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Management of Root Rot (*Rhizoctonia solani*) of Okra Through Novel Combined Formulations of Fungicides

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lady's finger or okra [*Abelmoschus esculentus* (L.) Moench] is known as "*Bhindi*" in Hindi, is one of the most important summer vegetables of Rajasthan as well as India and belongs to the family *Malvaceae.* This crop suffers harshly from the vagary of diseases caused by fungi and important one is root rot caused by *Rhizoctonia solani*, which is an important constraint to the crop and causes significant economic losses and fungicides are the major tool to overcome the disease incidence. During investigation, seven systemic and non-systemic fungicides were evaluated *In vitro* an d *in vivo* conditions for two consecutive years. All the tested fungicides showed highly inhibitory

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response at 100, 200 and 300 ppm concentrations. By treating the seeds with these fungicides, the highest disease reduction (85.91%), increased yield (30.65%) and maximum ICBR (1:215.89) was recorded with captan + hexaconazole (@ 0.2%) followed by tebuconazole + trifloxystrobin (81.59%, 30.10%, 1:140.55), penflufen + trifloxystrobin (78.82%, 29.11%, 1:209.08), fluxapyroxad + pyraclostrobin (75.58%, 27.67%, 1:111.12), hexaconazole (73.80%, 26.32%, 1:197.84), azoxystrobin (71.68%, 24.52%, 1:180.36) and least effective was copper hydroxide (69.38%, 22.75%, 1:169.18, respectively) over control. It is concluded that the use of combined formulations of fungicides may be the most powerful tools in managing root rot of okra with economical yield returns.

Keywords: Okra; root rot; Rhizoctonia solani; fungicides; seed treatment.

1. INTRODUCTION

"Okra [*Abelmoschus esculentus* (L.) Moench] is an important vegetable valued for its edible green pods. The geographical origin of okra is disputed, with supporters of South Asian, Ethiopian and West African origins. The plant is cultivated in tropical, subtropical and warm temperate regions around the world" [1]. "It considers as good source of proteins, carbohydrates, vitamins, calcium, enzymes and total minerals, as well as it has a therapeutic effect in the treatment of ulcer and relief of hemorrhoids, and as a substitute for blood plasma. Additionally, it is useful in the treatment of urinary and reproductive system disorders" [2].

"Furthermore, it can be beneficial from its fresh leaves, buds, flowers, horns, stems and seeds, and it is an immature fruit as vegetables. Its seeds are a source of oil, where the oil concentration range from 20% to 40% and contains tryptophan acid up to 47.4%, in the form of unsaturated fatty acid that necessary for human feed. Moreover, Okra helps to reduce cholesterol in blood, the other part of the okra is an insoluble fiber, which helps to maintain intestinal health, and it is rich in phenolic compounds with biological properties such as quartering and flavonol derivatives, catechin oligomers and hydroxycinnamic derivatives" [3].

"Due to the importance of this crop, its cultivation has expanded considerably and many difficulties have accompanied it, including many diseases such as damping-off and root rot, which causes a significant loss of the crop, which lead to the re-patching of agricultural sites in vain. Diseases are a determining factor in the production of okra and most of the pathogens endemic in soils are difficult to control through traditional methods such as the use of resistant or synthetic varieties" [4]. "The fruits are harvested at immature stage and eaten as a vegetable. The fruits of okra have reawakened beneficial interest in bringing this crop into commercial production" [5].

"Tests conducted in China suggest that an alcohol extract of okra leaves can eliminate oxygen free radicals, alleviate renal tubular interstitial diseases, reduce protein urea, and improve renal function" [6]. "The important diseases of okra are root rot (*Rhizoctonia solani* Kuhn*),* powdery mildew (*Oidium* spp.), Fusarium wilt (*Fusarium oxysporum*), charcoal rot (*Macrophomina phaseolina*), Cercospora leaf spot *(Cercospora abelemoschi*), damping off (*Pythium* spp.), root knot (*Meloidogyne* sp.) and yellow vein mosaic (*Bhindi Yellow Vein Mosaic Virus*) [7]. Amongest these diseases, root rot caused by *Rhizoctonia solani*, is an important constraint to the crop and causes significant losses. The pathogen mainly attacks the root and underground parts, but it is also capable of infecting the other plant parts like the green foliage parts, the seeds and the hypocotyls" [8].

"Among the initial symptoms of the disease, yellowing of leaves is a first symptom which in next two or three days, leaves droop and wither off. Infected plants may wilt within a week after the appearance of first symptom. When stem is examined closely, dark lesions can be observed on the bark near ground level. The roots of infected plants are poorly developed; finer roots are either not formed or rotted. Plants show stunted growth and can easily be pulled out. If the plants are pulled from soil, the basal stem along with main root, may show symptoms of rotting. The tissues are weakened and break off easily in advanced cases and sclerotial bodies can be seen scattered on the affected roots. The fungus is mainly a soil dweller and spreads from plant to plant through irrigation water and implements and cultural operations. The sclerotia and pycniospores may also become air borne and cause further spread of the pathogen" (Rangaswami and Mahadevan, 2008). "Crop losses by root rot of okra (*Rhizoctonia solani)* is ranged from negligible to 50-60 per cent depending on the extent of severity and different stages of crop" [9] and fungicides are the key tool to overcome this ailment. Crop losses by root rot of okra (*Rhizoctonia solani)* is ranged from negligible to 50-60 per cent depending on the extent of severity and different stages of crop [9]. For managing root rot disease, earlier plant pathologists conducted several experiments including chemical control. Application of fungicides is the most effective and satisfactory method to manage root rot through tetra methyl thiram disulphide (TMTD), mancozeb, carbendazim, zineb and copper oxychloride. The most of these fungicides alter only one or perhaps two steps in genetically controlled events into the metabolism of the fungus. Therefore, the present investigation was carried out to evaluate novel and combined formulations of fungicides for managing root rot of okra.

2. MATERIALS AND METHODS

The laboratory experiment was carried out in the Department of Plant Pathology while field experiments were conducted at Instructional Farm, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan) for two consecutive years. Jobner is situated at latitude 26°5' N, longitude of 75°20' E and altitude of 427 meters above MSL (mean sea level). The region falls under semi-arid eastern plain (Agro-Climatic Zone- Ill A) of Rajasthan.

The following seven systemic and non-systemic fungicides were evaluated against *R. solani* by poisoned food technique and seed treatment.

***In vitro* efficacy of fungicides:** *Efficacy of above mentioned seven systemic and non-systemic fungicides was tested against mycelial growth of R. solani by Poisoned Food Technique. Required quantity of each fungicide was added aseptically to 100 ml sterilized PDA medium in 150 ml flask so as to get concentration of 100, 200 and 300 ppm. Just before pouring in sterilized Petri plates, the flasks were shaken several times to ensure proper and uniform distribution of the fungicide. Poisoned medium was poured in sterilized Petri plates and allowed to solidify. Medium without fungicide served as control. Three replications were maintained for each treatment. Each plate was inoculated with 5 mm mycelial bit of the pathogen in the centre of plate. Inoculated plates were incubated at 28+1⁰C for 7 days.* The linear growth of test fungus was recorded and per cent growth inhibition was calculated *by Vincent's [10] formula:*

Per Cent Growth Inhibition = C - T / C X 100

Whereas,

C =Diameter of the colony in check (average of both diagonals)
T = Diameter of colony in treatment (average of both diagonals)

***In vivo* efficacy of fungicides in disease management:** A field experiment was conducted during Zaid 2022 and 2023 at Instructional Farm, *S.K.N. College of Agriculture, Jobner in randomized block design (RBD)* with three replications in 1.8 m x 2.25 m plots, using Pusa Bhindi-5 as test variety, under artificial inoculation conditions (20 g inoculum per meter row, multiplied on sorghum grains). All the recommended agronomic practices were followed to raise the crop. Above mentioned seven fungicides were used as seed treatment. Fungicide treated as well as untreated seeds were sown separately in plots with three replications. Observations on disease incidence (75 DAS) and pod yield (up to harvest) were recorded.

Incremental cost benefit ratio (ICBR): ICBR over the control was worked out to identify and judge the cost effectiveness of the respective treatments, incremental cost benefit ratio (ICBR) *i.e.* the ratio between changes in return and change in cost over control treatment in absolute terms for the respective treatment combinations were computed subsequently.

ICBR= [Additional income received (from the particular treatment)/Additional cost incurred for the particular treatment]

Incremental Cost-Benefit ratio: This was calculated separately for each treatment as per following formulae

Incremental Cost-benefit ratio = Net Return / Cost of Treatment

List 1. Systemic and non-systemic fungicides and their doses

S. No.	Common name	Dose	
		In vitro (ppm)	*In vivo* (%)
1.	Azoxystrobin 22% SC	100, 200 & 300	0.1
2.	Copper hydroxide 53.8% DF	100, 200 & 300	0.2
3.	Hexaconazole 5% SC	100, 200 & 300	0.2
4.	Captan 70% + hexaconazole 5% WP	100, 200 & 300	0.2
5.	Penflufen13.28% w/w + trifloxystrobin13.28% WP	100, 200 & 300	0.2
6.	Tebuconazole 50% + trifloxystrobin 25% WG	100, 200 & 300	0.2
7.	Fluxapyroxad G/L% + pyraclostrobin 250G/LSC	100, 200 & 300	0.2
8.	Control	-	-

Statistical Analysis: The data obtained in different experiments was transferred using angular transformation wherever necessary and was statistically analyzed using Completely Randomized Block Design (CRD) as per the procedures suggested by Panse and Sukhatme [11]. Statistical analysis was carried out as per the procedures given by Panse and Sukhatme [12]. Actual data in percentage were converted to angular transformed values, before analysis according to the table given by Walter [13]. Fischer's method of analysis of variance was used for analysis and interpretation of the data as outlined by Gomez and Gomez [14]. The level of significance used in 'F' and 'T' tests was p=0.05. Critical differences were calculated wherever 'F' test was significant. Other statistical analysis *viz.,* calculation of correlation coefficients, regression equations *etc.* were done using MS-excel.

Calculation and Statistical Analysis: Percent disease incidence (PDI) and disease control in various experiments were calculated as follows:

Disease incidence (%)= Number of diseased plants/ Total number of plants observed × 100

Disease control (%) = Disease incidence Disease incidence in inoculated control (%) - in treatment (%) / Disease incidence in inoculated control (%) × 100

Increase in yield over check (%) = Yield of plants Yield of plants in treatment (%) - in inoculated control (%) / Yield of plants in inoculated control × 100

3. RESULTS AND DISCUSSION

3.1 Efficacy of Fungicides (*in vitro*)

Seven systemic and non-systemic fungicides were evaluated against *R. solani* by poisoned food technique. All the tested fungicides showed significantly higher mycelial growth inhibition over control (Table-1, Plate-1 and Fig. 1). Among these fungicides, captan + hexaconazole was found the most effective in inhibiting mycelial growth (80.00, 89.00 and 98.00%) followed by tebuconazole + trifloxystrobin (70.00, 85.00 and 93.00%), penflufen + trifloxystrobin (49.00, 80.00 and 89.00%), fluxapyroxad + pyraclostrobin (45.00, 73.00 and 86.00%) and hexaconazole (43.00, 70.00 and 82.00%) at 100, 200 and 300 ppm concentration, respectively. Copper hydroxide 53.8% DF was found least effective in inhibition of mycelial growth (40.00, 65.00 and 81.00%) at 100, 200 and 300 ppm concentration, respectively. The data presented in Table-1 also reflected that mean mycelial growth inhibition was also maximum in case of captan + hexaconazole (89.00%) followed by tebuconazole + trifloxystrobin (82.67%), penflufen + trifloxystrobin (72.67%), fluxapyroxad + pyraclostrobin (68.00%), hexaconazole (65.00%), azoxystrobin (62.33%) and least effective was copper hydroxide (59.67%). Our results are in agreement with the findings of Deepthi *et al.* [15], Atia *et al.* [16], Basandrai *et al.* [17], Maruti *et al.* [18] and Yadav *et al.* [19] as they evaluated different fungicides under *in vitro* conditions against *M. phaseolina* in which carboxin + thiram and penflufen gave 100% inhibition at 500 ppm while tricyclazole gave 100 per cent inhibition at 1000 ppm. The seed treatment with Vitavax Power gave highest seed germination percentage and reduced seedling mortality, and lower yield losses. The Vitavax Power as seed treatment along with one foliar application of carbendazim was found most effective in increasing seed germination and reducing pre, post emergence mortality and lowering losses in yield of sesame.

3.2 Efficacy of Fungicides in Field Conditions (*In vivo*)

The results revealed that all fungicides tested significantly decreased the incidence of root rot

Table 1. *In vitro* evaluation of fungicides against *Rhizoctonia solani* at three concentrations

S. No.	Common name	Per cent inhibition of mycelial growth at various concentrations			Mean
		100 ppm	200 ppm	300 ppm	
1.	Azoxystrobin 22% SC	40.00 (39.23)	65.00 (53.73)	81.00 (64.90)	62.33 (52.14)
2.	Copper hydroxide 53.8% DF	38.00 (38.06)	63.00 (52.54)	78.00 (62.03)	59.67 (50.57)
3.	Hexaconazole 5% SC	43.00 (40.98)	70.00 (56.79)	82.00 (64.90)	65.00 (53.73)
4.	Captan 70%+hexaconazole 5% WP	80.00 (63.43)	89.00 (70.63)	98.00 (81.87)	89.00 (70.63)
5.	Penflufen13.28% w/w + trifloxystrobin13.28% WP	49.00 (44.43)	80.00 (63.43)	89.00 (70.63)	72.67 (58.48)
6.	Tebuconazole 50% + trifloxystrobin 25% WG	70.00 (56.79)	85.00 (67.21)	93.00 (74.66)	82.67 (65.40)
7.	Fluxapyroxad G/L% + pyraclostrobin 250 G/LSC	45.00 (42.13)	73.00 (58.69)	86.00 (68.03)	68.00 (55.55)
8.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		SEm±	**CD (p=0.05)**		
	F	0.60	1.66		
	C	0.91	2.53		
	F X C	1.58	4.38		

**Average of three replications. Figures in parentheses are angular transformed values*

of okra over control during both the years as well as pooled basis (Table-2 and Fig. 2). Among the tested fungicides, highest reduction in disease incidence (85.91%) was recorded by treating the seeds with captan + hexaconazole (@ 0.2%) followed by tebuconazole + trifloxystrobin (81.59%), penflufen + trifloxystrobin (78.82%), fluxapyroxad + pyraclostrobin (75.58%), hexaconazole (73.80%), azoxystrobin (71.68%) and least effective was copper hydroxide (69.38%) over control.

It was evident from the result that all the fungicides used in present experiment were significantly enhanced the pod yield of okra over control during both the years as well as under pooled basis (Table-2 and Fig. 2). Maximum increase in pod yield (30.65%) was observed by treating the seeds with captan + hexaconazole followed by tebuconazole + trifloxystrobin (30.10%), penflufen + trifloxystrobin (29.11%), fluxapyroxad + pyraclostrobin (27.67%), hexaconazole (26.32%), azoxystrobin (24.52%) and lowest with copper hydroxide (22.75%) over check.

3.3 Incremental Cost-benefit ratio (ICBR)

Incremental cost benefit ratio (ICBR) was calculated to interpretate the economics of seven fungicides. The data presented in Table-2 revealed that the highest ICBR was observed in captan + hexaconazole (1:215.89) followed by penflufen + trifloxystrobin (1:209.08), hexaconazole (1:197.84), azoxystrobin (1:180.36), copper hydroxide (1:169.18) and tebuconazole + trifloxystrobin (1:140.55) whereas, lowest ICBR was recorded with fluxapyroxad + pyraclostrobin (1:111.12).

Our results are in agreement with the findings of Atia *et al.* [16] who evaluated nine fungicides *viz.,* tebuconazole + trifloxystrobin, propiconazole, fenamidone + mancozeb, carbendazim, tebuconazole and hexaconazole + zineb, mancozeb, captan and metalaxyl + mancozeb against *R. solani* causing root rot of tomato *in vitro* and *in vivo* conditions. The fungicides, propiconazole and trifloxystrobin + tebuconazole were found to be highly effective (100% inhibition) at 250, 500 and 1000 ppm concentration. They also tested these fungicides in field conditions through seed treatment and found that captan (44.73%), carbendazim (58.36%) and propiconazole (61.60%) were reduced root rot incidence as compared to untreated check. Maruti *et al.* [18] tested combi fungicides against dry root rot of pigeon pea (*M. phaseolina*). Among combi products tested, carbendazim 12 per cent + mancozeb 63 per cent WP, trifloxystrobin 25 per cent + tebuconazole 50 per cent EC and carboxin 37.5 per cent + thiram 37.5 per cent WP showed total inhibition at 0.10, 0.20 and 0.30 per cent concentrations. Muhammad *et al*. [20] conducted an experiment to manage sesame charcoal rot caused by *M. phaseolina* under field conditions and found that tebuconazole + trifloxystrobin was exhibited minimum mean disease incidence. Yadav *et al*. [19] conducted an experiment to manage Rhizoctonia root rot of okra caused by *Rhizcotonia solani* and recorded that carbendazim was found most effective followed by propiconazole [21-26].

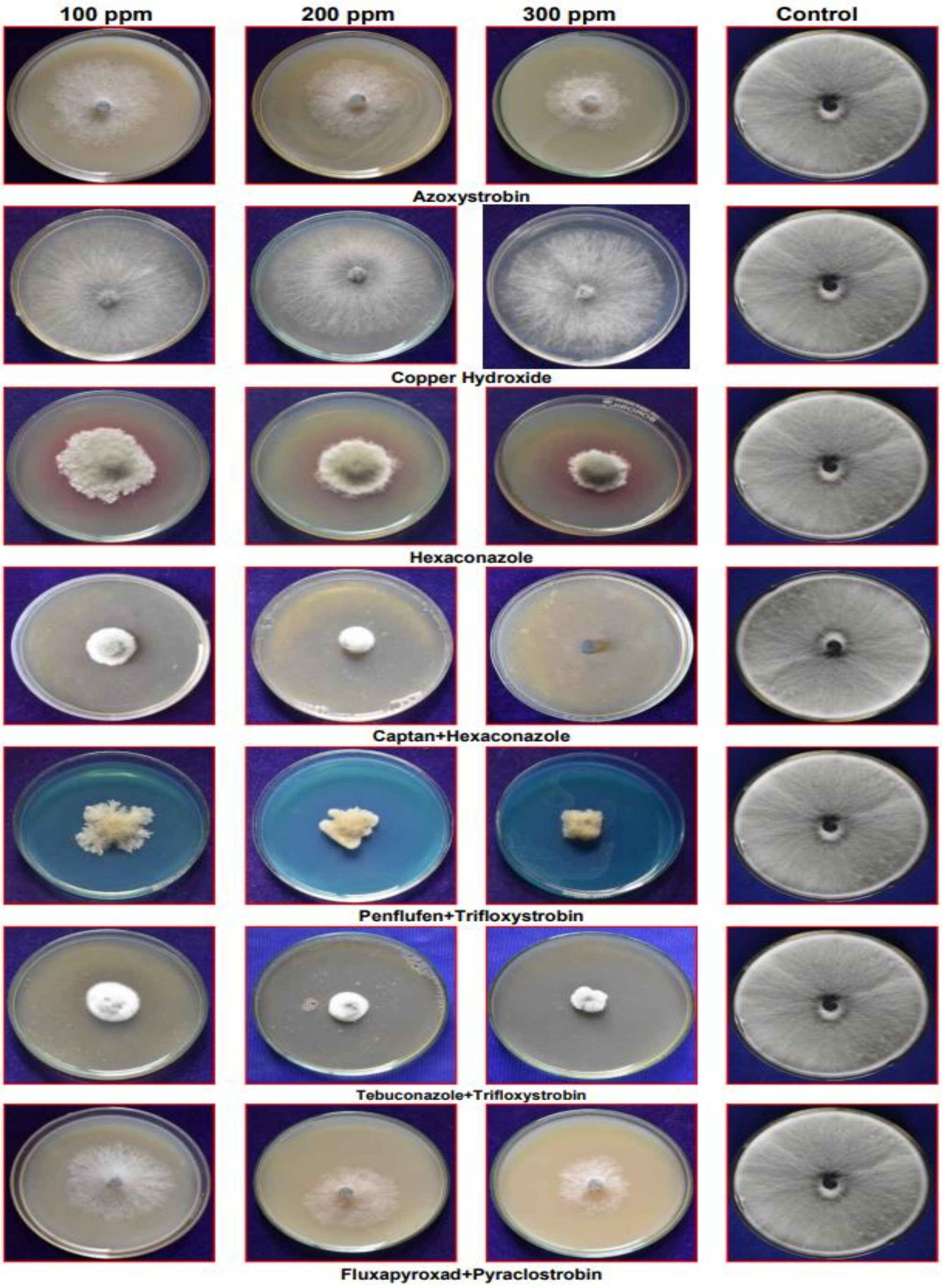

Plate 1. *In vitro* assessment of fungicides against *Rhizoctonia solani*

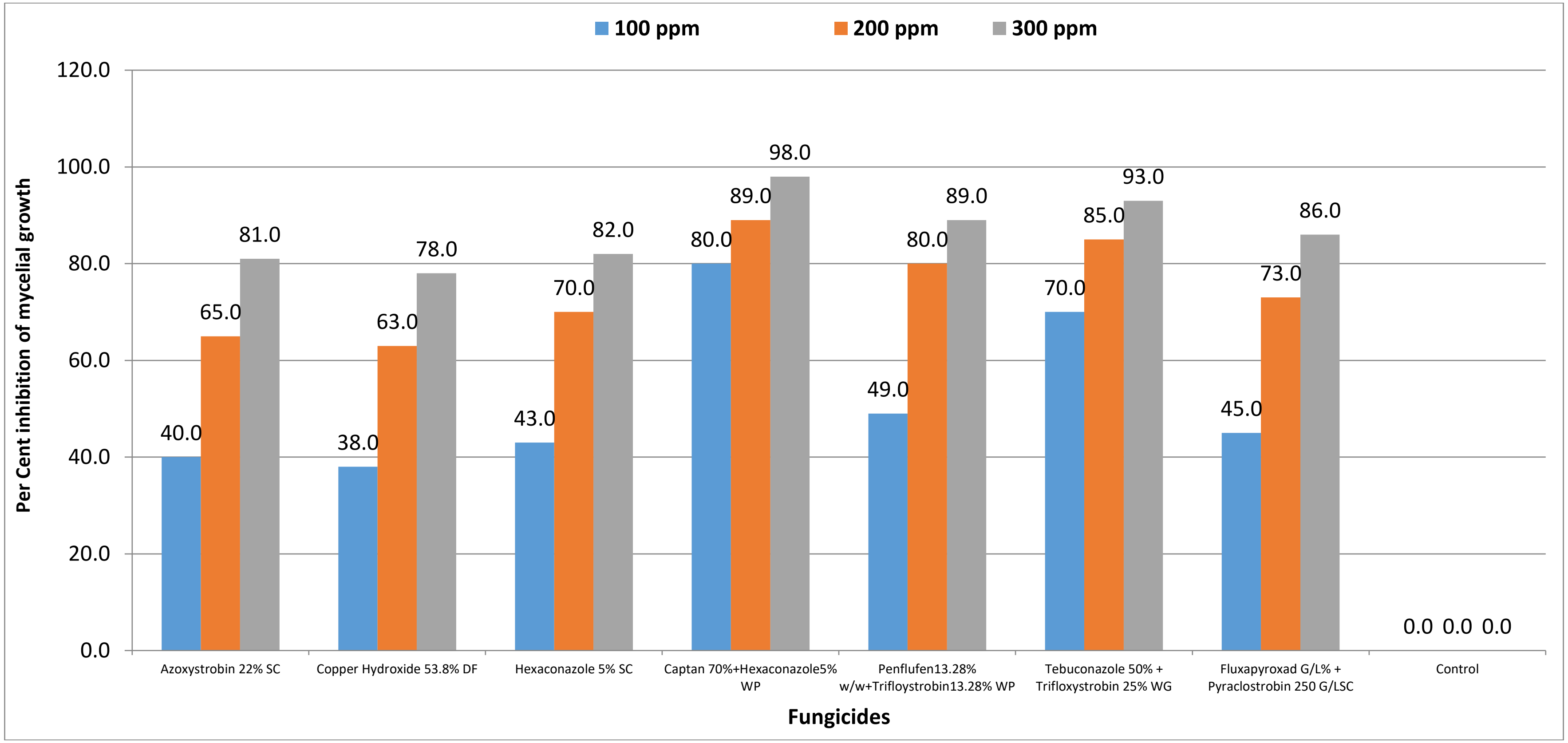

Fig. 1. ***In vitro*** **evaluation of fungicides against** ***Rhizoctonia solani***

Table 2. Efficacy of fungicides on root rot of okra applied through seeds under field conditions

S. No.	Common name	Dose *In vivo* (%)	PDI 2022	PDI 2023	Pooled	Disease reduction (%) over control	Yield (q/ha) 2022	Yield (q/ha) 2023	Pooled	Per cent increase in yield over check	Cost of treatment + Labour Charges (ha)	Gross return (ha)	ICBR ratio
1.	Azoxystrobin 22% SC	0.1	14.62 (22.48)	16.14 (23.69)	15.38 (23.09)	71.68	63.45	61.58	62.52	24.52	273	250080	1:180.36
2.	Copper hydroxide 53.8% DF	0.2	16.06 (23.63)	17.20 (24.50)	16.63 (24.07)	69.38	62.15	61.10	61.63	22.75	270	246520	1:169.18
3.	Hexaconazole 5% SC	0.2	13.10 (21.22)	15.36 (23.07)	14.23 (22.16)	73.80	64.35	62.49	63.42	26.32	267.08	253680	1:197.84
4.	Captan 70% + hexaconazole5% WP	0.2	6.20 (14.42)	9.10 (17.56)	7.65 (16.06)	85.91	66.74	64.45	65.60	30.65	285.14	262400	1:215.89
5.	Penflufen13.28% w/w + trifloxystrobin13.28% WP	0.2	9.80 (18.24)	13.20 (21.30)	11.50 (19.82)	78.82	65.78	63.86	64.82	29.11	279.5	259280	1:209.08
6.	Tebuconazole 50% + trifloxystrobin 25% WG	0.2	8.86 (17.32)	11.13 (19.49)	10.00 (18.43)	81.59	66.33	64.30	65.32	30.10	430	261280	1:140.55
7.	Fluxapyroxad G/L% + pyraclostrobin 250G/LSC	0.2	11.86 (20.14)	14.66 (22.51)	13.26 (21.35)	75.58	65.02	63.17	64.10	27.67	500	256400	1:111.12
8.	Control	-	53.01 (46.73)	55.60 (48.22)	54.31 (47.47)	0.00	51.29	49.12	50.21	0.00	-	200840	-
	SEm±		0.58	0.65	0.41		3.07	2.96	1.85				
	CD (P=0.05)		1.76	1.97	1.23		9.24	8.92	5.56				
	CV (%)		4.38	4.51	2.95		9.46	9.41	6.92				

**Average of three replications. Figures in parentheses are angular transformed values*

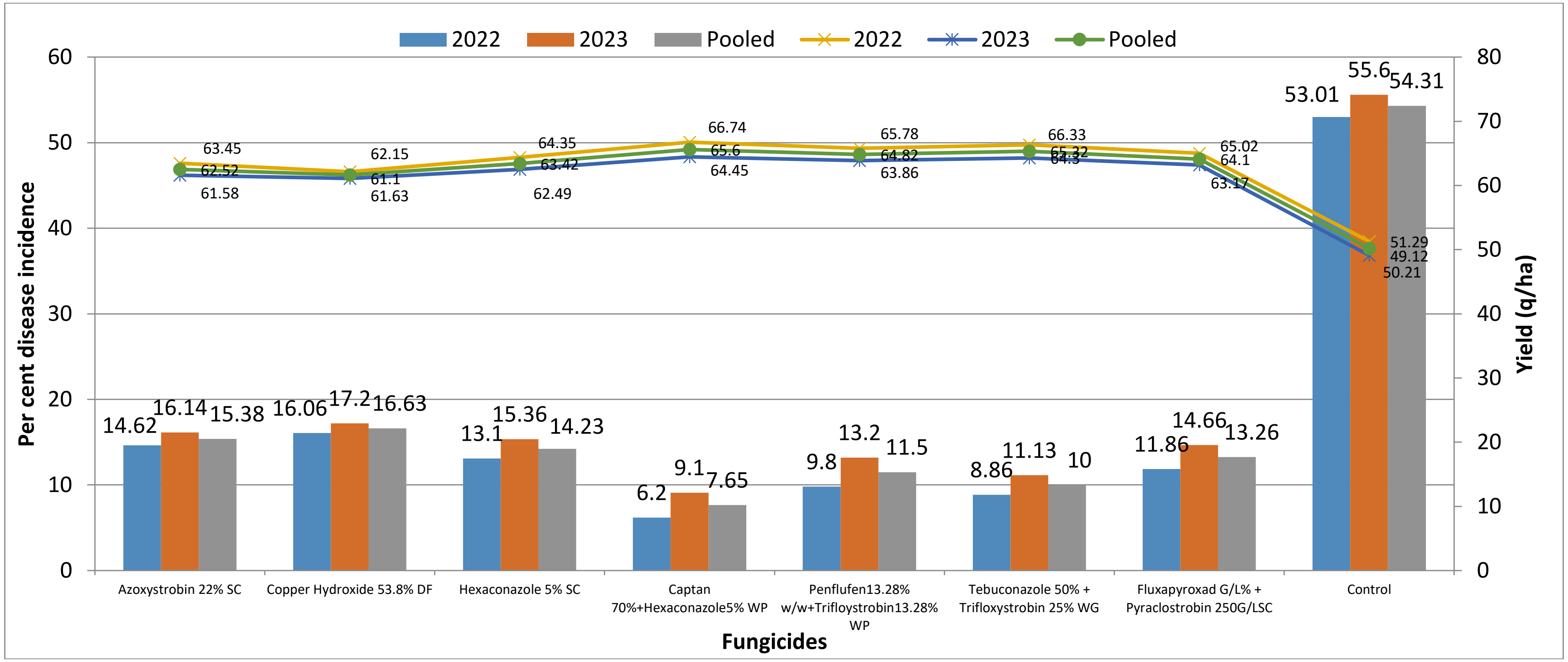

Fig. 2. Efficacy of fungicides on root rot of okra under field conditions

4. CONCLUSION

Conclusively, among evaluated seven fungicides, the seed application of captan + hexaconazole (2 g/kg seed) was recorded highly effective in reducing disease incidence and in increasing yield of okra followed by tebuconazole + trifloxystrobin (2 g/kg seed) and penflufen + trifloxystrobin (2 g/kg seed).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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