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Eco-Friendly Management of *Fusarium oxysporum* f.sp. *pisi* inciting wilt of Pea *in vitro*

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in vitro antifungal assay was conducted against *Fusarium oxysporum* f.sp.*pisi* (FOP), using plant extracts and biocontrol agents. Among six plants extract, maximum fungistatic effect was shown by Garlic plant extracts with a 100% inhibition at higher (4%) concentrations followed by Neem, Turmeric,Wild sedge, Ginger and *Aloe vera*. Eight isolates of *Trichoderma* spp. were tested for their ability to produce volatile and non-volatile metabolites against FOP. Studies indicated that volatile metabolites from isolate (NCIPMCAU-69) caused maximum reduction in mycelial growth 62.37% and minimum was recorded with isolate (NCIPMCAU-109) 18.57%. In case of non-volatile, the highest per cent inhibition at 7.5 and 15% v/v concentration was recorded with the isolate NCIPMCAU-69 where the inhibition percentage was 14.53% and 20.97% respectively. This research suggests that managing FOP with plant extracts and biocontrol agents could be a good choice for generating a profitable and risk-free product for the environment.

Keywords: Biocontrol agents, *Fusarium oxysporm,* plant extracts, *Trichoderma*

INTRODUCTION

Despite insufficient food supply, almost 800 million people in underdeveloped nations are losing 10% of their food owing to plant disease (Strange and Scott, 2005).Fungi, bacteria, viruses, and nematodes are essential candidates for plant disease production because they grow through soil-borne, above-ground infections. In some cases, they may be transmitted through insect feeding (Wani, 2011). However, fungi have the biggest influence on disease and crop output losses when compared to other plant parasites.

Pea (*Pisum sativum* L.) is a major vegetable-cum-pulse crop that is grown as a garden or field crop in temperate climates around the world.
It plays a significant role in the human diet as a source of high-quality proteins (7.2%), carbohydrates (15.8%), amino acids, sugars, vitamins A and C, calcium, and phosphorus, as well as a minor amount of iron. Manipur, a North-Eastern hill state of India, field pea is one of the most significant cash crops of farmers who have small holdings and rely mostly on rains for irrigation and grown in 26,000 ha of land, accounting for around 85% of the total pulse area (Anon., 2015). Rabi pulses were grown in roughly 27.07 Mha of land in 2017-18, generating 25.27 MT of pulses with a productivity of 0.93 MT/ha (Anon., 2017-2018). Regardless of other constraints in pea production, diseases are major biological constraints to production and this wilt disease caused by FOP is dominant and widespread and causes heavy damagepartial to complete losses of the crop (Persson *et al.,* 2007).The disease spreads through contaminated soil, causing the entire plant to wilt and shrivel. According to Maheshwari *et al.,* 1983 the incidence of wilt and root rot complex caused by *Fusarium oxysporum* f.sp. *pisi* and *Fusarium solani* f.sp. *pisi* was 25.0-100.0 %, with significant losses ranging from 13.9-

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95.0 %. Similarly, the disease incidence of pea wilt varied from 12.39 to 25.58% at various pea growing areas of Manipur viz., Andro, Yambem, Iroisemba, Thoubal Wangmataba, Wabagai, Kakching, Bishnupur and Moirang (Debbarma, 2015).During recent years, wilt of pea has become increasingly serious in several pea growing areas of Manipur, the disease is primarily soil borne as this pathogen and its numerous special forms harm a wide variety of economically important crops. It creates a bigger problem in terms of management because fungicides are expensive and not easily biodegradable, and they tend to stay in the environment for years, leading to pathogen resistance and indiscriminate use, which can cause a variety of problems for human and animal health as well as environmental issues. Likewise, many fungi and bacteria species have also been reported to be efficient bio-control agents against soil-borne plant diseases. Among the biocontrol agents, *Trichoderma* spp. has been discovered to be a promising bio-control agent against a variety of diseases, including *Fusarium* spp. again enhancement of the activity of the different volumes of plant extract were used to boost the action of different fungicides (Nikam *et al*. 2007).This scenario, therefore, calls for alternative approaches for the management of FOP are needed, such as using botanical or plant extracts and biocontrol agents, which have fewer negative effects on human and environmental health and are eco-friendly, as well as developing a long-term biocontrol strategy against Fusarium wilt disease and determining their relationships.

MATERIALS AND METHODS

The research was conducted in the Department of Plant Pathology's Laboratory at College of Agriculture, Central Agricultural University, Imphal, during 2019. The goal of this study was to isolate the causal organism of Fusarium wilt of pea and its management by using six plant extracts and eight native *Trichoderma* spp. The disease infected plants were collected from the field and brought to the laboratory to be subjected to various experiments. Symptoms of Fusarium wilt on pea plants were appeared as yellowing of lower leaves, stunting and dwarfing of plant growth.

Source of isolation

FOP was obtained from the diseased infected plants of pea which was collected from the farmer's field.

Sterilization of sample

Infected areas were cut into small pieces of 1mm size, then surface sterilized with 0.1 per cent NaOCI (Sodium Hypochlorite) solution for one minutes. Then it was washed many times in sterile distilled water, and the surplus water was pressed out.

Plating of fungi

The sterilised pieces were placed in pre-sterilized petri plates containing Potato Dextrose Agar (PDA) media and incubated in a BOD incubator at 27 ± 1^0C. Identified fungus was isolated separately, then inoculated on PDA slants and incubated.

Control measure

Plant extracts and bio-control agents were used as control measures.

Pathogenicity test

A pathogenicity test was performed on the isolated fungus. Soil inoculation with pathogen was done by means of rice seed inoculums technique of Weideman and Wehner (1993).

In vitro antifungal activity assay of plant extracts by using poison food technique on the growth of FOP

Six locally available botanicals including aloe vera (*Aloe barbadensis*), garlic (*Allium sativum*), neem (*Azadirachta indica*), wild sage (*Lantana camara*), ginger (*Zingiber officinales*) and turmeric (*Curcuma longa*) as well as check fungicide Mancozeb were tested in *in vitro* for their efficacy on fungus growth at three levels of concentration by using 'Poison food technique'. Fresh plant pieces were collected and washed for 2 mins in running water before being rinsed with sterile water. These samples were air dried on blotting paper and crushed individually in a mortar and pestle at a ratio of 1:1(w/v). These extracts were filtered through two layers of muslin fabric, centrifuged for 15 minutes at 1500 rpm, and the supernatants were collected. The extracts thus prepared were considered as 100% concentration. Three different concentrations were used for each treatment.

Plant extracts at various concentrations from each stock solution were applied to 20 ml of sterilised PDA per plate. The plates were then inoculated aseptically with 5mm mycelial disc from the actively growing culture of FOP in the centre of plate. As a negative control, the medium was supplied without any treatment. Each treatment was three times replicated and incubated at 27±1^{0}C.

Percentage inhibition on radial growth was estimated using the approach outlined below by Vincent (1927):

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where, C= Radial growth of the fungus in control
T= Radial growth of the fungus in treatment

In vitro antagonistic potential of some isolates of native Trichoderma species on the growth of FOP

In-vitro study on the antagonistic potential of eight native *Trichoderma* spp. viz., NCIPMCAU-96, NCIPMCAU-109, NCIPMCAU-131, NCIPMCAU-18, NCIPMCAU-69, NCIPMCAU-118, NCIPMCAU-7 and NCIPMCAU-123 (Source: Department of Plant Pathology, COA, CAU) were evaluated against the FOP through the production of non-volatile (Dennis and Webster, 1971a) and volatile antibiotics (Dennis and Webster, 1971b) of biocontrol agents.
The percent inhibition of the pathogen mycelial development by antagonists was estimated using Garcia's (1991) formula:
Per cent Inhibition of Radial Growth (% IRG) = 100 [(R_1-R_2) / R_1], where R_1 – the farthest radial distance grown by the pathogen in the direction of the antagonist.
R_2- the distance grown on a line between inoculation positions of the pathogen and antagonist.

Statistical analysis

A two-way analysis of variance (ANOVA) was used to assess the effect of plant extracts and biocontrol agents on the growth of FOP on PDA.

RESULTS AND DISCUSSION

In the laboratory of the Department of Plant Pathology, COA, CAU, Imphal, the causal fungus of pea wilt was isolated and purified. Then, on the basis of morphological traits and taxonomic keys found in the literature, it was determined to be *Fusarium oxysporum* f.sp. *pisi*. The pathogenicity of FOP was tested according to the materials and methods section.

Results presented in Fig.1 showed that there were considerable ranges of efficacies of aqueous plant extracts at three different concentrations against growth of the fungus. On linear growth, garlic demonstrated 74.12% inhibition at 1% concentration, 85.10% inhibition at 2% concentration, and 100% inhibition at 4% concentration (Fig. 4 A-G). The restof the plant extracts exhibited less inhibition on growth of the fungus at all three concentrations. Garlic plant extracts had the most fungistatic activity (100%) at higher doses, while *Aloe vera* had the least inhibition at lower concentrations (8.04%). With an increase in the concentration of plant extracts, there was an overall tendency of decreased mycelial growth of FOP. The current findings demonstrated that garlic extract fully inhibited the pathogen's mycelial growth at higher concentrations, and neem extract inhibited the pathogen's growth by more than 50%.The present findings were consistent with those of Devi and Chhetry (2012), who tested the antifungal effect of plant extracts against *F. udum* mycelial growth at four different concentrations of 5%, 10%, 15% and 20%. Among them, *A. sativum* has recorded 100% inhibition of mycelial growth at 20% concentrations. Similarly with the findings of Jamal-u-ddin *et al*. (2012). Neem leaf extract came in second place to garlic extract in terms of efficacy. This assertion is in line with the

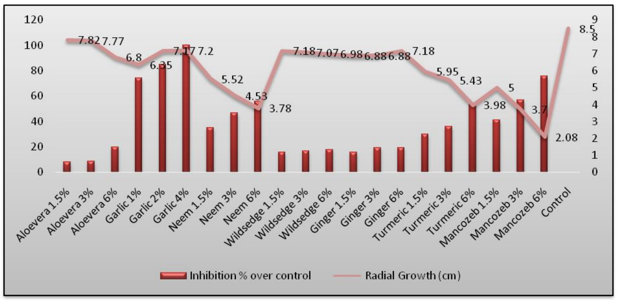

Fig. 1: Effect of Aqueous plant extracts and a check fungicide on growth of FOP at different level of concentrations

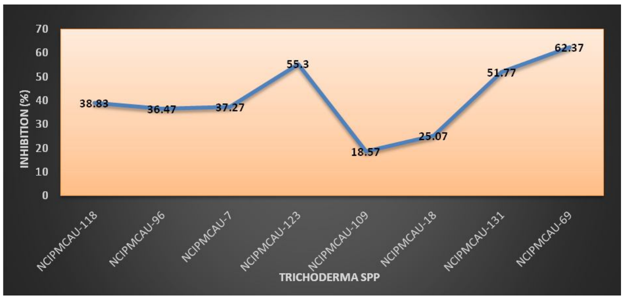

Fig. 2: Effect of volatile compounds of native *Trichoderma* spp. on growth of FOP

Fig.3: Effect of non-volatile compounds of native *Trichoderma* spp. on growth of FOP

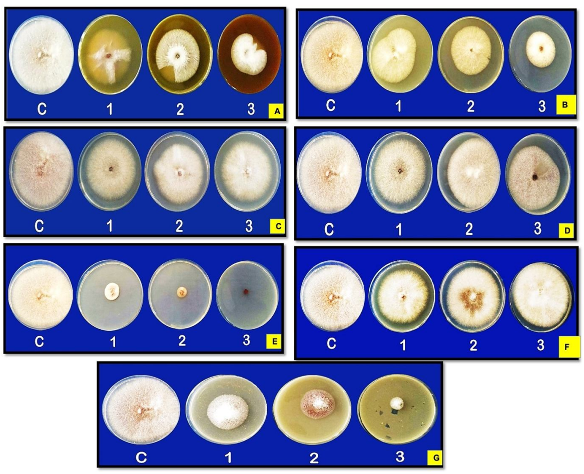

Fig. 4 :(A-G): Effect of plant extracts on the growth of FOP at different concentrations (A) Tumeric (1- 1.5%, 2- 3%, 3- 6%, C- Control), (B) Neem (1- 1.5%, 2- 3%, 3- 6%, C- Control),(C) Wild sedge (1- 1.5%, 2- 3%, 3- 6%, C- Control), (D) Ginger (1- 1.5%, 2- 3%, 3- 6%, C- Control) E) Garlic (1- 1%, 2- 2%, 3- 4%, C- Control, (F) *Aloe vera* (1- 1.5%, 2- 3%, 3- 6%, C- Control), (G) Mancozeb (1- 0.05%, 2- 0.1%, 3- 0.2%, C- Control).

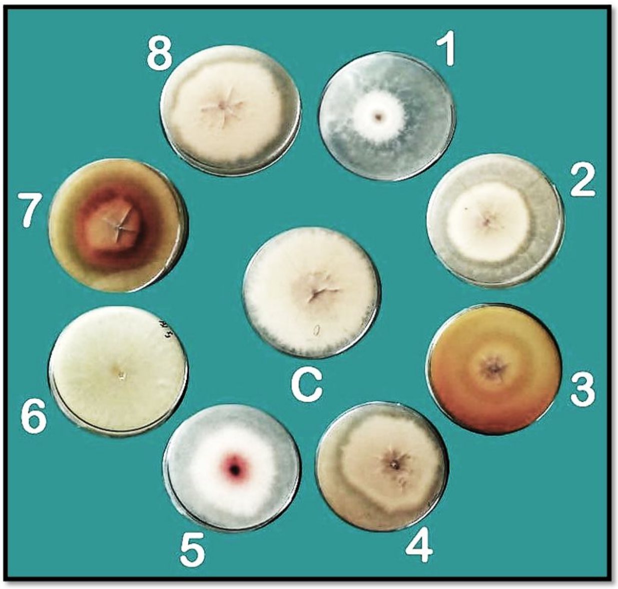

Fig.5: Effect of volatile compounds of native *Trichoderma* species on the growth of FOP. (1. NCIPMCAU-123, 2. NCIPMCAU-7, 3. NCIPMCAU-69, 4. NCIPMCAU-18, 5. NCIPMCAU-96, 6. NCIPMCAU-131, 7. NCIPMCAU-118, 8. NCIPMCAU-104, 9. Control)

findings of Khalell *et al*. (2014), who found that neem leaf extract was the most efficient (60.5% colony reduction), followed by Ginger extract (57.7%), and *Parthenium* leaf extract was the least effective (25.4%) at the highest concentration (1000 ìg/ml). None of the tested plant extracts and their concentrations tested has completely checked the mycelial growth of FOP. So, the effectiveness of garlic and neem extract in mycelial growth of the pathogen might be due to the presence of antifungal compounds like diallyl disulphide and diallyl trisulphide and azadirachtin. Similarly, different aqueous phytoextracts exhibited antifungal activity against *Colletotrichum dematium* and it was directly proportional to their concentrations (Suryawanshi *et al.* 2016).

Out of tested eight isolates of *Trichoderma* spp., the volatile compounds released by *T. harzianum* (NCIPMCAU-69) showed the greatest growth inhibition (62.37 %) against FOP and least inhibition (18.57 %) with isolate (NCIPMCAU-109) (Figs. 2 and 5). A wide range of volatile secondary metabolites are produced by *Trichoderma* spp., including ethylene, hydrogen cyanide, aldehydes, and ketones, all of which are useful in the control of plant diseases (Siddiquee *et al*. 2012; Bhagat *et al*. 2014; Chen *et al*. 2015).Volatile metabolites released by *Trichoderma* strains exhibited

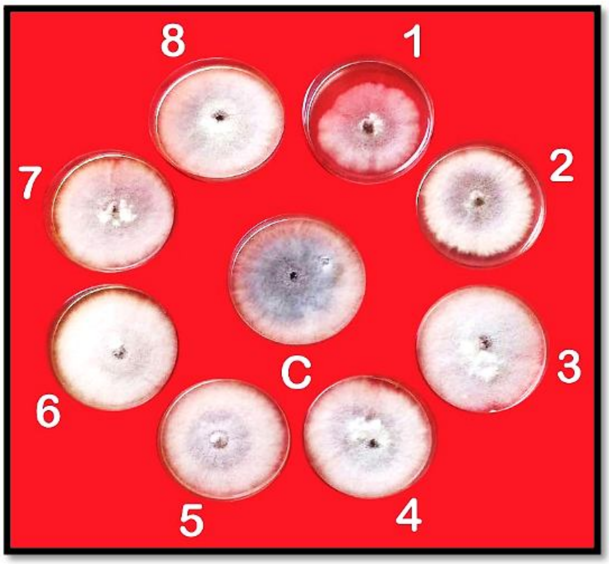

Fig.6: Effect of non-volatile compounds (7.5%) of native *Trichoderma* species on the growth of FOP. 1.NCIPMCAU-123, 2. NCIPMCAU-109 , 3. NCIPMCAU-7, 4. NCIPMCAU-1315. NCIPMCAU-118 6. NCIPMCAU-96, 7. NCIPMCAU-69, 8. NCIPMCAU-18 , C. Control

Fig. 7: Effect of non-volatile compounds (15%) of native *Trichoderma* species on the growth of FOP. 1. NCIPMCAU-123, 2. NCIPMCAU-109, 3. NCIPMCAU-7, 4. NCIPMCAU-131, 5. NCIPMCAU-118 6. NCIPMCAU-96, 7. NCIPMCAU-69, 8. NCIPMCAU-18, C. Control

inhibitory effects on the growth of *R. solani, S. rolfsii* and *P. ultimum* (Raut *et al*. 2014: Yan *et al*. 2006). Several researchers have documented the efficacy of diffusible volatile chemicals by *T. viride* and *T. harzianumin vitro*, including Stoppacher *et al*. (2010) and Pan *et al*. (2013).

The non-volatile substances secreted by NCIPMCAU-69 (*T. harzianum*) was found the most effective in lowering the greatest mycelial growth of tested FOP by 14.53% and 20.97% at 7.5% and 15% v/v concentrations, respectively. Moreover, revealed that per cent inhibition of radial growth of FOP were ranged from 6.82% to 14.53% at 7.5% v/v concentration and 6.08% to 20.97% at 15% v/v concentration (Figs. 3, 6 and 7). The level of inhibition differed from strain to strain. The possible reason may be due to their inherent potentiality to adapt well in introduced conditions and aggressiveness of the *Trichoderma* isolates towards certain plant pathogens (Bae and Knudsen, 2005; Pan and Jash, 2009).

The effect of volatile and non volatile compounds produced by the *Tricoderma* were evaluated and found to be effective in reducing FOP under *in vitro* as well as *in vivo* (Debbarma 2015) were more effective than volatile compounds. Antagonisim of *Trichoderma* species against numerous pathogens were described by Sundaramoorthy and Balabaskar (2013); Chanu *et al.* (2018) and Chakrapani *et al*. (2019). Several workers studied on the production of antifungal volatile and non-volatile compounds revealed that *T. harzianum* SQR-T037 strain inhibited *F. oxysporum* growth (Raza *et al.* 2013). Due to the presence of non-volatile substances, soil application with *T. hamatum, T. harzianum* or *T. viride* reduced the severity of wilt and root rot disease just as effectively as carbendazim (Khan *et al*. 2014). However, the inhibitory effect of *Trichoderma* spp. against FOC was possibly due to hyper-parasitism, mycoparasitism, competition for space and nutritional sources and antagonistic chemicals produced and dispersed into the environment. Antimicrobial metabolites produced by *Trichoderma* are efficient against a wide range of fungal phytopathogens including *Fusarium oxysporum, Rhizoctonia solani, Curvularia lunata, Bipolaris sorokiana* and *Colletotrichum* spp.,according to previous research of (Svetlana *et al.* 2010).The current data demonstrated that the test antagonists significantly suppressed the pathogen growth, which could be related to the production of various types of volatile and non-volatile compounds, as well as their amounts, which were very selective in their effect. As a result, this research emphasises the environmentally appropriate control of pea wilt.

CONCLUSION

The study's findings revealed that plant extracts and bio-control agents were effective at inhibiting the test pathogen's mycelial proliferation. The results of this study imply that, given the high expense of chemical fungicides and their potentially dangerous side effects, plant extracts and antagonistic bio-agents are major sources of compounds that are effective against some fungi and could be good fungicide substitutes. Therefore, field experiments should be conducted to confirm its efficacy before recommending it to pea farmers.

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DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

Anonymous. 2015. Annual Report (2014-15) on promotion of pulses in NEH region. ICAR Indian Institute of Pulses Research, Kanpur, Uttar Pradesh 208024 (India), pp. 39.

Anonymous. 2017-18. Area, production and yield in Manipur, Department of Agriculture, Government of Manipur.

Bhagat, S., Birah, A., Kumar, R., Yadav, M.S., Chattopadhyay, C. 2014. Plant disease management: prospects of pesticides of plant origin. In: *Advances in Plant Biopesticides* (Ed. D. Singh), DOI 10.1007/978-81-322-2006-0_7, Springer India, 119-129.

Chakrapani, K., Sinha, B., Chanu, W.T., Chakma, T., Siram, T. 2019. *In vitro* evaluation of antagonistic potential of native *Trichoderma* spp. against *Rhizoctonia solani* causing sheath blight of rice in Manipur. *Int. J. Chem. Stud.* **7**: 2207-2210.

Chanu, W.T., Sinha, B., Devi, P.S., Devi, N.O. and Devi, H.C. (2018). Molecular identification of pathogens associated with soil borne diseases of chickpea and their management by native *Trichoderma* spp. *Plant Dis. Res.* **33**: 50-59.

Chen, L.J., Sun, S.Z., Miao, C.P., Wu, K., Chen, Y.W., Xu, L.H., Guan H.L., Zhao, L.X. 2015. Endophytic *Trichoderma gamsii* YIM PH 30019: a promising biocontrol agent with hyperosmolar, mycoparasitism and antagonistic activities of induced volatile organic compounds on root-rot pathogenic fungi of *Panax notoginsen*g. *J. Ginseng Res.* **38**: 1-10.

Debbarma, D. 2015. Management of *Fusarium* wilt of pea (*Pisum sativum* L.). M.Sc. (Agri.) thesis, College of Agriculture, Central Agricultural University, Manipur, pp. 122.

Dennis, C., Webster, J. 1971a. Antagonists properties of species groups of *Trichoderma* 1. Production of non-volatile antibiotics. *T. Brit. Mycol. Soc*. **57**: 25-39.

Dennis, C., Webster, J. 1971b. Antagonistic properties of species groups of *Trichoderma* 11. Production of volatile antibiotics. *T. Brit. Mycol. Soc*. **57**: 41-48.

Devi, T.R., Chhetry, G.K.N. 2012. Evaluation of antifungal activities of certain plant against *Fusarium udum* Butler causing wilt in pigeonpea (*Cajanus cajan* (L.) Millsp.) *Int. J. Sci. Res*. 2: 1-4

Garcia, E.F. 1991. Screening of fungal antagonist to control *Sclerotium cepivorium.* In: New approaches in biological control of soil borne diseases (Eds. Jensen *et al.*). IOBCM/ PRS *Bull. Cph*., pp. 79-81.

Jamal-u-ddin Hajano, A. Mubeen Lodhi, M.A. Pathan, M.A. Khanzada, G.S. Shah. 2012. *In vitro* evaluation of fungicides, plant extracts and Bio-control agents against rice blast pathogen *Magnaporthe oryzae* couch. *Pak. J. Bot*. **44**: 1775-8.

Khaleel, M., Subhani, M.N, Ali, A., Sahi, S.T., Hussain, S., Abbas, W. 2014. *In vitro* evaluation of homeo-fungicides and methanolic plant extracts against mycellial growth of *Fusarium oxysporum* f.sp. *pisi* causing wilt disease in pea. *Pak. J. Phytopathol.* 26(02): 247-251.

Khan, M.R., S. Ashraf, F. Rasool, K.M.Salati F.A. Mohiddin and Z. Haque (2014). Field performance of *Trichoderma* species against wilt disease complex of chickpea caused by *Fusarium oxysporum* f.sp. *ciceri* and *Rhizoctonia solani*. *Turkish J. Agri. Forest.* **38**: 447-454.

Nikam, P.S., Jagtap, G.P., Sontakke, P.L. 2007. Management of chickpea wilt caused by *Fusarium oxysporium* f. sp. *ciceri. Afr. J. Agric. Res*. **2**: 692-697.

Pan, S., Mukherji , R., Bhagat, S. 2013. Evaluation of *Trichoderma* species against soil borne plant pathogens. *Ann. Plant Prot. Sci.* **21**: 176- 223.

Persson, L.L., Bodker, Wikstrom, M.L. 2007. Prevalence and pathogenicity of foot and root rot pathogen of pea in Southern Scandinavia. *Plant Dis.* **81**: 171-174.

Raut, I.. M. Doni-Badea, M. Calin, F. Oancea, G. Vasilescu, T. Sesan and L. Jecu (2014). Effect of volatile and non-volatile metabolites from *Trichoderma* spp. against important phytopathogens. *Revista de Chimie* **65**: 1285-1288

Raza, W., Faheem, M., Yousaf, S., Rajer, F.U. and Yameen, M. 2013. Volatile and non-volatile antifungal compounds produced by *Trichoderma harzianum* SQR-T037 suppressed the growth of *Fusarium oxysporum* f.sp. *niveum*. *Sci. Lett.* **1**: 2124.

Siddiquee, S., Cheong, B.E., Taslima ,K., Kausar, H., Hasan, M.M. 2012. Separation and identification of volatile compounds from liquid cultures of *Trichoderma harzianum* by GC-MS using three different capillary columns. *J. Chromatographic Sci.* **50**: 358-367.

Strange, R.N., Scott, P.R. (2005). Plant disease: a threat to global food security. *Annu Rev Phytopathol.* **43**: 83-116.

Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R., Schuhmacher, R. 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GCMS. *J. Microbiol.Methods* **81**: 187-193.

Sundara Moorthy, S., Bala Baskar, P. 2013. Biocontrol efficacy of *Trichoderma* species against wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*. *J. Appl. Bio. Biotechnol.* **1**: 36-40.

Suryawanshi, A.P., Somwanshi, S.D., Nikam, P.S. 2016. Bioefficacy of bioagents and botanicals against *Colletotrichum dematium* causing anthracnose of spinach; 6th International Conference "Plant, Pathogens and People" February 23-27, New Delhi, India.

Svetlana, Z., Stojanovic, S., Ivanovic, Z., Popovic, V.G.T., Balaz, J. (2010). Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*. *Arch. Biol. Sci.* **62**: 611-623.

Vincent, J.M. 1927. Distortion of fungal hyphae in presence of certain inhibitors. *Nature* **159**: 239-241.

Wani, A.H. 2011. An overview of the fungal rot of tomato. *Mycopath*ologia **9**: 33-38.

Weideman, H., Wehner, F.C. 1993. Green house evaluation of *Trichodrema harzianum* and *Fusarium oxysporum* for biological control of citrus root rot in soils naturally and artificially infected with *Phytophthora nicotianae. Phytophylactica,* **25**: 101-105.

Yan, S.X., Tao, S.Q., Tao, X.S., Ian, C.X., Yun, S.C., Zhong, Z.Y. 2006. Broad -spectrum antimicrobial activity and high stability of *Trichokonins* from *Trichoderma konongii* SMF2 against plant pathogens. *FEMS Microbial Lett.* **260**: 119-125.