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Evaluation of fungicides against stem rot of capsicum caused by *Sclerotium rolfsii* Sacc

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Abstract
Stem rot (*Sclerotium rolfsii* Sacc.) of capsicum which is also called as collar rot or southern blight is one of the serious diseases in capsicum that severely brings down the yield in all the major capsicum growing areas in Karnataka. Among the several management practices, managing the disease with fungicides is the most effective means. In the present investigation twelve fungicides that included three systemic, six combi products and three contact fungicides were evaluated against *S. rolfsii in vitro*. Among the fungicides, hexaconazole, difenoconazole, propiconazole, propiconazole + difenoconazole, tricyclazole + hexaconazole, hexaconazole + captan, zineb + hexaconazole and thiram were highly effective even at least concentration of 25 ppm tested and also in subsequent concentrations of 50 ppm, 100 ppm, 250 ppm, 500 ppm showing 100 per cent inhibition. Further, among the eight promising fungicides based on *in vitro* results, six fungicides were evaluated against stem rot of capsicum under polyhouse conditions. Among the treatments undertaken, propiconazole and difenoconazole each at 0.1 per cent were effective resulting in 97.05 per cent reduction in disease over control and can be concluded as promising fungicides against stem rot.

Keywords: Stem rot of capsicum, *Sclerotium rolfsii*, fungicides

Introduction
Capsicum (*Capsicum annuum* var. *Grossum* Sendt.) is a member of solanaceous family mainly grown as vegetable throughout world including India. It is known by other names such as Shimla mirch, green pepper, bell pepper *etc*. India contributes one fourth of world production of capsicum with average annual production of 288 thousand MT from area of 46 thousand hectares with the productivity of 80-100 tonnes per hectare. In India, capsicum is extensively cultivated in Andhra Pradesh, Karnataka, Maharashtra, Himachal Pradesh, Tamil Nadu and hilly areas of Uttar Pradesh. Himachal Pradesh shares 19.90 per cent of total production with a production of 57.41 thousand tones whereas Karnataka stands second with share of 19.66 per cent, with a production of about 56.70 thousand tonnes (APEDA, 2017-18). In Karnataka, it is grown largely in Bengaluru rural, Chikkaballapur and Kolar districts. The crop is cultivated in both open and protected type. Since good economic returns have been experienced, the cultivation under polyhouse conditions has created awareness among the farmers about its returns. However, the crop is suffered by many biotic and abiotic factors that have considerable impact on the yield. Among the biotic factors, the diseases caused by fungi, viruses and nematodes are the major constraints in the production. Some of the diseases include anthracnose, cercospora leafspot, murda complex, charcoal rot, damping off, root rot, downy mildew, fusarium wilt, powdery mildew, stem rot and verticilium wilt. Among these, stem rot or collar rot or southern blight of capsicum caused by a soil borne pathogen *Sclerotium rolfsii* Sacc. is the emerging disease. Recently, the incidence of stem rot (Sothern blight) has been noticed to the tune of 50 per cent in Karnal, Haryana (Bhat, *et al*., 2015) [3]. The disease has been found to cause considerable yield loss (Yaqub and Shahzad, 2009) [16].
In India, information on stem rot of capsicum and its management is scanty (Chowdary *et al*., 2000; Singh, *et al*., 2007; Rather *et al.*, 2012) [4, 13, 8]. Considering the soil borne nature of the pathogen, crop rotation is one among the effective management practices. However, the farmers in Karnataka rarely go for crop rotation as the practice will not fetch them good profit and above all the holding size of the land is very less. Thus, the farmers inevitably rely upon

the chemicals as a means of controlling the disease. In this background a study was taken up to evaluate the new fungicide molecules against the stem rot disease of capsicum.

Materials and Methods

Isolation and purification of the pathogen

Capsicum plants depicting typical symptoms of stem rot were collected from capsicum growing fields of Kolar. The affected collar regions were cut into small pieces and were used for the isolation of the pathogen. The isolation was done according to tissue segment methodology (Rangaswami, 1958) [7]. The culture of *S. rolfsii* was purified by hyphal tip technique (Riker and Riker, 1936) [9]. The identification was done through colony colour, morphology of the pathogen. The pure culture of the pathogen was maintained on Potato Dextrose Agar (PDA) slants at 27±1ºC.

In vitro evaluation of fungicides against *S. rolfsii*

Twelve fungicides comprising of three systemic, six combi products and three contact fungicides (Table.1) were evaluated *in vitro* for their efficacy against *S. rolfsii*, the causal agent of stem rot of capsicum at various concentrations of 25, 50, 100, 250 and 500 ppm of their active ingredient. The experiment was laid out statistically using Completely Randomized Design with three replications using Poisoned food technique (Shravelle, 1961) [12].

The fungus was grown on PDA medium for seven days prior to setting up the experiment. The PDA medium was prepared and melted. The required quantity of individual fungicide was added separately into molten and cooled PDA medium so as to get the desired concentration of the fungicides. Later, twenty ml of poisoned medium was poured in each sterilized Petri dishes. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of seven day old colony was placed in the center of Petri dishes and incubated at 27±1˚C for seven days. Radial growth of the fungus was measured when fungus attained maximum growth in control. Per cent inhibition of mycelial growth of the fungus was calculated using the following formula (Vincent, 1947) [15].

I= {(C-T)/C}X100
Where,
I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment (fungicide)

Evaluation of fungicides against stem rot disease of capsicum under polyhouse conditions

An experiment was conducted under polyhouse conditions at College of Horticulture, Bengaluru during *Kharif* 2018 to evaluate the efficacy of fungicides against stem rot of capsicum. Six effective fungicides based on the *in vitro* results were selected for this study. The capsicum hybrid Indra was used for this study. The experiment was laid out statistically using Completely Randomised Design with three replications per treatment.

Seeds were surface sterilized before sowing. Such surface sterilized seeds were sown in protrays containing moist coco peat and allowed the seeds to germinate. Thirty days old seedlings were transplanted to the pots containing sterilized soil. Required nutrients to the plants were provided by mixing the N: P: K at 10kg + 5kg + 10kg and mixed well in the soil. For each replication, ten plants were maintained in the pots individually. Seedlings at the stage of 40 days were inoculated with mass multiplied culture of the pathogen by stem inoculation. Five ml of inoculum was added at the collar region of the plant. Prior to this an artificial injury was made at collar region of the plant with a sterile needle and then inoculum was added and moist cotton was placed at point of injury after inoculation.

The treatments were imposed immediately after the appearance of disease *i.e.,* 40 days after planting. The observations were recorded at a day prior to the first application of the fungicide. Second observation was recorded 7 days after 1st application and subsequently third and fourth observations were recorded 7 days after second and third applications, respectively.

The per cent disease incidence was calculated by using the following formula

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Results and Discussion

In vitro evaluation of fungicides against *S. rolfsii*

Evaluation of some of the fungicides is useful to know their anti-fungal activity against the pathogen. In the present study, *in vitro* evaluation of three systemic, six combi products and three contact fungicides was carried out against *S. rolfsii* causing stem rot of capsicum at various levels of concentrations using poisoned food technique. The per cent inhibition over control was worked out based on the complete mycelial growth in control plate. The results thus obtained have been presented in Table 2 and plates 1a, 1b and 1c. The results from table revealed that there was a significant difference in per cent inhibition of mycelial growth of pathogen at different concentrations and different fungicide treatments. The mycelial growth of pathogen was found completely inhibited (100%) in the treatments involving the fungicides hexaconazole, difenoconazole, propiconazole, propiconazole + difenoconazole, tricyclazole + hexaconazole, hexaconazole + captan, zineb + hexaconazole and thiram at the lowest concentration of 25 ppm tested and also in subsequent concentrations of 50, 100, 250 and 500 ppm. The combi-product carbendazim + mancozeb showed 54.07 per cent inhibition at 25 ppm, 68.52 per cent at 50 ppm, 80.37 per cent at 100 ppm and 100 per cent inhibition both at 250 and 500 ppm concentrations whereas the combi-product tricyclazole + mancozeb showed 53.33 per cent inhibition at 25 ppm, 61.48 per cent inhibition at 50 ppm, and 83.70 per cent inhibition at 100 ppm followed by 100 per cent inhibition at 250 and 500 ppm concentrations. The mancozeb showed 51.85 per cent inhibition at 25 ppm and 100 per cent inhibition at subsequent concentrations. Among the fungicides evaluated, the fungicide captan was least effective showing 52.22, 55.19, 54.81, 67.78 and 72.96 per cent of growth inhibition at 25, 50, 100, 250 and 500 ppm, respectively.

The *in vitro* studies revealed that the fungicides hexaconazole, difenoconazole, propiconazole and the combi-products propiconazole + difenoconazole, tricyclazole + hexaconazole, hexaconazole + captan, zineb + hexaconazole and thiram showed complete inhibition at the lowest concentration (25 ppm) followed by the contact fungicide mancozeb which gave 100 per cent inhibition at 50 ppm. The least effective fungicide was captan which even at 500 ppm was not effective.

Manu *et al*. (2012) [5] found the fungicides hexaconazole, propiconazole, difenconazole were highly effective against *S. rolfsii*, the causal agent of foot rot of finger millet under *in vitro* conditions. Sahana *et al*. (2017) [10] reported the effectiveness of fungicides hexaconazole, tebuconazole and combi product tebuconazole + trifloxystrobin against foot rot pathogen of tomato (*S. rolfsii*). Similarly, Suneeta *et al.* (2017) [14] in their studies on *in vitro* evaluation of fungicides against *S. rolfsii*, the causal agent of collar rot of gerbera noticed 100 per cent inhibition of the growth of the pathogen by the fungicides propioconazole, tebuconazole + trifloxystrobin and tebuconazole. In another similar studies, the fungicides hexaconazole, propiconazole and combi products, captan + hexaconazole, propiconazole + difenoconazole completely inhibited the growth of the pathogen at all the concentrations tested whereas the contact fungicide mancozeb was found inhibitive only at higher concentrations (100 ppm) against *S. rolfsii*, the incitant of collar rot of chickpea under *in vitro* conditions (Shirsole *et al*., 2019) [11].

Evaluation of fungicides against stem rot disease of capsicum under polyhouse conditions

Six fungicides that were found effective under *in vitro* conditions evaluated for their efficacy against stem rot disease of capsicum caused by *S. rolfsii* under poly house conditions. The disease incidence was recorded before the imposition of treatments and subsequently seven days after each application of fungicide. The disease incidence before the imposition of treatments varied from 33.33 to 38.33 per cent and was statistically non-significant.

However, after the imposition of the treatments, the disease incidence varied significantly (Table 3 and Fig. 1). At seven days after first application of fungicides, it was found that the fungicides, propiconazole and difenoconazole recorded least disease incidence of 23.33 per cent. However, the treatments comprising of zineb + hexaconazole (25.00%) and hexaconazole (26.67%) were also statistically on par with the afore mentioned treatments. The least effective fungicide was hexaconazole + captan registering disease incidence of 30.00 per cent. Maximum disease incidence (41.67%) was noticed in untreated control.

The disease incidence varied significantly 7 days after second application of fungicides and there was significant reduction in the disease in treated plants. Further, the fungicides propiconazole and difenoconazole showed least disease incidence (8.33%) and were statistically significant over other treatments followed by the fungicides hexaconazole (18.33%), hexaconazole + captan (23.33%) and thiram (25.00%). Maximum disease incidence (48.33%) was noticed in untreated control.

Similar trend on disease incidence was noticed 7 days after third application. There was further significant decline in the disease in treated plants. The least disease incidence was observed in the plants treated with the fungicides propiconazole and difenoconazole (1.67%) and these treatments were statistically superior over other treatments. This was followed by the fungicides hexaconazole (11.67%). The fungicides zineb + hexaconazole with 13.33 per cent disease incidence and hexaconazole + captan with 15.00 per cent disease incidence and were statistically on par with each other. Maximum disease incidence (56.67%) was noticed in untreated control.

The data on per cent reduction in disease over control showed that the fungicides propiconazole and difenoconazole with 97.05 per cent reduction in disease were found promising and superior over other fungicides.

Among the treatments undertaken, propiconazole and difenoconazole was found effective with 97.05 per cent reduction in disease followed by hexaconazole with 79.40 per cent, zineb + hexaconazole with 76.48 per cent, hexaconazole + captan with 73.53 per cent reduction in disease and least effective fungicide among all the treatments was thiram with 70.58 per cent reduction in disease over the control.

The results are in agreement with earlier findings of Prabhu and Hiremath (2003) [6] who noticed the fungicides propiconazole and hexaconazole were effective against collar rot of cotton caused by *S. rolfsii*. In an another experiment on field evaluation of fungicides against collar rot disease of sunflower caused by *S. rolfsii*, the fungicides tebuconazole, propiconazole, hexaconazole were found promising in managing the disease (Maji *et al*., 2016). Similarly, Suneeta *et al.* (2017) [14] who observed 70 per cent inhibition of the disease over control in field evaluation of the fungicides propioconazole, tebuconazole + trifloxystrobin and tebuconazole against collar rot of gerbera caused by *S. rolfsii.*

Table 1: List of fungicides used for *in vitro* evaluation against *S. rolfsii* by poisoned food method

Sl. No.	Chemical name	Trade name	Chemical group	Mode of action	FRAC code
1	Hexaconazole 5%EC	Sitara plus	Triazole	Sterol Biosynthesis inhibitors	3
2	Difenoconazole 25% EC	Debut	Triazole	Sterol Biosynthesis inhibitors	3
3	Propiconazole 25% EC	Tilt	Triazole	Sterol Biosynthesis inhibitors	3
4	Propiconazole 13.9% EC + difenoconazole 13.9% EC	Taspa	Triazole	Sterol Biosynthesis inhibitors	3
5	Tricyclazole 45% WP + hexaconazole 10% WG	Impression	Triazolobenzo- Thiazole + Triazole	Melanin Biosynthesis Inhibitors– Dehydratase + Sterol Biosynthesis inhibitors	16.1+3
6	Tricyclazole 18% + mancozeb 62%	Merger	Triazolobenzo-Thiazole +Dithio-carbamates and relatives	Melanin Biosynthesis Inhibitors– Dehydratase+	16.1+M03
7	Carbendazim 12% + mancozeb 63%WP	Companion	Benzimidazoles+ Dithio-carbamates and relatives	ß-tubulin assembly in mitosis + Multi-site contact activity	1+M03
8	Hexaconazole 5% WP + captan 70% WP	Taqat	Triazole+ Phthalimides	Sterol Biosynthesis inhibitors + Multisite activity	3+M04
9	Zineb 68% + hexaconazole 4% WP	Avatar	Dithio-carbamates and relatives +Triazole	Multi-site contact activity + Sterol Biosynthesis inhibitors	M03+3
10	Captan 50% WP	Captra	Phthalimides	Multisite activity	M04

11	Mancozeb 75%WP	IndofilM-45	Dithio-carbamates and relatives	Multi-site contact activity	M03
12	Thiram 50% WP	Vaccinator	Dithio-carbamates and relatives	Multi-site contact activity	M03

Source: FRAC code list, 2020

Table 2: *In vitro* evaluation of fungicides against *Sclerotium rolfsii* causing stem rot of capsicum

Sl. No.	Treatments	25 ppm	50 ppm	100 ppm	250 ppm	500 ppm
1	Hexaconazole 5%EC	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
2	Difenoconazole 25% EC	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
3	Propiconazole 25% EC	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
4	Propiconazole 13.9% EC + difenoconazole 13.9% EC	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
5	Tricyclazole 45% WP + hexaconazole 10% WG	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
6	Tricyclazole 18% + mancozeb 62%	53.33 (46.91)	61.48 (51.64)	83.70 (66.21)	100.00 (89.54)	100.00 (89.54)
7	Carbendazim 12%+mancozeb 63%WP	54.07 (47.34)	68.52 (55.87)	80.37 (63.70)	100.00 (89.54)	100.00 (89.54)
8	Hexaconazole 5% WP + captan 70% WP	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
9	Zineb 68% + hexaconazole 4% WP	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
10	Captan 50% WP	52.22 (46.27)	55.19 (47.98)	54.81 (47.76)	67.78 (55.42)	72.96 (58.74)
11	Mancozeb 75%WP	51.85 (46.06)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
12	Thiram 50% WP	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)	100.00 (89.54)
13	Control	0.00 (0.46)	0.00 (0.46)	0.00 (0.46)	0.00 (0.46)	0.00 (0.46)
SEM±		0.17	0.31	0.30	0.22	0.54
CD @ 0.01		0.657	1.240	1.183	0.859	2.127

The figures in parenthesis are arcsine transformed values

Table 3: Evaluation of fungicides against stem rot of capsicum under polyhouse conditions

Treatment details		Dosage (%)	Disease incidence (%)				Disease reduction over control (%)
			DBA	7 Days after			
				First application	Second application	Third application	
T1	Hexaconazole 5%WP + Captan 70%WP	0.25	38.33 (38.24)	30.00 (33.21)^{c}	23.33 (28.85)^{bc}	15.00 (22.59)^{b}	73.53
T2	Propiconazole 25% EC	0.10	35.00 (36.23)	23.33 (28.85)^{a}	8.33 (16.59)^{a}	1.67 (4.72)^{a}	97.05
T3	Thiram 50%WP	0.10	35.00 (36.27)	28.33 (32.14)^{bc}	25.00 (30.00)^{c}	16.67 (23.74)^{b}	70.58
T4	Difenoconazole 25% EC	0.10	33.33 (35.25)	23.33 (28.85)^{a}	8.33 (16.59)^{a}	1.67 (4.72)^{a}	97.05
T5	Zineb 68%+ Hexaconazole 4%WP	0.25	35.00 (36.23)	25.00 (30.00)^{ab}	21.67 (27.71)^{bc}	13.33 (21.14)^{b}	76.48
T6	Hexaconazole 5%EC	0.10	36.67 (37.25)	26.67 (31.07)^{abc}	18.33 (25.30)^{b}	11.67 (19.88)^{b}	79.40
T7	Control	-	38.33 (38.24)	41.67 (40.19)^{d}	48.33 (44.04)^{d}	56.67 (48.83)^{c}	-
SEm±			1.19	0.91	1.30	2.94	
CD@1%			NS	3.84	5.49	12.41	

DBA: Day Before Application; Figures in parenthesis are arcsine transformed values

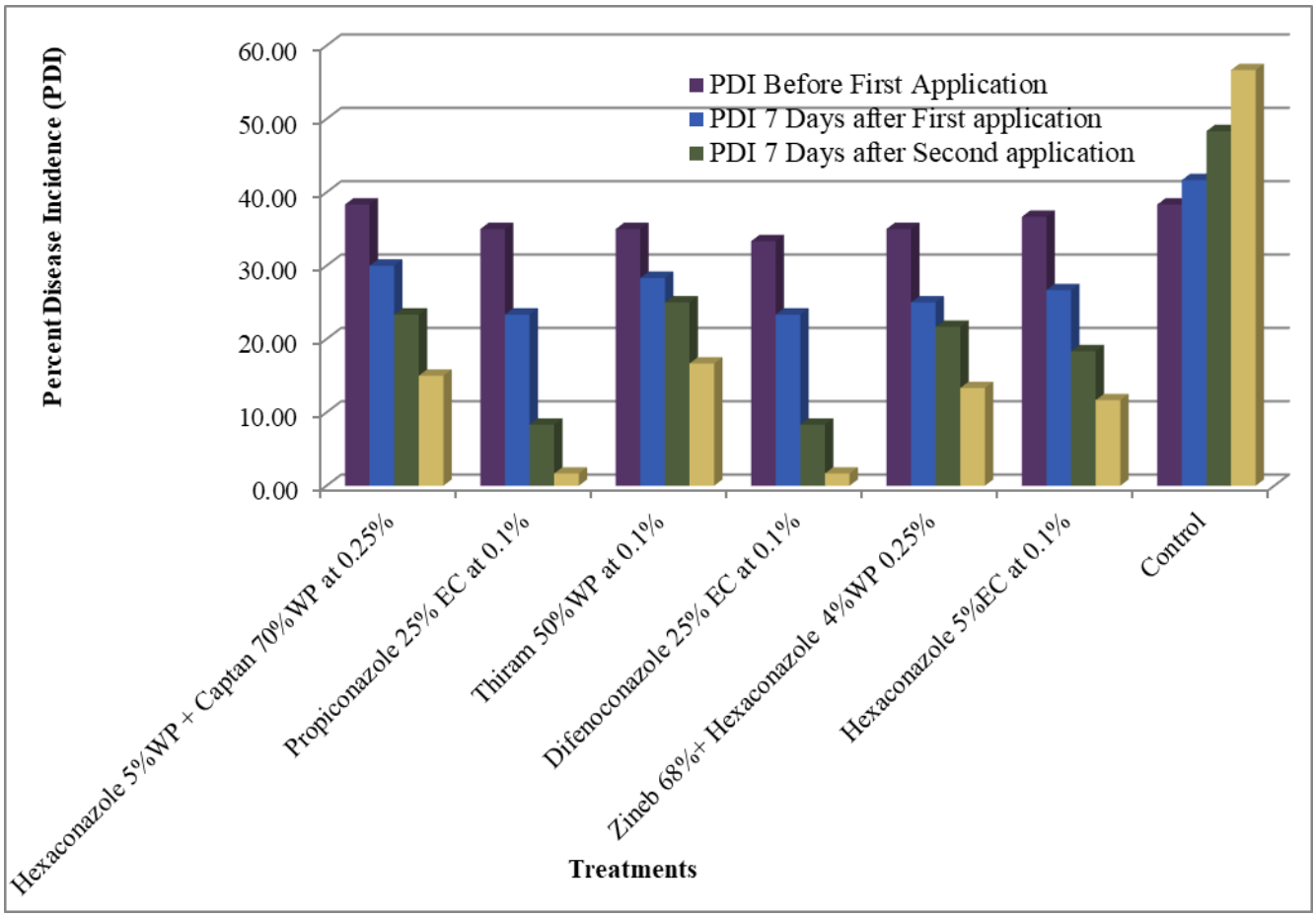

Fig 1: Evaluation of fungicides against stem rot of capsicum under polyhouse conditions

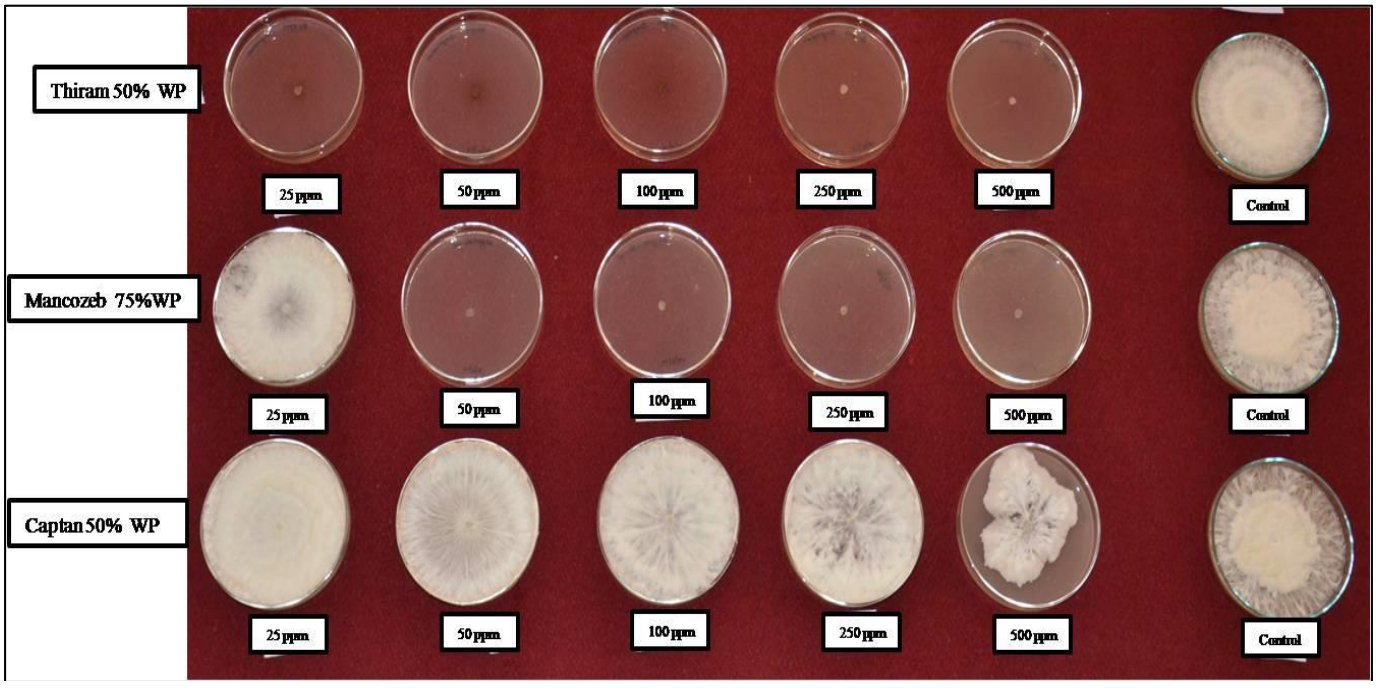

Plate 1(a): *In vitro* evaluation of contact fungicides against *S. rolfsii*

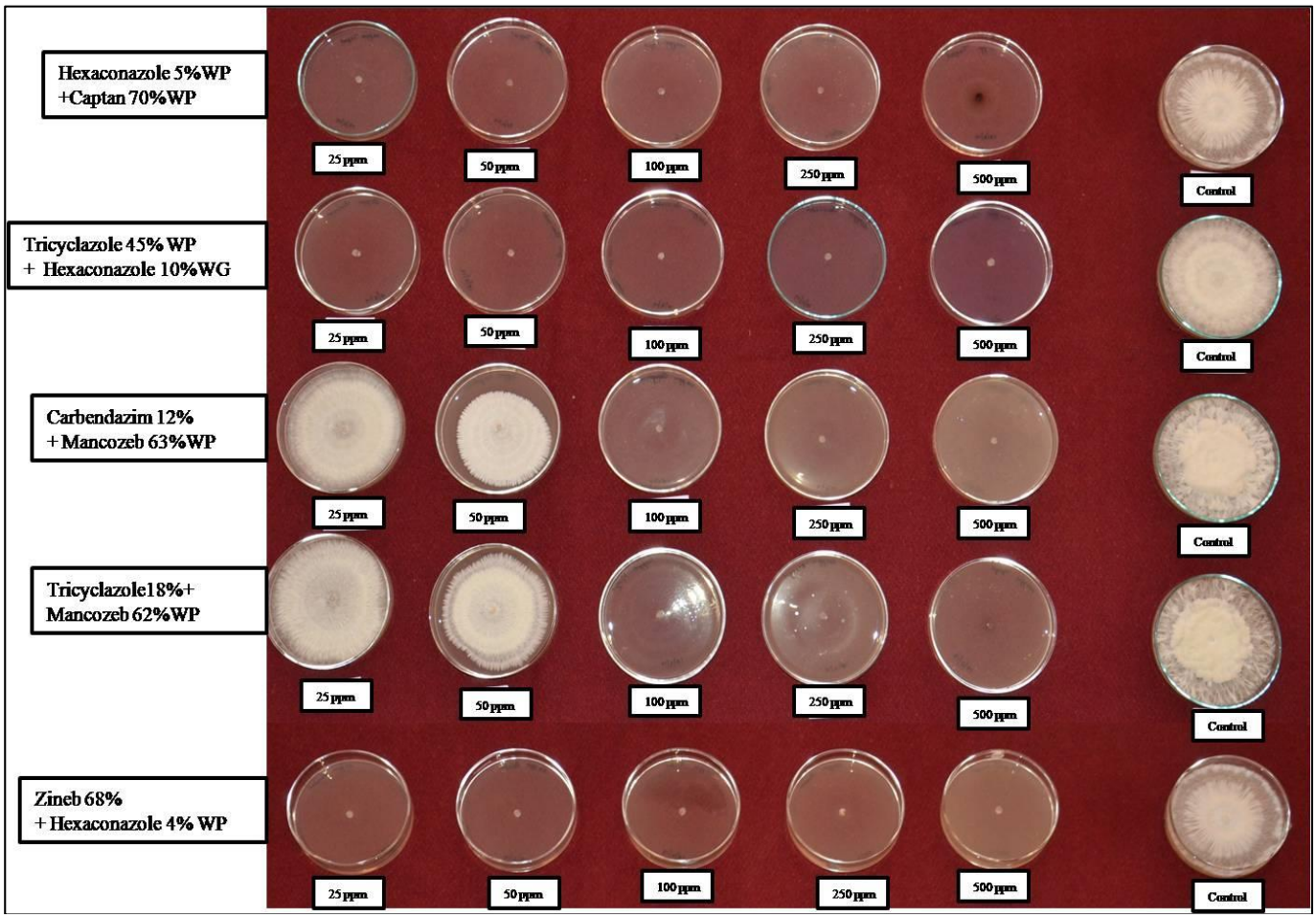

Plate 1(b): *In vitro* evaluation of combi-products against *S. rolfsii*

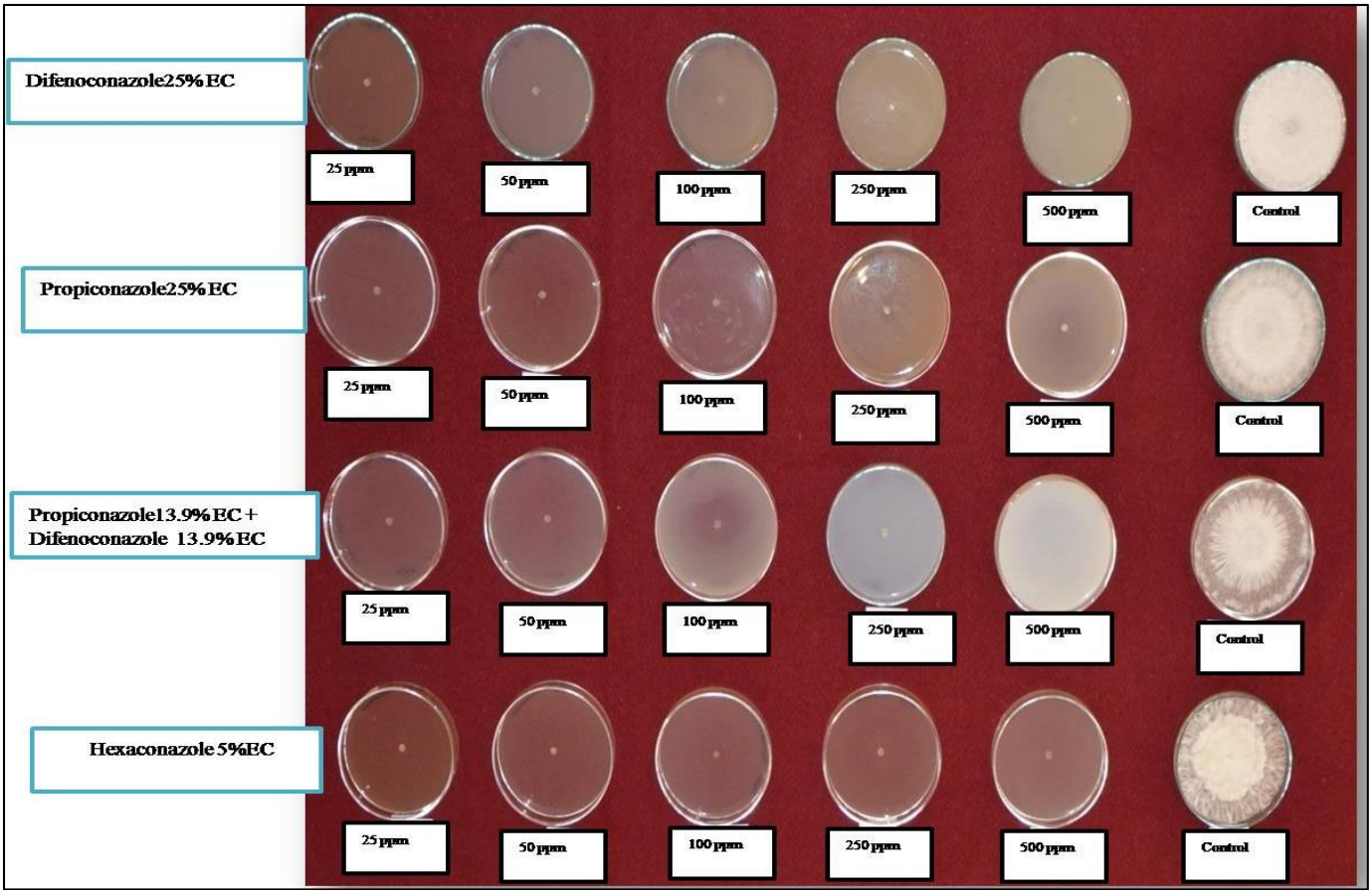

Plate 1(C): *In vitro* evaluation of systemic fungicides against *S. rolfsi*

Conclusion

In the present studies the fungicides hexaconazole, difenoconazole, propiconazole, propiconazole + difenoconazole, tricyclazole + hexaconazole, hexaconazole + captan, zineb + hexaconazole and thiram were highly effective even at least concentration of 25 ppm under *in vitro* conditions. The least per cent inhibition was observed in captan which was found ineffective even at 500 ppm tested.

The polyhouse studies revealed that propiconazole and difenoconazole each at 0.1 per cent dosage showed 97.05 per cent reduction in disease over control and can be concluded as promising fungicides against stem rot whereas least reduction in disease over the control (70.58%) was observed in thiram.

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