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J. Mycopathol, Res, 57(1) : 49-51, 2019;
ISSN 0971-3719

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Mycoparasitic *Trichoderma viride* and *T. harzianum* as biocontrol agents against fungal pathogens causing diseases of rhizome of Ginger (*Zingiber officinale* Rosc.)

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Received : 05.04.2019 Accepted : 05.04.2019 Published : 29.04.2019

Isolates of *Trichoderma viride* and *T. harzianum* isolated from soil of crop field of healthy plants were selected for screening the antagonistic effect against seven fungal pathogens causing diseases of rhizome of ginger by duel culture technique. Although both antagonists were proved as effective biocontrol agents, *T. viride* was found to be more effective than *T. harzianum* against all the fungal pathogens and maximum inhibition was reported in *F. oxysporum* as 76% and 57% respectively. Isolates of *Trichoderma viride* and *T. harzianum* isolated from soil of crop field of healthy plants were selected for screening the antagonistic effect against seven fungal pathogens causing diseases of rhizome of ginger by duel culture technique. Although both antagonists were proved as effective biocontrol agents, *T. viride* was found to be more effective than *T. harzianum* against all the fungal pathogens and maximum inhibition was reported in *F. oxysporum* as 76% and 57% respectively.

Key words: *Trichoderma*, antagonistic effect, ginger, dual culture technique

INTRODUCTION

Ginger (*Zingiber officinale* Rosc) (Family: Zingiberaceae) is a herbaceous perennial, the rhizomes of which are used as a spice and medicine in almost all parts of world. It has been traditionally used as antimicrobial, antioxidant, anti-inflammatory, anti-fungal agents. India is a leading producer of ginger in the world. In India, ginger is grown in almost all the states.

The main producing states in India are Assam, Kerala, Gujarat, Mizoram, Sikkim, Orissa, Arunachal Pradesh and Meghalaya. A huge amount of ginger is affected by fungi in storage condition despite of having its own antifungal property (Sharififar *et al*. 2009) It is reported that more than fifteen fungi are responsible for rhizome rot of ginger and *Aspergillus flavus*, *A. fumigatus, A. niger, Fusarium oxysporum, Monilia fructicola, Penicillium italicum* and *Rhizopus stolonifer* are among them. Fungicides are the primary means of controlling post-harvest diseases. Further, the use of synthetic chemicals to control postharvest deterioration has been restricted due to their carcinogenicity, teratogenicity, high and acute residual toxicity, long degradation period, environmental pollution and their direct effects on food and other side effects on humans.Another problem with these synthetic chemicals is that as their potency has been enhanced, so have been their side effects and their cost. In addition, synthetic fungicides can leave significant residues in treated commodities. Development of resistance to commonly used fungicides within populations of postharvest pathogens has also become a significant problem. The side-effects of synthetic fungicides means that alternative strategies need to be developed for reducing losses due to postharvest decay that are perceived as safe by the public and pose negligible risk to human health and environment. Recently, several promising biological approaches that include microbial antagonists which has been advanced as potential alternatives to synthetic fungicides to control postharvest decay of fruits and vegetables.

Antagonistic *Trichoderm*a species are considered as promising biological control agents against numerous phytopathogenic fungi. *Trichoderma*

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species are successful biocontrol agent of storage fungal pathogens and it shows its efficiency at relatively low concentration. *T. harzianum* strains were generally found to be effective at low concentration of 106-108 conidia/ml. These concentrations are even lower than the recommended concentrations of any other biocontrol agents. In the present study, potential of *T. viride* and *T. harzianum* were tested for the in vitro efficacy against isolated fungal pathogens from rhizome of ginger and found to be more or less effective against the pathogens.

MATERIALS AND METHODS

Isolation of *T harzianum* and *T viride*

Soil sample from crop field of healthy plants were collected. Soil samples collected in plastic bags were brought to the laboratory of IASST. It was dried at 20-25°C and the antagonistists were isolated on a selective culture media following serial dilution plate method with slight modification. *Trichoderma* spp. were identified on the basis of some important macro and microscopic characteristics (Table 1).

Test fungi

Isolation of fungi from infected rhizomes of ginger has been done according to the method described earlier. Later the predominating microfungi have been transferred to fresh agar slant for growth of pure culture and accordingly identification has been done by observing their morphological characteristics with the help of available literature. All the isolated pathogens have been subjected to pathogenecity test as to confirm whether the isolates (fungi) can actually cause disease or not.

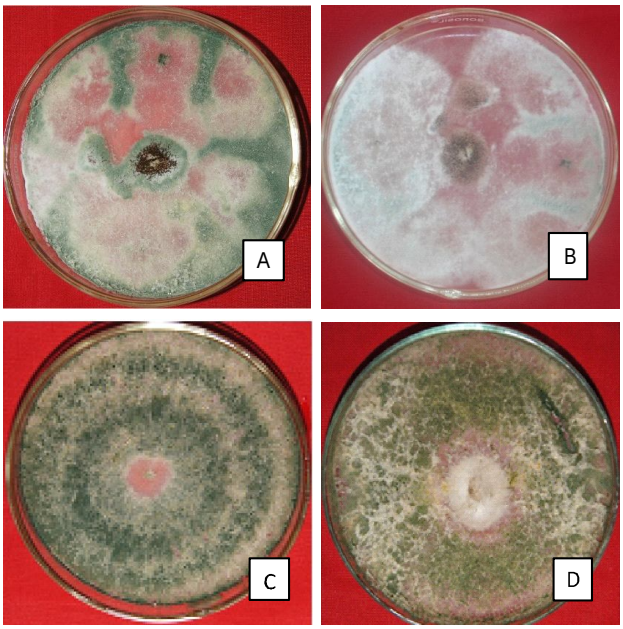

Fig. 1 : A-*In vitro* control of *A. niger* by *T. viride*, B-*In vitro* control of *A. niger* by *T. harzianum*, C-*In vitro* control of *F. oxysporum* by *T. viride*, D-*In vitro* control of *F. oxysporum* by *T. harzianum*

Preparation of fungal inocula

The organisms isolated from ginger were sub cultured and maintained in Potato dextrose agar (Himedia) slants at 4°C. For the experiment, freshly cultured slants were used for preparing spore suspension in 0.9% saline water. The fungal spore suspension was adjusted to give a final concentration of 1-5x10^5 cfu/ml.

In vitro evaluation of T. harzianum and T. viride against test fungi

In vitro evaluation of *T. harzianum* and *T. viride* for antagonistic activities against the fungi causing diseases of ginger was done by Dual Culture. Mycelial discs (5mm), each of bio-agents and the

Table 1: Characterizations and identification of *Trichoderma* species isolated from soil

Macro- and microscopic characteristics	*Trichoderma* spp.
At the early stage whitish to greenish mycelia appeared. Next, a deep green colour developed in central part and gradually extended to the periphery. Finally, it appeared a whitish green colour. Mostly globose to subglobose conidia size 2.8x2.6µm., developed on 5.0x2.6µm. flask shaped phialides, produced in the opposite direction in each point.	*T. harzianum*
Initially the colony colour was observed to be whitish to light green, watery in centre. Next, the colony gradually became deep grass green in colour and looked soft and leathery to the naked eye. The conidiophores were erect, smooth, penicillately branched; asymmetrical branches singly or vertically arranged at different levels, globose conidia, 3.0x2.8µm, phialides of 3.0x6.2µm. were slender, coverage toward the main branch, emphasizing the penicillate branching.	*T. viride*

Table 2: Per cent inhibition of the test fungi by the antagonistic activity of *T. harzianum* and *T. viride*

Phytopathogenic fungi	Per cent inhibition	
	T. harzianum	*T. viride*
Aspergillus flavus	31.5±0.6	41.8±1.1
A. fumigatus	26.5±0.6	32.4±0.7
A. niger	49.2±0.7	56.3±0.9
Fusarium oxysporum	56.7±0.8	75.6±0.4
Monilia fructicola	23.4±1.1	26.9±0.5
Penicillium italicum	28.9±0.7	38.2±0.7
Rhizopus stolonifer	45.3±1.1	52.4±0.7

All values are the mean ± SE (triplicate measurements)

pathogen were taken from the margins of their actively growing culture and transferred to PDA media in the Petriplates. Three replicas for each evaluation were prepared for duel culture. Control plates were set by the pathogens separately each in individual media. Dual petriplates were subsequently incubated at 28±2^{0}C. in BOD incubator. Culture diameters of the test fungus up to the inhibition are recorded in case of both bio-agents. The percentage growth inhibition of the test pathogens were calculated over control.

Per cent inhibition = (C – D) / C x 100, where, C-Area covered by the pathogen in control and D-Area covered by the pathogen in dual culture.

RESULTS AND DISCUSSION

In vitro evaluation of *T. harzianum* and *T. viride* against the tast fungi is presented in Fig 1. The antagonistic activity of *T. harzianum* and *T. viride against* the isolated fungi from diseased rhizome of ginger is presented in Table 2. *T. viride* was found to be more effective than *T. harzianum*. Maximum inhibition was reported in *F. oxysporum* (76% and 57%) followed by *A. niger* (56% and 49%), *R. stolonifer* (52% and 45%), *A. flavus* (42% and 32*%), P. italicum* (38% and 29%), *A. fumigatus* (32% and 27%) and *M. fructicola* (27% and 23%). The result of the ANOVA analysis of the data showed the variation on antagonists and their action on the pathogenic fungi was highly significant at 1% level.

The results indicated that *T. harzianum* and *T. viride* strains can be used as a biocontrol agent against the fungi isolated from diseased rhizomes of ginger. Antibiotic production, mycoparasitism, the production of cell wall-degrading enzymes and competition for nutrients or space are considered as the actions involved in biocontrol of pathogen (Zeilinger *et al*. 2007; Vinale *et al*. 2008). During mycoparasitic interactions between Trichoderma and fungal pathogen, a diffusible factor released from the host before physical contact was responsible for induction of hydrolytic enzymes(Zeilinger *et al*.2007). It proved the destructive mycoparasitic action of these antagonists against fungal pathogens. Further study can be aimed on the search of suitable inert and eco-friendly ingredients for novel formulations to make the production technology more promising.

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