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UNEARTHING A SOLUTION: NOVEL FUNGICIDE CANDIDATES FOR SOYBEAN COLLAR ROT (*SCLEROTIUM ROLFSII* SACC.) MANAGEMENT UNDER *IN VITRO* CONDITIONS

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ABSTRACT

The present laboratory experiment was carried out at the Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka during 2021-22. The purpose of the study was to evaluate the bio-efficacy of systemic and combination fungicides against *Sclerotium rolfsii* of soybean. Regardless of the concentration tested, the systemic fungicides hexaconazole 5% EC, propiconazole 25% EC, and tebuconazole 25.90% EC showed the greatest mycelial inhibition (100%) above control. However, the least inhibition was recorded in Carbendazim 50 % WP (0.29 %). With respect to combi product fungicides, cent percent inhibition was noticed in (Tricyclazole 18 % + Mancozeb 62 %) WP, (Carbendazim 12 % + Mancozeb 63 %) WP, (Carboxin 37.5 % + Thiram 37.5 %) WS, (Tebuconazole 50 % + Trifloxystrobin 25 %), (Penflupen 13.28 % + Trifloxystrobin 13.28 %), (Azoxystrobin 20 % + Difenoconazole 12.5 %) and (Carbendazim 25 % + Mancozeb 50 %) WS. Planning the application of fungicides in the field will be greatly aided by the evaluation of fungicides in a laboratory setting, which offers valuable information about the fungicides' efficacy against the test pathogen.

Keywords **:** Collar rot, soybean, *Sclerotium rolfsii*, Fungicides, *In vitro.*

Introduction

One such oilseed crop with significant global economic value is soybean (*Glycine max* L.), which are used as food, feed, and industrial by-products. The crop is often referred to as "Golden Nugget" because of high protein content (40%) and oil percentage (20%) content. Globally this crop is grown in an area of 132.26 mha with average production of 428.48 mt and productivity of 2882 kg/ha. Soybean is the crop of many countries and extensively growing in USA, Brazil, Argentina, China and India. With respect to India, this crop is grown in an area of 12.20 mha with a production of 11.92 m.t and a productivity of 989 kg/ha (Anon., 2022).

Though the area under soybean cultivation is large, India still suffers in productivity when compared to global levels. A loss of more than seven million tonnes of soybean has been reported worldwide due to diseases alone (Sinclair, 1988). Diseases are the main constraints hindering the production and productivity of soybean in India. Soil-borne plant pathogens are potential threats that affect soybean yield and quality as well. Collar rot, caused by *Sclerotium rolfsii* Sacc., is one of the soil-borne diseases that destroys plants at every stage of crop growth and has become more significant in recent years due to climate change

The pathogen is responsible for various diseases in different crops such as collar rot, sclerotium wilt, stem rot, charcoal rot, seedling blight, damping off, foot rot, stem blight, and root rot in many other economically valuable crops. It causes considerable loss to crops irrespective of the stage of infection

(Sangeetha, 2011). The first symptom of collar rot is pre- and post-emergence damping-off of the seedlings. Infection at the collar region produces a very small dark brown spot, and within 2–3 days white radiating mycelium can be observed from the spot. Sheaths of white mycelium of the pathogen are seen around the collar region of the affected plant or near the soil surface, giving a 'white-washed' appearance to the base of the affected plants. White sclerotial bodies are produced at the collar region of the plant, which later turn dark brown and black. Finally, it leads to yellowing and wilting of the lateral branch; the leaves of the affected branch become chlorotic and then turn brown as they rapidly dry out.

Fungicides have long been used to control plant diseases when resistant genotypes are not present, and they are among the best choices when a disease outbreak occurs. Finding novel molecular fungicides to combat soil-borne pathogens provides a better option for efficiently controlling the illness. Planning which fungicides to apply in the field is greatly aided by the evaluation of fungicides *in vitro*, which yields valuable information about the fungicides' efficacy against the test pathogen. The need, dosage, and kind of disease to be treated must all be taken into consideration when using these fungicides. Hence, the present study was undertaken to evaluate some systemic and combi product fungicides to find out the most effective fungicide under *in vitro* which can be further tested under the field condition as a chemical management strategy for the management of collar rot of soybean.

Material and Methods

Experimental site

The present laboratory experiment was carried out at the Department of Plant Pathology, University of Agricultural Sciences, Dharwad, Karnataka during 2021-22.

Isolation and identification of the test fungus

The standard tissue isolation procedure (Brunda, 2018) was used to isolate fresh infected soybean plant samples exhibiting typical collar rot symptoms. The samples were placed on sterile Potato Dextrose Agar (PDA) medium and incubated at 28 °C. The cultures were then purified by sub culturing and kept on Potato Dextrose Agar (PDA) slants for future use.

In vitro evaluation of fungicides against *Sclerotium rolfsii* Sacc.

The experiment was carried out in a completely randomized design (CRD) with three replications for each treatment. Eight systemic fungicides (at concentrations of 250, 500, and 1000 ppm) and eight combi product fungicides (at concentrations of 1500, 2000, and 2500 ppm) were tested in vitro against *Sclerotium rolfsii* Sacc. using the usual poisoned food approach (Sharvelle, 1961). Required quantity of the individual fungicide was added separately into molten and cooled potato dextrose agar so as to get the desired concentration of fungicides and mixed well to obtain a uniform mixture. Later 20 ml of the poisoned medium was poured into sterile Petri plates. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating. PDA plates without fungicide served as control. To keep the pathogen in constant contact with the poisoned medium, a five-millimeter mycelial disc that was removed from the edge of a seven-day-old fungal culture was positioned in the center of each plate in the opposite orientation. These plates were then incubated at 28 ±1 ^{0}C until the mycelium completely covered the control plate, at which point the colony's radial growth was measured. In a completely randomized design (CRD), three replications were kept for every fungicide concentration.

The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control and it was calculated using the formula given by Vincent (1947).

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

Where,

I = Inhibition of mycelial growth (%)

C = Radial growth of mycelium in control (cm).

T = Radial growth of mycelium in treatment (cm).

Statistical Analysis

The experiment was statistically analyzed using the method described by Panse and Sukhatme (1967). Before analysis, the actual percentage data was transformed into angular and square root transformed values using the table provided by Walter (1967). Then the data were analysed with ANOVA in factorial completely randomized design to test for significant difference among fungicides (F), concentrations (C) and their interactions (F×C). In this investigation, mycelial growth inhibition at various fungicide concentrations showed significant differences at the 1% level of significance (P value > 0.01).

Results and Discussion

Isolation and identification of the test fungus

Isolation of the pathogen was done from the plants showing typical symptoms of collar rot following standard tissue isolation method. The pathogen initially

produced dense, white radiating mycelium on potato dextrose agar medium and produced sclerotial bodies which were initiated from fifth day from inoculation. These sclerotial bodies were initially white in colour and later turned yellow, light brown to dark brown. The pure culture obtained was again sub cultured on PDA slants and kept in the refrigerator at 4 ^{0}C for further use (Figure 1).

***In vitro* evaluation of systemic fungicides**

A total of eight systemic fungicides *viz*., Hexaconazole 5 % EC, Propiconazole 25 % EC, Tebuconazole 25.90 % EC, Tebuconazole 60 % FS, Difenoconazole 25 % EC, Tricyclazole 75 % WP, Azoxystrobin 23 % SC and Carbendazim 50 % WP were selected for *in vitro* study at three different concentrations (250, 500 and 1000 ppm) against *Sclerotium rolfsii* and the results obtained are presented in Table 1 and Figure 2. All the fungicides significantly inhibited the mycelial growth of the pathogen, which increased with increase in concentrations of fungicides. Among the systemic fungicides tested, Hexaconazole 5% EC, Propiconazole 25% EC, and Tebuconazole 25.90% EC were found to be highly effective among the systemic fungicides tested, with 100% mean mycelial inhibition. They were also significantly better than the other treatments, indicating complete inhibition at all three tested concentrations. Besides, the treatments, Tebuconazole 60 % FS, Tricyclazole 75 % WP and Difenoconazole 25 % EC recorded the mean mycelial inhibition of 95.56, 91.08 and 90.29 per cent respect. The treatments, Tebuconazole 60 % FS and Difenoconazole 25 % EC at 1000 ppm, Tricyclazole 75 % WP both at 500 and 1000 ppm also recorded cent percent inhibition. Whereas, the least mycelial inhibition was noticed in Carbendazim 50 % WP (0.29 %). The efficacy of fungicides against *S. rolfsii* revealed the differential inhibitory action based on the concentration used and were less effective at lower concentration (250 and 500 ppm) as compared to their higher concentration (1000 ppm).

***In vitro* evaluation of combi product fungicides**

Eight combi-product fungicides *viz*., (Tricyclazole 18 % + Mancozeb 62 %) WP, (Carbendazim 12 % + Mancozeb 63 %) WP, (Carboxin 37.5 % + Thiram 37.5 %) WS, (Tebuconazole 50 % + Trifloxystrobin 25 %), (Penflupen 13.28 % + Trifloxystrobin 13.28 %), (Azoxystrobin 20 % + Difenoconazole 12.5 %), (Carbendazim 25 % + Mancozeb 50 %) WS and (Pyraclostrobin 5 % + Thiophanate methyl 45 %) were selected for *in vitro* study at three different concentrations (1500, 2000 and 2500 ppm) against *Sclerotium rolfsii* and the results obtained are presented in Table 2 and Figure 3. These fungicides had profound effect on reduction in mycelial growth of *S. rolfsii* and it was observed that the percent mycelial inhibition ranged from 62.74 to 100. Among the fungicide treatments, maximum mean mycelial inhibition (100 %) was recorded across all the fungicidal treatments, *viz*., (Tricyclazole 18 % + Mancozeb 62 %) WP, (Carbendazim 12 % + Mancozeb 63 %) WP, (Carboxin 37.5 % + Thiram 37.5 %) WS, (Tebuconazole 50 % + Trifloxystrobin 25 %), (Penflupen 13.28 % + Trifloxystrobin 13.28 %), (Azoxystrobin 20 % + Difenoconazole 12.5 %) and (Carbendazim 25 % + Mancozeb 50 %) WS. However, (Pyraclostrobin 5 % + Thiophanate methyl 45 %) recorded the mean mycelial inhibition of 62.74 per cent. The fungicides completely inhibited mycelial growth of the pathogen at their lowest concentration (1500 ppm), which needs to be proved with similar effects under field condition.

For the majority of plant diseases, fungicides are the main component of control strategies. Because of their rapid action, especially in the absence of resistant sources, the employment of novel compounds has grown in popularity in recent years. In the present study, three systemic fungicides *viz*., Hexaconazole 5 % EC, Propiconazole 25 % EC, Tebuconazole 25.90 % EC recorded cent per cent mycelial inhibition even at their lower concentration *i.e*., 250 ppm where other fungicides like Carbendazim 50 % WP recorded no mycelial inhibition at this concentration.

Triazoles are fungicides that block ergosterol, an important component of the cell wall whose absence results in irreversible damage to the cell wall and the fungus's demise. They impact the cytochrome P-450 enzyme, which is the inhibitor of sterol C-14 demethylation, as well as the sterol concentration and saturation of the polar fatty acids, which causes changes in membrane fluidity and membrane-bound enzyme behavior (Nene and Thapliyal, 1993). The outcome is consistent with previous research (Chowdhury *et al*., 1998; Virupaksha Prabhu and Hiremath, 2003; Arunasri *et al*., 2011), which found that the triazoles (Hexaconazole, Propiconazole, and Difenconazole) were very successful in inhibiting the growth of the pathogen *S. rolfsii*. In contrast, Johnson and Subramanyam (2000) found that carbendazim was the least effective against *S. rolfsii*. At lower concentrations, hexaconazole, tebuconazole, and propiconazole strongly inhibited the growth of *S. rolfsii* linked to finger millet (Manu *et al*., 2012). According to Das *et al*. (2014), propiconazole and mycobutanil were the next most effective against *S.*

rolfsii, after hexaconazole and tebuconazole at all dosages. Bavistin and thiophanate methyl showed the least amount of inhibition.

Among the combi product fungicides, Tricyclazole 18 % + Mancozeb 62 %) WP, (Carbendazim 12 % + Mancozeb 63 %) WP, (Carboxin 37.5 % + Thiram 37.5 %) WS, (Tebuconazole 50 % + Trifloxystrobin 25 %), (Penflupen 13.28 % + Trifloxystrobin 13.28 %), (Azoxystrobin 20 % + Difenoconazole 12.5 %) and (Carbendazim 25 % + Mancozeb 50 %) WS recorded the mycelial inhibition of 100 percent at all their tested concentrations. These findings are consistent with those of Virupaksha Prabhu and Hiremath (2003) and Arunasri *et al.* (2011), who found that the growth of *S. rolfsii* was significantly inhibited by combi products containing triazoles, namely (Hexaconazole 4% WP + Zineb 68% WG), (Tricyclazole 18% + Mancozeb 62% WP), and (Tebuconazole 50% + Trifloxystrobin 25%). Carboxin was found to be quite efficient against S. rolfsii by Vyas and Joshi (1977), Sujatha (1991), and Manu *et al.* (2012). Additionally, Carboxin 37.5% + Thiram 37.5% is proven to be highly effective on the growth of *S. rolfsii*, according to Das *et al.* (2014).

Conclusion

In the present investigation, eight systemic and eight combi product fungicides were evaluated for their potential of inhibition to the growth of the test pathogen (*S. rolfsii*) *in vitro* following poisoned food technique. The three fungicides, Hexaconazole 5 % EC, Propiconazole 25 % EC, Tebuconazole 25.90 % EC among the systemic and seven fungicides (Tricyclazole 18 % + Mancozeb 62 %) WP, (Carbendazim 12 % + Mancozeb 63 %) WP, (Carboxin 37.5 % + Thiram 37.5 %) WS, (Tebuconazole 50 % + Trifloxystrobin 25 %), (Penflupen 13.28 % + Trifloxystrobin 13.28 %), (Azoxystrobin 20 % + Difenoconazole 12.5 %) and (Carbendazim 25 % + Mancozeb 50 %) WS among combi product fungicides recorded the cent per cent mycelial inhibition at all the three concentrations tested. Hence, these treatments must be carried out at the field level to develop a technology with suitable delivery method.

Competing interests

No potential conflict of interest was reported by the authors.

Author contributions

SJ conceived the idea, designed the methodology and supervised the study; IGH conducted experiments, analysed the data and wrote original draft; KPU supervised the study; ASA, KDN contributed to supervision, reviewing and editing; PK contributed to reviewing and editing. All authors read and approved the final manuscript

Table 1 : *In vitro* bio efficacy of systemic fungicides against *Sclerotium rolfsii* Sacc. by poisoned food technique.

Sl. No.	Fungicides	Mycelial growth inhibition (%)			
		Concentration (ppm)			Mean
		250	500	1000	
1	Hexaconazole 5 % EC	100.00* (10.00)	100.00 (10.00)	100.00 (10.00)	**100.00 (10.00)**
2	Propiconazole 25 % EC	100.00 (10.00)	100.00 (10.00)	100.00 (10.00)	**100.00 (10.00)**
3	Tebuconazole 25.90 % EC	100.00 (10.00)	100.00 (10.00)	100.00 (10.00)	**100.00 (10.00)**
4	Tebuconazole 60 % FS	90.59 (9.52)	96.18 (9.80)	100.00 (10.00)	**95.59 (9.77)**
5	Difenoconazole 25 % EC	79.12 (8.89)	91.76 (9.58)	100.00 (10.00)	**90.29 (9.50)**
6	Tricyclazole 75 % WP	73.24 (8.56)	100.00 (90.00)	100.00 (10.00)	**91.08 (9.52)**
7	Azoxystrobin 23 % SC	19.41 (4.40)	29.12 (5.40)	36.76 (6.06)	**28.43 (5.28)**
8	Carbendazim 50 % WP	0.00 (0.00)	0.00 (0.00)	0.88 (0.65)	**0.29 (0.22)**
Mean		**70.29 (7.68)**	**77.13 (8.09)**	**79.70 (8.34)**	**75.70 (8.04)**

Sources	S. Em. ±	CD @ 1 %
Fungicide (F)	0.05	0.19
Concentration (C)	0.03	0.12
Fungicide × Concentration (F×C)	0.09	0.34

* Average of three replications

Figures in the parentheses are square root transformed values

Table 2 : *In vitro* bio efficacy of combi product fungicides against *Sclerotium rolfsii* Sacc. by poisoned food technique.

Sl. No.	Fungicides	Mycelial growth inhibition (%)			
		Concentrations (ppm)			Mean
		1500	2000	2500	
1	(Tricyclazole 18 % + Mancozeb 62 %) WP	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
2	(Carbendazim 12 % + Mancozeb 63 %) WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
3	(Carboxin 37.5 % + Thiram 37.5 %) WS	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
4	(Tebuconazole 50 % + Trifloxystrobin 25 %)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
5	(Penflupen 13.28 % + Trifloxystrobin 13.28%)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
6	(Azoxystrobin 20 % + Difenoconazole 12.5 %)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
7	(Carbendazim 25 % + Mancozeb 50 %) WS	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	**100.00 (90.00)**
8	(Pyraclostrobin 5 % + Thiophanate methyl 45 %)	52.94 (47.19)	63.53 (52.68)	71.76 (58.10)	**62.74 (52.65)**
Mean		**94.11 (84.65)**	**95.44 (85.33)**	**96.47 (86.01)**	**95.34 (85.33)**
Sources				**S. Em.±**	**CD @ 1%**
Fungicide (F)				0.08	0.29
Concentration (C)				0.05	0.17
Fungicide × Concentration (F×C)				0.13	0.50

* Average of three replications

Figures in the parentheses are angular transformed values

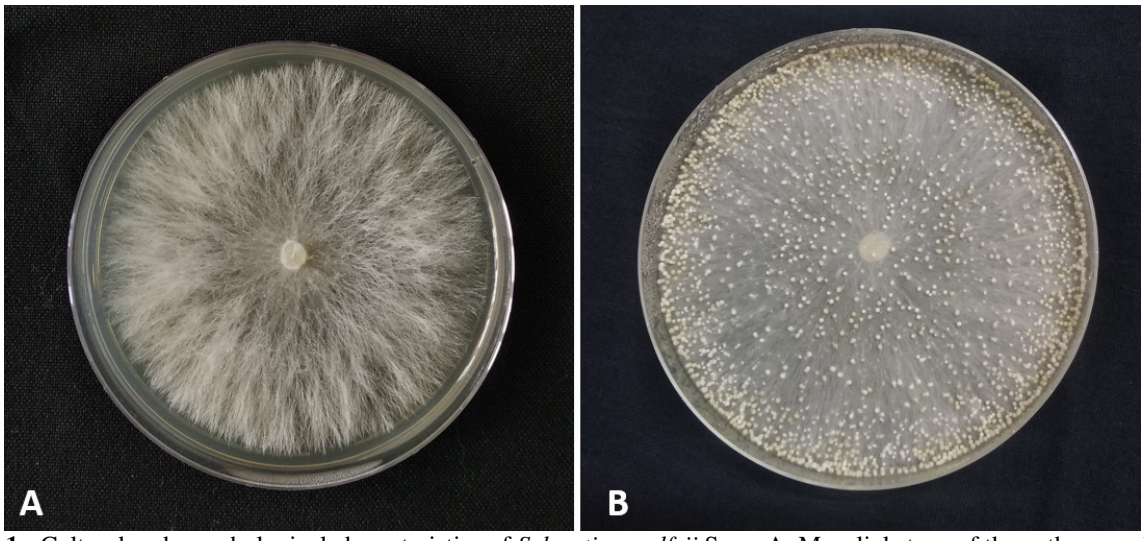

Fig. 1 : Cultural and morphological characteristics of *Sclerotium rolfsii* Sacc. A. Mycelial stage of the pathogen which produce dense, white radiating mycelium B. Mycelia aggregate to form sclerotial bodies which are initially white in colour and later turn to light to dark brown.

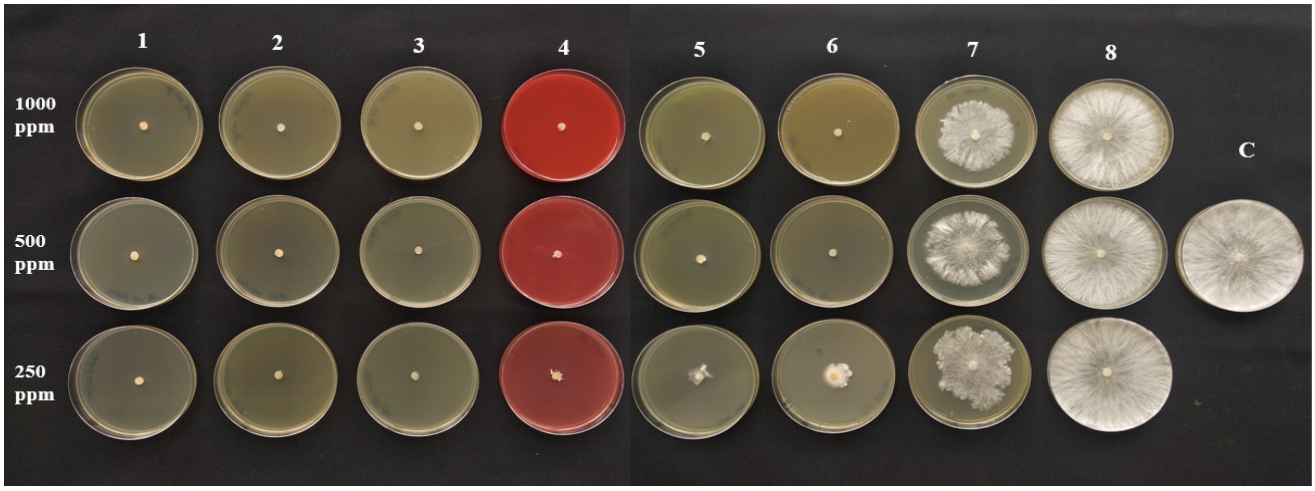

Fig. 2 : *In vitro* bio efficacy of systemic fungicides against *Sclerotium rolfsii* Sacc. of soybean. 1-8 are Hexaconazole 5 % EC, Propiconazole 25 % EC, Tebuconazole 25.90 % EC, Tebuconazole 60 % FS, Difenoconazole 25 % EC, Tricyclazole 75 % WP, Azoxystrobin 23 % SC and Carbendazim 50 % WP respectively, C – Control

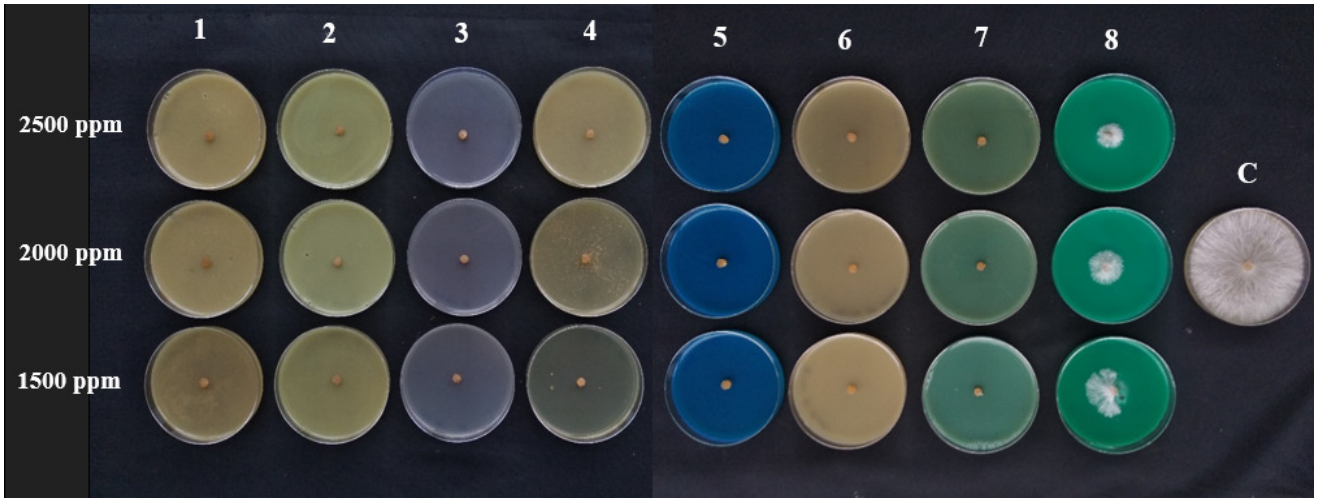

Fig. 3 : *In vitro* bio efficacy of combi product fungicides (1500, 2000 and 2500 ppm) against *Sclerotium rolfsii* Sacc. of soybean. 1-8 are (Tricyclazole 18 % + Mancozeb 62 %) WP, (Carbendazim 12 % + Mancozeb 63 %) WP, (Carboxin 37.5 % + Thiram 37.5 %) WS, (Tebuconazole 50 % + Trifloxystrobin 25 %), (Penflupen 13.28 % + Trifloxystrobin 13.28 %), (Azoxystrobin 20 % + Difenoconazole 12.5 %), (Carbendazim 25 % + Mancozeb 50 %) WS and (Pyraclostrobin 5 % + Thiophanate methyl 45 %) respectively, C- Control

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