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Efficacy of Bioagents against Sclerotinia Rot of Chickpea Incited by *Sclerotinia sclerotiorum*

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ABSTRACT

Background: *Sclerotinia sclerotiorum* (Lib.) de Barry is a soil-borne plant pathogen, capable of infecting more than 500 host plants worldwide. It is a major pathogen that plays a crucial role in reducing the yield of economically important crops. Sclerotinia rot also known as Stem rot or white mold, caused by *Sclerotinia sclerotiorum* is a serious disease of chickpea.

Methods: The antagonistic potential of four bioagents *i.e. Trichoderma harzianum* (Th-BKN), *Trichoderma viride* (Tv-BKN), *Pseudomonas fluorescens* (Pf-BKN) and *Bacillus subtilis* (Bs- BKN) were isolated from chickpea rhizospheric soil. The fungal bioagents were tested for their antagonistic potential against the pathogen *in vitro* by modified dual culture technique on potato dextrose agar (PDA) medium. While bacterial antagonists tested for their antagonistic potential against the pathogen *in vitro* by paper disc inoculation technique on Nutrient Agar (NA) and Pseudomonas Agar Fluorescens (PAF) media. Each treatment was replicated four times, incubated at 23±10°C, data on the antagonistic activity of different bioagents were recorded and percent inhibition was calculated for each antagonist.

Result: The fungal antagonists *T. harzianum* (Th-BKN) resulted in maximum growth inhibition of the pathogen (70.48%) and bacterial antagonists, *Pseudomonas fluorescens* (Pf-BKN) (37.56%) was more effective than the *Bacillus subtilis* (Bs- BKN).

Key words: Chickpea, Growth inhibition, *Sclerotinia sclerotiorum, Trichoderma*.

INTRODUCTION

Stem rot is also known as Sclerotinia wilt or white mold, caused by *Sclerotinia sclerotiorum* is a serious disease of chickpea. It infects all the economically important food and feeds legumes (Pratt and Knight, 1984). This fungus has a wide host range and has a worldwide distribution on numerous crops (Purdy, 1979: Boland and Hall, 1994). It is one of the deastructive pathogens associated with the root rot/wilt complex of chickpea and its occurrence is increasing in both incidence and severity on chickpea grown in the Mediterranean region (Anonymous, 1996).

The initial infection occurs in the late winter or early spring, and the fungal mycelia grow within and between plants. Patches like symptoms of dead plant parts enlarge and coalesce through spring and cause major losses in stands (Bolton *et al.,* 2006). The fungus produces many black fleshy structures called Sclerotia, which survive from one cropping season to the next. Over-wintered Sclerotia may germinate during the summer or may remain dormant for many years (Adams and Ayers, 1979). The etiology, biology and epidemiology of the fungus had been studied extensively by several workers (Philips, 1987; Purdy, 1979; Roberts *et al.,* 1982).

Cultivation of resistant varieties is the ideal and feasible control of the disease and no resistant varieties against this disease have been identified so far. Erect type cultivars can better withstand the disease and management can also minimize the crop losses. Stable resistance could not be achieved due to the prevalence of virulent isolates of *S. sclerotiorum* (Sharma *et al.,* 2002). Management of Sclerotinia rot of chickpea through chemical alone is less effective as *S. sclerotiorum* having a broad host range and survives in soil for long periods in the form of Sclerotia. The Sclerotia will survive up to ten years even in the absence of the host plants and beneath prevailing dry soil conditions. The use of host plant resistance is the most economical strategy for the management of Sclerotinia rot in chickpea. Apart from the plant resistance, biological control of stem rot of chickpea is emerging as a new approach, because it is environmentally safe biopesticide and effective. To keeping in mind, the present investigation was carried out to manage this pathogen by used fungal and bacterial bioagents.

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MATERIALS AND METHODS

The antagonistic potential of four bioagents *i.e. Trichoderma harzianum* (Th-BKN), *Trichoderma* viride (Tv-BKN), *Pseudomonas fluorescens* (Pf-BKN) and *Bacillus subtilis*

(Bs- BKN) against *S. sclerotiorum* was determined under laboratory conditions.

In vitro evaluation of fungal antagonists

Dual inoculation test

For evaluation of the antagonistic potential of *Trichoderma* spp. Dual inoculation technique was followed to ascertain the antagonistic capacity of *T. harzianum* and *T. viride*. One mycelial disc (5 mm diameter) each of the pathogen and antagonist was kept on the surface of potato dextrose agar (PDA) medium at 5 cm apart in Petri dishes. The inoculated Petri dishes were incubated at 26±1°C for 7 days. Three replications were kept for each fungal antagonist *i.e*. *T. harzianum* and *T. viride*. In the case of control, the Petri dishes were inoculated with mycelial discs of the test pathogen only. The mycelial growth of the test pathogen was measured after 7 days of inoculation. The inhibition of mycelial growth of the pathogen was calculated using the following formula as suggested by Bliss, 1934.

$$\text{Per cent inhibition (I)} = \frac{(C - T)}{C} \times 100$$

Where,

I = Per cent inhibition

C = Mycelial growth of *S. sclerotiorum* in control (mm)

T = Mycelial growth of *S. sclerotiorum* in the presence of antagonist (mm)

In vitro evaluation of bacterial antagonists

Paper disc inoculation method

Stock culture of *B. subtilis* was streaked on Nutrient Agar Media and stock culture of *P. fluorescens* was streaked on Pseudomonas Agar Fluorescens (PAF) slants and incubated at 27±1°C for 48 hours. Ten milliliters of sterilized distilled water were added to each slant containing the fresh colony of bacterial antagonist and suspension was prepared by scrapping the bacterial growth with the help of a sterilized inoculating needle. The suspension was then transferred to sterile Petri dishes. Sterilized filter paper discs (5 mm diameter) were dipped in the bacterial suspension. Four such inoculated discs were placed in opposite directions on the surface of Potato Dextrose Agar Media in Petri dishes. Mycelial discs (5 mm diameter) were taken from the periphery of actively growing culture of *Sclerotinia sclerotiorum* raised on Potato Dextrose Agar Medium was placed at the center of Petri dishes containing the inoculated paper discs. In the control, Petri dishes were inoculated with mycelial discs of the test pathogen only. Three replications were kept for each treatment. The inoculated Petri dishes having *P. fluorescens* and *B. subtilis*, discs were incubated at 27±1°C. Mycelial growth was recorded after 7 days of incubation. The inhibition of mycelial growth by the bacterial antagonist was calculated by using the following formula as suggested by Bliss, 1934.

$$\text{Percent inhibition (I)} = \frac{(C - T)}{C} \times 100$$

Where,

I = Per cent inhibition.

C = Mycelial growth of *S. sclerotiorum* in control (mm)

T = Mycelial growth of *S. sclerotiorum* in the presence of antagonist (mm).

RESULTS AND DISCUSSION

The efficacy of different fungal and bacterial bioagents was tested against *S. sclerotiorum* of chickpea by using different methods under *in vitro* conditions and the results obtained are presented below.

In vitro evaluation of fungal antagonists

Two native fungal bioagents *T. harzianum* (Th-BKN) and *T. viride* (Tv-BKN) isolated from chickpea rhizosphere were evaluated to ascertain the antagonistic potentiality against *S. sclerotiorum* by dual culture method. The results (Table 1, Plate 1 and Fig 1) revealed that both the fungal bioagents significantly inhibited the mycelial growth of *S. sclerotiorum.* The minimum mycelial growth of pathogen was recorded in *T. harzianum* (Th-BKN) (26.57 mm) followed by *T. viride* (Tv-BKN) (35.10 mm) as compared to control (90.00 mm). Both the fungal bioagents significantly inhibited the mycelia growth of the test pathogen as compared to control but

Table 1: Efficacy of different bioagents on mycelial growth of *S. sclerotiorum* after 7th days of incubation.

Treatment	Mycelial growth (mm)* ##	Growth inhibition (%)
T_1- *Trichoderma harzianum*	26.57 (31.03)**	70.48
T_2- *Trichoderma viride*	35.10 (36.33)	61.00
T_3- *Pseudomonas fluorescens*	56.20 (48.56)	37.56
T_4- *Bacillus subtilis*	59.30 (50.36)	34.11
T_5- Control	90.00 (71.57)	-
S.Em (±)	1.08	
C.D (P=0.05)	3.33	
C.V (%)	5.60	

**Figures in parenthesis are angular transformed value.

* Average of three replications.

##The data showed in above table were pooled both the years (*in vitro*).

maximum per cent growth inhibition of pathogen was shown in *T. harzianum* (Th-BKN) (70.48%) followed by *T. viride* (Tv-BKN) (61.00%) after 7 days of incubation.

In vitro evaluation of bacterial antagonists

The efficacy of two native isolates bacterial bioagents *viz.*, *P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN) were evaluated against *S. sclerotiorum* by using the paper disc inoculation method. The results revealed that the highest mycelia growth of *S. sclerotiorum* was suppressed by *P. fluorescens* (Pf-BKN) (56.20 mm) followed by *B. subtilis* (Bs-BKN) (59.30 mm) as compared to control (90 mm). Both the bacterial bioagents significantly reduced mycelia growth and were found superior in controlling *S. sclerotiorum.* The results showed that the *P. fluorescens* (Pf-BKN) found relatively more inhibitory and gave maximum (37.56%) mycelial growth inhibition of *S.* sclerotiorum followed by *B. subtilis* (Bs-BKN) (34.11%) after 7 days of incubation. It was observed that fungal bioagents were competitively more effective than bacterial bioagents (Table 1, Plate 1 and Fig 1).

In the present experiment, the Antagonistic potential of two fungal and two bacterial bioagents was evaluated against *S. sclerotiorum in vitro* employing dual inoculation and paper disc inoculation methods. The fungal antagonists *T. harzianum* (Th-BKN) and *T. viride* (Tv-BKN) and two bacterial antagonists *P. fluorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN) significantly inhibited the mycelia growth of isolates of *S. sclerotiorum in vitro*. The data showed that the obtained results revealed that all tested bioagents drastically reduced the linear growth of *S. sclerotiorum*. The antagonistic fungi *Trichoderma harzianum* (70.48%) showed superior inhibitory effect against the growth of pathogenic fungi as compared to bacterial isolates *B. subtilis* (34.11%) and *P. fluorescens* (37.56%), as the antagonistic fungi had a greater significant effect on the retardation of growth compared with the bacterial bioagents. The highest inhibitory

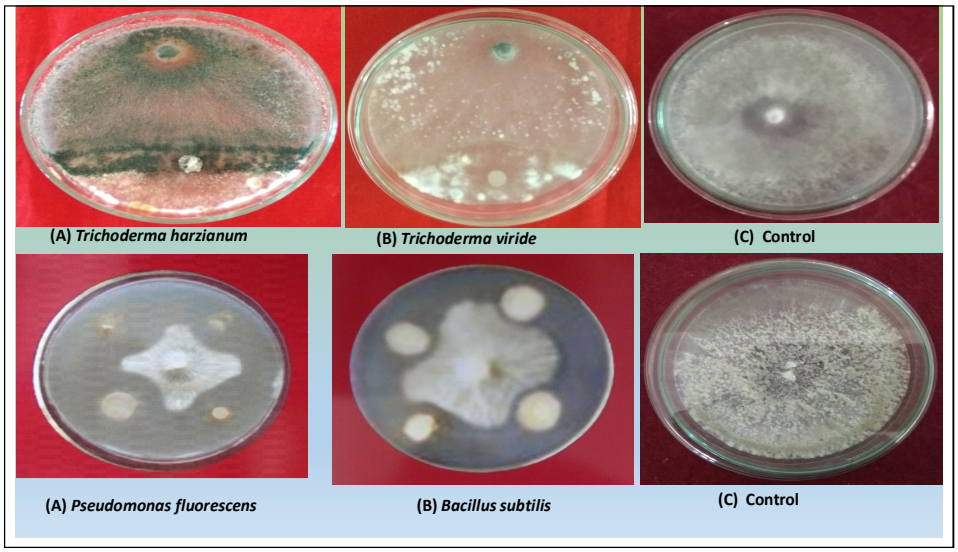

Plate 1: Antagonistic effect of different bioagents on *Sclerotinia sclerotiorum.*

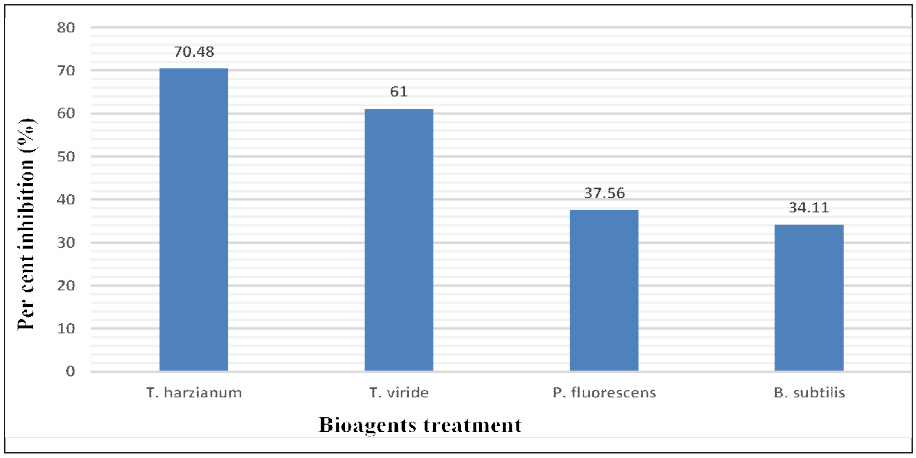

Fig 1: Efficacy of different bioagents on mycelial growth of *Sclerotinia sclerotiorum* after 7th days of incubation.

effect was recorded for *B. subtilis* (Table 1). This indicated that *T. harzianum* and *P. fluorescens* are potential bioagents in controlling the stem rot of chickpea. Similar observations were reported by earlier workers Sharma *et al*.,(1999) found that *Trichoderma harzianum* caused maximum inhibition (75%) of mycelial growth followed by *A. cylindrospora* and *T. viride*. Similar results were also reported by Sharma (1994). Kuar *et al*., (2003) also tested the *Trichoderma isolate viz*., *T. harzianum* (Th 38) and *T. viride* (Tv 34) by dual culture against *S. sclerotiorum* and observed that *T. harzianum* (Th 38) completely parasitized the pathogen. Cuong *et al*., (2007) evaluated the antagonistic potential of *Trichoderma harzianum* and observed the maximum inhibition of mycelia growth of the pathogen *in vitro*. Javeria *et al*., (2014) also reported that maximum inhibition was found by *T. harzianum* causing 70.82% inhibition of the mycelial growth of the *S. sclerotiorum*. Sharma *et al*., (2016) evaluated the antagonistic potential of *viz*., *Bacillus subtilis*, *Trichoderma harzianum*, *T. viride etc*. and they recorded the maximum inhibition was found by *T. viride* 69.8% and minimum mycelial growth inhibition 42.2% was recorded in *Bacillus subtilis*.

CONCLUSION

The fungal antagonist *T. harzianum* (Th-BKN) and *T. viride* (Tv-BKN) and bacterial antagonist *i.e*. *P. fiuorescens* (Pf-BKN) and *B. subtilis* (Bs-BKN) significantly suppressed the mycelial growth of *S. sclerotiorum* isolate *in vitro*.

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