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Evaluation of Fungicides against Growth of *Rhizoctonia solani* under *in vitro* condition

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ABSTRACT: *Rhizoctonia solani* is a multiphagous widely distributed plant pathogen. Web blight caused by *Rhizoctonia solani* causes huge yield loses in urdbean (*Vigna mungo*). All the commercially grown varieties were found susceptible during course of investigation. Being a typical soil borne fungus, its management through chemicals is expensive and not feasible, because of the physiological heterogeneity of the soil and other edaphic factors etc. Integrated approaches of the disease management are paying more attentiveness in terms of sustainability. A set of six fungicides namely Azoxystrobin 23% SC, Propiconazole 25% EC, Difenoconazole 25% EC, Tebuconazole 25% EC, Carbendazim 12% WP and Copper oxy chloride 50% WP were tested for their efficacy against *R. solani* under in vitro conditions using Poisoned food technique at five different concentrations of respective fungicide (250 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm). Among the different fungicides, two fungicides namely Tebuconazole 25% EC and Carbendazim 12% WP completely inhibited the growth of test fungi *R. solani* even at 250 ppm concentration and no mycelial growth of *R. solani* was recorded in these two treatment at 250 ppm concentration and above that. However, Propiconazole and difenoconazole were most effective at 1000 and 2000 ppm. Rest of the fungicides were effective over the control but not at par with each other.

Keywords: *Rhizoctonia solani*, fungicides, mycelial growth.

INTRODUCTION

Urdbean (*Vigna mungo* (L.) Hepper) is an important pulse crop of India. This crop is a major source of dietary proteins, minerals and vitamins for vegetarian population of India. It is also rich in phosphoric acid. Urd bean is also cultivated as mixed crop with finger millet or barnyard millet in the hills of Uttaranchal during the *kharif* season. In North India, it is grown in *kharif* and summer season. In India is its primary origin and is mainly cultivated in Asian countries including Pakistan, Myanmar and parts of Southern Asia. About 70 per cent of the world's blackgram production comes from India. India is the world's largest producer as well as consumer of blackgram. It produces about 24.5 lakh tonnes of Urad annually from about 4.6 million hectares of area, with an average productivity of 533 Kg per hectare in (2020-21 agricoop.nic.in). Blackgram area accounts for about 19 per cent of India's total pulse acreage which contributes 23 per cent of total pulse production. Despite being an important pulse crop its productivity has been quite low probably due to various biotic and abiotic constraints. Urdbean is vulnerable to a variety of diseases *viz.*, anthracnose (*Glomerella lindemuthianum*), dry root rot (*Macrophomina phaseolina*), leaf spot (*Cercospora canescens*), powdery mildew (*Erysiphe polygoni*), rust (*Uromyces phaseoli*), web blight (*Rhizoctonia solani*), Mosaic and leaf crinkle (Bara, 2007). Among the biotic constraints, web blight disease of urdbean caused by *Rhizoctonia solani* Kuhn [Teleomorph: *Thanatephorous cucumeris* (Frank) Donk] is considered as an important constraint accountable for losses in production as well as productivity in India up to 20-30% (Kumar *et al.*, 2018). The disease had been reported in other countries like Pakistan, Sri Lanka, West Indies, Japan, Philippines, Myanmar, North America, South America, Argentina, Brazil, and Mexico too beside India. The disease has been reported from various urdbean growing areas of India including; Punjab, Haryana, Bihar, Rajasthan, Uttarakhand, Madhya Pradesh, Uttar Pradesh, West Bengal, Himachal Pradesh and Jammu and Kashmir (Shailbala and Tripathi 2007). The disease appears about 21-25 days after sowing depending on

cultivars, environmental conditions, crop stages and cultivation practices (Dubey and Patel 2001; Shailbala and Tripathi 2007). Seed quality and grain yield are heavily affected in this disease. Web blight of urd bean is a seed and soil borne disease (Saksena and Dwivedi 1973; Dwivedi and Saksena 1975) and managed by chemical seed treatment (Dubey and Dwivedi 1988). The chemicals not only disturb the ecology of soil but also develop hazardous impact on surroundings including *Rhizobium* spp. Biological seed treatment with fungal antagonist has significant promise against such devastating pathogens (Mukhopadhyay, 1994) but suitable methods of seed treatment and optimum doses are ingredients for successful management. The first report of occurrence of web blight on urdbean caused by *Rhizoctonia solani* Kuhn [Teleomorph: *Thanatephorous cucumeris* (Frank) Donk] in India was reported by (Saksena and Dwivedi 1973). This disease is known to occur in other leguminous crops like mungbean (Dwivedi and Saksena 1975), pigeonpea (Dwivedi and Saksena 1975), cowpea (Lakshman *et al.* 1979), soybean (Verma and Thapliyal 1976), groundnut (Dwivedi and Dubey 1986) and rice bean (Jalali, 1989).

MATERIAL AND METHODS

Effect of fungicides on radial mycelium growth of *Rhizoctonia solani.* In order to find out suitable fungicides for management of *Rhizoctonia solani* of black gram six fungicides namely Azoxystrobin, propconazole, tebuconazole, Carbendazim and copper oxychloride along with control was evaluated against *Rhizoctonia solani* by following the poisoned food technique under *in vitro* condition. PDA poisoned with each fungicide quantity was poured into three sterilized petriplates @ 20 ml/plate and allowed to solidify. Plates containing PDA without fungicide served as check. After solidification each petriplate was inoculated with 5 mm mycelial disc aseptically. Plates were incubated at 28 + 10C and observation on radial mycelium growth of test fungus. Growth was measured at 48, 72 and 96 hours the colony in the control plate was covered with the growth of mycelium of pathogen. The details about fungicides have been give in table.

Table 1: List of fungicides with their trade name and chemical name.

Common Name	Trade Name	Chemical Name	Manufacturer
Azoxystrobin	Amistar	Methyl(2E)-2-(2-{[6-(2-cyanophenoxy)pyrimidin-4-yl]oxy}phenyl)-3-methoxyacrylate	Syngenta India Ltd.
Propiconazole	Tilt	1-[[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole dioxolan-2-yl]methyl]-1,2,4-triazole	Syngenta India Ltd.
Difenocnazole	Score	1-((2-(2-Chloro-4-(4-chlorophenoxy)phenyl)-4-methyl-1,3-dioxolan-yl)methyl)-1H-1,2,4-triazole	Syngenta India Ltd.
Tebuconazole	Folicur	1-(4-Chlorophenyl)- 4,4-dimethyl-3-(1H, 1,2,4-triazol-1-ylmethyl) pentan- 3-ol	Bayer Crop Science
Carbendazim	Quintal	Methyl1H-benzimidazol-2-ylcarbamate	Bayer Crop Science
Copperoxychloride	blitox	Copper oxychloride	Rallis India Ltd.

Table 2: Name of fungicides, formulation and their doses.

Treatment	Name of fungicides	Formulation	Doses (ppm)
T1	Azoxystrobin	Liquid	250,500,1000,1500,2000 ppm
T2	Propiconazole	Liquid	250,500,1000,1500,2000 ppm
T3	Difenocnazole	Liquid	250,500,1000,1500,2000 ppm
T4	Tebuconazole	Liquid	250,500,1000,1500,2000 ppm
T5	Carbendazim	Powder	250,500,1000,1500,2000 ppm
T6	Copperoxychloride	Powder	250,500,1000,1500,2000 ppm
T7	Control	-	-

Table 3: Skeleton of analysis of variance.

Source of variation	DF	SS	MSS	T.CAL	F.TAB (5%)
Treatments					
Error					
Total					

Statistical Analysis. The data were analyzed statistically using Complete Randomized Design (CRD). Treatments were compared by mean of critical differences at 5% level of significance.

Test of significance. To test the significance difference among the treatment means following formula were used for calculating the critical differences.

S.Em± = MSE
r
C.D. = S.Em × √2 ‘t’ at error d.f.
Where:
D.F. = Degree of Freedom
S.S. = Sum of square
M.S.S. = Mean sum of square
The significant difference between mean was determined by using critical difference.
S.Em ± = EMss/replication
C.D. = S.Ed. × t 5% at error d.f.

RESULTS

Effect of fungicides on mycelial growth of *Rhizoctonia solani.* A set of six fungicides namely Azoxystrobin 23% SC, Propiconazole 25% EC, Difenoconazole 25% EC, Tebuconazole 25% EC, Carbendazim 12% WP and Copper oxy chloride 50% WP were tested for their efficacy against *R. solani* under *in vitro* conditions using Poisoned food technique at five different concentrations of each fungicide (250 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm). Data were recorded for mycelial radial growth after three different incubation periods (48 hrs, 72 hrs and 96 hrs) and per cent inhibition in growth of *R. solani* was calculated above untreated control.

It was observed that after 48 hrs of incubation period, all the six fungicides significantly reduced the growth of *R. solani*. However, mycelial radial growth of *R. solani*varied from treatment to treatment. Two fungicides namely Tebuconazole 25% EC and Carbendazim 12% WP completely inhibited the growth of test fungi *R. solani* even at 250 ppm concentration and no mycelial growth of *R. solani* was recorded in these two treatments at 250 ppm concentration and above that. This showed the highest efficacy of Tebuconazole 25% EC and Carbendazim 12% WP among the tested fungicides against *R. solani*. However, maximum mycelial radial growth (7.66 mm) and minimum per cent inhibition (64.09%) of *R. solani* was recorded in Copper oxychloride 50% WP at 250 ppm concentration and after 48 hrs of incubation period. At 500 ppm concentration, average mycelial radial growth of *R. solani* ranged from 0.00 mm to 3.66 mm. However, at 1000 ppm concentration only two fungicides namely Azoxystrobin 23% SC and Copper oxy chloride 50% WP showed 1.83 mm and 2.50 mm mycelial radial growth respectively after 48 hrs of incubaition period. Further on increasing the concentrations of respective fungicides, the per cent inhibition kept on increasing and it was observed that at 1500 ppm concentration all the six tested fungicides completely inhibited the growth of *R. solani* and no mycelial growth was recorded in any treatment except control after 48 hrs of incubation period. In control plate of *R. solani*, where no fungicide was added, mycelial radial growth of 21.33 mm was recorded after 48 hrs of incubation period. The detailed data for mycelial radial growth and per cent inhibition in growth of *R. solani* in different treatments after 48 hrs of incubation period are presented in Table 1. The graphical representation for mycelial radial growth and per cent inhibition in growth of *R. solani* in different treatments after 48 hrs of incubation period are presented in Plate Further, mycelial radial growth of *R. solani* was recorded in different treatments and per cent inhibition in growth of *R. solani* was calculated after prolonged incubation of 72 hrs. It was observed that after 72 hrs of incubation period, 50.33 mm mycelial radial growth of *R. solani* was recorded in control where no fungicide was added. Among different treatments, two fungicides namely Tebuconazole 25% EC and Carbendazim 12% WP completely inhibited the growth of the test fungus *R. solani* even at 250 ppm concentration and no mycelial growth of *R. solani* was recorded in these two treatments at 250 ppm concentration and above that even after 72 hrs of incubation period. The maximum mycelial radial growth (8.83 mm) and minimum per cent inhibition (82.46%) of *R. solani* was recorded in Copper 78 oxychloride 50% WP at 250 ppm concentration and after 72 hrs of incubation period. At 500 ppm concentration, average mycelial radial growth of *R. solani* ranged from 0.00 mm to 6.33 mm.

Further, it was observed that at 1500 ppm concentration all the six tested fungicides completely inhibited the growth of *R. solani* and no mycelial growth was recorded in any treatment except control after 72 hrs of incubation period. The detailed data for mycelial radial growth and per cent inhibition in growth of *R. solani* in different treatments after 72 hrs of incubation period are presented in (Table 2). The graphical representation for mycelial radial growth and per cent inhibition in growth of *R. solani* in different treatments after 72 hrs of incubation period are presented in Plate 3.1.

After 96 hrs of incubation period, it was observed that *R. solani* covered full mycelial radial growth of 89.33 mm in control plate. Among different treatments, maximum mycelial radial growth of 89.33 mm was recorded in Copper oxychloride 50% WP at 250 ppm concentration. However, Tebuconazole 25% EC and Carbendazim 12% WP completely inhibited the growth of *R. solani* at all the tested concentrations and no mycelial growth of *R. solani* was recorded in these two treatments. At 500 ppm and 1000 ppm concentrations, more than 95 per cent inhibition was recorded in all the fungicides except Copper oxychloride 50% WP. Further, at 1500 ppm concentration all the six tested fungicides completely inhibited the growth of *R. solani* and no mycelial growth was recorded in any treatment except control after 96 hrs of incubation period. The detailed data for mycelial radial growth and per cent inhibition in growth of *R. solani* in different treatments after 96 hrs of incubation period are presented in (Table

4, 5 and 6). The graphical representation for mycelial radial growth and per cent inhibition in growth of *R. solani* in different treatments after 96 hrs of incubation period are presented in (Fig. 1, 2 and 3). The pictorial representation of mycelial radial growth of *R. solani* in different treatments after 96 hrs of incubation period is depicted in plate.

Table 4: Effect of different fungicides on radial growth of *Rhizoctonia solani* after 48 hours incubation period.

Fungicide	Mean radial growth (mm*)									
	Concentration (ppm)									
	250	% inhibition	500	% inhibition	1000	% inhibition	1500	% inhibition	2000	% inhibition
Azoxystrobin 23 % SC	4.00	81.25	2.66	87.53	1.83	91.42	0.00	100.00	0.00	100.00
Propiconazole 25 % EC	3.83	82.04	3.66	82.84	0.00	100.00	0.00	100.00	0.00	100.00
Difenoconazole 25 % EC	2.50	88.28	2.16	89.87	0.00	100.00	0.00	100.00	0.00	100.00
Tebuconazole 25 % EC	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Carbendazim 12 % WP	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
COC 50 % WP	7.66	64.09	3.00	85.94	2.50	88.28	0.00	100.00	0.00	100.00
Control	21.33	0.00	21.33	0.00	21.33	0.00	21.33	0.00	21.33	0.00
SEm±	A= 0.111, B= 0.093 C= 0.247									
CD at 5 %	A= 0.312, B= 0.264 C= 0.699									

*Mean of three replication

Table 5: Effect of different fungicides on radial growth of *Rhizoctonia solani* after 72 hours incubation period.

Fungicide	Mean radial growth (mm*)									
	Concentration (ppm)									
	250	% inhibition	500	% inhibition	1000	% inhibition	1500	% inhibition	2000	% inhibition
Azoxystrobin 23 % SC	4.16	91.73	2.43	95.17	2.50	95.03	0.00	100.00	0.00	100.00
Propiconazole 25 % EC	4.00	92.05	3.00	94.04	0.00	100.00	0.00	100.00	0.00	100.00
Difenoconazole 25 % EC	3.16	93.72	2.83	94.38	0.00	100.00	0.00	100.00	0.00	100.00
Tebuconazole 25 % EC	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Carbendazim 12 % WP	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
COC 50 % WP	8.83	82.46	6.33	87.42	3.66	92.73	0.00	100.00	0.00	100.00
Control	50.33	0.00	50.33	0.00	50.33	0.00	50.33	0.00	50.33	0.00
SEm±	A= 0.217, B= 0.184 C= 0.486									
CD at 5 %	A= 0.614, B= 0.519 C= 1.372									

*Mean of three replication

Table 6: Effect of different fungicides on radial growth of *Rhizoctonia solani* after 96 hours incubation period.

Fungicide	Mean radial growth (mm*)									
	Concentration (ppm)									
	250	% inhibition	500	% inhibition	1000	% inhibition	1500	% inhibition	2000	% inhibition
Azoxystrobin 23 % SC	5.00	94.40	3.00	96.64	2.50	97.20	0.00	100.00	0.00	100.00
Propiconazole 25 % EC	4.33	95.15	3.33	96.27	0.00	100.00	0.00	100.00	0.00	100.00
Difenoconazole 25 % EC	3.66	95.90	3.66	95.90	0.00	100.00	0.00	100.00	0.00	100.00
Tebuconazole 25 % EC	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
Carbendazim 12 % WP	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
COC 50 % WP	15.00	83.21	13.33	85.08	11.66	86.95	0.00	100.00	0.00	100.00
Control	89.33	0.00	89.33	0.00	89.33	0.00	89.33	0.00	89.33	0.00
SEm±	A= 6.132 B= 5.183 C= 13.712									
CD at 5 %	A= 16.994 B= 14.364 C= 38.758									

*Mean of three replication

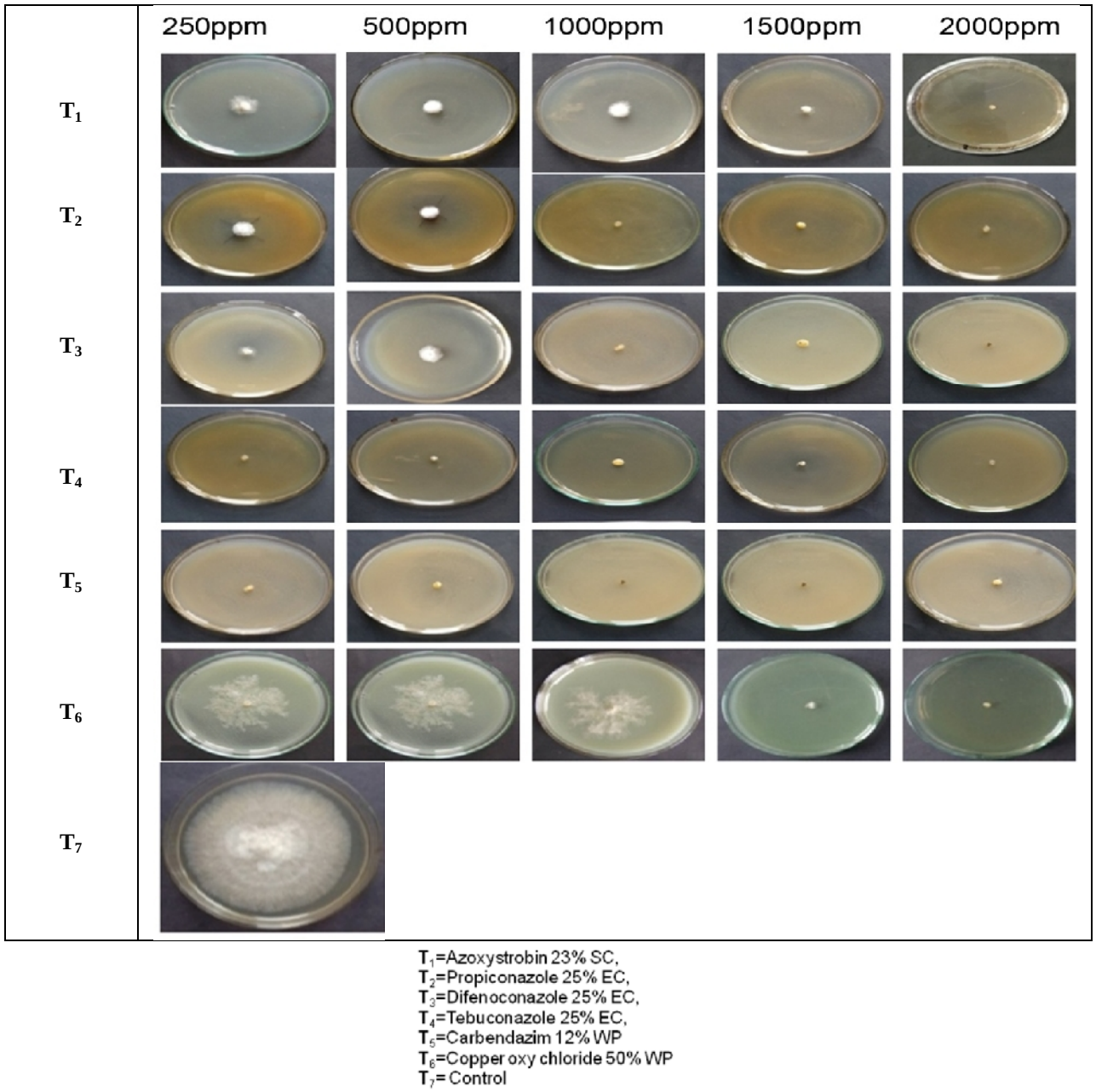

Plate 1: Effect of fungicides on mycelial growth of *Rhizoctoniasolani* after 96 hrs of incubation period.

DISCUSSION

A set of six fungicides namely Azoxystrobin 23% SC, Propiconazole 25% EC, Difenoconazole 25% EC, Tebuconazole 25% EC, Carbendazim 12% WP and Copper oxychloride 50% WP was tested for efficacy against *R. solani* under *in vitro* conditions using Poisoned food technique at five different concentrations of respective fungicide (250 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm). Among the different fungicides, two fungicides namely Tebuconazole 25% EC and Carbendazim 12% WP completely inhibited the growth of test fungus *R. solani* even at 250 ppm concentration and no mycelial growth of *R. solani* was recorded in these two treatments at 250 ppm concentration and above that. This showed the highest efficacy of Tebuconazole 25% EC and Carbendazim 12% WP among the tested fungicides against *R. solani*.

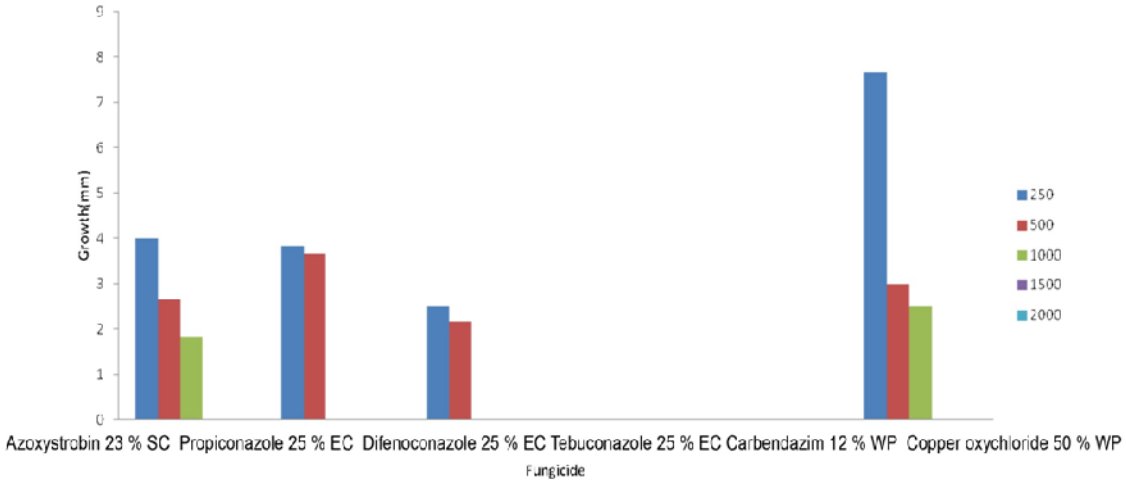

Fig. 1. Graphical representation of effect of fungicides on mycelial growth of *Rhizoctonia solani* after 48 hrs of incubation period.

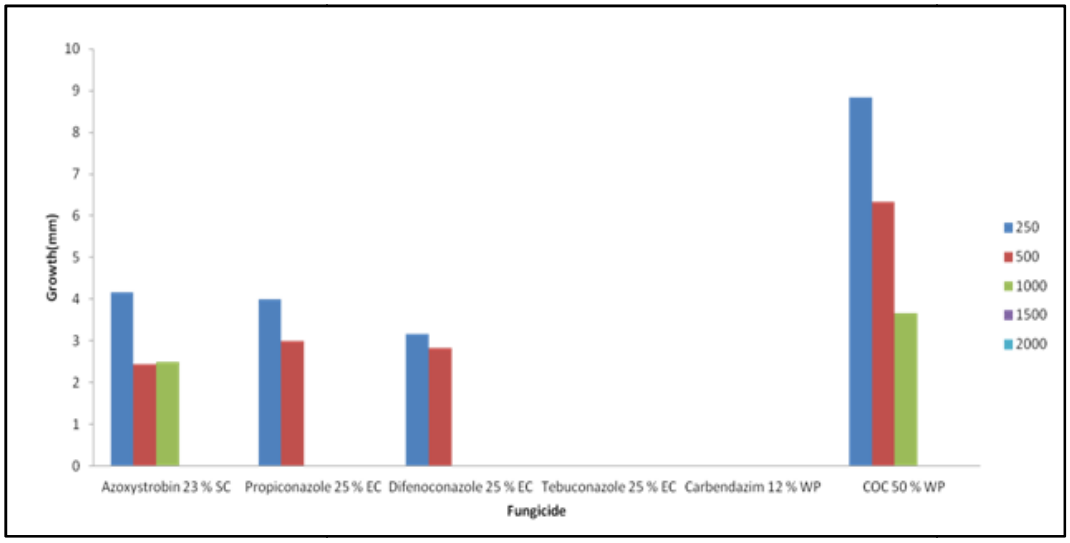

Fig. 2. Graphical representation of effect of fungicides on mycelial growth of *Rhizoctonia solani* after 72 hrs of incubation period.

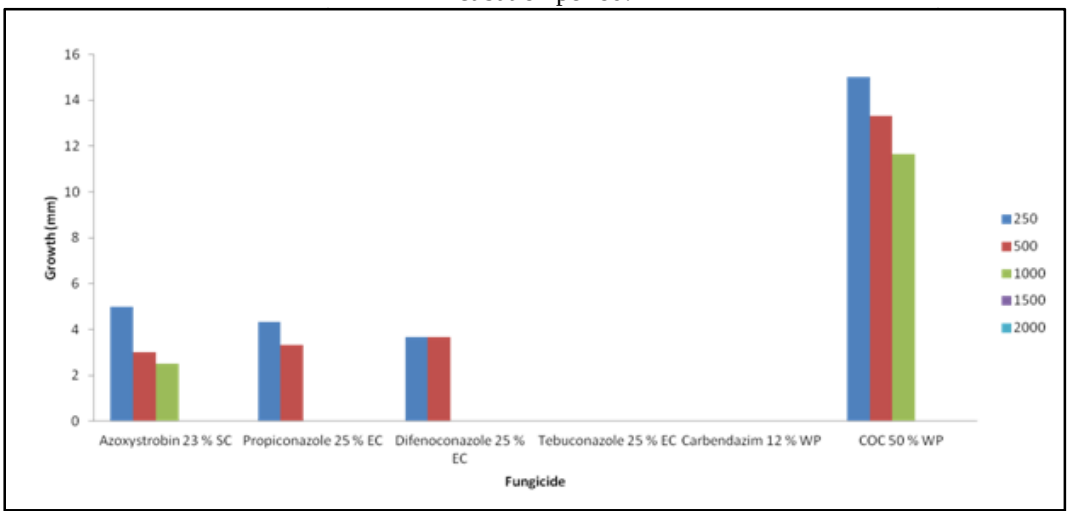

Fig. 3. Graphical representation of effect of fungicides on mycelial growth of *Rhizoctonia solani* after 96 hrs of incubation period.

The similar reports have been documented for use of fungicides and botanicals for the management of web blight of urd bean by Sharma and Tripathi (2001); Jhamaria and Sharma (2002); Shailbala and Tripathi (2004); Mishra *et al*., (2005); Shailbala and Tripathi (2007) and Shailbala and Tripathi (2010). Effect against new molecules of fungicides decreases the percent inhibition *in vitro* study may be good sign of management of *R. solani.* The importance of study revealed significance in respect their behavior against the host generated better approach in the management strategy.

In our study the fungicides Tebuconazole 25% EC and Carbendazim 12% WP completely inhibited the growth of test fungus *R. solani* even at 250 ppm concentration and no mycelial growth of *R. solani* was recorded in these two treatments at 250 ppm concentration. Other fungicides showed their efficacy against test fungus at higher concentrations.

CONCLUSION

Rhizoctonia solani cause serious problem and it is responsible for wide range of commercially significant plant diseases. *In vitro* study concluded that out of six fungicides tested at five concentrations with different formulations inhibited the growth of *Rhizoctonia solani*. The mycelial growth *of Rhizoctonia solani* were arrested *in vitro* study. Some of the fungicides are new molecules recommended against foliar and soil inhabitant fungi, hence effectively may not be as their chemical behaviour.

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Conflict of Interest. None.

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