Doi:

International Journal of Economic Plants

https://ojs.pphouse.org/index.php/IJEP

IJEP January 2025, 12(1): 01-07

Crossref

Article IJEP5785b

Full Research

Stress Management

Doi: HTTPS://DOI.ORG/10.23910/2/2025.5785b

Efficacy of Pre-mixed Fungicides Against *Rhizoctonia solani* and *Macrophomina phaseolina* Isolated from Soybean

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Article History

Received on 23rd September, 2024
Received in revised form on 27th December, 2024
Accepted in final form on 10th January, 2025
Published on 21st January 2025

Abstract

The experiment was conducted in 2022 (July to December) at J.N.K.V.V., Jabalpur, Madhya Pradesh, India to explore the efficacy of new combo agrochemicals against soil borne phytopathogenic fungi,i.e. *Rhizoctonia solani* and *Macrophomina phaseolina*. The pathogens were isolated from infected soybean plants and identified based on cultural and morphological characteristics. Five combo fungicides/ agro-chemicals were tested based on their active ingredients at 50 and 100 ppm in four replications. The mycelial development of *R. solani* was significantly inhibited by penflufen 13.28%+trifloxystrobin 13.28% FS (100%) and thiophanate methyl 45%+pyraclostrobin 5% FS (100%), carboxin 37.5%+thiram 37.5% DS (88.33%) and carbendazim 12%+mancozeb 63% WP (80.55%) at 100 ppm. Whereas mycelial development of *M. phaseolina* was entirely inhibited by penflufen13.28%+trifloxystrobin 13.28% FS, thiophanate methyl 45%+pyraclostrobin 5% FS and carboxin 37.5%+thiram 37.5% DS at 100 ppm. Penflufen 13.28%+trifloxystrobin 13.28% FS completely checked both pathogen's mycelial development even at 50 ppm. The significant lowest growth inhibition of *R. solani* (45.83%) and *M. phaseolina* (50.27%) was recorded by azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS at 50 ppm. Efficient combo fungicides in the present investigation could be tested against other fungal pathogens and applied as a seed treatment to reduce the fungal infection in the early stages of crops.

Keywords: Fungicides, *In vitro, Macrophomina phaseolina, Rhizoctonia solani*, soybean

1. Introduction

Soybean (*Glycine max* L.) is a seed legume that stands out for its richness in quality protein (42.8%), edible oil (20.2%), carbohydrate (35.2%) and several health-beneficial contents (Uikey et al., 2022; Banerjee et al., 2023). Soybean is the leading food crop that contributes to the global edible oil (about 25%) and protein concentrate (about two-thirds) in the form of de-oiled cake for livestock feeding (Anonymous, 2022; Amrate et al., 2023). Despite India being the top fifth soybean producer, it imports soybean oil (around 25%) to fulfil its domestic demand (Sagarika et al., 2023). There are several biotic and abiotic constraints in soybean production in India (Sharma et al., 2014; Rajput et al., 2021; Amrate and Shrivastava, 2021; Amrate et al., 2021; Bhamra and Borah, 2022. Nataraj et al., 2023).

Rhizoctonia solani Kuhn is a polyphagous necrotrophic soil-borne fungus that causes root rot, root lesions, hypocotyl rot and aerial blight in soybean (Nelson et al., 1996; Wrather et al., 2010; Ajayi-Oyetunde and Bradley, 2017). Rhizoctonia damage may occur at any time during the growing season, but it is more severe on young seedlings.Fourteen anastomosis groups (AGs) of *R. solani* have been reported wherein AG 1 to 4 causes economically significant diseases in many crop plants (Senapati et al., 2022; Ajayi-Oyetunde and Bradley, 2018; Hosseini et al., 2023). It infects a diverse range of hosts (around 250) and causes seed rot, hypocotyl rot, crown rot, stem rot, sheath blight, pod rot, stem canker, black scurf, seedling blight, and pre and post-emergence damping off in several economic crop plants like corn, rice, wheat, soybean, chickpea, lentil, groundnut, arhar, mung bean, tomato, brinjal, chillies, and okra tobacco, potato, sugar beet and cotton (Ajayi-Oyetunde and Bradley, 2018; Senapati et al., 2022).

Another soil-inhabitant fungus *Macrophomina phaseolina* (Tassi.) Goid. [=*Rhizoctonia bataticola* (Taub.) Butler], the causal agent of charcoal rot, is also a devastating soybean pathogen (Smith and Wyllie, 1999; Amrate et al., 2023). It is a worldwide disease that causes significant yield

losses in soybeans (Wrather et al., 2010). Yield reduction due to charcoal rot is very high, up to 100% (Amrate et al., 2019). In addition to soybean, this pathogen has a wide host range (about 500), including some significant economic crops of India like maize, sorghum and chickpea (Mengistu et al., 2011; Amrate et al., 2024). *M. phaseolina* causes complete mortality often during the reproductive stages of the crop and is characterized by the charcoal-like black appearance of the vascular tissues and lower stem and root of the plant (Gupta et al., 2012; Amrate et al., 2020). It survives in the soil as small black fungal structures called microsclerotia (Amrate et al., 2024) and may also be present on the seed coat of soybean (Sagarika et al., 2023).

R. solani and *M. phaseolina* affect the crop seriously, and their management under field conditions is complex since their inoculum remains in the soil (Belkar and Gade, 2013; Sagarika et al., 2023; Patidar et al., 2023; Amrate et al., 2024). Efforts have been made to control this disease by antagonistic microorganisms (Khaledi and Taheri, 2016; Sagarika et al., 2023). Fungicides always play an essential role in reducing inoculum present in or on the seed. Most fungicides have been reported efficacy in reducing both these diseases in the field and in vitro testing (Reznikov et al., 2016; Patidar et al., 2023; Sagarika et al., 2023). Application of combo agrochemicals in the form of soil drenching and seed treatment might effectively reduce disease severity. Hence, the present investigation was undertaken to reveal the *In vitro* efficacy of combo agrochemicals/fungicides recently available in the market against both targeted pathogens.

2. Materials and Methods

2.1. Collection of infected materials

Root rot-affected plants were identified based on the typical field symptoms such as rusty brown, dry, sunken lesions forming on hypocotyl near the soil area of young seedlings, along with some dead seedlings scattered throughout the field during the early stage of soybean (Nelson et al., 1996) (Figure 1). Likewise, charcoal rot-affected plants were identified (Amrate et al., 2020) (Figure 1). Affected samples were collected from the research field of JNKVV, Jabalpur, Madhya Pradesh (482 004), India during *kharif,* 2022 (latitude 23°12'42"N and longitude 79°56'53"E).

2.2. Media preparation and sterilization

The target pathogen was isolated on Potato Dextrose Agar (PDA) medium. For preparation of PDA medium, 200 g peeled potatoes were cut into slices and boiled in 1000 ml of distilled water. The extract was strained through a piece of muslin cloth, and then 20 g dextrose and 20 g agar were added and heated to melt properly. Finally, the volume was made up to 1000 ml by adding distilled water. The medium was poured into flasks and plugged with non-absorbent cotton plugs. The culture media was then sterilized in an autoclave at 121°C temperature (15 lb square inch^{-1}) for 15 minutes.

2.3. Isolation,identification and purification of target fungal agent

Root rot affected portion and tap roots of charcoal rot-affected plants were cut into small pieces. These were surface sterilized in 1% sodium hypochlorite for 1 minute, rinsed in distilled water thrice, and then placed on a paper towel. After that, pieces were placed in the Petri plates containing autoclaved PDA medium amended with streptomycin sulphate. Inoculated Petri plates were incubated in a BOD incubator at 26±2°C for 5–7 days. A small portion of the fastest-growing colony of test fungus was transferred to other Petri plates. Sclerotia/ microsclerotia were separated and transferred to other Petri plates containing PDA medium. After that, both the fungi (*Rhizoctonia solani* and *Macrophomina phaseolina*) were identified based on typical cultural and morphological characteristics (Dhingra and Sinclair, 1978; Nelson et al., 1996; Gupta et al., 2012; Oyetunde and Bradley, 2018; Amrate et al., 2024). The culture was maintained in refrigerator at 4–5°C and used for further studies.

2.4. In vitro testing of agrochemicals

The experiment was conducted at the Department of Plant Pathology, J.N.K.V.V., Jabalpur, Madhya Pradesh, India in 2022. Five combo fungicides/agrochemicals, i.e. penflufen 13.28%+trifloxystrobin 13.28% FS (Evergol xtend), carbendazim 12%+mancozeb 63%WP (Saaf), carboxin 37.5%+thiram 37.5% DS (Vitavax Power), azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS (Electron) and thiophanate methyl 45%+pyraclostrobin 5% FS (Xelora) were tested based on their active ingredients at 50 and 100 ppm. The poisoned food technique was employed to test fungicides' mycelial growth inhibition efficacy (Nene and Thapliyal, 1982). In this, the required quantity of agrochemicals was mixed thoroughly in 100 ml autoclaved PDA by using serial dilution and micropipettes. The PDA medium was also amended with a small quantity of streptomycin sulphate (100 ppm) to check for unwanted bacterial contamination. This poisoned medium (16–18 ml) was poured into a sterilized Petri dish. After solidification, the medium was inoculated with a five mm disc of actively growing pure culture of target pathogens. PDA plates without fungicide were used as a control. Each treatment/chemical was replicated four times in a complete randomized design (CRD). All these were incubated at 26±2°C in a BOD incubator for different periods. Mycelial growth (mm) of *Rhizoctonia solani* (at the third and fourth days of inoculation) and *Macrophomina phaseolina* (third and fifth days of inoculation) were recorded from different treatments at 50 and 100 ppm concentrations. Fungicidal efficacy in per cent growth inhibition was calculated using Vincent's formula (1947).

$$I=(C-T)/C\times100$$

I=Per cent inhibition, C=Mycelial growth in untreated, T=Mycelial growth in treatment

Per cent, inhibition data was transformed and statistically

analysed using online OP stat software.

3. Results and Discussion

3.1. Identification of pathogens

The two-day-old culture of *Rhizoctonia solani* exhibited whitish growth of culture, and later, the culture turned creamish white. Light brown irregularly shaped sclerotia were noticed in 7 days old growth, and later, sclerotia were mixed. Right angle branching of hyphae, septation and constriction at the origin of septation was noticed (Figure 1). Initially, the colony colour of *M. phaseolina* was dirty white but later started turning greyish black with age. Initially, hyphae were hyaline and turned honey or brown-black with septation later. Numerous dark brown to black microsclerotia varying in size (50–100 µm) were formed from aggregation and coiling of hyphae (Figure 1).

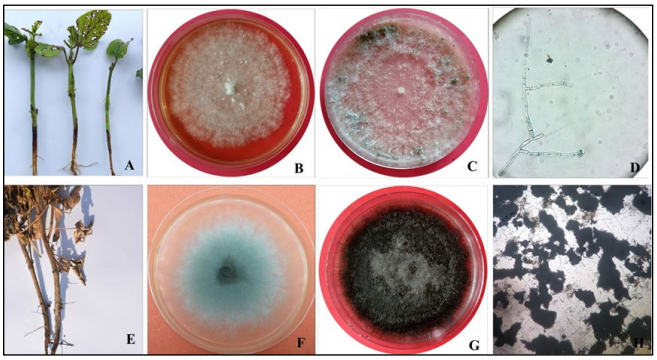

Figure 1: Identification of *Rhizoctonia solani* [Root rot affected lower stem and soybean root (A), 3 days old culture of *R. solani* (B), formation of sclerotia in 10 days old culture of *Rhizoctonia solani* (C) and branching pattern of *R. solani* (D)], and *Macrophomina phaseolina* [Charcoal rot affected lower stem and root (E), 3rd days (F) and 6 days old culture of *M. phaseolina* (G) and microsclerotia presence in old culture (H), respectively

3.2. Inhibitory effect of agrochemicals

3.2.1. Rhizoctonia solani

The mycelial growth of *R. solani* at three and four days varied among treatments. *R. solani* attained 64.0 and 90.0 mm mycelial growth on 3rd and 4th days after inoculations, respectively (Figures 2, 3 and 4). Per cent growth inhibition was calculated for both the concentrations 50 and 100 ppm. Results revealed significant differences in growth inhibition of *R. solani* among treatments (Table 1). At 100 ppm, complete growth inhibition (100%) was recorded by penflufen 13.28%+trifloxystrobin 13.28% FS and thiophanate methyl 45%+pyraclostrobin 5% FS. carboxin 37.5%+thiram 37.5% DS (92.25% and 88.33%) and carbendazim 12%+mancozeb 63% WP (83.17% and 80.55%) also recorded high growth inhibition at 3rd and 4th day inoculation, respectively. Azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS (73.27% and 70.14%) recorded the lowest significant

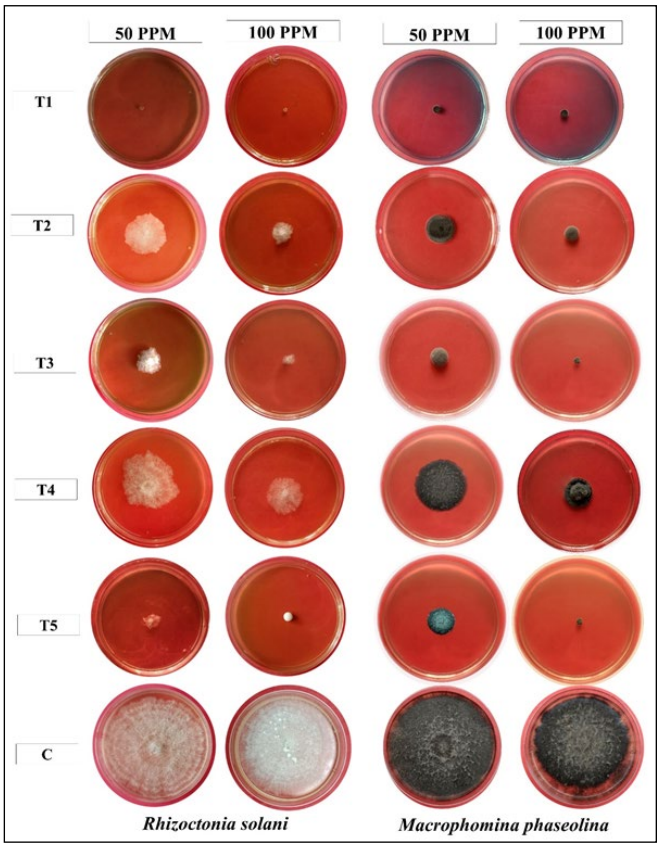

Figure 2: Mycelial growth of *Rhizoctonia solani* and *Macrophomina phaseolina* in PDA amended with 100 and 50 ppm of different of fungicides/insecticides (T_1) Penflufen13.28%+Trifloxystrobin 13.28% FS, (T_2) Carbendazim 12%+Mancozeb 63%WP, (T_3) Carboxin 37.5%+Thiram 37.5% DS, (T_4) Azoxystrobin 2.5%+Thiophanate methyl 11.25%+Thiamethoxam 25% FS, and (T_5) Thiophanate methyl 45%+Pyraclostrobin 5% FS and (C) without chemical (Control)

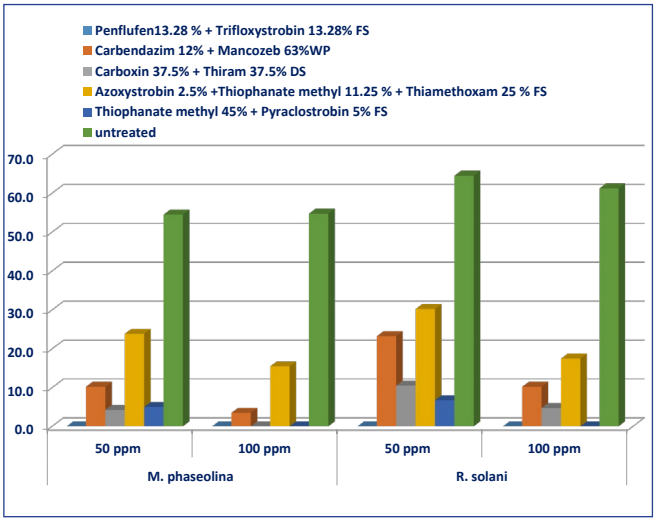

Figure 3: Mycelial growth of *M. phaseolina* and *R. solani* 3rd days of inoculation in PDA amended with 50 and 100 ppm concentration of fungicides

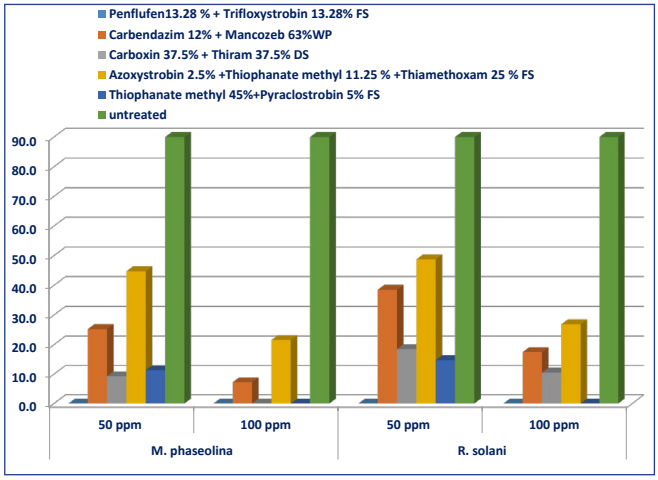

Figure 4: Mycelial growth of *M. phaseolina* (5 days) and *R. solani* (4 days) in PDA amended with 50 and 100 ppm concentration of fungicides

growth inhibition at 3rd and 4th day, respectively. In the case of 50 ppm, highest percent growth inhibition (100%) was recorded by penflufen 13.28%+trifloxystrobin 13.28% FS followed by thiophanate methyl 45%+pyraclostrobin 5% FS (89.56% and 83.61%), carboxin 37.5%+thiram 37.5% DS (83.67% and 79.44%) and carbendazim 12%+mancozeb 63% WP (63.94% and 57.22%) at 3 and 4 days of inoculation, respectively. At the same time, the lowest per cent growth inhibition was recorded by azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS (53.18% and 45.83%) at 3 and 4 days, respectively. Dutta and Kalha (2011) found carbendazim 12%+mancozeb 63% as the most effective fungicide inhibiting the mycelial growth of *R. solani*, followed by carbendazim (98.9%) and vitavax (98.2%).hexaconazole and carbendazim showed 100% inhibition of *R. solani* isolated from soybean at 25 ppm (Ray and Kumar, 2008). Seed dressing with azoxystrobin+*T. viride* showed the highest significant effect on germination per cent against root rot of soybean incited by *R. solani* (Kashyap et al., 2020). Magar et al. (2020) reported the mycelial growth inhibition of *Fusarium oxysporum* with tebuconazole, carboxin + thiram and carbendazim + mancozeb.

3.2.2. *Macrophomina phaseolina*

The mycelial growth of *M. phaseolina* at three and five days varied among treatments (Figure 2). *M. phaseolina* attained 54.0 and 90.0 mm mycelial growth at 3rd and 5th days after inoculation, respectively (Figure 2, 3 and 4). Per cent growth inhibition varied significantly among the fungicidal treatments of both the concentrations, i.e. 100 and 50 ppm. Hundred per cent growth inhibition was recorded in case of penflufen 13.28%+trifloxystrobin 13.28% FS, thiophanate methyl 45%+pyraclostrobin 5% FS and carboxin 37.5%+thiram 37.5% DS at 100 ppm. Carbendazim 12%+mancozeb 63%WP also recorded high growth inhibition (93.69% and 91.94% at 3rd and 5thday, respectively). At the same time, the lowest per cent growth inhibition was recorded in azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS (76.03% and 71.71%) at 3rd and 5th day of inoculation, respectively. In case of 50 ppm, highest significant growth inhibition was recorded by penflufen13.28%+trifloxystrobin 13.28% FS (100%) followed by carboxin 37.5%+thiram 37.5% DS (92.17% and 89.72%), thiophanate methyl 45%+pyraclostrobin 5% FS (90.90% and 86.94%), and carbendazim 12%+mancozeb 63% WP (81.25% and 71.94%) at 3rd and 5th day of inoculation, respectively. At the same time, lowest per cent growth inhibition was recorded in azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS (56.12% and 50.27%) on the 3rd and 5th day, respectively. Previous to our findings, many researchers also demonstrated

Table 1: Percent growth inhibition of *Rhizoctonia solani* by new generation agrochemicals on PDA media

Sl. No.	Treatments	Trade name	3 days		4 days	
			50 ppm	100 ppm	50 ppm	100 ppm
T_1	penflufen13.28%+trifloxystrobin 13.28% FS	Evergol xtend	100.0 (90.00)	100.00 (90.00)	100.0 (90.00)	100.0 (90.00)
T_2	carbendazim 12%+mancozeb 63% WP	SAAF	63.94 (53.09)	83.17 (65.83)	57.22 (49.14)	80.55 (63.83)
T_3	carboxin 37.5%+thiram 37.5% DS	Vitavax power	83.67 (66.23)	92.25 (73.98)	79.44 (63.05)	88.33 (70.06)
T_4	azoxystrobin2.5%+thiophanate methyl 11.25%+ thiamethoxam 25 %FS	Electron	53.18 (46.81)	73.27 (58.85)	45.83 (42.59)	70.14 (56.85)
T_5	thiophanate methyl 45% +pyraclostrobin 5% FS	Xelora	89.56 (71.21)	100.0 (90.00)	83.61 (66.13)	100.0 (90.00)
CD (p=0.05)			3.095	2.735	2.544	1.905
SEm±			1.018	0.899	0.836	0.626
CV			3.109	2.375	2.690	1.689

*Values in brackets are angular transformed

the efficacy of these kind of molecules against *M. phaseolina*. Bankoliya et al. (2022) recorded high growth inhibition of *Rhizoctonia bataticola* by carboxin+thiram (100%) and thiophanate methyl+pyraclostrobin (100%) at 300 and 100 ppm, respectively. This experimental finding also corroborated the results of Sagarika et al. (2023) that penfufen 13.28%+trifoxystrobin 13.28% FS (100%), carboxin 37.5%+thiram 37.5% DS (90.00%) and thiophanate methyl 45%+pyraclostrobin 5% FS (86.90%) were highly efficient in mycelial inhibition *M*. *phaseolina* at 50 ppm. Tebuconazole 50%+trifloxystrobin 25% WG (100%), carboxin 37.5%+thiram 37.5% WP (84.17%) and carbendazim 12%+mancozeb 63% WP (88.60%) were also highly efficient in mycelial growth of *M. phaseolin* inciting agent of charcoal rot of fenugreek at 100 ppm (Kumari et al., 2023). These fungicides also showed a similar efficacy trend against *M. phseolina* inciting agent of dry root rot of chickpea (Malagi et al., 2023). Carbendazim 12%+mancozeb 63% and thiophanate methyl and other fungicides also showed high mycelial growth inhibition in case of root rot of sesame caused by *M. phaseolina* (Bairwa et al., 2022). Tonin et al. (2013) revealed that carbendazim and penflufen+trifloxystrobin were the most powerful in controlling *M. phaseolina*. Thiophanate methyl 45%+pyraclostrobin 5% FS (84.17%) inhibited the most mycelial development of *M. phaseolina* and also showed *in vivo* efficacy in reducing root rot of sunflower (Thikare et al., 2023) (Table 2) .

In our finding, all the combo fungicides containing two different kinds of groups have shown very high efficacy against soil-borne fungi. One compound agrochemical, i.e., azoxystrobin 2.5%+thiophanate methyl 11.25%+thiamethoxam 25% FS, only had a low inhibitory effect on both funguses. This might be due to the presence of one insecticidal ingredient, i.e., Thiamethoxam.

Table 2: Percent growth inhibition of *Macrophomina phaseolina* by new generation agrochemicals on PDA media

Sl. No.	Treatments	Trade name	3 days		4 days	
			50 ppm	100 ppm	50 ppm	100 ppm
T_1	penflufen13.28%+trifloxystrobin 13.28% FS	Evergol xtend	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
T_2	carbendazim 12%+mancozeb 63%WP	SAAF	81.25 (64.35)	93.69 (77.42)	71.94 (58.01)	91.94 (73.61)
T_3	carboxin 37.5%+thiram 37.5% DS	Vitavax power	92.17 (74.0)	100.0 (90.0)	89.72 (71.40)	100.0 (90.00)
T_4	azoxystrobin2.5%+thiophanate methyl 11.25%+ thiamethoxam 25% FS	Electron	56.12 (48.50)	76.03 (60.67)	50.27 (45.14)	71.71 (57.87)
T_5	thiophanate methyl 45% +pyraclostrobin 5% FS	Xelora	90.90 (72.59)	100.0 (90.00)	86.94 (68.85)	100.00 (90.00)
CD (p=0.05)			3.738	5.934	2.951	2.271
SEm±			1.229	1.951	0.970	0.747
CV			3.516	4.780	2.910	1.860

*Values in brackets are angular transformed

4. Conclusion

All the combo fungicides, i.e. penflufen 13.28%+trifloxystrobin 13.28% FS and thiophanate methyl 45%+pyraclostrobin 5% FS, carboxin 37.5%+thiram 37.5% DS and carbendazim 12%+mancozeb 63% WP were showed high efficacy in inhibiting mycelial development of both the pathogens *R. solani* and *M. phaseolina* at 50 and 100 ppm concentrations.

5. Acknowledgement

The authors sincerely acknowledge the laboratory and agrochemicals' support from the Department of Plant Pathology and the Department of Plant Breeding and Genetics, J.N.K.V.V., Jabalpur.

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