IN VITRO EVALUATION OF DIFFERENT CHEMICAL FUNGICIDES FOR THE CONTROL OF *Rhizoctonia solani* KUHN

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ABSTRACT

An in vitro experiment was conducted for an evaluation of different chemical fungicides against *Rhizoctonia solani* using poisoned food technique at National Plant Pathology Research Centre, Nepal Agricultural Research Council (NARC) Khumaltar, Lalitpur. Pathogen was isolated from infected broad leaf mustard. The experiment was conducted in Completely Randomized Design (CRD) with three replications for each treatment. Seven fungicides as treatments, namely, Saaf (carbendazim 12% + mancozeb 63%), Bavistin (carbendazim 50%), Vacomil Plus (metalaxyl 15% + copper oxychloride 35%), Nativo (tebuconazole 50% + trifloxystrobin 25%), Sectin (fenamidone 10% + mancozeb 50%), Kingstival (dimethomorph 50%) and Topcare (azoxystrobin 50%) were taken for the evaluation. Two levels of concentrations (100 ppm and 200 ppm) were used for each fungicide and concentration was calculated based on active ingredients (a.i.) of the fungicides. All fungicides were used individually to study the rate of inhibition of *R. solani* on PDA media. At higher concentration 200 ppm, Bavistin, Nativo and Saaf showed complete inhibition of the mycelial growth of the pathogen whereas at lower concentration 100 ppm, Bavistin was only completely effective against the pathogen. Sectin and Topcare were also highly effective in inhibiting the growth of *R. solani*. The efficaces of Kingstival and Vacomil Plus against the pathogen were much lesser than others. The active ingredients carbendazim, tebuconazole, mancozeb and trifloxystrobin showed the highest degree of mycelial growth inhibitions compared to others. The fungicides that found to be effective for inhibition of mycelial growth of *R. solani* in this study should be further tested in the field conditions in order to verify their efficacies as well as to determine their optimum application doses.

Key words : Fungicides, in vitro experiment, poisoned food technique, *Rhizoctonia solani*

INTRODUCTION

R. solani produces damping-off, a disease that kills or weakens seeds and seedlings. Pre-emergence damping off occurs when the seed does not germinate, and post-emergence damping off occurs when the seed germinates but the seedling dies. Stems frequently decompose fast, beginning with dark to reddish brown lesions that grow and produce deep cankers along the soil line on plants. This results in withering, particularly during the hotter parts of the day, as well as nutritional deficits (Lawson, 2021). *R. solani* may live as sclerotia in soil, pathogenically on alternate hosts and weeds, and saprophytically on crop residues (Schwartz and Gent, 2016)

Implementing an integrated disease management approach and understanding each stage of *Rhizoctonia* illness are required for effective disease management. Although cultural approache is most essential control strategy, chemical management is still an important tool to decrease *R. solani*

problem (Tsror, 2010). Soil disinfection and the use of resistant cultivars are the best ways to control it. It can also be managed by removing contaminated seedlings. Pentachloronitrobenzene-based fungicides are efficient in killing the pathogen.Tebuconazole and carbendazim are known to be effective against *Rhizoctonia* (Karkee & Mandal, 2020).It can also be controlled by contact fungicides such as iprodione and chlorothalonil, as well as systemic fungicides such as carboxin, triadimefon, and thiophanate-methyl (Chaube and Pundhir, 2022). A combination of resistant cultivars and fungicide treatment, according to Bartholomäus *et al.* (2017), can prevent the infected field buildup of *R. Solani*. A laboratory experiment was conducted in the National Plant Pathology Research Centre, Khumaltar to determine the efficiency level of seven different chemical fungicides against the pathogen *R. solani*.

MATERIALS AND METHODS

In Vitro Efficacy of Fungicides on Mycelia Growth of *R. solani*

A seven-day old mycelial culture of *R. solani* isolated from broad leaf mustard was used for the study. Poisoned food technique was used to evaluate the efficacy of fungicides to inhibit the mycelial growth of *R. solani*. Seven fungicides were used at concentrations of 100 ppm and 200 ppm which are listed in Table 1. Selection of fungicides was done on the basis of availability in market. The required numbers of fungicides for specific concentrations were calculated based on active ingredients (a.i.) of the pesticides as mentioned on each pesticide label. Around 60 ml of PDA solution was taken each on 15 conical flasks, and the flasks were autoclaved at 121 ºC and 15 lbs pressure for 25 minutes. The calculated and weighed fungicides were amended in respected conical flasks at lukewarm conditions and 20 ml media was poured into 9 cm sterilized petri plates under aseptic conditions and allowed to cool. PDA plates without chemicals served as control.

5 mm mycelial discs of *R. solani* was excised with sterile cork borer from one week old culture and placed at the center of each PDA plates. Three petri plates were chosen as three replications for each treatment and the plates were arranged in completely randomized design (CRD) at 26 ºC ± 1 inside an incubator. Measurement of the colony diameter of pathogens was taken on 24 hr, 48 hr, 72 hr, 96 hr and 120 hr in each plate with the help of measuring scale. Measurement was done from two perpendicular diameters of the plates and their average was taken. Percent growth inhibition of the pathogen was calculated by using the following formula of Vincent (1947).

$$I\ (\%) = (C - T) / C \times 100$$

where, I = Inhibition percentage
C = Colony diameter in control
T = Colony diameter in treatment

Table 1. Quantity of the fungicides used in 60 ml of the PDA medium

Commercial Name	Common Name	Active ingredients (a.i.)	100 ppm (mg)	200 ppm (mg)
Saaf	Carbendazim 12% + mancozeb 63%	75%	8	16
Bavistin	Carbendazim 50%	50%	12	24
Vacomil Plus	Metalaxyl 15% + copper oxychloride 35%	50%	12	24
Nativo	Tebuconazole 50% + trifloxystrobin 25%	75%	8	16
Sectin	Fenamidone 10% + mancozeb 50%	60%	10	20
Kingstival	Dimethomorph 50%	50%	12	24
Topcare	Azoxystrobin 50%	50%	12	24

Statistical Analysis

STAR software (Version 2.0) and Ms-Excel (Version 2013) were used for the analytical part of the study. Analysis of variance (ANOVA) was performed to test the significance of treatment effect on mycelial growth of *R. solani*. Duncan's multiple range test (DMRT) was used to seperate means at 5% level of significance.

RESULTS AND DISCUSSION

The data recorded from in vitro study were analyzed and the obtained results were presented with the help of tables and figures wherever necessary. The results were discussed and supported by literature.

Effect of Different Fungicides on *R. solani*

Table 2 shows all the tested fungicides exhibited control over the mycelia growth of the pathogen in varying degrees and they were significantly different ($P<0.05$) from control at both concentrations of 100 ppm and 200 ppm. The growth of mycelia in fungicide amended petri-plates was significantly lesser than that of control plates. There was no growth of pathogen in Bavistin at both concentrations and in Nativo and Saaf at 200 ppm. There was also minimal growth in Topcare and Sectin but the pathogen easily grew in Vacomil and Kingstival. The variations in mycelial growth were significant also in between the concentrations of each fungicide.

Sriraj *et al.* (2014) reported that Nativo WG 75 and Bavistin 50 WP completely inhibited the mycelial growth of *R. solani* and sclerotial formation even at lower concentration. Persaud *et al.* (2019) also found Nativo to be highly effective against *R. solani*. According to Kumar *et al.* (2017) and Adhikari *et al.* (2018), copper oxychloride was found more effective in 50 ppm, 100 ppm and 200 ppm by inhibiting greater than 90% of the growth of *R. solani* but the results obtained in this study and also by Karkee and Mandal (2020) were in contrast to this as copper oxychloride was not able to show greater inhibition of mycelial growth. Such contrast in the results may occur due to different formulation mixtures as well as due to different brands of fungicides. Vacomil Plus (metalaxyl 15% + copper oxychloride 35%) was used in this study whereas Adhikari *et al.* (2018) used Allcop (Copper oxychloride 50 w/w).

Table 2. Radial growth rate of *R. solani* in different fungicides amended medium

Fungicide	Mean colony diameter (mm)				
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**
Vacomil 100 ppm	13.33^{b}	35.33^{a}	60.67^{a}	78.67^{b}	85.33^{b}
Vacomil 200 ppm	5.33^{d}	14.00^{c}	34.33^{c}	59.00^{c}	83.00^{bc}
Saaf 100 ppm	7.33^{e}	10.00^{d}	14.33^{f}	16.00^{g}	19.33^{g}
Saaf 200 ppm	0.00^{e}	0.00^{e}	0.00^{h}	0.00^{i}	0.00^{i}
Sectin 100 ppm	8.00^{c}	11.33^{cd}	19.33^{e}	26.67^{e}	34.00^{e}
Sectin 200 ppm	0.00^{e}	0.00^{e}	7.67^{g}	12.33^{gh}	17.33^{g}
Topcare 100 ppm	7.67^{c}	12.00^{cd}	19.33^{e}	21.67^{f}	24.67^{f}
Topcare 200 ppm	0.00^{e}	0.00^{e}	7.33^{g}	9.00^{h}	10.67^{h}
Bavistin 100 ppm	0.00^{e}	0.00^{e}	0.00^{h}	0.00^{i}	0.00^{i}
Bavistin 200 ppm	0.00^{e}	0.00^{e}	0.00^{h}	0.00^{i}	0.00^{i}
Kingstival 100 ppm	13.33^{b}	26.33^{b}	44.33^{b}	62.67^{c}	80.33^{c}
Kingstival 200 ppm	0.00^{e}	13.00^{c}	27.00^{d}	46.00^{d}	65.33^{d}
Nativo 100 ppm	8.33^{c}	12.67^{cd}	20.00^{e}	26.00^{ef}	32.67^{e}
Nativo 200 ppm	0.00^{e}	0.00^{e}	0.00^{h}	0.00^{i}	0.00^{i}
Control	17.67^{a}	36.00^{a}	63.33^{a}	83.33^{a}	90.00^{a}
Mean	5.4	11.38	21.18	29.42	36.18
P-value	<0.05	<0.05	<0.05	<0.05	<0.05
LSD	1.7925	2.6307	3.1639	4.4328	3.6702
CV %	19.91	13.87	8.96	9.04	6.08

Comparison of Growth of *R. solani* at Different Treatments

Pathogens showed different growth patterns from 1st to 5th day after incubation at both 100 ppm and 200 ppm concentrations. At 100 ppm, Bavistin exhibited no growth of the pathogen throughout the study period. Slow growth of the pathogen was observed in the plates treated with Saaf and Topcare. The growth patterns in Nativo and Sectin were found to be similar with each other. Kingstival, Vacomil and control exhibited significant increase in the diameter of the fungal mycelia each day where the growth was comparatively less in Kingstival and it was almost similar in Vacomil and control. On the 5th day after incubation, the control plates were fully covered by the fungal pathogen.

At 200 ppm, there was no growth of pathogens in the plates treated with Bavistin, Saaf and Nativo till the 5th day after incubation. Sectin and Topcare also exhibited no growth upto 2nd day but slow growth was seen from the 3rd day onwards. Gradual increase in the growth was observed in Kingstival and Vacomil from the 1st day to the last day of the experiment. It was also observed that the growth of the pathogen was slightly inhibited by the increase in concentration of the fungicides.

Table 3. Percentage of inhibition of *R. solani* in 120 hr after incubation

Fungicide	100 ppm	200 ppm
	Inhibition percentage	Inhibition percentage
Vacomil	5.19	7.78
Saaf	78.52	100.00
Sectin	62.22	80.74
Topcare	72.59	88.15
Bavistin	100.00	100.00
Kingstival	10.74	27.41
Nativo	63.70	100.00
Control	0.00	0.00

Bavistin, Saaf, Nativo, Topcare and Sectin individually showed great effectiveness against the pathogen by inhibiting the growth of mycelia. At higher concentration 200 ppm, Bavistin, Nativo and Saaf showed complete inhibition of the mycelial growth whereas at lower concentration 100 ppm, complete inhibition was exhibited only by Bavistin (Table 3). These were followed by Topcare and then Sectin implying significantly less effectiveness and having significant results between each other too. Although Vacomil and Kingstival showed minimal inhibition of the mycelial growth, the results were significant from the control.

From the obtained result, it was found that the fungicides carbendazim and tebuconazole were highly effective in controlling the fungal growth followed by azoxystrobin and mancozeb which also showed good inhibition in the growth. Gupta (2002) also reported that carbendazim inhibited 95-100 percent radial growth of *R. solani*. The mixture of carbendazim + mancozeb and tebuconazole + trifloxystrobin were also significantly effective. Similar result was obtained from the study of Hunjan *et al.* (2011). It was also noted that all the fungicides showed more mycelial inhibition at higher concentration. The result was also supported by the study performed by Karkee and Mandal (2020) which showed that carbendazim and tebuconazole were highly effective against *R. solani.*

Therefore, an increase in the concentration of fungicides also increased their efficacy in controlling the growth of pathogen fungi. But being said this, use of higher concentration than the optimum concentration may not be beneficial as it only increases unnecessary cost in long term and excessive use of chemicals is hazardous to the health and environment as well.

CONCLUSIONS

From the study, some fungicides showed significant control against *R. solani* though their efficacy varied among fungicides. It can be concluded that fungicide containing active ingredient carbendazim is best for an inhibition of the growth of *R. solani*. Tebuconazole at comparatively higher concentration is also effective against *R. solani*. Thus, it implies that those fungicides could be used in the management of *Rhizoctonia* diseases. Since carbendazim is documented as an effective fungicide against *Rhizoctonia*, and tebuconazole was found to be as effective as carbendazim at higher concentration, it can be used as an alternative to carbendazim in controlling the growth of *R.*

solani. However, these findings need verification in vivo conditions in seedling stage and thereafter in the field conditions to determine the degree of disease control caused by *R. solani.*

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