In vitro evaluation of antifungal activity of different *Trichoderma* spp. and plant extracts against *Sclerotinia sclerotiorum* (Lib.) de Bary causing stem rot of mustard

Abstract

Sclerotinia stem rot (SSR) is one of the most destructive disease of mustard which affects the quality and quantity of the crop and resulting in severe yield losses. The present study was conducted to evaluate the bio-efficacy of different *Trichoderma* spp. and plant extracts against *Sclerotinia sclerotiorum* under laboratory conditions. All the tested *Trichoderma* spp. and plant extracts showed a significant effect on growth inhibition of test fungus over control. Among different *Trichoderma* spp., maximum growth inhibition (73.33%) was observed with *T. viride* followed by *T. harzianum* (67.77%). While as the least efficacy was found in *T. atroviride* (38.37%) against the pathogen. Of all, *Allium sativum* was proved to be most potent at all three tested concentrations (5, 10, and 15%), thereby registering 59.26, 67.41, and 100.0% growth inhibition followed by *Azadirachta indica* and *Lantana camara*, respectively. However, *Argemone maxicana* was least effective at all concentrations in this study.

Keywords: *Trichoderma* spp., plant extracts, rapeseed-mustard, Sclerotinia stem rot, *in vitro*

Introduction

India is one of the leading edible oil economy after the USA, China, and Brazil, which contributes about 10 per cent to the global oilseed oil. Rapeseed-mustard is the third major oilseed crop after soybean and palm in the world, and about 25 per cent of total oilseed produced by this crop (Yadav *et al.*, 2019; Chand *et al*., 2021). This crop used as an important source of edible oil, industrial oil, livestock feed, vegetable, and soil amendment. The mustard has nutritional value, *viz.*, sugar 1.41g, carbohydrates 4.51g, fat 0.47g, protein 2.56g per 100 g (3.5 oz), and dietary fiber 2g. India holds second place in the area (20.50%) and fourth rank in yield production (10.72%) of rapeseed and mustard, contributing 6.23 m ha and 9.34 mt, respectively, with an average productivity of 1499 kg ha^{-1} (Agricultural Statistics at a Glance, 2021). A wide gap occurs between the probable yield and the actual yield at the farmer's field, which is mainly because of biotic and abiotic factors to which this crop is exposed. Amongst, diseases caused by fungi are posing a severe menace. These fungal diseases cause deterioration in oilseed quality and also reduce production. About thirty diseases are known to occur on crops in India (Saharan, 1992). Some of the economically critical diseases of rapeseed-mustard reported in India or abroad are white rust, downy mildew, Alternaria blight, stem rot, powdery mildew, Rhizoctonia leaf blight, black leg/canker, grey mold, Fusarium wilt, damping off, black rot and mosaic (Saha *et al.* 1989).

Amongst, Sclerotinia stem rot (SSR) has become one of the most ubiquitous and devastating soil-borne disease distributed worldwide. The disease occurs commonly in cool and wet regions on a number of plants that are predominantly grown in temperate and subtropical regions all over the world (Purdy, 1979; Saharan and Mehta, 2008; Sharma *et al*., 2014). Earlier, this disease was considered to be of lesser importance, but due to newer crop practices and large cultivation of susceptible cultivars by the farmers, it has now achieved the next place to Alternaria blight in their economic importance (Kolte, 1985; Parveen *et al*., 2007). In India, this disease has been reported in serious proportion in states like Uttar Pradesh, Rajasthan, Madhya Pradesh, Punjab, Haryana, and Bihar (Saharan and Mehta, 2002). In Rajasthan, about 60 per cent yield loss has been observed in heavily infected plants (Ghasolia *et al.*, 2004). According to Shukla (2005), plants infected before initiation of flower can result in 100 per cent crop losses, whereas the plants attacked after flowering suffer 50 per cent yield loss.

The pathogen appears to be non-specific and attacks different plant parts, *viz.*, leaf, stem, and pods. The characteristic symptoms appeared as elongated water-soaked lesions at stem base, which usually enlarge quickly, become necrotic, and subsequently develop patches of white fluffy mycelium (Bolton *et al.*, 2006). Later, the stem is girdled completely and covered by a cottony mycelium growth; it breaks from where it shows rotting and drying. The pathogen survives through latent sclerotia which can remain viable for relatively longer time as they are resistant to adverse conditions (physically and chemically), as well as to degradation by another beneficial microorganism (Wu *et al*. 2008).

The management of Sclerotinia rot is absolutely difficult because of the broad host range of the pathogen, variability, and soil-inhabitant nature (Mondal *et al.*, 2015; Smolinska and Kowalska, 2018). The management of stem rot needs a proper understanding of pathogen etiology and epidemiology. Application of fungicides is the most popular method used by the farmers for the management of *S. sclerotiorum*. But extensive use of fungicides causes hazardous effect on health of human, animals and also pollutes the environment. So the best alternative to fungicides is use of plant extracts and bio-control agents because it is cheap, effective, and environmentally safe. Keeping in mind the significance of crop, the current experiment was conducted *in vitro* to the effectiveness of different *Trichoderma* spp. and plant extracts in managing the disease.

Material and Methods

In vitro evaluation of antagonistic effect of *Trichoderma* spp. against *S. sclerotiorum*

Four *Trichoderma* spp. namely, *T. harzianum*, *T. atroviride*, *T. viride*, and *T. virens* were tested to check their inhibitory effect on the growth of *S. sclerotiorum* using a dual culture method (Dennis and Webster, 1971). Twenty ml of sterilized medium (PDA) was transferred into a sterile Petri plate and allowed for solidification. Five mm diameter discs from fresh colonies of the pathogen were cut with the help of a sterile cork borer and positioned near the periphery of the plate having PDA. Similarly, *Trichoderma* spp. was also placed on another side, i.e., at a 180° angle. Plates with no antagonists are treated as a control for the pathogen. Each treatment was replicated thrice and incubated at 25±2°C for seven days. The antagonistic activity by *Trichoderma* spp. was recorded after an incubation period of 7 days by assessing the growth of the fungus in both treated and untreated plates. The growth inhibition of the pathogen was measured by using the method given by Vincent (1947).

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

Where,

C=pathogen growth in control plate

T=pathogen growth in dual culture plate

In vitro evaluation of antifungal activity of plant extracts against *S. sclerotiorum*

Antifungal activity of four plant extracts *i.e.,* Neem (*Azadirachta indica*), Lantana (*Lantana camara*), Satyanashi (*Argemone maxicana*) and Garlic (*Allium sativum*) were also evaluated against *S. sclerotiorum* at three different concentrations (5%, 10% and 15%) by poison food technique (Dubey and Patel, 2001). About 100 gm of fresh leaves/clove were used and carefully washed in distilled water. Such plant parts were cut into small pieces and then grinded in a mortar and pestle by including 100 ml of distilled water. The basic material was then filtered through muslin cloth (double layer), and then the filtrate was filtered through Whatman no. 1 filter paper. The prepared plant extracts were autoclaved at temperature of 40°C for 5 minutes to prevent contamination.

The requisite amount of plant extracts was incorporated into sterilized potato dextrose agar medium (non-solidified) and stirred well to make it standardized. Thereafter, 20 ml of amended medium was then poured into 90 mm measuring Petri plates. The observation, thus, recorded the growth of fungus at all tested concentrations until the growth of test fungus fully covered the un-poisoned Petri plates (check). The percent inhibition in radial growth (T) over control (check) was assessed by using the following method given by Vincent, 1947.

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

Where,
C= growth of the pathogen in control plates
T= growth of the pathogen in treated plates

Result and Discussion

In-vitro* efficacy of *Trichoderma* spp. against *S. sclerotiorum

All *Trichoderma* spp. showed a significant impact on radial growth inhibition of test fungus over control. Among all tested *Trichoderma* spp., significant maximum growth inhibition (73.33%) was recorded due to *T. viride* which was followed by *T. harzianum* (67.77%), *T. virens* (44.44%). While as, the least efficacy was found in *T. atroviride* (38.37%) against the pathogen (Fig 1, Plate 1).

It is clear from the present observations that among all *Trichoderma* spp., *T. viride* was the most efficient in radial growth inhibition of the fungus followed by *T. harzianum, T. virens,* while *T. atroviride* was the least effective in this study (Fig 1, Plate 1). The present findings are in testimony to the results of several earlier reports that also indicated the efficacy of *T. virens, T. viride,* and *T. harzianum* against *S. sclerotiorum* in reducing the mycelial growth and sclerotial development (Pandey *et al*., 2011; Yadav *et al*., 2011; Sharma *et al*., 2017). In a study, Mehta *et al.* (2012) also tested the effect of several antagonists against *S. sclerotiorum* (stem rot of mustard) and reported that *T. harzianum* was most superior as compared to other strains of

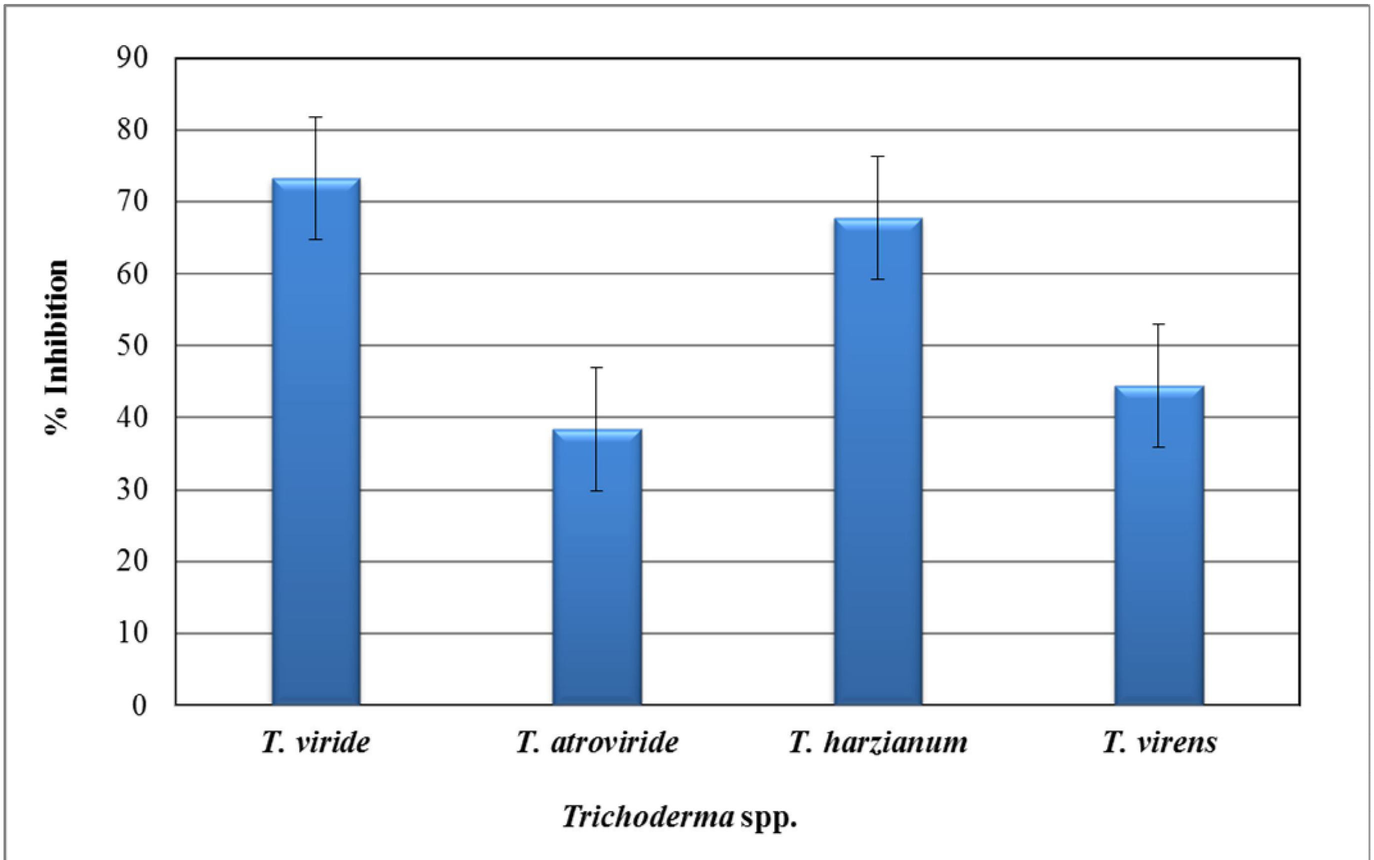
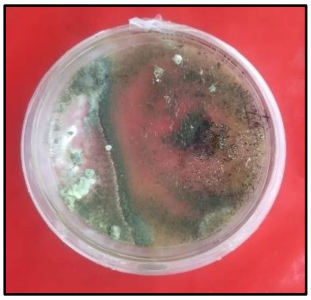


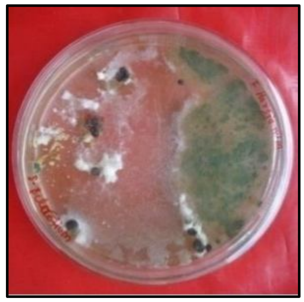
Fig 1. Efficacy of different *Trichoderma* spp. on growth inhibition of *S. sclerotiorum*

Trichoderma in vitro and it was also found to be excellent in checking length of lesions and post emergence damping-off *in vivo*. The effectiveness of bio-control agents in minimizing the mycelial growth and sclerotial development against *S. sclerotiorum* has also been reported by several scientists and researchers **(**Shivpuri and Gupta, 2001; Mehta *et al.*, 2012).

Different *Trichoderma* spp. are known to suppress *S. sclerotiorum* by coiling their hyphae rather than sclerotia by mechanisms involved mycoparasitism, antibiosis, and systemically induced resistance (Vinale *et al*. 2008; Geraldine *et al*. 2013**).** For parasitizing the pathogen, a number of enzymes like glucanases, chitinases, cellulases and proteases are produced by *Trichoderma* spp. which results in disintegration of fungal cell wall (Lopez-Mondejar *et al*. 2011).



(A)



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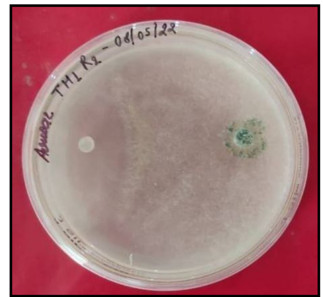

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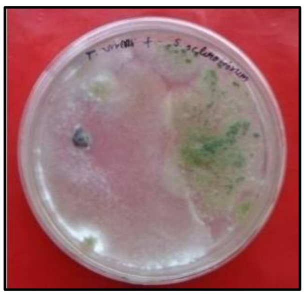

(D)

(E)

Plate 1. Efficacy of different *Trichoderma* spp. on growth of *S. sclerotiorum*, *T. viride* (A), *T. atroviride* (B), *T. harzianum* (C), *T. virens* (D) and control (E).

In vitro* efficacy of different plant extracts against *S. sclerotiorum

All plant extracts significantly reduced the growth of fungus at all concentrations over control. Of all, *Allium sativum* was found to be most effective at all three concentrations of 5, 10, and 15 per cent, thereby registering 59.26, 67.41, and 100 per cent radial growth inhibition, followed by *Azadirachta indica* (51.11, 65.55, and 82.6 %) and *Lantana camara* (43.33, 64.44 and 77.77%), respectively. However, *Argemone maxicana* was least effective at all concentrations in comparison to other plant extracts and

Table 1. Effect of different plant extracts on growth inhibition of *S. sclerotiorum*

Plant extracts	Concentrations					
	5%		10%		15%	
	Radial growth (mm)	% Inhibition	Radial growth (mm)	% Inhibition	Radial growth (mm)	% Inhibition
Azadirachta indica	51.00	51.11[b]	31.00	65.55[d]	15.66	82.6[b]
Lantana camara	36.66	43.33[c]	32.00	64.44[c]	20.00	77.77[c]
Argemone maxicana	60.00	33.33[d]	43.66	51.48[b]	33.00	63.33[d]
Allium sativum	44.00	59.26[a]	29.33	67.41[a]	00.00	100.0[a]
Control	90.00	-	90.00	-	90.00	-
L.S.D. (P≤0.05)	4.28	4.76	3.54	3.93	2.83	3.14
S.E(m)±	0.94	1.04	0.78	0.86	0.62	0.69

*Each value is an average of three replicates. Values within a column followed by different alphabets are significant and some alphabets are non-significant according to Tukey's Test at P≤0.05

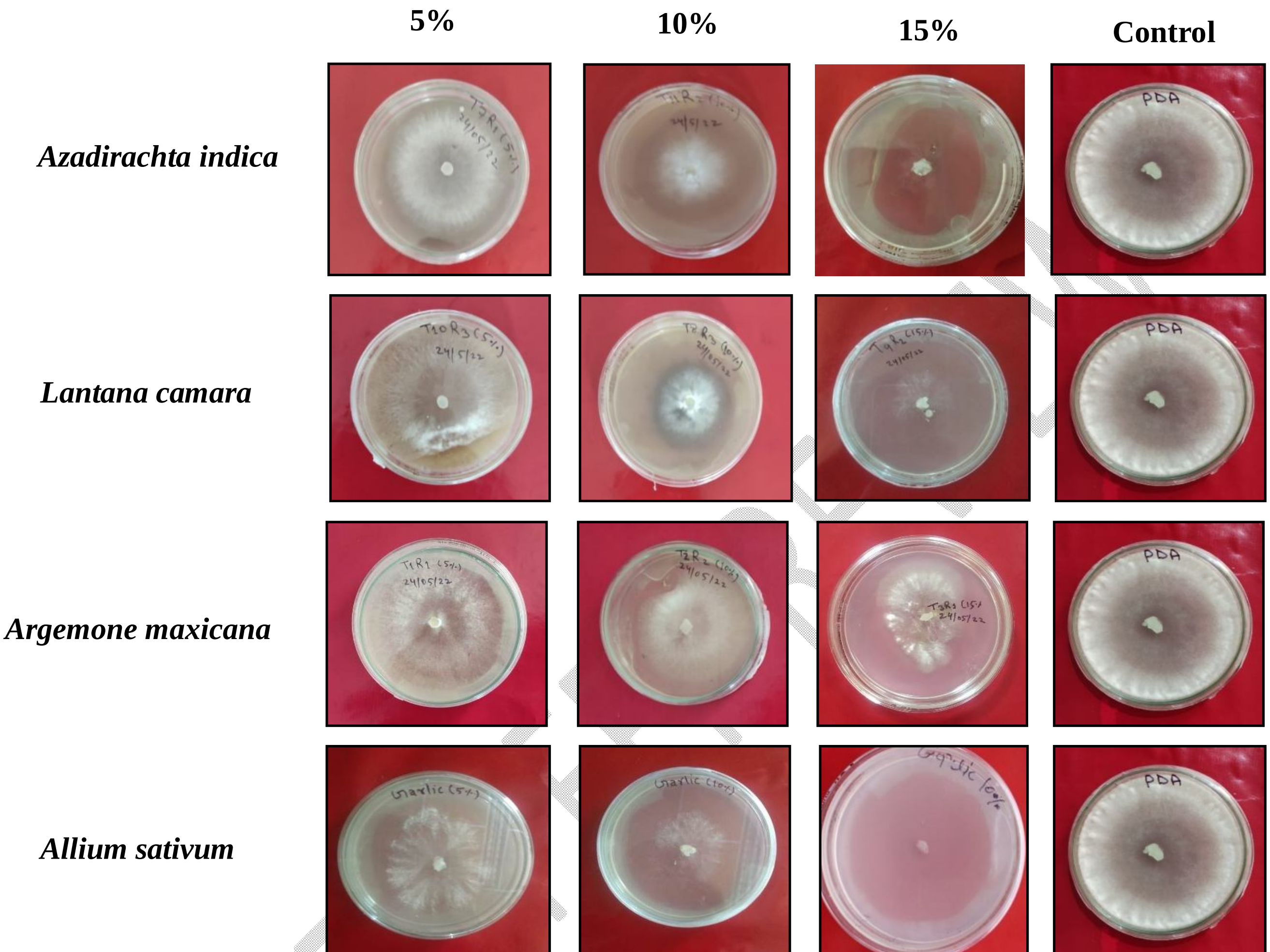

Plate 2. Effect of different plant extracts on growth of *S. sclerotiorum*

resulted in 33.33, 51.48, and 63.33 per cent inhibition of growth at 5, 10, and 15 per cent concentrations, respectively (Table 1, Plate 2).

It is, thus, clear from this study *Allium sativum* was the most effective plant extracts, followed by *Azadirachta indica* and *Lantana camara*. However, *Argemone maxicana* was the least effective in this study (Table 1, Plate 2). The results, thus, obtained in the present study are in agreement with the findings of other researchers, who have also noted the efficacy of garlic and neem extracts for inhibition of mycelial growth and sclerotial formation of *S. sclerotiorum* (Kapil and Kapoor, 2005; Tripathi and Tripathi, 2009; Mehta *et al*., 2011; Yadav *et al*., 2011; Meena *et al*., 2013; Fatehpuria *et al*., 2017). In a study, Fagodia *et al.* (2017) also investigated the efficacy of different botanicals on the growth of *S. sclerotiorum* causing stem rot of coriander and found that *Allium sativum* extract was most potent in inhibiting mycelial growth of fungus, followed by Eucalyptus leaf extract.

Conclusion

All *Trichoderma* spp. significantly inhibited the growth of *S. sclerotiorum*. However, *T. viride* and *T. harzianum* was noted to be most effective antagonist in inhibiting the maximum radial growth of the pathogen. While as, *T. atroviride* proved to be least efficacious in this study. Of all plant extracts, *Allium sativum* was found superior over all others at all concentrations followed by *Azadirachta indica* and *Lantana camara*. However, *Argemone maxicana* was found least effective in this study. The biopesticides should be used by the farmers alone or in combinations with other cultural practices because it is economical and eco-friendly than chemicals.

Conference disclaimer:

Some part of this manuscript was previously presented in the conference: 3rd International Conference IAAHAS-2023 "Innovative Approaches in Agriculture, Horticulture & Allied Sciences" on March 29-31, 2023 in SGT University , Gurugram, India. Web Link of the proceeding: https://wikifarmer.com/event/iaahas-2023-innovative-approaches-in-agriculture-horticulture-allied-sciences/

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