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In-vitro and *in-vivo* Evaluation of Fungicides Against Collar Rot of Chickpea (*Sclerotium rolfsii*)

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ABSTRACT: The efficacy of fungicides was tested at different concentrations of 100ppm, 500ppm, 1000ppm and 2000ppm against *Sclerotium rolfsii* on PDA by poisoned food technique *in-vitro* condition and seed treatment with fungicides under pot experiment. It was concluded that fungicides like, Metiram 70% WG, Tricyclazole 75% WP, Propiconazole 25% EC, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Hexaconazole 5% SC, Iprobenfos 48% EC, Azoxystrobin 11% +Tebuconazole 18.3% SC, Thifluzamide 24% SC, Mancozeb 75% WP, Propineb 70% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Tebuconazole 38.39% w/w SC, Hexaconazole 4% + Zineb 68% WP and Metiram 55% + Pyraclostribin 5% WG were completely inhibitory at 100ppm concentration showing high potential against *Sclerotium rolfsii.* While, Kasugamycin 3% SL showed complete inhibition of the pathogen at 500ppm concentration. Fungicide *i.e.* Validamycin 3% showed partial inhibition of pathogen at 500ppm, 1000ppm and 2000ppm concentrations respectively. Similar results was found in case of seed treatment with fungicides under pot experiment. Fungicides which include phenylpyrroles, anilinopyrimidines, strobilurin analogues etc with effects on respiration, cell membranes components, protein synthesis, signal transduction and cell mitosis.

Keywords: Fungicides, *Sclerotium rolfsii,* collar rot, chickpea.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the world's third most important food legume after dry bean and pea India is the largest producer of chickpea, contributing more than 70 per cent of the total world production. One of the major constraints limiting agriculture production is difficult in managing disease caused by soil borne pathogens. Among the soil borne diseases of chickpea, Collar rot disease caused by *Sclerotium rolfsii* Sacc. is a serious threat to chickpea that may cause 55-95% mortality of the crop at seedling stage under favourable environment conditions (Gurha and Dubay 1982). Collar rot caused by soil borne fungus *Sclerotium rolfsii* Sacc is a major threat when weather conditions are conducive. It is an important diseases especially in paddy - chickpea or soybean - chickpea based cropping systems. The disease is favoured by good soil moisture, high soil temperature (25-30^{o}C) and low organic matter in the soil (Mathur and Sinha 1968). Collar rot is a fast spreading and destructive disease of chickpea. The disease is confined at seedling stage (upto 45 days) resulting in reduced plant population. Collar rot can cause 54.7-95% mortality of chickpea seedlings (Kotastthane *et al*., 1976; Nirmalkar *et al*., 2017). Diseases caused due to *S. rolfsii* requires warm climates, occurs more frequently at high moistures and high temperatures (Al-Askar *et al.,* 2013) *S. rolfsii* initiates the rotting of seeds and seed surfaces are covered with white mycelia mat in soil. Germinated seedlings were killed within 7 days after emergence. Symptoms were evident as yellowing and collapse. The affected roots showed rotting at the collar region and down ward with the whitish mycelium in earlier stages of infection. Rapeseed like *sclerotia* can be observed attached to mycelium around the collar (Nene *et al.,* 1978).

MATERIALS AND METHODS

Isolation of pathogen *Sclerotium rolfsii* from infected plant samples. Fresh samples of collar rot were brought to the laboratory in paper bags and washed under tap water to remove dust and other inert materials. In Small pieces of specimen were then cut, with each piece containing half infected and half healthy portions. These pieces were disinfected with a sodium hypochlorite (0.1%) solution for 1 minute followed by washing with sterilized distilled water. The pieces were placed on blotting paper and allow to dry. Once properly dried the pieces were transferred into culture slants and subsequently transferred into petri dishes containing PDA media (Neha *et al*., 2016).

***In-vitro*, evaluation of fungicides against *Sclerotium rolfsii*by poison food technique.** The efficacy of 16 fungicides (Metiram 70% WG, Tricyclazole 75% WP, Propiconazole 25% EC, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Validamycin 3% L, Hexaconazole 5% SC, Iprobenfos 48% EC, Azoxystrobin 11% + Tebuconazole 18.3% SC, Thifluzamide 24% SC, Mancozeb 75% WP, Propineb 70% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Kasugamycin 3% SL, Tebuconazole 38.39% w/w SC, Hexaconazole 4% + Zineb 68% WP and Metiram 55% + Pyraclostribin 5% WG) was evaluated *in-vitro*at different concentrations of 100, 500, 1000 and 2000ppm on growth of *Sclerotium rolfsii*on Potato dextrose agar (PDA) medium using poisoned food technique (Nene and Thapliyal 1982).

The pathogen *Sclerotium rolfsii* was grown on Potato Dextrose Agar (PDA) medium for a period of seven days. The PDA medium was prepared and melted. Concentrations of fungicide were added to the cooled melted medium. Each sterilized petri plate was poured with 25-30 ml of the fungicide containing medium. To prevent bacterial contamination, 500 ppm of streptomycin was added to the medium while pouring it into the petri plates. A ycelia disc with a diameter of 5 mm was taken from the periphery of a fresh culture of test plant pathogens *i.e. Sclerotium rolfsii* and placed in the centre of each petri plate. Three replications were maintained for each treatment. The inoculated plates were then incubated at 28 ± 2°C for 4 days. The percentage of inhibition was calculated using the formula developed by Vincent (1947).

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

Where,
I = per cent inhibition
C = growth in control
T = growth in treatment

***In-vivo*, Testing the efficacy of fungicides as seed treatment for the control of collar rot of chickpea (*Sclerotium rolfsii*).** Pot experiment was conducted during *Rabi* season in 2023-2024 to assess the efficacy of different fungicides as seed treatment applied for the control of collar rot of chickpea. Different fungicides were used at 3g/seed as seed treatment to treat the chickpea seeds. The experiment was laid out in completely randomized design (CRD) and each treatment was replicated thrice.

Treatment Details

	Treatments	Doses (g/kg seeds)
T_1	Metiram 70% WG	3g
T_2	Hexaconazole 4% + Zineb 68% WP	3g
T_3	Tebuconazole 38.39% w/w SC	3g
T_4	Iprobenfos 48% EC	3g
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG	3g
T_6	Propineb 70% WP	3g
T_7	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	3g
T_8	Metiram 55% + Pyraclostribin 5% WG	3g
T_9	Propiconazole 25% EC	3g
T_{10}	Mancozeb 75% WP	3g
T_{11}	Azoxystrobin 11% + Tebuconazole 18.3% SC	3g
T_{12}	Thifluzamide 24% SC	3g
T_{13}	Hexaconazole 5% SC	3g
T_{14}	Tricyclazole 75% WP	3g
T_{15}	Control	-

Preparation of inoculums. Fresh inoculum of collar rot pathogen (*Sclerotium rolfsii*) was prepared in the laboratory of Plant Pathology on sorghum grains which were boiled with water sealed and packed in polythene bag @ 200 g/bag and kept for sterilization in autoclave (121.6°C for 15 PSI for 15 min) after sterilized bag were allowed with mycelial disc to cool at room temperature followed by inoculation with mycelial disc 8 to 10 disc (5 mm) of 6 days old culture of *Sclerotium rolfsii* were aseptically inoculated into sterilized seed and incubated for the seven days in BOD incubator at 28 ± 2°C temperature. The cement pots (25 cm diameter) containing sterilized sandy loam soil, were inoculated with two weeks old culture of *Sclerotium rolfsii* (multiplied on sorghum grain medium) @ 25g/kg soil and allowed to incubate for fifteen days. Pots were regularly observed for the sclerotia formation and top layer of soils along with sclerotia were removed from the pot after Sclerotial formation and mixed with the soil a definite proportion. Number ofsclerotia were determined by the adding 10 gm of sick soil into water. Fresh pot further inoculated with 200g of sick soil which contain and covered and mixed with the soil with the definite proportion @ (920) number of sclerotia. Treated chickpea seeds (variety RVG- 202) were sown in pots (28 × 28 cm) @ 50 seeds / pot.

Observations recorded:

• Observations on seed germination and pre-emergence were recorded seven days after sowing, while post emergence seedling mortality (PESM) at 30 days after sowing.

• The percentage seed germination, pre emergence and post emergence seedling mortality was calculated by the formulas.

$$\text{Germination (\%)} = \frac{\text{Number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Pre emergence seedling mortality (\%)} = \frac{\text{Number of seed ungerminated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Post emergence seedling mortality (\%)} = \frac{\text{Number of seedlings dead}}{\text{Total number of seedlings}} \times 100$$

$$\text{Reduction (\%) in PESR and PESM} = \frac{C - T}{C} \times 100$$

Where, C = Per cent rot/ mortality in treatment pots

T = Per cent mortality in treated pots

RESULTS AND DISCUSSION

Data presented in Table 1 indicated that the different fungicides tested at different concentrations (100ppm, 500 ppm, 1000 ppm and 2000 ppm) significantly reduced the mycelial growth of *Sclerotium rolfsii* over untreated control (90.00 mm). However fungicides *i.e.* Metiram 70%WG, Tricyclazole 75% WP, Propiconazole 25% EC, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Hexaconazole 5% SC, Iprobenfos 48% EC, Azoxystrobin 11% +Tebuconazole 18.3% SC, Thifluzamide 24% SC, Mancozeb 75% WP, Propineb 70% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Tebuconazole 38.39% w/w SC, Hexaconazole 4% + Zineb 68% WP and Metiram 55% + Pyraclostribin 5% WG were completely inhibitory at 100ppm concentration showing high potential against *Sclerotium rolfsii*compared to other fungicides. Moreover, fungicides *i.e.* Validamycin 3% L (90.00, 65.83, 62.67 and 49.00 mm) and Kasugamycin 3% SL (60.67, 0.00, 0.00 and 0.00 mm) respectively at 100ppm, 500ppm, 1000ppm and 2000ppm. Present study also indicated that the new generation fungicides having combination of two molecules and formulated either in wettable powder (WP), emulsifiable concentrate (EC) and suspension concentrate (SC) were found to be significantly more effective compared to other systemic or non-systemic fungicides already recommended against *Sclerotium rolfsii.*

The results of present study all in accordance with the findings of Bhat and Shrivastava (2003) who reported the highly effectiveness of triazole fungicides *i.e.* hexaconazole, propiconazole and penconazole were also found result with higher inhibitory rates ranging from 63.0 per cent to 100 per cent against *Sclerotium rolfsii* at concentrations of 250, 500, and 1000 ppm. Similarly, Shirsole *et al.* (2019) also reported the effectiveness of fungicides used under the present study. The higher efficacy of systemic and combo fungicides (combination of two molecules) under the present study might be due to synergistic action of both molecules in inhibiting the mycelial growth of *Sclerotium rolfsii.* Sangeeta *et al.* (2022); Singh, *et al.* (2017) also reported that Hexaconazole (0.05%), Propiconazole (0.15%), and Mancozeb showed 100per cent inhibition.

Testing the efficacy of fungicides as seed treatment for the control of collar rot of chickpea (*Sclerotium rolfsii*). The pot experiment was carried out to assess the efficacy of fungicides for management of collar rot of chickpea. Fungicides were used as a seed treatment for management of collar rot mortality in chickpea.

The data recorded in Table 2 on per cent seed germination from various treatments indicated that seeds treated with Hexaconazole 4% + Zineb 68% WP (95.33%) followed by Tebuconazole 50% + Trifloxystrobin 25% WG (93.33%) and Hexaconazole 5% SC (90.33%) were found significantly superior over all other treatments including control (39.33%) for enhancing the per cent seed germination of chickpea. Seed treated with other fungicides *i.e.* Tebuconazole 38.39% w/w SC (88.33%), Thifluzamide 24% SC (86.33%), Metiram 55% + Pyraclostribin 5% WG (85.66%), Azoxystrobin 11% +Tebuconazole 18.3% SC (85.33%), Azoxystrobin 18.2% + Difenoconazole 11.4% SC (83.00%), Propineb 70% WP (81.33%), Metiram 70% WG (76.66%), Propiconazole 25% EC (71.33%), Iprobenfos 48% EC (67.33%), Mancozeb 75% WP (66.67%) and Tricyclazole 75% WP (62.00%) had also significant effect on increasing the per cent germination over control(39.33%).

Data represent in Table 2 indicated that the lowest per cent pre emergence mortality was observed in treatment Hexaconazole 4% + Zineb 68% WP (4.67%) which was at par with Tebuconazole 50% + Trifloxystrobin 25% WG (6.67%) and found significantly superior over other treatments including control (60.67%). Other fungicides *i.e.* Hexaconazole 5% SC (9.67%), Tebuconazole 38.39% w/w SC (11.67%), Thifluzamide 24% SC (13.67%), Metiram 55% + Pyraclostribin 5% WG (14.33%), Azoxystrobin 11% +Tebuconazole 18.3% SC (14.67%), Azoxystrobin 18.2% + Difenoconazole 11.4% SC (17.00%), Propineb 70% WP (18.67%), Metiram 70% WG (23.33%) and Propiconazole 25% EC (28.67%) were also found significant in controlling pre emergence mortality compare to control (60.67%). Whereas, Iprobenfos 48% EC (32.67%), Mancozeb 75% WP (33.33%) and Tricyclazole 75% WP (38.00%) were found least effective. Moreover these fungicides were found significantly superior over control (60.67%).

Data from Table 2 indicated that the treatment *i.e.* Hexaconazole 4% + Zineb 68% WP (0.00%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.00%), Hexaconazole 5% SC (0.00%), Tebuconazole 38.39% w/w SC (0.00%), Thifluzamide 24% SC (0.00%), Azoxystrobin 11% +Tebuconazole 18.3% SC (0.00%), and Propiconazole 25% EC (0.00%) were found significantly highly effective and completely checking the post emergence mortality of chickpea. Whereas, other fungicides *i.e.* Azoxystrobin 18.2% + Difenoconazole 11.4% SC (3.27%), Propineb 70% WP (28.00%), Metiram 70% WG (34.83%), Iprobenfos 48% EC (44.33%), Mancozeb 75% WP (28.73%) and Tricyclazole 75% WP (13.33%) were also found significantly effective for suppressing the post emergence mortality over control (75.00%).

The results of present study are in accordance with the findings of Shirsole *et al.* (2019), who reported that the efficacy of seventeen fungicides (systemic - seven, non-systemic - four and combo - six) as seed treatment under pot experiment (*in-vivo*). Seed treatment with Hexaconazole 5% EC, Propiconazole 25% EC and Azoxystrobin 35% EC exhibited complete protection from collar rot mortality and 100% reduction in disease incidence over control. Charde *et al.* (2002) also found that seed treatment with Propiconazole and Hexaconazole were superior in checking stem rot of groundnut caused by *S. rolfsii* and increasing the shoot and root biomass.

Table 1: *In-vitro,* evaluation of fungicides at different concentrations (ppm) of fungicides against mycelial growth of *Sclerotium rolfsii,* the causal agent of collar rot of chickpea (at 96 hour).

	Treatments	Mycelial growth (mm)				Mean
		100 ppm	500 ppm	1000 ppm	2000 ppm	
T_1	Metiram 70% WG	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_2	Tricyclazole 75% WP	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_3	Propiconazole 25% EC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_4	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_5	Validamycin 3% L	90.000 (71.56)	65.83 (54.23)	62.67 (52.34)	49.00 (44.42)	66.88
T_6	Hexaconazole 5% SC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_7	Iprobenfos 48% EC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_8	Azoxystrobin 11% + Tebuconazole 18.3% SC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_9	Thifluzamide 24% SC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{10}	Mancozeb 75% WP	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{11}	Propineb 70% WP	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{12}	Tebuconazole 50% + Trifloxystrobin 25% WG	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{13}	Kasugamycin 3% SL	60.67 (51.16)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	15.17
T_{14}	Tebuconazole 38.39% w/w SC	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{15}	Hexaconazole 4% + Zineb 68% WP	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{16}	Metiram 55% + Pyraclostribin 5% WG	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00 (0.34)	0.00
T_{17}	Control	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00 (71.56)	90.00
	Mean	14.16	9.17	8.98	8.18	
		Fungicide	Concentration	F x C		
	C.D.5%	0.11	0.24	0.48		
	CV%	3.54				

*Figures in the parentheses indicate arc sine transformed

Table 2: Effect of different fungicides on percent germination of chickpea seed and controlling pre-emergence and post-emergence collar rot mortality of chickpea caused by *Sclerotium rolfsii*.

	Treatments	Germination %	Per cent pre emergence mortality	Reduction per cent in pre emergence mortality	Per cent post emergence collar rot mortality	Reduction per cent in post emergence collar rot mortality
T_1	Metiram 70% WG	76.66 (61.14)	23.33 (28.85)	61.47 (51.66)	34.83 (36.16)	53.49 (42.00)
T_2	Hexaconazole 4% + Zineb 68% WP	95.33 (77.64)	4.67 (12.35)	92.33 (74.05)	0.00 (0.42)	100.00 (89.57)
T_3	Tebuconazole 38.39% w/w SC	88.33 (70.05)	11.67 (19.94)	80.73 (64.00)	0.00 (0.42)	100.00 (89.57)
T_4	Iprobenfos 48% EC	67.33 (55.15)	32.67 (34.84)	46.13 (42.77)	44.33 (41.74)	40.50 (39.46)
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG	93.33 (75.28)	6.67 (14.92)	89.03 (70.69)	0.00 (0.42)	100.00 (89.57)
T_6	Propineb 70% WP	81.33 (64.49)	18.67 (25.56)	69.18 (56.31)	28.00 (31.93)	62.50 (52.26)
T_7	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	83.00 (65.69)	17.00 (24.34)	71.95 (58.03)	3.27 (10.31)	95.58 (78.01)
T_8	Metiram 55% + Pyraclostribin 5% WG	85.66 (67.78)	14.33 (22.22)	76.34 (60.92)	0.00 (0.42)	100.00 (89.57)
T_9	Propiconazole 25% EC	71.33 (57.64)	28.67 (32.35)	52.69 (46.54)	0.00 (0.42)	100.00 (89.57)
T_{10}	Mancozeb 75% WP	66.67 (54.75)	33.33 (35.23)	45.02 (42.11)	28.73 (32.41)	61.63 (51.72)
T_{11}	Azoxystrobin 11% + Tebuconazole 18.3% SC	85.33 (67.77)	14.67 (22.44)	75.77 (60.60)	0.00 (0.42)	100.00 (89.57)
T_{12}	Thifluzamide 24% SC	86.33 (68.34)	13.67 (21.66)	77.44 (61.68)	0.00 (0.42)	100.00 (89.57)
T_{13}	Hexaconazole 5% SC	90.33 (72.08)	9.67 (18.09)	84.05 (66.48)	0.00 (0.42)	100.00 (89.57)
T_{14}	Tricyclazole 75% WP	62.00 (51.94)	38.00 (38.05)	37.31 (37.63)	13.33 (21.39)	82.20 (65.06)
T_{15}	Control	39.33 (38.83)	60.67 (51.16)	0.00 (0.42)	75.00 (60.07)	0.00 (0.42)
	CD	4.20	2.73	4.25	2.06	2.81
	CV	4.00	6.11	4.82	7.82	2.40

*Figures in the parentheses indicate arc sine transformed

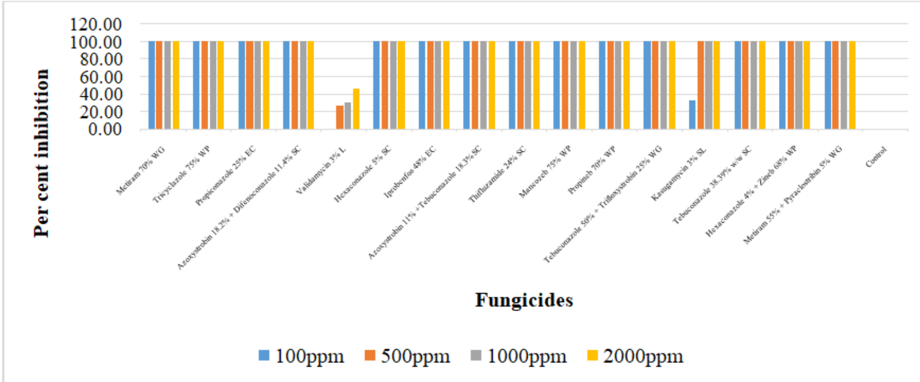

Fig. 1. *In-vitro,* evaluation of fungicides against *Sclerotium rolfsii*at different concentrations (ppm).

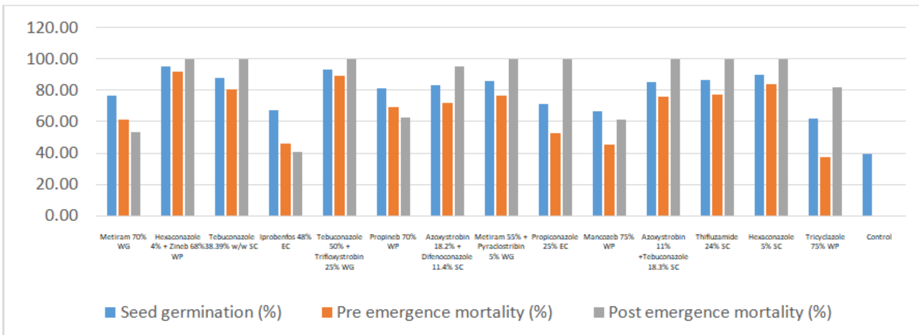

Fig. 2. Effect of seed treatment with fungicides on seed germination, pre emergence mortality and post emergence mortality of chickpea against collar rot.

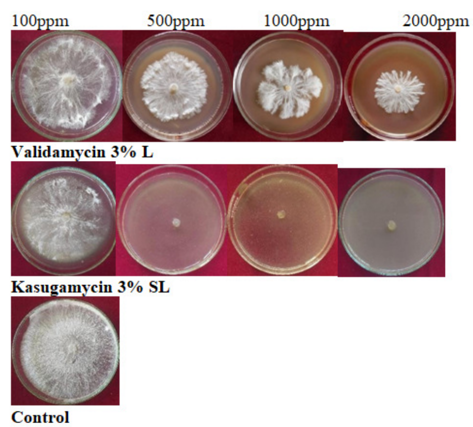

Plate 1. *In-vitro,* evaluation of fungicide against *Sclerotium rolfsii*at different concentrations (ppm).

CONCLUSIONS

In-vitro study on the efficacy of fungicides against *Sclerotium rolfsii* indicated that fungicides Metiram 70%WG, Propiconazole 25% EC, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Hexaconazole 5% SC, Azoxystrobin 11% +Tebuconazole 18.3% SC, Mancozeb 75% WP, Propineb 70% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 4% + Zineb 68% WP and Metiram 55% + Pyraclostribin 5% WG were found highly effective and can be used as seed treatment for the control of disease caused by *Sclerotium rolfsii*.

Findings of pot experiment conducted on the efficacy of fungicides as a seed treatment for the control of collar rot of chickpea indicated that fungicides *i.e.* Hexaconazole 4% + Zineb 68% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 5% SC, Tebuconazole 38.39% w/w SC, Thifluzamide 24% SC, Azoxystrobin 11% + Tebuconazole 18.3% SC, Propiconazole 25% EC were highly effective in controlling pre emergence and post emergence mortality caused by *Sclerotium rolfsii* causal agent of collar rot of chickpea.

FUTURE SCOPE

Fungicides found most effective against *Sclerotium rolfsii* under *in-vitro* and pot condition may be tested for their efficacy under natural field condition with different combination of novel fungicides and in combination of bioagents *i.e. Trichoderma* and

Pseudomonas as seed and soil treatment and their combination, Integrated management strategy for collar rot of chickpea that is combined the use of microbial agents by fortification techniques. The efficacy of the fungicides may be tested at lower dosages. Compatibility scale should be prepared according to toxic level.

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Conflict of Interest. None.

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