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Efficacy of biocontrol agents against *Sclerotium rolfsii* causing collar rot disease of chickpea, under *in vitro* conditions

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ABSTRACT

Chickpea is known in this country since ancient times. It is a widely grown major pulse crop in India, accounts for nearly 75 per cent of the total pulse production in the world. Chickpea crop is prone to many diseases. Among them, collar rot caused by *Sclerotium rolfsii* which is gaining importance. *S. rolfsii* is a soil borne plant pathogen causing root rot, stem rot, collar rot, and foot rot diseases on more than 500 plant species of agricultural and horticultural crops throughout the world. Most of the first symptom associated with *S. rolfsii* are usually yellowing and wilting of leaves following collar rot infections. Pathogenecity was proved by Koch's postulates. Biological management of the disease through antagonists is an eco-friendly approach apart from better alternative to the use of chemicals. In the present study, the nine antagonistic micro-organisms were evaluated by dual culture technique for their antagonistic effect against *S. rolfsii* under *in-vitro* conditions. Maximum inhibition of mycelial growth (71.67%) was noticed in *T. harzianum* (Bacteriology lab isolate) which was followed by *T. viride* (Microbiology lab) (63.33%). Least inhibition was observed in *T. harzianum* GKVK isolate (31.67%). The results indicated that the application of these micro-organisms successfully decreases the stem rot incidence and also increases the growth of the chickpea plants.

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INTRODUCTION

Chickpea is known in this country since ancient times. It is a widely grown major pulse crop in India, accounts for nearly 75 per cent of the total pulse production in the world. Chickpea crop is prone to many diseases *viz.*, *Fusarium* wilt, dry root rot, collar rot, *Ascochyta* blight, *Verticillium wilt*, black root rot, *Phytophthora* root rot, wet root rot, foot rot, *Pythium* rot and seed rot etc. Among these, collar rot caused by *Sclerotium rolfsii* which is gaining importance. *S. rolfsii* is an economically important pathogen on numerous crops worldwide. It has an extensive host range; at least 500

species in 100 families are susceptible, the most common hosts are legumes, crucifers and cucurbits, and commonly occurs in the tropics, subtropics, and other warm temperate regions (Punja, 1985).

Humid weather is conductive to sclerotial germination and mycelial growth. Consequently the diseases caused by the fungus are more serious in tropical and subtropical regions than in temperate regions. The large number of sclerotia produced by *S. rolfsii* and their ability to persist in the soil for several years, as well as the profuse growth rate of the fungus make it well suited facultative parasite and a pathogen of major importance throughout the world (Punja, 1985). The first confirmed report of losses due to the pathogen in USA was made by Rolfs in 1892 on tomato (*Lycopesicon esculentum* Miller) in Florida (Aycock, 1966).

The fungus survives in soil mainly as sclerotia, which represent the main source of inoculums and it remains viable in soil for several months (Higgins, 1927). Most of the first symptom associated with *S. rolfsii* are usually yellowing and wilting of leaves following stem rot infections.

Biological management of the disease through antagonists is an eco-friendly approach apart from better alternative to the use of chemicals. Among the soil micro-organisms, there are forms that inhibit the growth of other microbes; these are called antagonists (Campbell, 1989; Morang *et al*., 2013). *Trichoderma* sp. has been reported to be potential antagonists and these gained considerable success for the control of plant diseases (Dennis and Webster, 1971; Dutta, 1981; Upadhya and Mukhyopadhayay, 1986).

The idea of a sustainable agricultural practice and environmental protection is enhancing the need of biocontrol as an alternative technique to avoid chemical hazards on both human beings and beneficial soil micro-organisms (Campbell, 1989). The application of biocontrol agents is the key elements for sustainable agriculture. Therefore, the adoption of a sustainable agricultural practice, using strategies that are environmentally friendly, less dependent on agricultural chemicals is gaining worldwide recognition. In view of the above findings the present study was carried out by some of the beneficial bioagents collected from different institutions and tested against the *S. rolfsii* to determine their antagonistic potential *in vitro.*

MATERIAL AND METHODS

Isolation and identification of the pathogen:

The infected specimens was cut into small bits and washed in running water. These bits was surface sterilized with 1 per cent sodium hypochlorite solution for one minute, washed thoroughly with three changes of water for three times to remove the traces of mercuric chloride if any and then aseptically transferred to Petriplates containing sterilized PDA medium. The plates were incubated at 27±1°C for three days. The fungal growth on fourth day, which arose through the infected tissue was taken by inoculation loop and transferred aseptically to the PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture method under aseptic conditions (Rangaswami, 1988).

The colony and sclerotial characters morphology was the principal characters considered for identification of pure culture isolates of *S. rolfsii.*

Proving the pathogenicity :

Sterilized soil was taken in earthen pots of size 45 x 30 cm. Thirty days old culture grown on sorghum grains was mixed thoroughly with soil to get sick soil. Then apparently healthy chickpea seeds (A-1 variety) were planted in pots filled with sick soil. chickpea seeds sown in pots without inoculum served as control. Soil moisture was maintained at 25 per cent moisture holding capacity of soil by adding water on weight basis throughout the period. Re-isolation was made from such affected portion of the plant tissue and compared with that of original culture.

Evaluation of bio-agents :

In vitro evaluation was carried out with nine bioagents (Table A) were collected from different institutions against CBSr (Chickpea Bengaluru *S. rolfsii*) isolate of *S. rolfsii* through dual culture technique.

For this study both bioagents and test fungus were cultured on potato dextrose agar in order to get fresh and active growth of fungus.

Dual culture technique :

Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. For evaluation of fungal biocontrol agents, mycelial discs of test fungus were inoculated at one end

Table A : List of bio-agents used for in vitro evaluation against *Sclerotium rolfsii* causing collar rot of chickpea

Sr. No.	Bio agents	Source / isolate
1.	*Trichoderma harzianum*	NBAII*
2.	*Trichoderma viride*	NBAII
3.	*Trichoderma harzianum*	GKVK
4.	*Trichoderma viride*	Microbiology lab
5.	*Trichoderma asperullum*	Bacteriology lab
6.	*Trichoderma harzianum*	Bacteriology lab
7.	*Trichoderma harzianum*-1	IIHR**
8.	*Trichoderma harzianum*-6	IIHR
9.	*Trichoderma harzianum*-16	IIHR

* NBAII: National Bureau of Agriculturally Important Insects
** IIHR: Indian Institute of Horticultural Research

of the petriplate and antagonistic fungus was placed opposite to it on the other end. The plates were incubated at 27±1°C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

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

where,
I = per cent inhibition
C = growth in control
T = growth in treatment

RESULTS AND DISCUSSION

The pathogencity test of *S. rolfsii* to chickpea was proved by soil inoculation method, carried out under glasshouse conditions and for this mass multiplication of the pathogen was done (Plate 1a to e) as per the procedure described in 'Material and Methods'. Control was maintained with sterilized soil. The pathogen infected first at collar region. Leaves of such infected plants became pale green followed by yellowing. During advanced stage of infection the white mycelium grew around the collar region and completely covered it. The plant gradually dried and toppled. The sclerotial bodies were formed on infected parts. The fungus was re-isolated from affected plant tissue and compared with

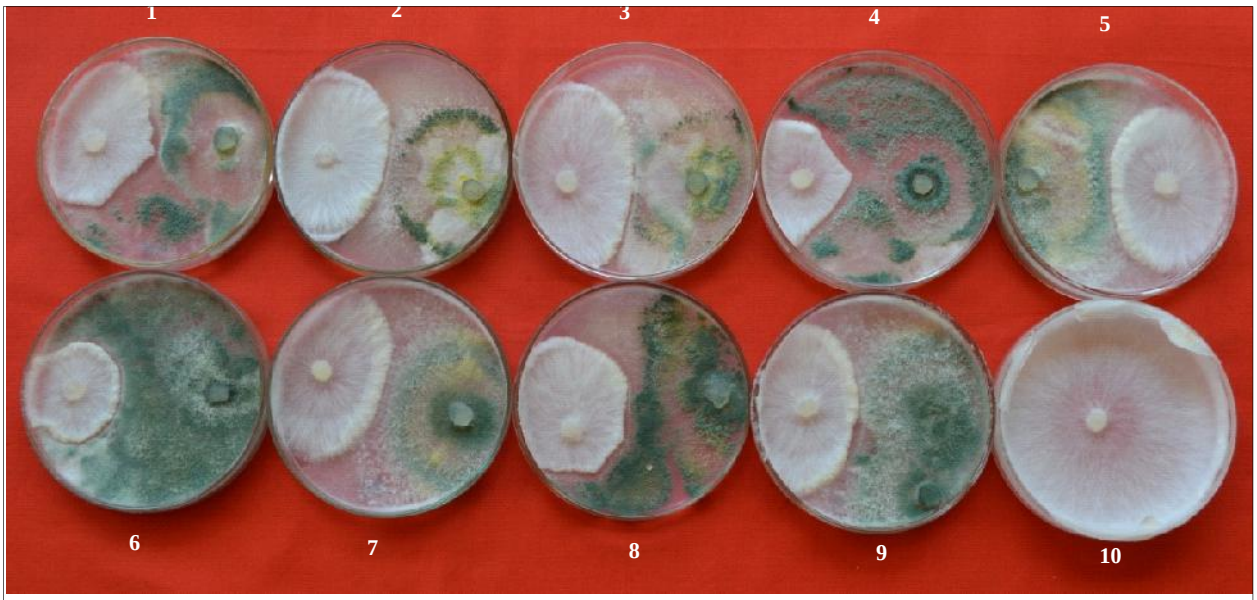
Plate 1 : Symptoms of collar rot of chickpea

the original culture (Plate f).

The nine antagonistic micro-organisms were evaluated by dual culture technique for their antagonistic effect against *S. rolfsii* under *in-vitro* conditions. Inhibition zone in mm was recorded and the per cent inhibition was calculated.

At 7 days after inoculation maximum inhibition of mycelial growth 71.67 per cent was observed in *T. harzianum* (Bacteriology lab isolate), which was followed by *T. viride* (Microbiology lab) 63.33 per cent and *T. harzianum* NBAII 60.00 per cent. Least inhibition was recorded in *T. harzianum* GKVK isolate 31.67 per cent respectively (Table 1, Plate 2).

Some soil fungi isolated from the agricultural field soil were found to grow fast in dual culture with the pathogen *i.e., S. rolfsii*. In the present study the slow growth rate of the pathogen suggested a more rapid utilization of nutrients by the antagonists when grown together. Nutrient depletion, space and production of toxic substances (antibiotic and antibiotic like substances) by the fungi are known to play a dominant role in antagonism and these factors are usually governed by the physico-



1.*Trichoderma harzianum* (NBAII),2. *Trichoderma viride* (NBAII), 3. *Trichoderma harzianum (*GKVK), 4. *Trichoderma viride* (Microbiology lab),5. *Trichoderma asperullum* (Bacteriology lab),6. *Trichoderma harzianum* (Bacteriology lab), 7. *Trichoderma harzianum* (Th-1), 8. *Trichoderma harzianum*-(Th-6), 9. *Trichoderma harzianum*-(Th-16), 10. Control

Plate 2 : Mycelial inhibition by different bioagents in dual culture

Table 1 : Antagonistic effect of different bioagents on *Sclerotium rolfsii* (CBSr isolate)

Sr. No.	Bio agents		Per cent inhibition of mycelial growth
1.	*Trichoderma harzianum* (NBAII)		60.00
2.	*Trichoderma viride* (NBAII)		53.33
3.	*Trichoderma harzianum (*GKVK)		31.67
4.	*Trichoderma viride* (Microbiology lab)		63.33
5.	*Trichoderma asperullum* (Bacteriology lab)		56.00
6.	*Trichoderma harzianum* (Bacteriology lab)		71.67
7.	*Trichoderma harzianum* (Th-1)		50.00
8.	*Trichoderma harzianum*-(Th-6)		53.33
9.	*Trichoderma harzianum*-(Th-16)		55.00
10.	Control		0.00
		S.E.±	1.22
		C.D (P=0.01)	4.91

chemical nature of the environment (Burgess and Griffin, 1967). The present *in vitro* study results showing the positive antagonistic effect of the soil fungi which have restricted the growth of the pathogen under *in vitro* condition (*i.e.*, *Trichoderma* sp.)

Member of *Trichoderma* species are known to be active hyperparasites of several soil fungi and hence they are used as a biocontrol agents (Ekefan *et al*., 2009). Control of plant diseases by the use of antagonistic micro-organisms can be an effective means (Cook, 1993). Various plant diseases have been successfully controlled through bacterial and fungal antagonists (Cook and Baker, 1983; Campbell, 1989). Antagonistic micro-organisms reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions and enzyme secretion. The exploitation of biocontrol agents for the management of plant diseases have achieved greater significance in the recent time due to its readily available nature, antimicrobial activity, easy biodegradability, non phytotoxicity, besides inducing resistance to the host.

Antagonistic micro-organisms, such as *Trichoderma*, reduce growth, survival or infections caused by the pathogens by different mechanisms like competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion (Ponnusamykonar *et al*., 2011).

Biological control is an effective, ecofriendly and alternative approach for management of any disease. Similar trend was observed when the test pathogen was placed at periphery where, maximum per cent inhibition of mycelial growth in *T. harzianum* (Dharwad isolate) (59.81%), followed by *T. harzianum* of PDBC (57.97%) and least inhibition of mycelial growth was observed in *Bacillus subtilis* (10.74%). Similarly Basamma (2008) and Manu (2012) reported least inhibition by *B. subtilis* and *P. fluorescens* as against higher inhibition by *Trichoderma* spp. Parmar *et al.* (2015) screened the six *Trichoderma* strains among them *T. viride* (NBAII Tv 23) inhibited 61 per cent growth of *S. rolfsii* followed by *T. harzianum* (NBAII Th 1) 55 per cent, respectively

Conclusion:

From the *in vitro* findings, it can be suggested that the antagonists *Trichoderma species*. can be used as a bio-control agent against *S. rolfsii* under field condition. It is also revealed that the microorganisms that naturally remain in the soil are having more or less similar potential antagonistic effect on the various crop disease caused by various pathogens. And some of them can be used as a potential bio- control agent under field condition to decrease the disease incidence and to increase crop productivity. Therefore, further work should be taken up to explore the possibility of the use of the antagonists study under field condition.

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