

Original Research Article

In vitro evaluation of botanicals, bio-agents and fungicides against *Rhizoctonia solani* causing Web blight disease of mungbean

Abstract

Mungbean [*Vigna radiata* (L.) Wilczek] is the important source of proteins, minerals, and vitamins of the predominantly vegetarian Indian diet. It belongs to the family Leguminaceae. Web blight caused by *Rhizoctonia solani* (Kuhn) is one of the most important fungal diseases which come every year with different intensity and causes huge losses in yield. The losses in grain yield is more when the plants get infected earlier *i.e.* 25 days after sowing (DAS) than 35 and 40 DAS. It causes losses in yield and weight 33.40 to 37.80 per cent and 23.12 to 28.60 per cent in different varieties. The present investigations were carried out in the laboratory, ~~department~~ Department of Plant Pathology A.N.D.U.A. & T., Kumarganj, Ayodhya to test the efficacy of different treatments against *Rhizoctonia solani* Kuhn under lab conditions (*In vitro*). Botanicals and Fungicides were tested through Poisoned food technique and Bio-agents were tested through dual culture technique. Fungus were isolated from diseased mungbean plant and further tested against different treatments. Radial growth and percent inhibition were recorded at 24, 36 and 48 hours of intervals. Minimum radial growth and maximum percent inhibition were recorded in Propiconazole 1.45 mm, 1.85 mm and 2.16 mm at 24, 36 and 48 hours of intervals respectively.

Keywords: Efficacy, Botanicals, Bio-agents, *Vigna radiata*, *Rhizoctonia solani*, *In vitro*

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is the important source of protein, vitamins and minerals. It belongs to the family Leguminaceae. Among the pulses mungbean also called as green gram or golden gram. It is mainly grown in Rajasthan, Maharashtra, Karnataka, Andhra Pradesh, Orissa, Bihar, Tamil Nadu, Madhya Pradesh, and Uttar Pradesh (Anonymous, 2019). In Uttar Pradesh, it is cultivated on 93000 ha, with a production of 9480 tonnes. Compared, the productivity of mung bean in India and the U.P. is 567 kg/ha and 536 kg/ha, respectively, which

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Provide information about the importance and history of disease where the study is conducted.

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Comment [A7]: Abstract needs to be revised carefully.
Add results about botanicals and bioagents also.

Comment [A8]: Key words should be specific words.
Add name of crop/botanical/fungicide/bioagents used in this study.

Comment [A9]: The introduction should provide a more comprehensive background on the significance of mungbean and its cultivation in India. The introduction lacks proper citations, and it is crucial to provide credible sources to support the claims made.

Comment [A10]: Citation needs to be changed.

is very low compared to the genetic potential of 1500–2000 kg/ha (Anonymous, 2019). The major limiting factors for its poor yield are the attacks of various biotic and abiotic stresses. Among them, diseases caused by fungi, bacteria, and viruses are major potential threats that adversely affect the productivity of mung bean. In 1924, web blight was reported for the first time on mung bean from the Philippines (Nacien, 1924). While in India, Dwivedi and Saksena (1974) first reported it on mung beans from Kanpur, Uttar Pradesh. Further, it has also been reported from Assam (Saikia, 1976), Punjab (Bains *et al.*, 1988), Madhya Pradesh (Tiwari and Khare, 1998), Bihar, Rajasthan, Haryana, Himachal Pradesh, and Jammu & Kashmir (Anonymous, 2004). Web blight caused by *Rhizoctonia solani* (Kuhn) is one of the most important fungal diseases which appear every year in varying intensity and causes heavy reduction in yield. The losses in grain yield is more when the plants get infected earlier i.e. after 25 days after sowing (DAS) than 35 and 40 DAS. Gupta *et al.* (2010) reported losses in yield and lost weight were 33.40 to 37.80 per cent and 23.12 to 28.60 per cent respectively. Though, the web blight could be managed by the use of fungicide but due to the emergence of several problems like environmental pollution, residual effect in grains, killing non targeted organisms its use should be discouraged. Hence, for minimizing the losses caused by web blight need inexpensive and environmentally safe management practices. Many botanicals and bio-agent have been found effective against *Rhizoctonia solani* in different crops, therefore keeping in view the importance of the crop and seriousness of diseases present research work carried.

Comment [A11]: Rewrite it.

Comment [A12]: disease

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Comment [A14]: Add source

Comment [A15]: Provide recent reports on losses.

Comment [A16]: Need of study should be better

Comment [A17]: Why you selected the used botanicals and bioagents. Give references.

Comment [A18]: This section lacks clarity and detail. It is essential to provide a comprehensive description of the methodology used, including specific procedures for testing botanicals, bioagents, and fungicides.

Comment [A19]: Add the information about the statistical analysis.

Comment [A20]: Add year of study

Comment [A21]: Add the source of bioagents

Comment [A22]: Write the scientific names of the botanical

Comment [A23]: Add more specific information

Comment [A24]: Which part of these Extracts is used and also provide methodology for extraction and preparation of suspensions.

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Materials and Methods

The present investigations were carried out in the laboratory, ~~department~~ Department of Plant Pathology A.N.D.U.A. & T., Kumarganj, Ayodhya to test the efficacy of different treatments against *Rhizoctonia solani* Kuhn under ~~lab conditions~~ (*in vitro*). Botanicals and Fungicides were tested through Poisoned food technique and Bio-agents were tested through dual culture technique. Fungus were isolated from diseased mungbean plant and further tested against different treatments. In order of find out the efficacy of various plants extracts against *R. solani*, five plants extract viz., Neem, Garlic, Tulsi, onion and Ginger, were used. By adding the necessary quantity of sterilized PDA medium, the concentrations of 10.0 percent were created. Two fungicides were tested *in vitro* against *R. solani* i.e., Propiconazole and Hexaconazole. Prior to pouring, both fungicides Propiconazole and Hexaconazole were added at a concentration of 20

ppm to PDA. The flasks were thoroughly shaken to ensure an even mix of the extract under aseptic conditions. Twenty ml of sterilized melted PDA was aseptically poured in sterilized Petri dishes and allowed to solidify. The efficacy of *T. asperellum* and *T. harzianum* against *Rhizoctonia solani* were assessed by using dual culture technique by measuring the radial growth of *R. solani* as well as that of *Trichoderma* spp. Three-day-old *R. solani* culture discs were cut into five mm pieces with a sterilized cork borer and positioned in the centre of Petri dishes with plant extracts and fungicides added. Five mm disc of each antagonist and *R. solani* cut with the help of sterilized cork borer from the age of three days old culture and were placed in Petri dishes having solidified PDA in such a manner that they lie opposite to each other 60 mm apart. Control (Check) Petri dishes were inoculated only with *R. solani* bits. Each treatment and control was repeated four times to make four replications. These Petri dishes were kept in BOD incubator at 28⁰ C. The observations on radial growth were made at 24, 36, and 48 hours of incubation in Petri dishes amended with different treatments as well as in control. Per cent growth inhibition was calculated by using formula (Vincent, 1947).

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Comment [A29]: Why used 3 days old culture

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

Where,

I = Per cent inhibition of fungal growth.

C = Radial growth of control.

T = Radial growth in treated Petri dish.

Comment [A31]: The presentation of results is confusing. It is unclear how the data in tables and figures correspond to the treatments and time intervals discussed in the text.

Comment [A32]: Results presentation is very poor. All the figures are the repetition of the tables. All tables can be merged in the single table. Subheadings should be changed.

Comment [A33]: • The paper lacks a discussion section where the results are interpreted and placed in the context of previous research. This is a critical component of a research paper.

• The paper lacks a clear conclusion section where the main findings and their implications are summarized.

• The paper does not offer suggestions for future research, which is typically expected in a research paper.

Results and Discussion

A. Botanicals

1. Effect of botanicals against *R. solani* on mycelial growth at 24, 36 and 48 hours.

At 24 hours of incubation, the minimum mycelial growth was obtained in Garlic (10.18 mm) followed by Ginger (11.43 mm), Neem (12.90 mm), Onion (15.42 mm), Tulsi (17.63 mm) as

compared to control (45.47 mm). Each treatment was significantly different to each other (Table 1, Fig 1). At 36 hours of incubation, the minimum mycelial growth was obtained in Garlic (14.04 mm) followed by Ginger (16.07 mm), Neem (18.63 mm), Onion (20.00 mm), Tulsi (22.00 mm) as compared to control (71.86 mm). Garlic and Ginger, Neem and Onion, Onion and Tulsi were found at par to each other (Table 1, Fig 1). At 48 hours of incubation, the minimum mycelial growth was observed in Garlic (15.49 mm) followed by Ginger (18.00 mm), Neem (19.95 mm), Onion (22.33 mm) and Tulsi (24.66 mm) as compared to control (85.42 mm) which were significantly differed to each other (Table 1, Fig 1)

2. Efficacy of botanicals against *R. solani* on per cent inhibition at 24, 36 and 48 hours

At 24 hours of incubation, the maximum per cent inhibition was recorded in Garlic (77.45%) followed by Ginger (74.67%), Neem (71.43%), Onion (65.79 %) and Tulsi (60.95%). However, each treatment was significantly differed to each other (Table 2, Fig 2). At 36 hours of incubation, the maximum per cent inhibition was recorded in Garlic (80.42%) followed by Ginger (77.63%), Neem (74.09%), Onion (72.20%) and Tulsi (69.39%). However, Ginger and Garlic were at par to each other (Table 2, Fig 2). At 48 hours of incubation, the maximum per cent inhibition was recorded in Garlic (81.89 %) followed by Ginger (78.98 %), Neem (76.52 %), Onion (73.81 %) and Tulsi (71.08%). However, each treatment was statistically differed to each other (Table 2, Fig 2.) Plate 1. The present findings are supported by Shinde and Patel (2004) discovered that bulb gather of garlic gave 100% inhibition of mycelial growth of *R solani* causing black scurf of potato, followed by Ginger, Tulsi, Eucalyptus, and Neem. Meena *et al.* (2002) also discovered that extract of garlic at 5.0 per cent concentration (w/v) completely inhibited the mycelial growth of *R. solani* causing sheath blight of rice.

B. Bio-agents

1. Effect of bio-agents against *R. solani* on mycelial growth at 24, 36 and 48 hours.

The efficacy of bio-agents *T. asperellum* and *T. harzianum* were tested for mycelial growth and per cent inhibition of *R. solani* by using dual culture technique. At 24 hours of incubation results clearly indicated that mycelial growth was minimum in *T. asperellum* (18.58 mm) followed by *T. harzianum* (23.14 mm) as compared to control (44.99 mm). Each treatment was significantly differed to each other (Table 3 Fig 3). Similar results were also obtained at 36 hours of

incubation. However, the mycelial growth was 23.24 mm, 31.58 mm and 71.83 mm in *T. asperellum*, *T. harzianum* and control respectively. Each treatment was significantly differed to each other (Table 3 Fig 3). At 48 hours of incubation the mycelial growth was 25.60 mm, 39.55 mm and 82.00 mm in *T. asperellum*, *T. harzianum* and control respectively. Each treatment was statistically differed to each other (Table 3 Fig 3).

2. Efficacy of bio-agents against *R. solani* on per cent inhibition at 24, 36 and 48 hours.

T. asperellum and *T. harzianum* significantly inhibit the growth of *R. solani* as compared to control at 24, 36 and 48 hours of incubation. *T. asperellum* showed higher per cent inhibition as compared to *T. harzianum* (Plate 2). The per cent inhibition in *T. asperellum* and *T. harzianum* were 58.71% and 48.53% at 24 hours, 67.64% and 56.02% at 36 hours and 68.77% and 58.32% at 48 hours, respectively. Each treatment was significantly differed to each other (Table 4, Fig. 4). The present findings are supported by Dubey and Patel (2001) and Dubey (2002) reports that *T. asperellum* was more effective than *T. harzianum* at inhibiting the mycelial growth of *R. solani* in vitro. *T. harzianum*, as opposed to *T. asperellum*, was found to be more effective at preventing the mycelial growth of *R. solani* by Meena *et al.* (2002) and Khan and Sinha (2007).

C. Fungicide

1. Effect of fungicides against *R. solani* on mycelial growth at 24, 36 and 48 hrs.

The efficacy of Propiconazole and Hexaconazole were tested for mycelial growth and per cent inhibition of *R. solani* by using poison food technique. At 24 hours of incubation, the minimum mycelial growth was recorded in Propiconazole the mycelial growth 1.45 mm followed by Hexaconazole 3.65 mm and control 45.17 mm, respectively. Each treatment was statistically differed to each other (Table 5, Fig. 5). At 36 hours of incubation, the mycelial growth was recorded 1.85 mm, 4.85 mm and 71.86 mm in Propiconazole, Hexaconazole and Control respectively. Each treatment was statistically differed to each other (Table 5, Fig. 5). At 48 hours of incubation, the results clearly indicated that mycelial growth was minimum in Propiconazole

(2.16 mm) followed by Hexaconazole (5.93 mm) as compared to control (85.42 mm). Each treatment was significantly differed to each other (Table 5, Fig. 5).

2. Efficacy of fungicides against *R. solani* on per cent inhibition at 24, 36 and 48 hours.

At 24 hours of incubation, results clearly indicated that maximum percent inhibition in Propiconazole (96.80%) followed by Hexaconazole (91.92%) as compared to control (0.00%). Each treatment was significantly differed to each other (Table 6, Fig. 6). At 36 hours of incubation, maximum percent inhibition was recorded in Propiconazole (97.41%) followed by Hexaconazole (93.25%) as compared to control (0.00%). Each treatment was significantly differed to each other (Table 6, Fig. 6). At 48 hours of incubation, maximum percent inhibition was recorded in Propiconazole (97.47%) followed by Hexaconazole (93.05%) as compared to control (0.00%). Each treatment was significantly differed to each other (Table 6, Fig. 6 Plate 3). Propiconazole and Hexaconazole significantly inhibit the growth of *R. solani* as compared to control. Propiconazole showed higher per cent inhibition as compared to Hexaconazole. (Table 6, Fig. 6). The present findings are supported by Vipin Kumaret al. (2001) reports that Propiconazole was more effective than Hexaconazole at inhibiting the mycelial growth *in vitro*.

Table 1: Effect of Plant extracts against *R. solanion* mycelial growth at 24, 36 and 48 hours.

Treatment	MycelialGrowth(mm)		
	Duration in Hours		
	24 Hours	36 Hours	48 Hours
Neem	12.90	18.63	19.95
Garlic	10.18	14.04	15.49
Tulsi	17.63	22.00	24.66

Onion	15.42	20.00	22.33
Ginger	11.43	16.07	18.00
Control	45.17	71.86	85.42
CD at5%	1.18	2.27	1.30
SE(m)	0.39	0.76	0.44

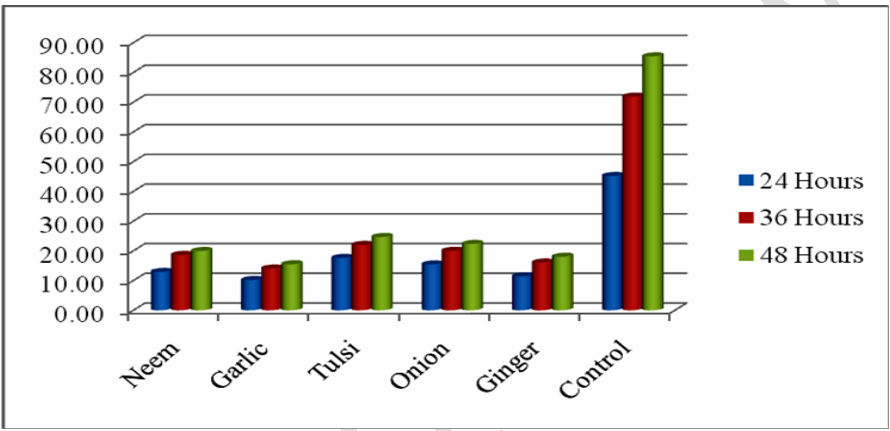


Fig. 1: Effect of Plant extracts against *R. solanion* mycelial growth at 24, 36 and 48 hours.

Table 2: Efficacy of Plant extracts against *R. solanion* percent inhibition at 24, 36 and 48 hours.

Treatment	Percent inhibition		
	Duration in Hours		
	24 Hours	36 Hours	48 Hours
Neem	71.43 (57.67)	74.09 (59.40)	76.52 (61.07)
Garlic	77.45 (61.62)	80.42 (63.76)	81.89 (64.79)

Tulsi	60.95 (51.31)	69.39 (56.40)	71.08 (57.48)
Onion	65.79 (54.20)	72.20 (58.20)	73.81 (59.23)
Ginger	74.67 (59.77)	77.63 (61.75)	78.98 (62.64)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at5%	2.34	2.88	1.47
SE(m)	0.78	0.96	0.49

Figure given in parenthesis are transformed value

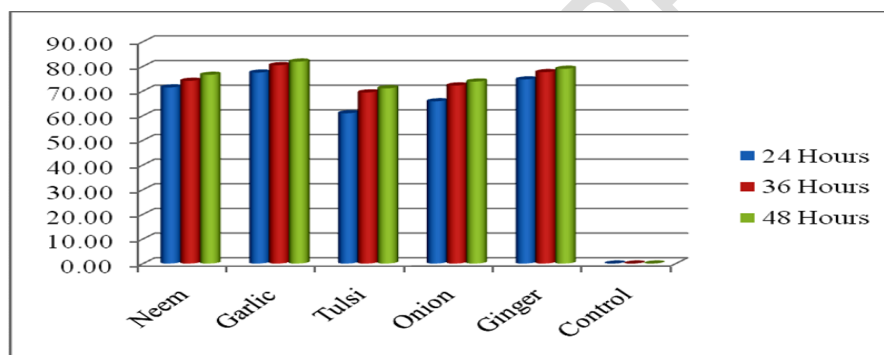


Fig. 2: Efficacy of Plant extracts against *R. solanion* percent inhibition at 24, 36 and 48 hours.
Table 3: Effect of bio-agents against *R. solanion* mycelial growth at 24, 36 and 48 hours.

Fungal antagonist	Mycelial Growth (mm)		
	24 Hours	36 Hours	48 Hours
<i>T. asperellum</i>	18.58	23.24	25.60
<i>T. harzianum</i>	23.14	31.58	39.55
Control	45.17	71.86	85.42
CD at 5 %	1.58	1.56	1.17
SE(m)	0.49	0.48	0.36

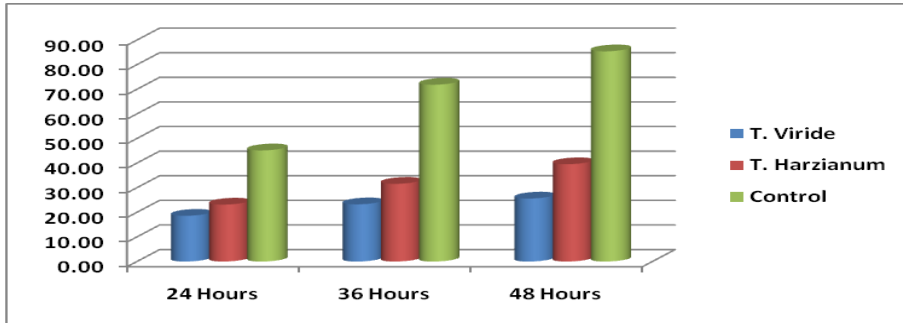


Fig. 3: Effect of bio-agents against *R. solanion* mycelial growth at 24, 36 and 48 hours

Table 4. Efficacy of bio-agents against *R. solanion* per cent inhibition at 24, 36 and 48 hours.

Fungal antagonist	Percent inhibition		
	24 Hours	36 Hours	48 Hours
	58.87	67.65	70.82
	(.28)	(.30)	(.45)
	(.00)	(.00)	(.09)
SE(m)	0.48	0.48	0.64

Figure given in parenthesis are transformed value

Fig. 4: Efficacy of bio-agents against *R. solanion* percent inhibition at 24, 36 and 48 hours.

Table 5: Effect of Fungicides against *R. solanion* mycelial growth at 24, 36 and 48 hours.

Treatment	Mycelial Growth (mm)		
	Duration In hours		
	24 Hours	36 Hours	48 Hours

Propiconazole	1.45	1.85	2.16
Hexaconazole	3.65	4.85	5.93
Control	45.17	71.86	85.42
CD at 5%	1.43	1.37	1.01
SE(m)	0.44	0.42	0.31

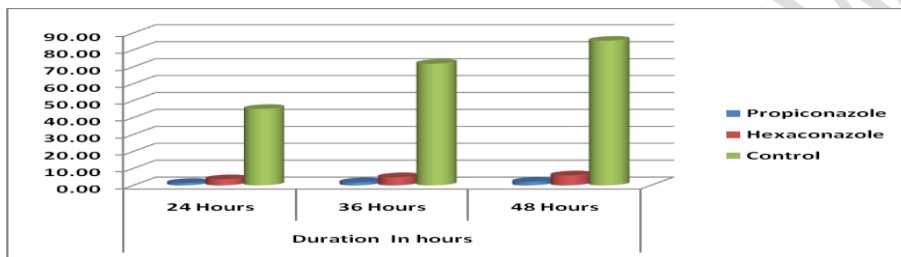


Fig. 5: Effect of fungicides against *R. solanion* mycelial growth at 24, 36 and 48 hours.

Table 6: Efficacy of Fungicides against *R. solanion* percent inhibition at 24, 36 and 48 hours.

Treatment	Percent inhibition		
	Duration In hours		
	24 Hours	36 Hours	48 Hours
Propiconazole	96.80 (79.67)	97.42 (80.74)	97.47 (80.81)
Hexaconazole	91.92 (73.46)	93.25 (74.91)	93.05 (74.70)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
CD at 5%	0.66	0.23	0.76
SE(m)	0.20	0.07	0.23

Figure given in parenthesis are retransformed value

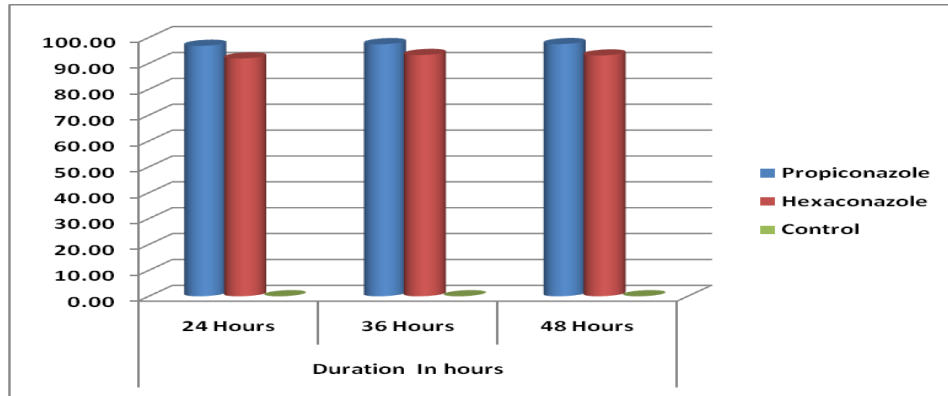
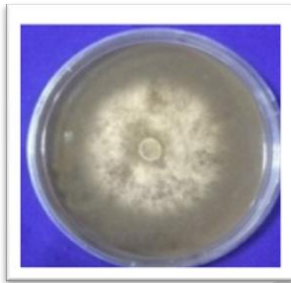


Fig. 6: Efficacy of fungicides against *R. solanion* inhibition percent at 24, 36 and 48 hours.



NeemGarlicTulsi



OnionGinger



control

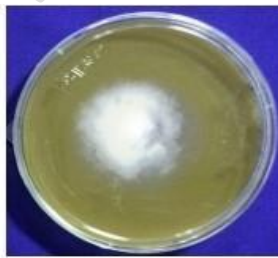
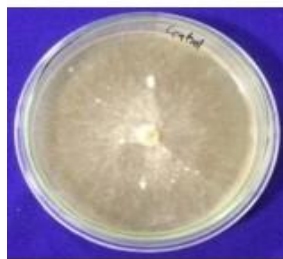


Plate:1. Response of botanicals against *R. solanion* mycelial growth.

Comment [A34]: Very shameful thing for the author. They used fake photographs for different treatments. The photograph used for Tulsi and Onion is the same. Even some photographs are with the label "Control"

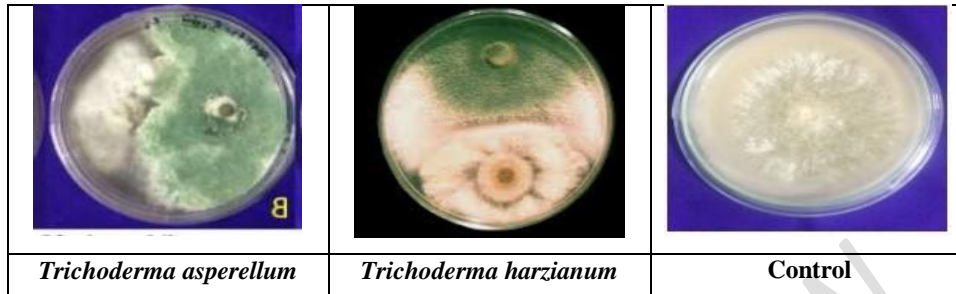


Plate:2.Response of bio-agents against *R. solanion* mycelial growth.

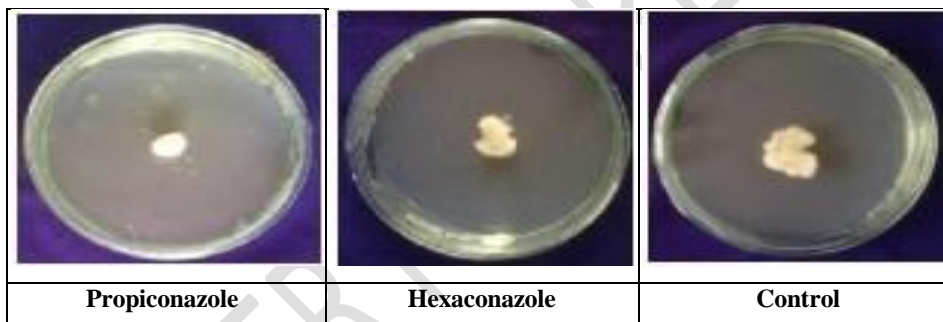


Plate:3. Response of fungicides against *R. solanion* mycelial growth.

References

- Anonymous (2019). *All India Coordinate Research Project on MullaRP, IIPR. Kanpur*, 108 pp.
- Nacien, C.C. (1924). Studies on *Rhizoctonia blight* of beans. *Philippine Agriculturist*, 8:315-321.
- Dwivedi, R.P. and Saksena, H.K. (1974). Occurrence of web blight disease caused by *Thanatephorus cucumeris* on mungbean. *Indian J. Farm Sci.* 2:100.
- Saikia, U.N. 1976. Blight of mung caused by *Corticium sasakii* a new disease recorded from Assam. *Indian Phytopath.* 29: 61-62.

Comment [A35]: Treatments and control having the almost same growth

Comment [A36]: The references cited in the paper need to be formatted correctly, following a standardized citation style.

Comment [A37]: Occurrence

- Bains, S.S., Dhaliwal, H.S. and Basandrai, A.K. 1988. A new blight of Mung and Mash in Punjab. *Ann. Biol. Ludhiana*. 4: 113–114.
- Tiwari, A. and Khare, M.N. (1998). Variability among isolates of *Rhizoctonia solani* infecting mungbean. *Indian phytopath.*, 51:334-337.
- Anonymous (2004). *Annual Report (kharif)*. All India Co-ordinated Research Project on MullaRP (ICAR), IIPR, Kanpur, 112 pp.
- Gupta, R.P. Singh, S.K. and Singh, R.V. (2010). Assessment of losses due to web blight and weather effects on disease development in mungbean. *Indian Phytopath.* 63 (1) : 108-109.
- Vincent, J.M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 159:850-853.
- Dubey, S.C. and Patel, B. (2001). Evaluation of fungal antagonists against *Thanatephorus Cucumeris* causing web blight of urd and mungbean. *IndianPhytopath.* 54(2) : 206- 209.
- Dubey, S.C. (2002). Evaluation of *Glioclodiumvirens* and *Trichoderma viride* as foliar spray against web blight of urd and mungbean. *J. Mycol. Pl. Pathol.* 32 (2):236-237
- Meena, R.L., Rathore and Mathur, K. (2002). Evaluation of fungicides and plant extracts against banded leaf and sheath blight of maize (*Zea mays*). *J. Mycol. PL. Pathol.* 32 (3):397.
- Khan, A.A. and Sinha, A.P. (2007). Screening of *Trichoderma* spp. against *Rhizoctonia solani* the causal agent of rice sheath blight. *Indian Phytopath.* 60 (4):450-456
- Kumar, Vipin., Chaudhary, V.P., Kumar, Dharmendra., Kumar, Ajay., Sagar, Sushma and Chaudhary, Sorabh. (2017) Efficacy of botanicals and fungicides against *Rhizoctonia solani* inciting sheath blight disease on Rice (*Oryza sativa* L.) *Journal of Applied and Natural Science* 9 (4): 1916 -1920(2017)
- Shinde, G.R. and Patel, R.L. (2004). Evaluation of plant extract against *R. solani* incitant of black scurf Disease in Potato. *J. Mycol. Pl. Pathol.* 34 (2) 28:4- 285