhary observed that the pectinolytic and cellulolytic enzymes produced by *Fusarium* spp. dissolve the middle lamella and diffuse into the xylem vessels

resulting in xylem parenchyma maceration, middle lamella distortion, vessel blockage and disease symptoms. The pectin enzyme is produced primarily to dissolve the cell wall of the host and to reach the interior tissue. During colonization it secretes cellulase to attack the primary and secondary cell wall resulting in thinning of cell wall followed by disintegration [26]. Aktar and Dimond brought out the fact that the cellulolytic enzymes secreted by *Fusarium* sp. attack the primary and secondary cell wall of the host (tomato) and disintegrate them. The degraded products may get into the transpirational stream, which block the vessels leading to wilt symptoms formation [27]. The treatment might inhibit the activity of lytic enzymes of the pathogen either by antibiotic action or ISR in the host cell such as thickening cell wall or it induces the host cell to produce lytic enzymes which are able to inactivate or inhibit the pathogen's lytic enzymes. The accumulation of phenolic compounds in the treated leaves may be toxic to the enzyme activity of the pathogen. The present result substantiates the observation made by Borowitz, Stankie- Diez, Lewicka, and Zukowska. who reported that *P. fluorescens* treatment degraded the lytic fungal pathogen's cellulase, pectinase and xylanase mainly by the action of different antibiotics [28]. Frindlender, Inbar and Chet. observed the activity of chitinase and β -1, 3-glucanase of *Pseudomonas* participating in the inhibition of lytic enzymes of different fungal pathogens [29]. Similar reports are available on *F. solani* [30], *F. oxysporum* in tomato [31] and *Pythium* sp. in cucumber [32].

Trichoderma spp. are reported to produce many antifungal lytic enzymes such as chitinases, proteases, glucanases, lipases, laminarinase and xylanase, which are responsible for the degradation of lytic enzymes of other fungal species. This was observed in *Trichoderma* spp.-treated bean plant against *Pythium* sp. [33]. Similar degradation has been reported in *S. sclerotiorum* by *Trichoderma* treatment [34] and *Pythium ultimum* by *Trichoderma harzianum* treatment [35] and *B.cinerea* by *Trichoderma harzianum* treatment [36]-[38].

The treatment might inhibit the activity of lytic enzymes of the pathogen either by antibiotic action or ISR in the host cell such as thickening cell wall or it induces the host cell to produce lytic enzymes which are able to inactivate or inhibit the pathogen's lytic enzymes. The accumulation of phenolic compounds in the treated leaves may be toxic to the enzyme activity of the pathogen [39].

IV. CONCLUSION

P. fluorescens treatment degraded the lytic fungal pathogen's cellulase, pectinase and xylanase mainly by the action of different antibiotics. *Trichoderma* spp. produces many antifungal lytic enzymes such as chitinases, proteases, glucanases, lipases, laminarinase and xylanase, which are responsible for the degradation of lytic enzymes of fungal species. Experiments were conducted in various other biochemical parameters, total sugars, reducing sugars, nonreducing sugars, amino acid, protein DNA, RNA which reveals that maximum inhibition of *Fusarium*

oxysporum was noticed in *T.viride*-sprayed plants, followed by *T.harzianum*-sprayed plants and *P.fluorescens*-sprayed plants. Of all the treatments, *T. viride* treatment showed higher rate of inhibition of *Fusarium oxysporum*. This study concludes that the culture filtrate of *T.viride*(1%) is the best biocontrol agent in the inhibition of *Fusarium oxysporum* causing *Fusarium* wilt of *Arachis hypogaea*.L.

REFERENCES

- [1] Beckman, C.H. (1987) *The nature of wilt diseases in plants*. Amer. Phytopathol. Soc. St. Paul MN., USA. 175pp
- [2] Agrios, G.N. (1997) *Plant Pathology (4th ed.)* Academic Press, New York.
- [3] Rombouts, F. M. and Pilnik, W. (1980) Pectic enzymes. In: *Microbial Enzymes and Bioconversions, Economic Microbiology* (Ed.) A.H.Rose. Academic Press, New York, pp.228-282.
- [4] Kawano, C.Y., Chellegatti, M.A.S.C., Said, S. and Fonseca, M.J.V. (1999) Comparative study of intracellular and extracellular pectinases produced by *Penicillium frequentans*. *Biotechnology and Applied Biochemistry* 29:133-140.
- [5] Verlent, I., Van loey, CA., Smout, C., Duvetter, T. and Hendriclox, ME. (2004) Purified tomato polygalacturonase activity during thermal and high pressure treatment. *Biotechnol. Bioeng.* 86: 63-71.
- [6] Mac Hardy, W.E. and Beckman, C.H. (1981) Vascular wilt *Fusarium*: Infections and Pathogenesis. In: *Fusarium: Diseases, Biology and Taxonomy*, P.E. Nelson, T.A. Toussoun, and R.J. Cook (Eds.). The Pennsylvania State University Press, University Park and London. pp. 365-390.
- [7] Posada, M. L., Patino, B., Mirete, S., Munoz, M. C., Vazquez, C. and Gonzalez-Jaen, M.T. (2001) Comparative analysis of polygalacturonases in isolates of seven species of *Fusarium* from *Pinus pinea*. *Mycol.Res.* 105:100-104
- [8] Ibid.
- [9] Bateman, D.F. and Bashman, H.G. (1976) Degradation of plant cell walls and membranes by microbial enzymes. In: *Encyclopedia of Plant Physiology*. (Eds.) Heitfuss, R. and Williams, P.H. Springer Verlag, Berlin. pp. 316-355.
- [10] Walton, J.D. (1994) Deconstructing the cell wall. *Plant Physiol.* 104:191-196.
- [11] Cooper, R.M. (1983) The mechanisms and significance of enzyme degradation of host cell walls by parasites. In: *Biochemical Plant Pathology*. (Ed.) Callow, J. John Wiley and Sons, Chichester. pp. 101-135.
- [12] Mac Hardy, W.E. and Beckman, C.H. (1981) Vascular wilt *Fusarium*: Infections and Pathogenesis. In: *Fusarium: Diseases, Biology and Taxonomy*, P.E. Nelson, T.A. Toussoun, and R.J. Cook (Eds.). The Pennsylvania State University Press, University Park and London. pp. 365-390
- [13] Najar, A.G., Anwar, Ali., Masoodi, Lubna. and Khar, M.S. (2011) Evaluation of native biocontrol agents against *Fusarium solani* f.sp.*melongenae* causing wilt disease of Brinjal in Kashmir. *J. Phytol.* 3: 31-34
- [14] Satyaprasad, K., Desai, B.B. and Ramarao, P.(1998) Biological control of fusarial wilt diseases. In: *Integrated Pest and disease Management*, (Eds.) Upadhyay, R.K., Mukherji, K.C., Chamola, B.P. and Dubey, O.P., A.P.H. Publishing Corporation, New Delhi. pp.563-579.
- [15] Anwar, Ali., Bhat G.N. and Bhat, K.A. (2008) Mycoparasitic behaviour of certain bioagents against sheath blight pathogen (*Rhizoctonia solani*) of rice. *J. Mycol. Pl. Pathol.* 38: 135-137.
- [16] Sivasithamparam, K. and Ghisalberti, E.L. (1998) Secondary metabolism in *Trichoderma* and *Gliocladium*. In: *Trichoderma and Gliocladium* Volume1, *Basic biology, taxonomy and genetics*, (Eds.) Harman GE, Kubicek C.P., London, UK: Taylor and Francis Ltd. pp. 139-192.
- [17] Howel, C.R. (1998) The role of antibiosis in biocontrol. In: *Trichoderma and Gliocladium, Vol. 2, Enzymes, biological control and commercial application*. (Eds.) Harman G.E., Kubicek CP. London, UK: Taylor and Francis Ltd, pp.173-180.

- [18] Kubicek, CP. and Penttila, M.E. (1998) Regulation of plant polysaccharide degrading enzymes by *Trichoderma* In: *Trichoderma and Gliocladium, Vol. 2, Enzymes, biological control and commercial application.* (Eds.)Harman G.E and Kubicek C.P. London, Taylor and Francis Ltd. pp. 49-67.
- [19] Lorito, M. (1998) Chitinolytic enzymes and their genes In: *Trichoderma and Gliocladium, Vol.2, Enzymes, Biological control and Commercial Application* (Eds.) Harman G.E, Kubicek C.P. London, Taylor and Francis Ltd. pp.73-92.
- [20] Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S.L. and Lorito, M. (2008) *Trichoderma* –plant-pathogen interactions. *Soil Biol. Biochem.* 40: 1-10.
- [21] Mohandas, Sukhada., Manmohan, M., Rawal, R.D., Chakraborty, Saikat., Sreekantappa, Manjula, R.,H. and Lakshmiikantha.(2004) Interaction of *Fusarium oxysporum f.sp. cubense* with *Pseudomonas fluorescens* precolonized to banana roots. *World J. Microbio. Biotech.* 20: 651-655.
- [22] Muse, R.R., Couch. H. B., Moore, L.D. and Muse, B. D. (1972) Pectinolytic and cellulolytic enzymes associated with *Helminthosporium* leaf spot on Kentucky bluegrass. *Can. J. Microbiol.* 18: 1091-1098.
- [23] Mahadevan, A. and Sridhar, R. (1986) *Methods in physiological plant pathology* (3rd ed.) Sivakami Publications, Madras, India.
- [24] Ibid.
- [25] Wang, G., Michailides, T.J. and Bostock, R. M. (1997) Improved detection of polygalacturonase activity due to *Mucor piriformis* with a modified Dinitrosalicylic Acid Reagent. *Phytopathol.*87: 161-163.
- [26] Chakrabarti, D.K. and Basuchaudhary, K.C.(1979) Enzymes produced by *Fusarium oxysporum f.sp. carthami* incitant of safflower wilt and their effect on host tissue. *Indian Phytopath.* 32: 202-205
- [27] Aktar, Husain. and Dimond, A.E. (1960) Role of cellulolytic enzyme in pathogenesis of *Fusarium oxysporum f. sp. lycopersici*.*Phytopathol.* 50: 329-331.
- [28] Borowitz, J.J., Stankie- Diez, M., Lewicka, T. and Zukowska, Z. (1992) Inhibition of fungal cellulase, pectinase and xylanase activity of plant growth promoting fluorescent Pseudomonads. *Bull. OILB/SROP.* 15:103-106.
- [29] Frindlender, M., Inbar, J. and Chet, I. (1991)Biological control of soil -borne plant pathogen by a glucanase producing *Pseudomonas cepacia*. *Soil Biol. Biochem.* 25: 1211-1221
- [30] Lim, H., Kim, Y. and Kim, S. (1991) *Pseudomonas stutzeri* YLP-1 genetic trans formation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot. *Appl. Environ. Microbiol.* 57: 510-516.
- [31] M 'Piga., Belanger, R.R., Paulitz, T.C. and Bennhamou, N.(1997) Increased resistance to *Fusarium oxysporum f.sp. radicyloperisici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. *Physiol. Mol. Plant Pathol.* 50: 301-320.
- [32] Chen, C., Belanger, R.R., Benhamou, N. and Paulitz, T. (2000) Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria and *Pythium aphanidermatum*. *Physiol.Mol.Plant Pathol.* 56: 13-23.
- [33] Cotes, A.M., Lepoivre, P. and Semal, J. (1996) Correlation between hydrolytic enzyme activity measured in bean seedlings after *Trichoderma kiningii* treatment combined with pre germination and the protective effect against *Pythium splendens*. *Eur.J.Plant Pathol.* 102: 497-506.
- [34] Jones, D., Gardon, H.H. and Bacon, J.S.D. (1974) Co-operative action by endo and exo - (1-3) glucanases from parasitic fungi in degradation of cell wall glucan of *Sclerotinia sclerotiorum* (Lib.) De berry. *J. Biochem.* 140: 47-55.
- [35] Haran, S., Schickler, H. and Chet, I. (1996) Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiol.* 142: 2321-2331.
- [36] Lorito, M., DiPietro, A., Hayes, C.K., Woo, S.L and Harman, G.E.(1993a) Antifungal synergistic interaction between chitinolytic enzymes from *Trichoderma harzianum* and *Enterobacter cloacae*. *Phytopathol.* 83: 721-728.
- [37] Lorito, M., Harman, G.E, Hayes, C.K., Broadway, R.M.,Tronsmo, A., Woo,S.L.and Di Pietro, A. (1993b) Chitinolytic enzymes produced by *Trichoderma harzianum*: antifungal activity of purified endochitinase and chitobiosidase. *Phytopathol.* 83: 302-307.
- [38] Lorito, M., Hayes, C.K., Di Pietro, A., Woo, S.L. and Harman, G.E.(1994) Purification, characterization and synergistic activity of a glucan 1,3- β - glucosidase and an N-acetyl - β - glucosaminidase from *Trichoderma harzianum*. *Phytopathol.* 84: 398-405.
- [39] Rajeswari ,P (2014) Role of phenols and antioxidant enzymes in the biocontrol of *Fusarium oxysporum* causing Fusarium wilt on *Arachis hypogaea* L.(Groundnut) *International Journal of Agricultural Science and Research* 4:95-104

AUTHOR'S PROFILE



Dr. P. Rajeswari

Doctorate from the Department of Biochemistry and Molecular Biology, Pondicherry University, Pondicherry, India. Her research work focused on Biocontrol of Fusarium wilt of Groundnut. She published papers in International Journals.