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Effect of chemicals and antagonists on the growth of *Rhizoctonia solani* (Kuhn) causing black scurf of potato (*Solanum tuberosum* L.)

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Abstract

Rhizoctonia solani is the cause of potato black scurf disease, which has become a serious hazard in the majority of India's potato-growing regions. The effectiveness of fungicides was verified *in vitro* for the per cent mycelial growth inhibition of *Rhizoctonia solani*. Monoceren entirely inhibited the mycelial growth at 100 ppm as compared to MEMC and boric acid, which inhibited mycelial growth up to 81 per cent and 76 per cent, respectively at 100 ppm concentration. Among tested fungicides, mancozeb was least effective which checked 45.00 to 74.33 per cent of fungal growth, followed by hexaconazole (70.00 to 99.33%) and propiconazole (72.33 to 99.66%) relatively at 100 to 1000 ppm concentrations. Amongst the four bioagents, *Trichoderma harzianum* showed superior results, inhibiting mycelial growth by 71.11 percent, followed by *T. viride* (64.44%). *Bacillus subtilis* and *Pseudomonas fluorescens*, the bacterial bio-agents, showed the least effectiveness, inhibiting mycelial growth by 55.27 and 47.22 percent, respectively.

Keywords: Black scurf, Bioagents, Fungicides, Potato and *Rhizoctonia solani*

1. Introduction

The fungus '*Rhizoctonia solani*' attacks tubers, underground stems and stolons of potato (*Solanum tuberosum* L.). In potato, this disease may cause both qualitative & quantitative loss to the potato crop. In northern areas, where farmers frequently plant potato in cold soils, *Rhizoctonia* is a more stable problem. *Rhizoctonia* disease is characterized by poor standing, stunted plant growth, reduced tuber quantity and size, and deformed tubers. While stem canker causes quantitative losses due to stolon, sprout, and root infections, primarily in the early stages of the season, reducing tuber size and number, black scurf increases during plant senescence and is associated with the development of sclerotia on progeny tubers and their deformities.

Poor standing, limited plant development, decreased tuber number and size, and malformed tubers are the hallmarks of *Rhizoctonia* disease. Black scurf increases during plant senescence and is linked to the development of sclerotia on progeny tubers and their deformities, whereas stem canker reduces tuber size and number through quantitative losses caused by stolon, sprout, and root infections, mostly in the early stages of the season.

Black scurf of potato caused by isolates of AG-3 group does not physically damage tubers but affects their market value (Banville *et al.*, 1996) ^[1]. *R. solani* is transmitted by infected seed tubers, providing a mechanism for its long-distance dispersal. Once existed in soil, the sclerotia and mycelium of the pathogen can then provide an additional source of primary inoculum. (Kumar *et al.*, 2017) ^[14].

Xiao-Yu Zhang *et al.* (2014) ^[31] observed that a few hyphae might penetrate in the potato tuber epidermis and infect the internal tissue in field test which provides the basis for the tuber piece inoculation. On tuber surface, brownish to blackish irregular hard-masses of sclerotia were formed. Though these structures adhere firmly to the tuber skin, they are superficial and do not cause any injury, even in storage and help in spread of the disease from one season to the next. Disease intensity is not always associated with the reduction of yield. Potato black scurf disease was reported to cause marketable yield losses up to 30-50 % (Keiser, 2008) ^[10].

Since there is no universally accepted inoculation technique or disease categorization system, there are few resistant sources of the *Rhizoctonia* pathogen in potato germplasms, therefore it is difficult to screen for and identify resistant potato cultivars and/or lines (Naz *et al.*, 2008; Djébali and Belhassen, 2010) [21,4]. Therefore, chemical methods and antagonists can be used to manage disease. Among the several options for disease control are the application of fungicides and bio-agents. Numerous countries that produce potatoes have conducted extensive research on the chemical management of black scurf (Jeger *et al.*, 1996,) [8]. Bioefficacy of different fungicides, *i.e.*, Monceren 250 EC, Penflufen 240 FS, Emisan 6 FS and Carbendazim 50 WP, were evaluated against black scurf disease of potato by dip treatment on Kufri Bahar variety (Kumar and Raj, 2016) [13]. For the control of black scurf, the use of harmful organo-mercurial compounds was replaced with non-harmful ecofriendly chemical boric acid (Somani, 1988) [28]. Pencycuron affects the metabolic activity of *Rhizoctonia solani* and inhibits its further growth. Pencycuron and boric acid are the two chemicals which are regularly used by Indian farmers for the management of black scurf (Khurana *et al.*, 2001) [11]. Many isolates of *Trichoderma* spp. have been reported to effectively inhibit soil-borne plant pathogenic fungus, including *Fusarium* spp., *R. solani*, *Pythium* spp., and *Sclerotium rolfsii*. (Samuels, 1996: Singh J.K *et al.*, 2017) [25, 27]. *R. solani* isolates' mycelial growth is considerably inhibited by *Trichoderma viride* and *Trichoderma harzianum* (Hussain *et al.*, 2014) [7]. *Bacillus*, *Pseudomonas fluorescence*, and actinomycetes are among the bacteria and fungi that have been used to suppress *R. solani*. Antagonistic bacteria, particularly those in the *Bacillus* genus, are among the most widely utilized biological agents to combat a variety of plant diseases (Elhamshary and Khattab, 2008) [6] including *Rhizoctonia* (Mizumoto *et al.*, 2007) [19]. *Bacillus* strains are known to produce bioactive natural peptides that may have antifungal, antibacterial, and anti-enveloped viral properties. (Li and Yang, 2005; 2002; Jing *et al.*, 2009; Bizani and Brandelli, Ongena *et al.*, 2009) [15, 9, 3, 22]. Moreover, endophytic plants can be colonized by *Bacillus* bacteria (Mahaffee and Kloepper, 1997) [16], and these bacteria can create compounds that influence the growth of bacteria and fungi. (Stein, 2005) [29]. While *P. fluorescence* produces a group of antifungal metabolites like siderophores and various antibiotics like 2,4-diacetyl-phloroglucinol (2,4-DAPG) and phenazine-1 carboxylic acid that stop the fungus from growing further, the bacterial antagonist *B. subtilis* produces a wide range of antifungal compounds, including subtilin, subtilosin, TasA, mycobacillin, bacilysin, and even some enzymes that may break down the fungal cell wall (Berg *et al.*, 2001) [2]. Therefore, it was suggested that the current study examine how well next generation fungicides work against *Rhizoctonia solani* and naturally occurring antagonists that are frequently found in soil ecosystems to ensure environmental safety.

2. Materials and Methods

2.1 Isolation and purification of *Rhizoctonia solani* isolates

As sclerotia of black scurf were gathered at harvest, potato tubers of the susceptible variety "Kufri Bahar" show typical symptoms. The black scurf-covered potato tuber peels were taken off, chopped into little pieces, and properly cleaned

with sterile water to get rid of any dust, etc. After being purified using the single hyphal tip approach (Rangaswami and Mahadevan, 2004) [24], the pathogen cultures were kept on PDA slants and refrigerated at 4°C for future research. Pathogen thus isolated from the samples were identified as *Rhizoctonia solani* Kuhn and confirmed through the available literature.

2.2 In vitro evaluation under laboratory conditions

2.2.1 Evaluation of chemicals/fungicides against *Rhizoctonia solani*

Using the standard procedure of poison food technique as provided by Mayer (1962) [18], the effectiveness of six chemicals/fungicides *viz.*, boric acid, methoxy ethyl mercuric chloride (MEMC), mancozeb, monceren, hexaconazole, and propiconazole on the growth of *Rhizoctonia solani* was tested *in vitro* at four different concentrations, namely, 100, 250, 500, and 1000 ppm, on the radial growth inhibition of *R. solani*. Under lab conditions, the study employed a completely randomized design (CRD) with three replications for every fungicide.

2.2.1.1 Observations recorded

The colony diameter (mm) was measured every 24 hours until the control treatment's fungus had completely taken over the Petri plate's growth area. Using Vincent's equation (1947) [30], the percentage inhibition of mycelial growth over control was determined.

$$\text{Growth inhibition (\%)} = \frac{(C - T)}{C} \times 100$$

Where,

C= Radial growth of *Rhizoctonia solani* mycelium in control

T= Radial growth of *Rhizoctonia solani* mycelium in treatment

2.2.2 Evaluation of antagonists against *Rhizoctonia solani*

The antagonists utilized in this investigation, which included *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma viride*, and *Trichoderma harzianum*, were collected from the Department of Plant Pathology at CCS HAU, Hisar. They were evaluated using the dual culture approach (Morton and Stroube, 1955) [20] to determine their effectiveness against *R. solani*. Until the control plate reached full development (45.0 mm), the test pathogen's radial expansion toward the antagonists was evaluated in the dual culture assay. The formula from Vincent (1947)) [30] was used to determine the percentage inhibition of radial growth, as previously mentioned. (section 2.2.1.1).

Statistical analysis

The data of *in vitro* experiments was studied by using statistical package of programs OPSTAT (Sheoran, 2006) [26]. The factorial experiment in CRD was conducted for laboratory. Angular (arcsine) transformation was done for analysis of the per cent data in CRD design. Analysis of variance (ANOVA) in one way or two ways for the analysis of the data was used to calculate the critical difference (CD) and coefficient of variations (CV) for the significance of the treatments.

3. Results

The current study was conducted *in vitro* to assess several environmentally friendly management strategies against potato black scurf. In the Department of Plant Pathology at CCS Haryana Agricultural University in Hisar, Haryana, India, the study was conducted. Here are the findings from several laboratories regarding the control of *Rhizoctonia solani*, which causes potato black scurf.

3.1 Efficacy of different chemicals/ fungicides

Using the poisoned food technique, six fungicides *viz.*, boric acid, methoxy ethyl mercuric chloride (MEMC), mancozeb, monceren, hexaconazole, and propiconazole were tested *in vitro* at four different concentrations *viz.*, 100, 250, 500, and 1000 ppm to determine how effective they were at stopping the radial growth of *Rhizoctonia solani* (section 2.2.1). Following the fifth day of incubation, the colony diameter was measured, and the percentage of *R. solani* mycelial growth inhibition for each fungicide concentration was

computed. Results (Table 1) showed all fungicides inhibited *R. solani*'s radial growth, with a significant decrease in growth as fungicide concentration increased.

All six fungicides, however, differed in the degree of growth inhibition. Table 1's experimental results demonstrated unequivocally that, at a concentration of 100 ppm, the monceren fungicide completely inhibited mycelial growth up to 100 percent, followed by MEMC (81.0% inhibition). In contrast, boric acid, propiconazole 25%, hexaconazole 5%, and mancozeb 75% were responsible for 76.0, 72.3, 70.0, and 45.0 percent inhibition of *Rhizoctonia solani* mycelial. Boric acid decreased mycelial development 97.3%, whereas MEMC at 500 ppm concentration entirely inhibited it (100%). Boric acid and MEMC concentrations of 250 ppm showed an inhibition of 89.7% and 94.3%, respectively. Increasing concentrations (100-1000 ppm) enhanced growth inhibition of *R. solani* as across all fungicides.

Table 1: *In vitro* evaluation of different chemicals/fungicides against mycelial growth of *R. solani*

Chemicals	Per cent inhibition of mycelial growth				
	Concentration (ppm)				
	100	250	500	1000	Mean
Boric Acid	76.00 (60.65)*	89.67 (71.31)	97.33 (84.11)	100.00 (89.39)	90.66 (75.93)
MEMC -6 FS % w/v	81.00 (64.17)	94.33 (76.34)	100.00 (89.39)	100.00 (89.39)	93.83 (79.82)
Mancozeb 75% WP	45.00 (42.10)	59.00 (50.18)	71.67 (58.55)	74.33 (59.57)	62.50 (52.60)
Monceren 250 EC	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)	100.00 (89.39)
Hexaconazole 5% EC	70.00 (56.04)	85.33 (67.51)	91.33 (73.14)	99.33 (86.87)	86.50 (71.04)
Propiconazole 25% EC	72.33 (58.25)	86.67 (68.64)	97.00 (80.62)	99.66 (87.67)	89.00 (74.23)
Control	---	---	---	---	---
Mean	74.06 (53.13)	85.83 (67.97)	92.88 (70.56)	95.55 (71.83)	-
	Fungicides (F)		Concentrations (C)		Interaction (F x C)
SEm ±	0.962		0.727		1.923
CD (p = 0.05)	2.732		2.065		5.464

All values represent means of three replications; *Figures in parenthesis indicate angular transformed values

At 1000 ppm, all fungicides strongly inhibited *R. solani*'s growth. Complete growth inhibition was observed with monceren 250 EC at 100 ppm, MEMC at 500 ppm, and boric acid, propiconazole 25%, and hexaconazole 5% at 1000 ppm. Mancozeb 75% was the least effective, with 74.33% inhibition, indicating its limited efficacy in checking radial growth of the fungus.

The *in vitro* evaluation of the fungicides against the test fungus revealed monceren 250 EC and MEMC-6 FS as the most promising fungicides against *R. solani*. Boric acid, propiconazole 25%, and hexaconazole 5% showed intermediate efficacy, while mancozeb 75% was the least effective at all concentrations. Statistical analysis confirmed significant effects of fungicides, concentrations, and their interactions.

Similar types of findings under *in vitro* were also found by Dutta and Kalha (2011) [5] while working with *Rhizoctonia solani*. They have observed that hexaconazole and propiconazole had inhibited the mycelial growth of the pathogen. Hexaconazol (Contaf) gave good result against *R.*

solani under *in vitro* study (Rai *et al.*, 2007) [23]. The same trend of results was found in the seed treatment with 3% boric acid and 3% Dithane M-45 which reduced the disease incidence of black scurf reported by Kiptoo *et al.*, 2021 [12].

3.2 Efficacy of different bioagents

The antifungal activity of four bioagents *viz.*, *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* against *Rhizoctonia solani* was evaluated under *in vitro* conditions and results thus obtained are shown in table 2.

The bioagents significantly inhibited *R. solani*'s growth through overgrowth or inhibition zones. Both the *Trichoderma* spp. showed considerable antifungal activity by checking the radial growth of *R. solani* as compared to the bacterial antagonist *B. subtilis* and *P. fluorescens*. The inhibition of radial growth observed in *T. harzianum* (71.11%) was significantly higher to *T. viride* (64.44%). While the bacterial antagonist *B. subtilis* was able to inhibit radial growth up to 55.27 per cent and *P. fluorescens*

inhibited 47.22 per cent radial growth of the test fungus. In comparison to *T. viride*, faster growth inhibition of the test fungus was obtained with *T. harzianum*. These results are in agreement with Malik *et al.* (2014) [17] and Hussain *et al.* (2014) [7] who demonstrated that *T. harzianum* revealed prominent antagonism against *R. solani*, followed by *T. viride* which significantly suppressed the mycelial growth of pathogen.

Table 2: Efficacy of different bio-agents against mycelial growth inhibition of *R. solani* under *in vitro* condition

Bio-agents	Radial growth (mm)	Growth Inhibition (%)
<i>T. harzianum</i>	26.00	71.11
<i>T. viride</i>	32.00	64.44
<i>P. fluorescence</i>	40.20	55.27
<i>B. subtilis</i>	47.50	47.22
Control	90.00	-
SEm (±) :- 1.815, CD (p = 0.05) :- 5.52, CV(%) :- 7.7		

All values represent means of four replications

4. Conclusion

Efficacy of chemicals and bioagents were evaluated *in vitro* for their ability to reduce *Rhizoctonia solani* mycelial growth by a percentage. At 100 ppm, Monceren was the chemical that entirely prevented mycelial development, whereas *Trichoderma harzianum* was the most effective bioagent, decreasing mycelial growth by 71.11 per cent.

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