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IN VITRO EVALUATION OF COMPATIBILITY OF INDIGENOUS *TRICHODERMA HARZIANUM* WITH BOTANICALS

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Abstract: The plant leaf extracts were evaluated for their compatibility with *Trichoderma harzianum* at 15 and 30 % concentrations under *in vitro* conditions for their possible use in integrated disease management. The plant leaf extracts had little inhibitory effect on the mycelia growth of *T. harzianum.* However, the plant extracts reduced the sporulation of antagnostic fungus to varying degree. Least inhibition in sporulation was observed with *Mentha arvensis* (mentha)*, Vitex negundo* (banna) and *Adhatoda zeylanica* (basuti). Hence, there is scope to integrate *T. harzianum* with *Mentha arvensis*, *Adhatoda zeylanica* and *Vitex negundo* for ecofriendly management of plant diseases. Sporulation of *T. harzianum* was either completely or severely inhibited with *Allium sativum* (garlic) and *Melia azedreck* (darek). Hence *T. harzianum* is not compatible with *A. sativum* and *M. azedreck*. The present results will help delineate the possibility of combining *T. harzianum* biocontrol agent and plant extracts for use in an integrated disease management approach.
Keywords: *Trichoderma*, compatibility, plant extract, sporulation, botanicals.

Introduction

Soil microorganisms influence ecosystems by contributing to plant nutrition, plant health, soil structure and soil fertility. *Trichoderma*, commonly available in soil and root ecosystems has gained immense importance since last few decades due to its biological control ability against several plant pathogens. Different species of *Trichoderma* reduce growth, survival or infections caused by plant pathogenic fungi and bacteria by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion (MacKenzie and Starman, 1995, Suleman et al., 2008, Chakraborty et al., 2013). Extracts of many botanicals are also used against many plant pathogenic fungi and bacteria. Bioagents and botanicals are important components in integrated disease management programme and sustainable agriculture. The combined use of biocontrol agents and phytoextracts has attracted much attention as a way to obtain synergistic effects in the control of plant pathogens. With this view, a compatibility study was conducted to investigate the effect of

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various plant extracts on growth of *Trichoderma harzianum*. This compatibility study will be helpful in designing framework of integrated disease management program in future.

Material and Methods

Preparation of botanical extracts

Fresh leaves of test plants namely *Allium sativum (*garlic), *Melia azedreck* (darek), *Allium cepa* (onion), *Mentha arvensis* (mentha), *Adhatoda zeylanica* (basuti) and *Vitex negundo* (banna) were taken for preparing crude extracts. The leaves were thoroughly washed with water and fine slurry was prepared by taking 100g leaves with minimum quantity of distilled water and grinding in sterilized Pestle and Mortar. The resultant slurry was filtered through double layered muslin cloth and final volume of the extracts was made to 100 ml by adding required sterilized water. These were used as stock solution of 100 % concentraion. The plant extracts were evaluated at two concentrations i.e., 15%, and 30% against the tested antagonistic fungus.

Compatibility of the botanical extracts with *T. harzianum*

The soil from the rhizosphere of tomato plants grown at the experimental farm of the department of Plant Pathology, Dr YS Parmar University of Horticulture & Forestry, Nauni, Solan, HP and used for isolation of *Trichoderma harzianum* through serial dilution method on Potato Dextrose Agar (PDA) medium. The identification was confirmed according to the identification key (Rifai, 1969) based on the branching of conidiophores, shape of phialides, emergence of phialospores and shape of phialospores. The culture was maintained on the same medium at 28± 2°C. The plant extracts were tested *in vitro* by using the Poisoned Food Technique (Falck, 1907) in Completely Randomized Design (CRD) to study their inhibitory effect on mycelia growth and sporulation of *T. harzianum*. Double strength potato dextrose agar medium was prepared in distilled water and sterilized in autoclave at 15 psi pressure and 121^0 C for 20 minutes. Simultaneously, double concentrations of plant extracts were also prepared in sterilized distilled water and were mixed with double strength potato agar medium aseptically to achieve the desired concentrations i.e. 15 % and 30 % and poured into Petri plates. After the solidification of medium, these plates were inoculated with the 2 mm diameter mycelial bit of antagonistic fungus taken from actively growing 5-days old culture. A control treatment was also maintained in which only plain sterilized distilled water was added to double strength medium. Each treatment was in five replicates. The inoculated plates were incubated at 28± 2°C. The observation was recorded in the form of radial growth of fungus in millimeter (mm) until the control plates were fully covered with the mycelium. The percent growth inhibition was calculated as described by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

where, I = Per cent mycelia growth inhibition

C = Mycelial growth of fungus in control (mm)

T = Mycelial growth of fungus in treatment (mm)

The effect of plant extracts on the sporulation of the fungus was also recorded. Five disc of 5 mm diameter were cut out from each of the replication of each treatment using sterile cork borer and mixed with 5 ml sterile water, for a uniform concentration. The spore count was taken using a haemocytometer and number of spores per ml was counted. Percent reduction in sporulation was calculated as per the above mentioned formula of Vincent (1947).

Statistical Analysis

The data recorded were subjected to statistical analysis. The differences exhibited by the treatments in various experiments were tested for their significance by employing Completely Randomized Design (CRD) as per the details given by Gomez and Gomez (1983).

Results and Discussion

The extracts of different plants were found to have little inhibitory effect on the mycelial growth of the native *T. harzianum* which was isolated from tomato rhizosphere soil (Table 1). Least inhibition (1.03%) of mycelial growth of the test antagonist was observed with *Adhatoda zeylanica* leaf extract at 15 % concentration which was found to be highly compatible with antagonist. *Mentha arvensis* and *Vitex negundo* extracts were also compatible with the test antagonist with 2.31 % and 2.56 % mycelia inhibition respectively at 15 % concentration. The inhibition in mycelia growth was maximum with *Allium sativum* (27.18 %) followed by *Allium cepa* and *Melia azedreck* at 30 % concentration. No significant difference was observed between *M. arvensis*, *A. zeylanica* and *V. negundo* for percent inhibition in mycelia growth at 30 % concentration. The sporulation of *T. harzianum* was completely inhibited with garlic at 30 % concentration (Table 1) while sporulation was severely reduced (77.55 %) at 15 % concentration. This was followed by *M. azedreck* (73.58 %) at 30 % concentration. Least inhibition (4.61 %) in sporulation was observed with *V. negundo* at 15 % followed by *V. negundo* (15.68 %) at 30 % concentration. This was followed by *M. arvensis* (18.39 %) and *A. zeylanica* (23.17%). This indicates that *M. arvensis*, *A. zeylanica* and *V. negundo* are compatible with *T. harzianum* even at 30 % concentration while *A. sativum* and *M. azedreck* were not compatible with the test antagonist. Few plant extracts are reported to be compatible with the growth of *T. harzianum* (Rajappan et al., 2000; Meena Devi and Paul, 2005). *T. viride, T. harzianum* and *Glioctadium virens* were found to be compatible with *Clerodendron* leaf extract at a concentration of 15 per cent while *Bougainvillea* leaf extract, lantana leaf extract, neem leaf extract, garlic bulb extract and onion bulb extract were found to inhibit the growth of the test antagonists (Verma and Gandhi, 2007). Leaf extract of karanj leaves (*Pongamea pinnata*) and cumin leaves inhibited the growth of *T. harzianum* and *T. viride* whereas neem oil, leaves extract of neem and wild sorghum enhanced the growth of antagonists (Bagwan, 2010). Water extracts of botanicals viz., *Parthenium hysterophorus, Urtica dioeca, Polystichum squarrosum* and *Adiantum venustum* were compatible with *T. viride* but not with *Cannabis sativa* (Tapwal et al., 2012). Neem seed kernel extract, Eucalyptus leaf extract and Pongamia leaf extract were compatible and nimbicidine and Prosophis leaf extract were inhibitory for growth of *T. viride* and *T. harzianum* (Bheemaraya et al., 2012).

Table1. *In vitro* evaluation of compatibility of *T. harzianum* to plant extracts

Plant extract	**Per cent inhibition in mycelial growth**			**Per cent reduction in sporulation**		
	15 %	**30 %**	**Mean**	**15 %**	**30 %**	**Mean**
Allium sativum	18.97 (25.81)	27.18 (31.41)	23.08	77.55 (61.70)	100.00 (89.96)	88.84
Mentha arvensis	2.31 (8.73)	6.67 (14.96)	4.49	18.39 (25.39)	37.17 (37.55)	27.85
Allium cepa	17.44 (24.67)	25.64 (30.41)	21.54	34.41 (35.90)	52.33 (46.32)	43.43
Vitex negundo	2.56 (9.21)	8.21 (16.64)	5.38	4.61 (12.41)	15.68 (23.32)	10.28
Melia azedreck	17.95 (25.06)	22.05 (27.99)	20.00	53.39 (46.93)	73.58 (59.05)	63.48
Adhatoda zeylanica	1.03 (5.81)	5.64 (13.73)	3.33	23.17 (28.76)	40.43 (39.47)	31.88
CD	11.59	14.34		10.17	8.98	

Conclusions

It is essential to adopt an integrated approach involving antagonistic organisms, fungicides and aqueous plant extract for effective disease management. This finding indicates that seed treatment, furrow and foliar applications of *Trichoderma harzianum* is compatible to most of the botanicals except *A. sativum* and *M. azedreck* therefore commercial farmers can use it in the integrated disease management to safe guard the environment, reduce the human health hazards and also cut down the unwanted pesticide cost especially against oil-borne pathogens which are otherwise difficult to manage.

References

[1] Bagwan, N.B. 2010. Evaluation of *Trichoderma* compatibility with fungicides, pesticides, organic cakes and botanicals for integerated management of soil borne diseases of soybean [*Glycine max* (L.) Merril]. *International Journal of Plant Protection* 3(2): 206-209.

[2] Bheemaraya, Patil, M.B., Ramesh, Vendan, K.T., Amaresh, Y.S. and Rao, K. 2012. Compatibility of *Trichoderma* spp. with commonly used fungicides, insecticides and plant extracts. *Indian Journal of Plant Protection* 40(2): 118-122.

[3] Chakrabarty, R., Acharya, G.C. and Sarma, T.C. 2013. Effect of fungicides, *Trichoderma* and plant extracts on mycelial growth of *Thielaviopsis paradoxa*, under *in vitro* condition. *The Bioscan* 8(1): 55-58.

[4] Falck, R. 1907. Wachstumgesetze, Wachstm-taktoren und temperature Wertder helzersterenden. *Mycelien* 1:43-154.

[5] Gomez, K.A. and Gomez, A. 1983. Statistical procedure for agricultural research. IInd Ed. John Wiley & Sons, New York pp 357-427.

[6] MacKenzie, A.J. and Starman, T.W. 1995. Enhanced root and shoot growth of chrysanthemum cuttings propagated with the fungus *Trichoderma harzianum*. *Hort Science* 30(3):496–498.

[7] Meena Devi and Paul Y. S. 2005. Management of pea wilt/ root rot complex by integrating plant extracts and biocontrol agents. Integrated plant Disease management Challenging problems in horticultural and forest pathology, Solan, India, 14-15 November 2003. Pp 101-105.

[8] Rajappan, K., Mariappan, V. and Kareem, A.A. 2000. Effect of solvent free EC formulation of neem oil on beneficial microorganisms and fungal pathogen of rice. *Annals of Plant Protection Science* 8: 98-100.

[9] Rifai, M.A. 1969. A revision of the genus *Trichoderma*, Mycological paper, No. 116. Commonwealth Mycological Institute, Association of Applied Biologists, Kew, Surrey, England.

[10] Suleman, P., Abdullah, M.T. and Ali, Y.N. 2008. Biological control of *Sclerotinia aclerotiarum* (Lib) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquifaciens*. *Crop Prot*ection 27: 1354-1359.

[11] Tapwal, A., Kumar, R., Gautam, N. and Pandey, S. 2012. Compatability of *Trichoderma viride* for selected fungicides and botanicals. *International journal of Plant Pathology* 3(2): 89-94.

[12] Verma, P.K. and Gandhi, S. K. 2007. Bioefficacy of some plant extracts and biocontrol agents against *Alternaria solani* and their compatibility. *Plant Disease Research* 22 (1): 12-17

[13] Vincent, J.M. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 150:850.