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ANTIFUNGAL POTENTIAL OF PLANT EXTRACTS AND *TRICHODERMA* SPP. AGAINST FUSARIUM WILT OF TOMATO CAUSED BY *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*

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ABSTRACT

Fungal attack on tomato is a serious problem in tomato growing areas. Among many fungal diseases, fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* cause a significant loss in yield. In the current study, the pathogen was isolated from an infected tomato plant and identified on the basis of morphological characteristics. Due to the harmful effect of fungicides, the present study was designed to adopt the alternative methods. For this purpose, four plant extracts viz; *Syzygium aromaticum*, *Allium Sativum*, *Euclyptus globulus* and *Lantana camara* and two bio-control agents (*Trichoderma harzanium* and *Trichoderma viridi*) were evaluated to check their antifungal activity against this pathogen. It was observed that all the plant extracts showed significant results. *S. aromaticum* was the most effective in control of *F. oxysporum* f. sp. *Lycopersici* followed by *A. Sativum*, *E. globulus* and *L. camara*. Both the bio-control agents inhibited the growth of fungus *T. harzianum* showed 42.60% growth inhibition and *T. viride* exhibited 36.69% growth inhibition. Application of plant extracts and bio-control agents are cost effective, easily available and eco-friendly for the management of fusarium wilt disease.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is the most important solanaceous vegetable crop after potato. It is cultivated in 140 countries of the world and it is native to South America (Gondal et al., 2012). It stands first in ranking among the processing crops in the world acreage. Tomato is a vital source of antioxidants and is used ordinarily in our daily life (Sgherri et al., 2008). It comprises a water (95.3%), niacin and calcium (0.07%) due to which it has an important role in human metabolic activities. In addition to, it has high nutritional contents providing a source of Vitamin A, C and E, which maintains the health of human being (Jaramillo et al., 2007; Olaniyi et al., 2010). Tomato crop is prone to a number of viral, nematode, bacterial and fungal diseases. Among the fungal diseases, Fusarium wilt is the most devastating disease in the world. It is caused by the fungus *F. oxysporum* f. sp. *lycopersic*i (Sacc.) and causes huge economic losses (Charoenporn et al., 2010). By the attack of this pathogen wilting of seedling and adult plants occurs. Older leaves of plants infected with this pathogen turn into yellow colour. On one side of the plant, yellowing of leaf can occur and progressively many leaves wilt (Ramaiah and Garampalli, 2015). The fungus infects the crop of tomato by contaminating the seed.

Pathogen enters the xylem vessels of plant and cause break down and blockage leading to yellowing, wilting and finally death of plant (Agrios, 2000; Bennett et al., 2008). Conventionally, fungicides are used to control the disease, which increases the resistance of pathogen and have health hazardous effects on human and also on the environment (Özgönen et al., 2001). By application of chemical fungicides *F. oxysporum* f. sp. *lycopersici* becomes resistant to those fungicides, which are used repeatedly. So, there is a dire need to develop of alternative methods to control the disease. For this, plant extracts and bio-control agents are used which are eco-friendly.

Bio-control agents have the ability to control this disease by different mechanisms such as suppression, competition, antibiosis, hypovirulance, direct parasitism, predation and induced resistance. Secondary metabolites production is also involved in the antagonistic activity (Haggag and Mohamed, 2007; Larkin and Fravel, 1998). Application of plant extracts is an alternative method to control the disease and have no toxic effects. Plant extracts have antifungal compounds which inhibit the fungus production. So the current studies were designed to investigate antifungal activities of bio-control agents and plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*.

MATERIALS AND METHODS

For the management of *F. oxysporum* f. sp. *lycopersici* two fungal antagonists and four plant extracts were used under in vitro conditions. The fungal bio-control agents, including *T. harzianum* and *T. viride* were obtained from Plant Pathology Department, University of Agriculture, Faisalabad. The cultures of *Trichoderma* species were retained on Potato Dextrose Agar (PDA) medium at 25 ±1 °C for further use.

Isolation of pathogen: Samples were collected from the tomato plants showing the symptoms of wilting from the vegetable area of Institute of Horticultural Sciences, University of Agriculture Faisalabad. Isolation of pathogen was performed by following the standard methods illustrated by (Machado et al., 2002; Mathur and Kongsdal, 2003). The samples were washed gently with tap water to remove the soil and dirt particles from the surface. The tissues were then cut into small pieces (5mm), surface sterilized with 3% solution of sodium hypochlorite (NaOCL), transferred aseptically on PDA medium and were incubated at 25 ±1°C for 24 hrs. After 24 hrs growth of pathogen was detected and was purified by using single spore technique.

Inhibition of mycellial growth of *F. oxysporum* through plant extracts: The antifungal ability of methanol of various plant extracts was assessed by poisoned food technique described by (Nene and Thapliyal, 1993). Fresh samples from various plants including (*S. aromaticum*, *A. Sativum, E. globulus* and *L. camara*) were obtained and washed with tap water followed by distilled water. The samples were disinfected by using sodium hypochlorite (5%). The samples were then dried in an incubator at 70 ºC for 48 hrs. After drying samples were ground to make powder and 10g of each sample was dissolved in 100 ml of methanol. The samples were placed for 48 hrs in methanol which was then filtered by passing them through double layer filter papers. All the extracts were maintained at 6.5 pH by addition of basic buffer (NaOH) or acid (HCL) solutions. The PDA medium was poisoned by adding 10 ml of each extract into 100 ml of medium. The medium was poured into sterilized Petri dishes and after solidification, these were inoculated with 5 mm block of pathogenic culture in the center of plates. The plates were incubated at 25 ºC and each treatment was replicated three times. However, control was retained by mixing the medium only with distilled water. After 24 hrs the growth of the pathogen in each Petri plate was detected and colony diameter was measured (Vincent, 1947).

Inhibition of *F. oxysporum* by using dual culture technique: The antagonistic bio-control potential of *Trichoderma* spp. was estimated by using dual culture technique (Evans et al., 2003). For this purpose, 5mm disk from a freshly growing culture of the fungal pathogen was taken and was placed aseptically at one corner of Petri plate containing PDA medium and were placed at room temperature (30 °C) for 48 hours. After 48 hrs, the 5mm disk from *Trichoderma* spp. was cut and placed at opposite corner of same plates. The same procedure was repeated for both fungal antagonists to be tested. Petri plates inoculated only by pathogenic fungus served as a control treatment. All the treatments were replicated three times and data was recorded on daily basis. The percent growth inhibition by each antagonist was measured by applying the method of (Korsten and De Jager, 1995).

$$\% \text{ Growth Inhibition (PGI)} = \frac{R - R1}{R} \times 100$$

Where,

R= distance (cm) calculated from point of inoculation to colony edge in control plate and

R_1= distance of colony growth from the point of inoculation to colony margins in the treated petri dish towards the direction of the antagonist.

RESULTS

In vitro evaluation of different plant extracts against *F. oxysporum* f. sp. *lycopersici:* Four plant extracts were used to check their antifungal efficacy against *F. oxysporum* f. sp. *lycopersici*. Mycelial growth diameter was measured after 3, 5 and 7 days. All the tested plant extracts significantly inhibited the mycelial growth of the pathogen. After 7 days, minimum mycelial growth (28.66 mm) was observed by *S. aromaticum* and *E. globulus* (38.66mm), *A. cepa* (34mm) and *L. camara* (45.66 mm) as compared to control (81.33 mm) Figure 1.

Antifungal activity of bio-control agents against fusarium wilt of tomato: Two antagonistic fungal species including *T. harzianum* and *T. viride* were evaluated against *F. oxysporum* under *in vitro* conditions. It was observed that *T. harzianum* has great potential to inhibit the growth of *F. oxysporum*. Among both of the species, after 168 hrs, *T. harzianum* proved best and gave maximum inhibition of pathogenic fungus. After 168 hrs, *T. harzianum* was found best with 42.60% inhibition followed by *T. viride* with 36. 68% inhibition as shown in Table 1.

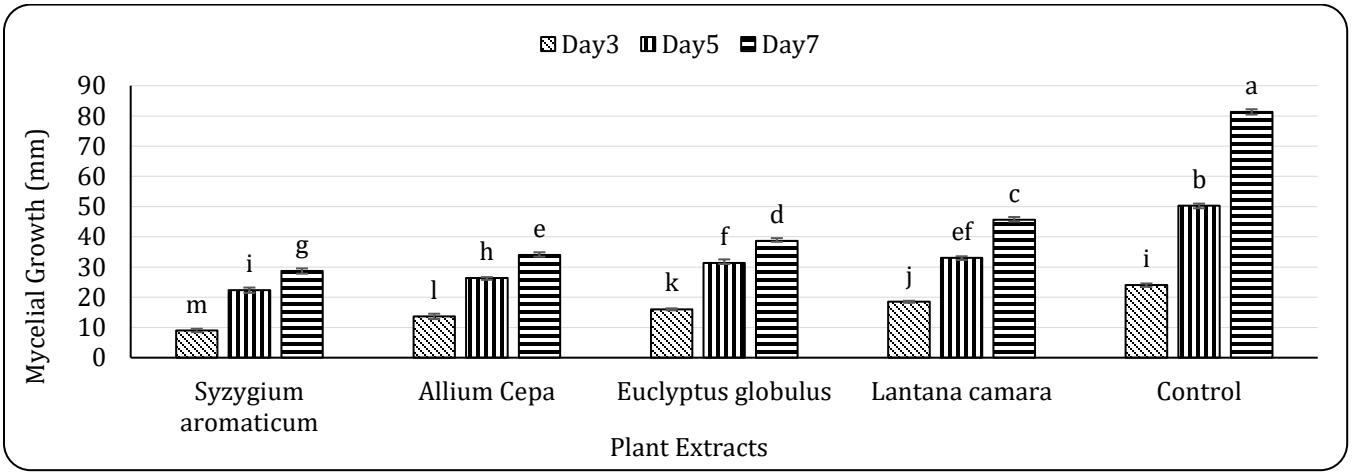

Figure 1. Antifungal activity of different plant extracts against *F. oxysporum* f. sp. *lycopersici.*

Table 1. Antifungal activity of biocontrol agents against *F. oxysporum* f. sp. *lycopercici.*

Treatment	Time (hrs)	Mean Mycelial Growth (cm)	Inhibition (%)
T. harzianum	48	1.95 k	16.30 e
T. viride	48	2.04 j	12.44 f
Control	48	2.33 i	00.00 h
T. harzianum	96	2.79 h	15.45 e
T. viride	96	3.04 f	7.87 g
Control	96	3.30 c	00.00 h
T. harzianum	120	2.94 g	25.75 c
T. viride	120	3.11 e	21.46 d
Control	120	3.96 b	00.00 h
T. harzianum	168	2.97 fg	42.60 a
T. viride	168	3.21 d	36.68 b
Control	168	5.07 a	00.00 h

Mean values sharing similar letters are not significantly different from each other at P≤ 0.05.

DISCUSSION

Currently, fungal diseases are managed through chemicals, but they have toxic effects on environment. So it is need of the time to develop alternative methods for the control of disease. For this reason, the present study was planned to check the antifungal efficacy of different

plant extracts and bio-control agents against *Fusarium oxysporum* f. sp. *lycopersici.*

Present study depicted that all the plant extracts used significantly inhibited the growth of the pathogen. *S. aromaticum* showed the best results, similar results were obtained by the studies of (Mathur et al., 2010). Similar studies were done and it was observed that *S. aromaticum* has more antifungal activity than other extracts (Abo-Elnaga and Ahmed, 2006). *S. aromaticum* has antifungal activities due to the presence of bioactive terpenes, hydrocarbans, phenolic compounds, alcohols and aldehydes (Burt, 2004; Singh et al., 2002). *A. cepa* and *E. globulus* also decreased the growth of the fungal pathogen. (Ramaiah and Garampalli, 2015) also reported the antifungal activity of *A. cepa* against *F. oxysporum* f. sp. *lycopersici. A. cepa* contains antimicrobial activities due to the presence of dimethyl trisulfide and methyl propyl trisulfide (Kocić-Tanackov et al., 2016; Pyun and Shin, 2006; Romeilah et al., 2010).

T. harzianum showed a strong mycoparasitism against *F. oxysporum*. The ability of Trichoderma spp. for the management of plant pathogens has been reported by numerous workers (Sharon et al., 2001; Wells, 1972). *In vitro* impact of *Trichoderma* spp. against species of *Fusarium* has also been reported (Padmodaya and Reddy, 1996). The isolates of *Trichoderma* were also found to inhibit the growth of *Fusarium* in several plant species including muskmelon, wheat and cotton (Sivan et al., 1987). The results of present study are also confirmatory with previous findings made by different scientists. *Trichoderma* species have characteristic to grow rapidly and have ability to suppress the pathogen by competing them for food and habitat (Devi et al., 2012) and also by inhibit pathogen through mycoparasitism (Khirood and Jite, 2012). In the current study, a greatest mycoparasitism was illustrated by *T*. *harzianum* against *F. oxysporum* f. sp. *lycopersici*. *Trichoderma* species produce different kinds of volatile and non-volatile compounds through which they inhibit the growth and development of pathogens (Sumana and Devaki, 2012; Tapwal et al., 2011).

CONCLUSION

Fusarium wilt of tomato can cause huge losses in tomato. It has been depicted from the present studies certain bio-control agents and plant extracts are cost-effective and eco-friendly fungicides against *F. oxysporum* f. sp. *Lycopersici*. So these plants extracts and bio-control agents have good antifungal activity and may be used for formation of new and eco-friendly fungicides.

REFERENCE

Abo-Elnaga, H.I.G., Ahmed, N.G., 2006. Effect of some essential oil and plants extract on controlling *Botrytis allii* the causal pathogen of onion neck rot disease, 9th Arab Congress of Plant Protection, pp. 19-23.

Agrios, G.N., 2000. Significance of plant diseases, Plant Pathology. Academic Press, London, pp. 25-37.

Bennett, R.S., Hutmacher, R.B., Davis, R.M., 2008. Main content area Seed Transmission of *Fusarium oxysporum* f. sp. *vasinfectum* Race 4 in California. Journal of cotton science 12, 160-164.

Burt, S., 2004. Essential oils: Their antibacterial properties and potential applications in foods: A review. International Journal of Food Microbiology 94, 223-253.

Charoenporn, C., Kanokmedhakul, S., Lin, F., Poeaim, S., Soytong, K., 2010. Evaluation of bio-agent formulations to control Fusarium wilt of tomato. African Journal of Biotechnology 9.

Devi, S.S., Sreenivasulu, Y., Saritha, S., Kumar, M.R., Kumar, K.P., Sudhakar, P., 2012. Molecular diversity of nativeTrichoderma isolates against *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.). A casual agent of Fusarium wilt in tomato (*Lycopersicon esculentum* Mill.). Archives of Phytopathology and Plant Protection 45, 686-698.

Evans, H.C., Holmes, K.A., Reid, A.P., 2003. Phylogeny of the frosty pod rot pathogen of cocoa. Plant Pathology 52, 476-485.

Gondal, A.S., Ijaz, M., Riaz, K., Khan, A.R., 2012. Effect of different doses of fungicide (Mancozeb) against alternaria leaf blight of tomato in tunnel. Journal of Plant Pathology and Microbiology 3, 125.

Haggag, W.M., Mohamed, H.A.A., 2007. Biotechnological aspects of microorganisms used in plant biological control. American-Eurasian Journal of Sustainable Agriculture 1, 7-12.

Jaramillo, J., Rodriguez, V., Guzman, M., Zapata, M., Rengifo, T., 2007. Technical manual: Good agricultural practices in the production of tomato under protected conditions. Food and Agriculture Organization, Rome, Italy.

Khirood, D., Jite, P.K., 2012. In-vitro efficacy of *Trichoderma viride* against *Sclerotium rolfsii* and

Macrophomina phaseolina. Notulae Scientia Biologicae 4, 39-44.

Kocić-Tanackov, S., Dimić, G., Mojović, L., Gvozdanović-Varga, J., Djukić-Vuković, A., Tomović, V., Šojić, B., Pejin, J., 2016. Antifungal activity of the onion (*Allium cepa* L.) essential oil against *Aspergillus*, *Fusarium* and *Penicillium* species isolated from food. Journal of Food Processing and Preservation 41, e13050.

Korsten, L., De Jager, E.S., 1995. Mode of action of *Bacillus subtilis* for control of avocado postharvest pathogens. South Africa Avocado Growers' Association Yearb, pp. 124-130.

Larkin, R.P., Fravel, D.R., 1998. Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. Plant Disease 82, 1022-1028.

Machado, J.C., Langerak, C.J., Jaccoud-Filho, D.S., 2002. Seed-borne fungi. Bassersdorf, Switzerland.

Mathur, A., Bhat, R., Prasad, G.B.K.S., Dua, V.K., Verma, S.K., Agarwal, P.K., 2010. Antimicrobial activity of plants traditionally used as medicines against some pathogens. Rasayan Journal of Chemistry 3, 615-620.

Mathur, S.B., Kongsdal, O., 2003. Common laboratory seed health testing methods for detecting fungi.

Nene, Y.L., Thapliyal, P.N., 1993. Fungicides in plant disease control, 3rd edition ed. International Science Publisher.

Olaniyi, J.O., Akanbi, W.B., Adejumo, T.A., Ak, O.G., 2010. Growth, fruit yield and nutritional quality of tomato varieties. African Journal of Food Science 4, 398-402.

Özgönen, H., Biçici, M., Erkiliç, A., 2001. The Effect of salicyclic acid and endomycorrhizal fungus *Glomus etunicatumon* plant development of tomatoes and Fusarium wilt caused by *Fusarium oxysporum* f. sp *lycopersici*. Turkish Journal of Agriculture and Forestry 25, 25-29.

Padmodaya, B., Reddy, H.R., 1996. Screening of *Trichoderma* spp. against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt in tomato. Indian Journal of Mycology and Plant Pathology 26, 266-270.

Pyun, M.S., Shin, S., 2006. Antifungal effects of the volatile oils from allium plants against *Trichophyton* species and synergism of the oils with ketoconazole. Phytomedicine 13, 394-400.

Ramaiah, A.K., Garampalli, R.K.H., 2015. In vitro antifungal activity of some plant extracts against *Fusarium oxysporum* f. sp. *lycopersici*. Asian Journal of Plant Science and Research 5, 22-27.

Romeilah, R.M., Fayed, S.A., Mahmoud, G.I., 2010. Chemical compositions, antiviral and antioxidant activities of seven essential oils. Journal of Applied Science and Research 6, 50-62.

Sgherri, C., Kadlecová, Z., Pardossi, A., Navari-Izzo, F., Izzo, R., 2008. Irrigation with diluted seawater improves the nutritional value of cherry tomatoes. Journal of Agricultural and Food Chemistry 56, 3391-3397.

Sharon, E., Bar-Eyal, M., Chet, I., Herrera-Estrella, A., Kleifeld, O., Spiegel, Y., 2001. Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Phytopathology 91, 687-693.

Singh, G., Singh, O.P., Maurya, S., 2002. Chemical and biocidal investigations on essential oils of some Indian *Curcuma* species. Progress in Crystal Growth and Characterization of Materials 45, 75-81.

Sivan, A., Ucko, O., Chet, I., 1987. Biological control of Fusarium crown rot of tomato by *Trichoderma harzianum* under field conditions. Plant Disease 71, 587-592.

Sumana, K., Devaki, N.S., 2012. In vitro evaluation of some bioagents against tobacco wilt pathogen. Journal of Biopesticides 5, 18-22.

Tapwal, A., Singh, U., Singh, G., Garg, S., Kumar, R., 2011. In vitro antagonism of *Trichoderma viride* against five phytopathogens. Pest Technology 5, 59-62.

Vincent, J.M., 1947. Distortion of fungal hyphæ in the presence of certain inhibitors. Nature 159, 850-850.

Wells, H.D., 1972. Efficacy of *Trichoderma harzianum* as a biocontrol for *Sclerotium rolfsii*. Phytopathology 62, 442-447.