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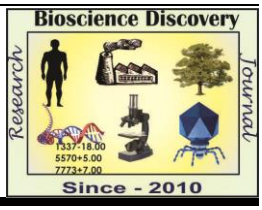

Antagonistic Capability combined *Trichoderma harzianum and T. Pseudokoningii* against *Alternaria alternata* (Fries) Keissler causing leaf spot of Gerbera

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Abstract

Gerbera is the ornamental plant suffering from the leaf spot caused by *Alternaria alternata*. Present ecofriendly studies have confirmed the importance and effectiveness of the *Trichoderma* species against many fungal phytopathogens In the present study investigation has been made on the bio control effects of *Trichoderma harzianum* alone ,in combination with *Trichoderma pseudokoningii* and alternate use were evaluated against the *Alternaria alternata* causing leaf spot of gerbera. In the continuous passage *Trichoderma harzianum* used alone against the pathogen up to seventh passage. In alternate passage *Trichoderma harzianum* used alternately with *T. pseudokoningi* and in the Mixed passage both the *Trichoderma* Sp. used combindly and Screened against the pathogen by dual culture technique. Among these, alternate and combined used of *Trichoderma sp.* shown the significant reduction in the growth of the pathogen. 84.33% and 78.66 % of growth inhibition was found in the combined and alternate use *Trichoderma harzianum*. While in continuous passage or alone use increased the *Trichoderma harzianum resistance* in the Pathogen.

INTRODUCTION

Gerbera (*Gerbera jamesonii* H. Bolux ex J. D. Hook) is an important ornamental plant cultivated throughout the India. All floricultural plants increasing the economy of the world. Among all gerbera is one of them. The cut flowers of gerbera are 5th rank in the use. Such commercially important ornamental plant is affected by several fungal diseases. Leaf spot caused by *Alternaria alternata* (Fries) Keissler is problematic to the plant. This disease is managed by carbendazim and other fungicides, but indiscriminate use of fungicides increased resistance in pathogen and disturbed the ecological balance (Dekker, 1982; Gangawane, 1990). So cultivars turned towards eco friendly disease management. *Trichoderma* is used for better management of various foliar and soil borne plant pathogens. (Mohanan, 1996). Number of workers reported the fungicide resistance in the *Trichoderma* (Bikila , 2015; Tapwal, 2012; Bhai and Thomas, 2010; Priti and Venkataravanappa 2017). In the present work study has been made on the effect of different passages on the on the antagonistic potentia l of Trichoderma sp. against the *Alternaria Alternata* causing leaf spot of gerbera.

MATERIALS AND METHODS

Isolation of pathogens: Naturally infected samples of gerbera were collected from widely cultivated fields and polyhouses.

They were brought to the laboratory in clean, sterilized polythene bags. Infected leaves were cut into 2-3mm pieces, surface disinfected with 70% alcohol for 2-3 minute washed three times with sterile deionized H_2O and inoculated on rose leaf agar medium plates amended with 30mg/L streptomycin sulphate. The plates were incubated under 12h cycles of lightness and darkness for 7days. On 8th day the plates were screened for the pathogen. The fungi were identified with the help of relevant Mycological literature (Subramanian, 1972; Barnett and Hunter, 1972). Culture tubes were maintained at 4ºC and used for study whenever necessary.

Isolation of *Trichoderma* sp.

Rhizosphere soils from garden were collected from and *Trichoderma* sp. were isolated by dilution plate technique (Johnson,1957) and grown on CDA medium (Ricker and Ricker, 1936). The isolated species of Trichoderma were identified by following the available literature(Nagamani and Manoharachary, 2002) . Pure cultures of *Trichoderma* were maintained on the CDA medium for further study in BOD Incubator

Screening of *Trichoderma* sp. against pathogen by dual culture method

Antagonistic potential of *Trichoderma* spp. was evaluated against *Alternaria alternata* by dual culture technique Followed (Morton and Stroube,1955)**.** The growth inhibition was calculated followed the formula (Vincent ,1947)

I= 100 x C-T/C,

Where I= Inhibition of growth,

C= Radial growth of pathogen in control set,

T= Radial growth of pathogen in treated set

Continuous passage-

In the continuous passage used the alone *Trichoderma harzianum* against the pathogen up to seven passage. For this 8 mm diameter agar disc from the seven days old actively growing mycelium of *Trichoderma harzianum* and *Alternaria alternata* was placed on the solidified CDA plate at the opposite sides. Each set was made in the triplicate. After 5 days of the inoculation measured the radial growth of both *Trichoderma* and *Alternaria* and find the percent growth inhibition followed by (Vincent 1974).

Alternate passage

To study the effect of alternate passage used the *Trichoderma harzianum* and *T. Pseudokoningii* alternately against the pathogen and find out the growth inhibition percentage. In the alternate use the 8mm mycelium disc of *Alternaria alternata* was selected from the plate which was first screened with *T. harzianum.*

Mixed passage

In this 10 ml of spore suspension of each Trichoderma harzianum and T. pseudokongii welmixed into 100 ml conical flask. After 10 minutes inoculate the mixed spore suspension on the Solidified agar plate and obtained the combined mycelium of both Trichoderma sp. in a single plate. After four days took the 8mm combined mycelium disc of Trichoderma sp and Alternaria alternata placed on the opposite side of the another freshly prepared CDA plate. After 7 days of the inoculation measured the radial mycelial growth of mycelium and find out the percent growth inhibition.

RESULT AND DISCUSSION

The results of the antagonistic potential of use of different combination of *Trichoderma harziaunm* shown in (Table1.) Data showed that combined and alternate use of both *Trichoderma harzianum* and *T. pseudokoningii* shown the good antagonistic potential than the alone use of *Trichoderma* against *Alernaria alternata.* These results are in agreement with the Mohammad and et.al (2011).similarly Elad and et .al. (1993) studied the alternate and combined use of *Trichoderma harzianum* with other fungicide against the *Botrytis cinerea* causing the cucumber gray mold.

Table 1. Antagonistic Capability of different combination of *Trichoderma Harzianum* against *Alternaria alternata*.

Mode of use of *Trichoderma harzianum* against Pathogen	Percent of Growth Inhibition
Continuous or alone use of *Trichoderma Harzianum*	72.00%
Alternate Use of Trichoderma harzianum and T. Pseudokongii	78.66 %
Mixed or Combined use of Both Trichoderma sp.	84.33%

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