RESEARCH ARTICLE

Efficacy of *Trichoderma* against violet root rot disease of tea caused by *Sphaerostilbe repens*

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ABSTRACT

Trichoderma viride (*T. viride*) and *Trichoderma harzianum* (*T. harzianum*) were screened against *Sphaerostilbe repens* (*S. repense*) causing violet root rot disease of tea through in vitro and in vivo method. In dual culture method, *T. viride* lysed *S. repense* and *T. harzianum* also ceased the growth of the pathogen. In case of field trials three varieties of tea TV-26 (Tocklai Variety), TV-18 and T-78 were taken. Antagonists and pathogen were applied in the rhizosphere in different combination and disease index was made after different days of interval. Rhizosphere of potted plants of one susceptible variety Teenali-17 were treated with same combination of antagonist and pathogen and efficacy of antagonists were proved through immunodiagnostic method like ELISA and DOT-BLOT.

Key words: Biocontrol agents**,** Tea, *Trichoderma*, *Sphaerostilbe repens*.

INTRODUCTION

Tea (*Camellia sinensis*) (L) O. Kuntze is perennial plant with an average life span of more than sixty years. During this long span of life it is prone to attack by many pathogens, pest, deficiencies which call for timely remedial measure. One common root disease of tea in the area of water logging with poor aeration is violet root rot caused by *Sphaerostilbe repens.* The most common means to check the disease by using fungicides but frequent and indiscriminate use of fungicide leads to pollution of environment and development of fungicides resistance in pathogens. In this context biological control is an alternative strategy for disease management which is also ecology conscious and environment friendly. Several organisms like *Trichoderma* spp, *Gliocladium*, and many other soil inhabitants are used to control a number of soil borne plant pathogens (Papavizas 1985). Hence, in the present study attempts were made to assess the effect of *Trichoderma viride* and *T. harzianum* on *S. repense* causes violet root rot of tea in Lab and land condition.

MATERIAL AND METHODS

***In-vitro* evaluation of biocontrol agents:** Antagonistic properties of *T. viride* and *T. harzianum* were evaluated against test pathogen through dual culture technique (Morten, and Stroube, 1955). Mycelial discs of 6mm diameter cut from the margin of 5 days old cultures both test pathogen (*S. repense*) and antagonists were placed opposite to each other on PDA in petriplates (9 cm dia.). The distance between inoculum blocks was 6cm. The pathogens and antagonists were placed in the same day. Control plates were also prepared both for pathogen as well as for antagonists and three replicates were performed for each treatment.

Preparation of inoculums and rhizosphere infestation :

The inoculum of pathogen and antagonists were prepared in sand maize meal media (media prepared as described by Biswas and Sen, 2000). Two year old tea plants (Teenali-17) were taken in earthen pots (12") containing 5 kg soil and allowed to establish for two weeks with regular watering and 100gm of *Trichoderma* inoculum was added carefully in the rhizosphere of each plant. According to the design of the experiment 100 gm of pathogen's inoculum was added to rhizosphere of tea plant 10 days after inoculation of antagonists. Experiments were designed considering different combination as follows:

a) pathogen only, b) *T. viride*, c) *T. harzianum*, d) *S. repense* + *T. viride*, e) *S. repense* + *T. harzianum* and f) healthy plants. Disease assessment and serological detection was done after 30 days of pathogen inoculation. Disease assessment were done maintaining the following disease index : 0-No symptoms 1-plants look sick, root surface sarteted roughening in patches, 2-leaves look yellowish with light black patches appeared on roots, 3- defoliation started and random inky black patches on root, 4-random defoliation with 70% blackening of root, 5- total defoliation with 70-85% blacking of root, 6-total defoliation, 85-100% blackening with drying of roots. Inoculation of field grown plants were done in the same way as that of potted plants where 300gm of inoculum was taken instead of 100gm and three varieties of tea TV-26, TV-18 and T-78 were taken for field experiment. Disease assessment done after 15, 30 and 45 days after pathogen inoculation and serological detection was performed preparing antigen 30 days after pathogen infestation.

Serological Detection:

DAC-ELISA (Direct Antigen Coating- Enzyme Linked Immuno Sorbent Assay) of root (root of inoculated potted plants) antigen and soil antigen were done following the method of Chakraborty *et al* (1995). Dot-blot of soil antigen was performed following the method Lange *et al* (1989).

RESULTS AND DISCUSSION

***In-vitro* and *In-vivo* evaluation of biocontrol agents**

Both *T. viride* and *T. harzianum* in dual culture inhibit the mycelial growth of the pathogen. *T. viride* overgrew the pathogen and lysed it over a period of time while *T. harzianum* form an inhibition zone around it so that pathogen was not able to grow further (Plate1:D&E). The mechanism of parasitism by *T. viride* and *T. harzianum* was also observed by Padmodaya and Reddy (1996), Kumar and Dubey (2001) and so many other authors.

Table 1: DAC-ELISA of tea root antigens with PAb of *S. repens* following treatment with biocontrol agents

Antigen source (Tea root)	Absorbance at 405nm			
	Expt. 1	Et. 2	Expt. 3	Mean
Healthy	0.492	0.501	0.500	0.497 ± 0.07
S. repens	0.837	0.850	0.823	0.837 ± 0.067
T. harzianum+ S. repens	0.605	0.600	0.616	0.607 ± 0.052
T. viride + S. repens	0.552	0.549	0.546	0.549 ± 0.031
T. harzianum	0.500	0.508	0.503	0.504 ± 0.036
T. viride	0.495	0.494	0.500	0.497 ± 0.03

Root antigens were made 40 days after amendment with biocontrol agents and 30 days after inoculation with *S. repens* of potted Teenali-17 variety of 2 years old tea plants.
± Standard error.

Table 2: Dot-blot of different soil antigen collected from root rhizosphere (pots) infested with different combination of *Trichoderma* and *S. repens*

Antigen source	Intensity of colours		
	PAbs		
	S. repens	*T. harzianum*	*T. viride*
Mycelia			
S. repens	++++	+	+
T. harzianum	++	++++	++
T. viride	++	+	++++
Soil inoculated with *S. repens*	++++	–	–
T. harzianum	–	++++	–
T. viride	–	–	–
T. harzianum+S. repens	–	+++	–
T. viride+S. repens	–	–	+++
Uninfested soil	–	–	–

++++ Deep coloured dot, +++ Medium coloured dot, ++ Light coloured dot, + very light coloured dot and – no dot. BCIP used as substrate; PAb (40µg/ml).

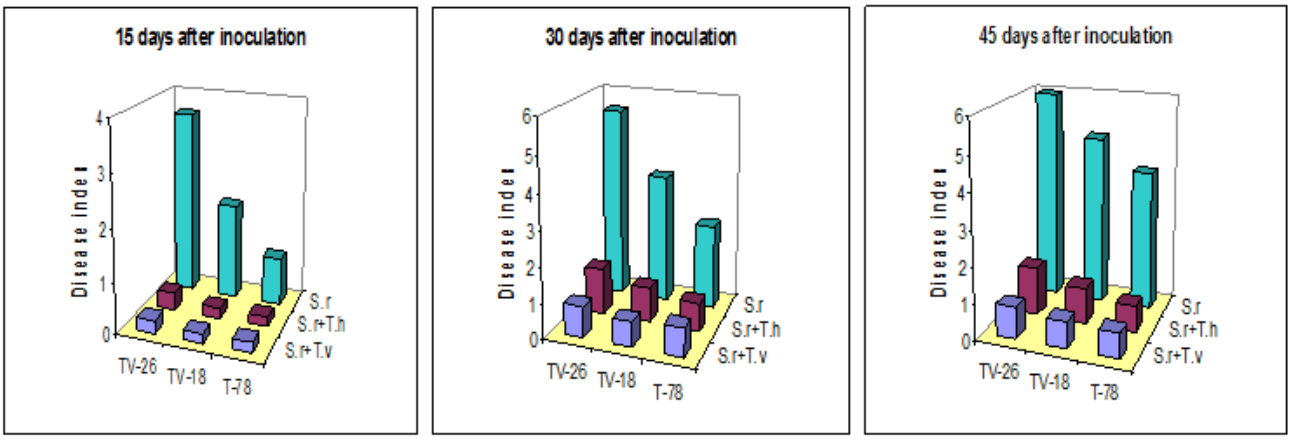

Fig 1: Effect of *Trichoderma harzianum* and *Trichoderma viride* on *S. repens* infection in field condition

The data presented on disease assessment in the Fig1 showed the effect of antagonists were very significant in reducing disease development. Attempts had been made for controlling certain root and stem diseases with *Trichoderma* bioagents in North East and South India (Barthakur and Dutta,1992; Chandra Mouli, 1993). Baby and Chandra Mouli (1996) tested antagonistic potential of *Trichoderma sp.* and *Gliocladium virens* against primary root pathogens of tea viz. *Fomes noxius, Poria hypolaterita Rosellinia arcuata* and *Armillaria mellea in vitro*.

DAC-ELISA:

Root antigens prepared from experimental potted tea plants were used with PAb raised against mycelia of *S. repense* in DAC-ELISA. Results showed (Table 1) that in case of roots treated with *T. viride* and *T. harzianum* ELISA values were almost same as that of the healthy root antigen. Antigens of roots from the *S. repense + T. viride* and *S. repense + T. harzianum* treated soil showed slightly higher absorbance than healthy, while only pathogen treated root showed almost double absorbance value as that of healthy tissue.

Dot-blot:

The dot immunobinding technique is a sensitive method for detection pathogen Lange *et al* (1989) Wakeham and White (1996) had previously detected the technique for detection *Plasmodium brassicae* in soil. In the present study, soil antigen prepared from rhizosphere soil subjected from various treatment were reacted with anti-*S. repense* PAb only *S. repense* mycelia antigen and *S. repense* inoculated rhizosphere soil gave dark violet coloured spots (Table 2). Mycelial antigen of *T. harzianum* and rhizosphere treated with *T. harzianum,* and antigen of *T. viride* mycelia and *T. viride* infested soil gave deep coloured dot when the same combinations treated with PAb of *T. harzianum* and *T. viride* respectively.

The present investigations are in resemblance with those of Bunker and Mathur (2001); Pandey *et al* (2005); Manjunath and Naik (2010) and Anjum *et al* (2011).

CONCLUSION

Green Revolution improved crop yield at the cost of adverse environmental effects. Environmental degradation, loss of biodiversity, spoilage of land and water, drug resistance in bacteria, the tremendous increase in the incidence of fungal infections add to problems facing mankind. Therefore, whole world has already started to convert the green revolution into an 'evergreen revolution' which is environmentally safe, economically viable and socially sustainable. So, the present study is relevant to the necessity in respect of modern context.

Conflicts of interest: The authors stated that no conflicts of interest.

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