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Assessing fungicides for the control of Polysora rust disease in maize (*Zea mays* L.) across the hilly landscape of Manipur, North-eastern India: an *in vitro* and *in vivo* study

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ABSTRACT

Maize holds the second position among the cereal crops produced in the state of Manipur, India, covering all the districts of the state. Polysora or southern rust, is a troublesome, sporadic foliar disease of maize caused by *Puccinia polysora* Underw. Due to the fungi's temperature sensitivity, the disease is a threat in tropical and subtropical areas of the world. Previously, it had not posed a hindrance to maize production in Manipur. Nevertheless, due to climate change, it is currently on the rise in a few regions, presenting fresh challenges in production in the late *Kharif* season and resulting in substantial yield loss. In order to analyze and manage the disease's impacts on crop loss, both *in vitro* and *in vivo* studies were carried out. Among all the nine fungicides (six systemic and three non-systemic) tested *in vitro*, significantly lowest mean spore germination percentage was observed in Kresoxim methyl (13.19%), followed by Mancozeb 75% WP (14.57%). The least effective fungicide was Metalaxyl 35% WS (26.79%). The results of fungicide treatment in vivo showed that Mancozeb 75% WP at 2 ml l^{-1} was most effective where lowest PDI (22.20%), maximum yield (70.71 q ha^{-1}) and maximum profit (B:C ratio was 2.52) were observed as compared to the control plot. Whereas, Metalaxyl 35% WS at 1 ml l^{-1} spray was found to be the least effective, showing highest PDI (36.31%) with lowest grain yield (52.55 q ha^{-1}) and comparatively less amount of profit (B:C ratio was 1.67).

KEY WORDS: *Polysora rust*; *Uredospore Germination Percentage*; *Fungicides*; *in vitro*; *in vivo*

1. Introduction

Maize is the second most important cereal crop cultivated in the state of Manipur, both in the hills and valley districts. According to the statistical indices of 2019–20, in terms of area, average yield, and production of maize in the state, the state holds a record of 2.22 thousand hectares, 2228.35 kg per hectare, and 4.94 thousand metric tons, respectively (ESM, 2020). Polysora rust, or southern rust, is a troublesome, sporadic foliar disease of maize caused by *Puccinia polysora* Underw. The disease is restricted to tropical and subtropical regions of the world due to the pathogen's temperature sensitivity. In India, it is found in the states of Karnataka, Andhra Pradesh, and Tamil Nadu. Traces of this disease have been reported to be highly frequent in the Manipur region during the late *Kharif* season, resulting in significant yield losses. Of the three rusts that occur on corn worldwide, polysora rust of corn has been reported as the most destructive among

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all the reported rusts of maize, where 80 to 84% yield losses were observed (Reyes, 1953). Growing disease-resistant varieties is one of the best ways to manage the disease. Due to the irregular nature of this fungus, farmers have had no access to resistant varieties yet. As a result, integrating available disease management methods, like the use of available cultural practices that reduce initial inoculum and fungicidal spray, is one of the most successful and cost-efficient approaches to reducing losses caused by this disease. Several fungicides, *viz*. Dithane M-45, Bavistin, Benlate, and Kitazin, were proven to be effective against *P. sorghi*, which causes common rust in maize (Gupta, 1978). The date-back records hold no list of chemicals that are effective against polysora rust disease. With the advent of finding an effective chemical fungicide against the disease, the current study was lined up to test various chemical fungicides to develop an effective management strategy against the recurring disease. In addition, an in vitro study on the effect of different concentrations of fungicides on uredospore germination of *P. polysora* was carried out, and the best concentration of each fungicide was used in the field experiment.

2. Material and Methods

In vitro examinations were conducted in the Department of Plant Pathology, College of Agriculture, CAU, Imphal, Manipur, India. In vivo experiments were conducted at Andro Research Farm (CAU), Imphal East, Manipur. Both the experiments were conducted between 2021 and 2022.

2.1 Collection of Spores

Naturally infected samples showing the typical characteristics of polysora rust were collected during November 2021 at regular intervals. A standard protocol was followed for the collection and storage of the uredospores. The spores were collected by a vacuum pump from the infected areas of the leaf. Freshly collected spores were subjected to air drying for 12 to 14 hours devoid of light at a temperature of 21 °C prior to the study of the germination test. Artificial inoculation was done with uredospore suspension of 1×10^5 spores/ml and Haemocytometer was used to prepare the exact spore concentration.

2.2 *In vitro* evaluation of fungicides against *P. polysora* uredospore

Nine fungicides, consisting of three non-systemic fungicides, namely Mancozeb 75% WP, Kresoxim methyl, and Zineb 75% WP, and six systemic fungicides, namely Hexaconazole 5% SC, Tebuconazole 25.9% EC, Propiconazole 25% EC, Metalaxyl 35% WS, Azoxystrobin + Difenoconazole, and Pyraclostrobin + Epoxiconazole, were evaluated against the germination of *P. polysora* uredospores in the laboratory. Five different concentrations, *viz*. 0.05, 0.1, 0.15, 0.2, and 0.25%, were tried for each fungicide. For each concentration, one drop of uredospore suspension and one drop of fungicide were put in the cavity slide. They were mixed thoroughly with the help of a needle. Uredospore suspension in sterile water without fungicide was kept as the control treatment. Slides were kept inside the BOD incubator (25°C–10°C). After that, the germination percentage of the uredospore was observed after 24 hours. Each concentration was replicated three times.

2.3 *In vivo* evaluation of different fungicides for the control of polysora rust disease in maize

All nine fungicides tried for the *in vitro* study were also tested in the *in vivo* experiment. In the *in vitro* experiment, each fungicide exhibited a rising trend in germination inhibition as their concentration increased. However, during the field trial, we opted to utilize only the recommended dose of each fungicide to minimize residue hazards. The inoculation method was taken up following the procedure of Hooda *et al* (2018). To conduct large-scale inoculation, we gathered naturally infected leaves exhibiting a high density of uredospore pustules from various locations, ensuring the incorporation of all prevalent races in the region for screening the plants against the *P. polysora* fungus. The infected leaves were thoroughly macerated in between the two palms of the hands dipped in a bucket of water until the water became brown. The uredospores thus obtained were kept in the freezer at a lower temperature, *i.e*., 5-7 °C, and carried to the research field for inoculation purposes. A knapsack sprayer was used for inoculating the plants in a field. The spore suspension was sprayed over the plants during the second half of the day, when the sun became mild. While spraying inoculum, the sprayer's nozzle kept passing over the plant's whorl, and all the leaves were sprayed thoroughly. The spore suspension is stirred continuously during spraying as the light spores aggregate together on the upper surface of the water. Inoculation was repeated twice to get the best result.

For each fungicide, two sprays were given: the first one three days after inoculation of the pathogen and the second one eighteen days after inoculation. Plots without any fungicide and only water-sprayed were used as control plots. Several parameters, *viz*. disease scoring, per cent disease incidence (PDI), Per cent Disease Control (PDC), Grain Yield (q/ha), and per cent increase in yield, were taken during this experiment. The treatment plots along with the fungicides are presented in Table 1.

Table 1 - List of fungicides used against *P. polysora*

Treatments	Fungicides
T1	Hexaconazole 5% SC
T2	Tebuconazole 25.9% EC
T3	Propiconazole 25% EC
T4	Metalaxyl 35% WS
T5	Azoxystrobin 18.2 w/w + Difenoconazole
T6	Pyraclostrobin 133gl^{-1} + Epoxiconazole 50gl^{-1}
T7	Zineb 75% WP
T8	Kresoxim methyl 44.3% SC
T9	Mancozeb 75% WP
T10	Untreated control

2.4 Statistical Analysis

Two factorial randomized block design (2FRBD) with three replications was used for evaluating the effect of different fungicides on uredospore germination under *in vitro* condition. For *in vivo* experiment, a Randomized block design (RBD) was laid where the plot size was 3.6 × 2 m^2. Six rows of 2 m each was made in each plot and the spacing of the plants was 60 × 20 cm^2. Three replications were kept for each treatment. R studio was used to analyze the data and to make an interaction plot.

2.5 Economics

For the purpose of calculating the experiment's economics, all inputs and outputs were considered. Net Benefit-cost ratio was calculated according to the following equation –

$$\text{Net Benefit-cost ratio (B:C)} = \frac{\text{Net return}}{\text{Total cost of cultivation}}$$

3. Results and Discussion

The efficacy of six systemic and three non-systemic fungicides was evaluated against uredospore germination of *P. polysora*. Among all the nine fungicides, the significantly lowest mean spore germination percentage was observed in Kresoxim methyl (13.19%), followed by Mancozeb 75% WP (14.57 %), Azoxystrobin + Difenoconazole (14.95%), Zineb 75% WP (15.08 %), Hexaconazole 5% SC (15.57%), Tebuconazole 25.9% EC (16.09%), Propiconazole 25% EC (16.10%), Pyraclostrobin + Epoxiconazole (21.08 %) and Metalaxyl 35% WS (26.79 %) as compared to control (59.37%). Non-systemic fungicide Kresoxim methyl was found to be the best in the inhibition of spore germination at all five different concentrations and systemic fungicide Metalaxyl 35% WS was found to be the least effective in inhibition of uredospore germination at all five different concentrations. The results are presented in Table 2 and germination of spores against different doses of fungicides under a compound microscope is depicted in Fig. 1.

3.1 *In vivo* efficacy of different fungicides for control of polysora rust disease of maize

A field (*In vivo*) experiment on the efficacy of different fungicides for control of polysora rust disease of maize was observed also. Among these, Mancozeb 75% WP (0.2%) was found to significantly be the most effective treatment. The lowest disease score (2.2), lowest PDI (22.20%), were observed as compared to 3.9 disease score, 42.96 % PDI and 1.47 B:C ratio in the control plot. Also, the highest disease control efficacy (48.32 %), highest per cent increase in yield (42.76 %) and highest profitability (2.52 B:C ratio) were observed in Mancozeb treatment. Kresoxim methyl 44.3% SC (0.1%) was found to be the second most effective fungicide where disease score, PDI, PDC, B:C ratio, grain yield, and per cent increase in yield were 2.6, 24.64 %, 42.64%, 2.16, 61.80 q ha^{-1}, and 24.77 % respectively. Among all fungicide treatments, Metalaxyl 35% WS (1 ml l^{-1}) spray was found to be least effective in combating polysora rust disease with the highest disease score (3.6) and PDI (36.31 %), lowest PDC (15.48 %) and a one of the lowest B:C ratio (1.67). The economics are presented in Table 4 and other results are presented in Table 3.

4. Discussion

In this experiment, all the tested fungicides considerably reduced the rate of spore germination compared to the control. In vitro results from this experiment indicated that non-systemic fungicide Kresoxim methyl was found to be the best in inhibition of spore germination at all five different concentrations, followed by Mancozeb 75% WP (14.57%), and systemic fungicide Metalaxyl 35% WS was found to be the least effective in inhibition of uredospore germination at all five different concentrations. Almost similar results were observed while studying the efficacy of eight systemic, four non-systemic, and two combi-product fungicides against uredospore germination of *Puccinia sorghi* (Dey *et al*., 2013). Their data indicated that Tebuconazole (0.1%) showed the least percent spore germination (4.49%), which is statistically on par with Difenoconazole, Propiconazole, Mancozeb, Penconazole, and Azoxystrobilurin.

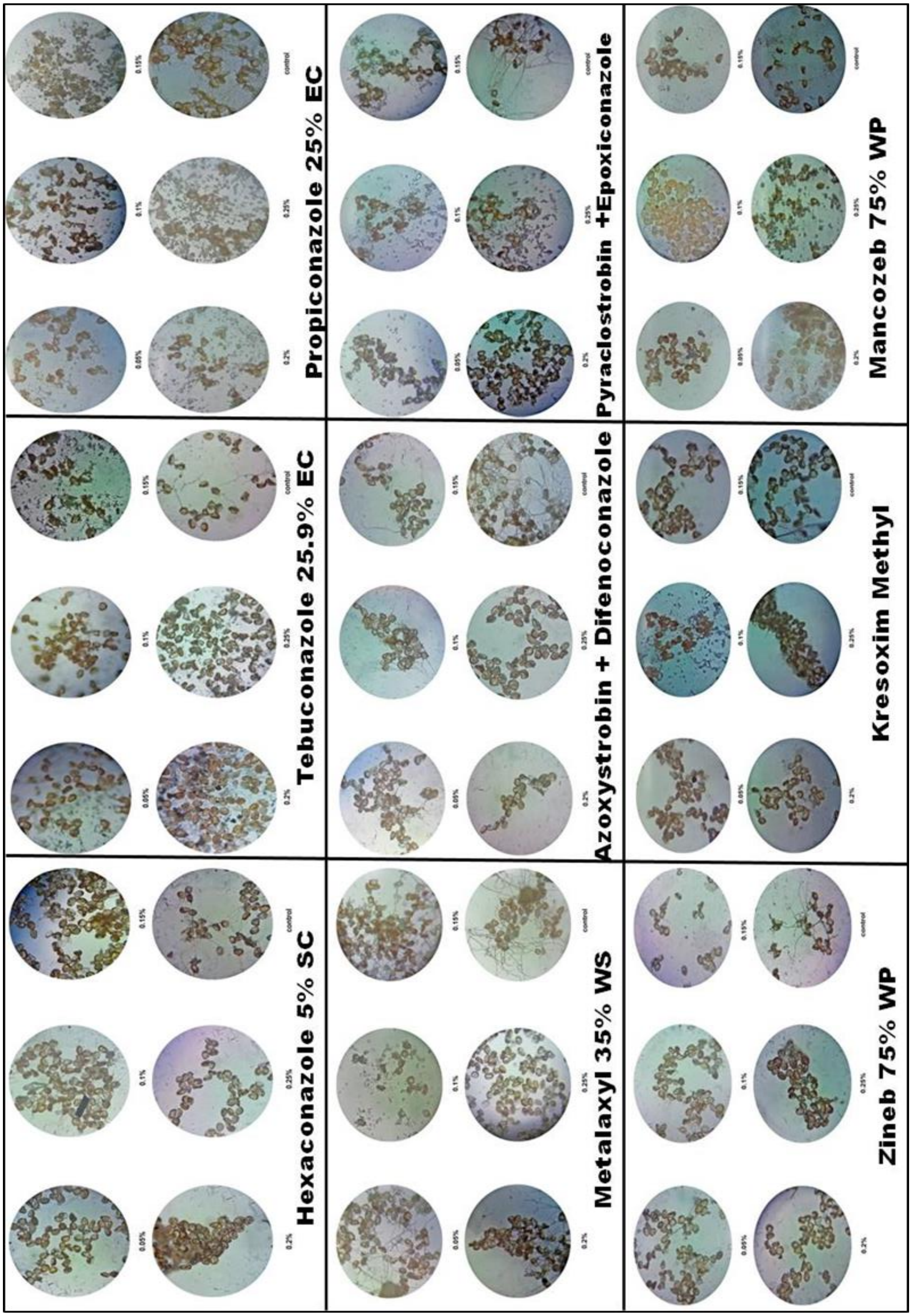

Fig. 1 - *In vitro* evaluation of different fungicides against uredospore germination of *P. polysora*

Table 2 - *In vitro* evaluation of different fungicides against uredospore germination of *P. polysora*

Fungicides	0.05%	0.10%	0.15%	0.20%	0.25%	Mean
Hexaconazole 5% SC	22.10 (14.16)	16.93 (8.51)	13.18 (5.21)	10.88 (3.66)	9.00 (2.67)	20.40 *(15.57)**
Tebuconazole 25.9% EC	29.34 (24.18)	14.48 (6.29)	11.42 (3.96)	8.29 (2.14)	5.05 (0.77)	19.82 (16.09)
Propiconazole 25% EC	27.21 (20.94)	16.67 (8.27)	12.27 (4.54)	9.55 (2.88)	4.42 (0.73)	20.07 (16.10)
Metalaxyl 35% WS	41.84 (44.50)	26.68 (20.26)	24.63 (17.41)	20.78 (12.61)	14.93 (6.72)	29.86 (26.79)
Azoxystrobin 18.2 w/w + Difenoconazole 11.4% w/w SC	20.83 (12.65)	17.60 (9.16)	14.02 (5.89)	8.41 (2.14)	3.78 (0.65)	19.16 (14.95)
Pyraclostrobin 133g/l + Epoxiconazole 50g/l SC	31.27 (27.00)	23.25 (15.61)	19.41 (11.07)	16.43 (8.15)	13.42 (5.41)	25.68 (21.08)
Zineb 75% WP	20.29 (12.05)	16.37 (7.96)	13.71 (5.66)	11.55 (4.01)	7.05 (1.58)	19.88 (15.08)
Kresoxim methyl 44.3% SC	19.73 (11.44)	12.11 (4.45)	8.99 (2.46)	6.04 (1.14)	3.53 (0.43)	16.79 (13.19)
Mancozeb 75% WP	21.01 (12.95)	16.30 (7.99)	10.91 (3.59)	8.02 (1.98)	7.40 (1.68)	19.00 (14.57)
Untreated control	50.32 (59.22)	50.51 (59.56)	50.38 (59.33)	50.47 (59.47)	50.34 (59.27)	50.40 (59.37)
Mean	28.40 (24.08)	21.09 (14.80)	17.90 (11.91)	15.04 (9.82)	11.37 (7.99)	
Comparing the mean of	S.Em (±)				CD @ 0.01	
Treatment (A)	0.40				1.47	
Concentration (B)	0.32				1.04	
A × B	0.96				3.29	

*Arcsine transformed values; **Data in parenthesis are original values

Table 3 - *In vivo* management of polysora rust of maize caused by *P. polysora*

	Fungicide	Disease Score	Percent Disease Incidence (PDI)	Percent disease control (PDC)	Grain Yield (q ha^{-1})	Percent increase in yield
T1	Hexaconazole 5% SC 1 ml l^{-1} spray at 3 days & 18 days after inoculation	2.8	30.95	27.96	60.23	21.60
T2	Tebuconazole 25.9% EC 1 ml l^{-1} spray at 3 days & 18 days after inoculation	3.5	35.46	17.46	50.30	1.55
T3	Propiconazole 25% EC 1 ml l^{-1} spray at 3 days & 18 days after inoculation	3.3	35.24	17.97	50.40	1.75
T4	Metalaxyl 35% WS 1ml l^{-1} spray at 3 days & 18 days after inoculation	3.6	36.31	15.48	52.55	6.08
T5	Azoxystrobin 18.2 w/w + Difenoconazole 11.4% w/w SC @ 0.10% spray at 3 days & 18 days after inoculation	2.9	26.16	39.11	61.21	23.58
T6	Pyraclostrobin 133 g l^{-1} + Epoxiconazole 50 g l^{-1} SC spray at 3 days & 18 days after inoculation.	3.5	35.30	17.83	57.47	16.02
T7	Zineb 75% WP @ 0.20% spray at 3 days & 18 days after inoculation	3.1	32.03	25.44	60.00	21.12
T8	Kresoxim methyl 44.3% SC @ 0.10% Spray at 3 days & 18 days after inoculation	2.5	24.64	42.64	61.80	24.77
T9	Mancozeb 75% WP @ 0.20 spray at 3 days & 18 days after inoculation	2.2	22.20	48.32	70.71	42.76
T10	Untreated control (water spray)	3.9	42.96	-	47.20	-
	SE.m (±)	0.26	2.52	-	3.39	-
	C.D. @ 0.05	0.77	7.48	-	10.08	-
	C.V. %	14.42	13.57	-	10.24	-

Table 4- Effect of different fungicides on the economics of maize

Treatments	Cost of Cultivation (×10⁴ ₹/ha)	Gross returns (×10⁴ ₹/ha)	Net returns (×10⁴ ₹/ha)	Net B:C
Hexaconazole 5% SC (T1)	4.63	14.64	10.01	2.16
Tebuconazole 25.9% EC (T2)	4.68	14.16	9.48	2.03
Propiconazole 25% EC (T3)	4.65	14.39	9.74	2.09
Metalaxyl 35% WS (T4)	4.68	12.51	7.82	1.67
Azoxystrobin 18.2 w/w + Difenoconazole (T5)	5.16	14.20	9.05	1.75
Pyraclostrobin 133g l^{-1} + Epoxiconazole 50 g l^{-1} (T6)	4.93	12.32	7.39	1.50
Zineb 75% WP (T7)	4.85	12.22	7.37	1.52
Kresoxim methyl 44.3% SC (T8)	4.98	12.66	7.68	1.54
Mancozeb 75% WP (T9)	4.69	16.52	11.83	2.52
Untreated Control (T10)	4.53	11.18	6.64	1.47
SE.m (±)	-	1.00	1.00	0.15
C.D. @ 0.05	-	1.85	1.84	0.38
C.V. %	-	7.98	12.36	12.18

Another experiment on uredospore germination of *Puccinia recondita* f. sp. *tritici* against different fungicides revealed that maximum inhibition of uredospore germination was observed in mancozeb (54.85%), followed by chlorothalonil (40.89%) (Chaudhary *et al*., 2015).

In vivo results indicated that Mancozeb 75% WP (2 ml l^{-1}) was found to be significantly the most effective in controlling polysora rust disease, and Metalaxyl 35% WS (1 ml l^{-1}) spray was found to be the least effective. Similar findings were noticed that the fungicides Bavistin (Carbendazim), Bayleton (Triadimefon), and Dithane M-45 (Mancozeb) are most effective against maize rust (Kumar and Jaglan, 1989). Also, several kinds of protectant chemicals - e.g., HOE 2873, HOE 6052, HOE 6053, sulfur, and zineb—have been used on experimental bases to control *P. polysora* infection (Ellis, 1954; HH, 1958; Onofeghara and Kapooria, 1975). All delayed rust development and substantially increased grain yields under conditions favorable for rust development. Another experiment that results in an almost similar finding is that fungicide applications provided the greatest increase in yield when disease severity levels of *Puccinia sorghi* were high (Wegulo *et al*., 1998). Results levels indicated that when conditions are favourable for the development of common rust (cool, wet weather), foliar applications of chlorothalonil, mancozeb, or propiconazole minimize yield losses due to foliar diseases in hybrid corn seed production.

Our historical research records failed to yield any evidence of fungicides showing inhibitory effects on the Manipur isolates. In our study, we utilized both systemic and non-systemic fungicides to combat the germination of uredospores. Interestingly, the non-systemic fungicides proved to be more effective in inhibiting spore germination compared to their systemic counterparts. However, it is essential to emphasize that the results of these fungicides' application showed variability when tested against different pathogens. This underscores the importance of considering the specific pathogen when selecting and applying fungicides for disease control.

5. Conclusion

The better results on protective and curative actions of test fungicides and their efficacy are one of the essential steps to optimizing and developing a suitable management strategy for the control of the disease and maximizing the yield of the crop. In an *in vitro* experiment, it was observed that the non-systemic fungicide Kresoxim methyl was found to be the best in inhibiting spore germination at all five different concentrations, followed by Mancozeb 75% WP (14.57%), and the systemic fungicide Metalaxyl 35% WS was found to be the least effective in inhibiting uredospore germination at all five different concentrations. *In vivo* results indicated that Mancozeb 75% WP (0.2%) was found to be significantly the most effective in controlling polysora rust disease, and Metalaxyl 35% WS (1 ml l^{-1}) spray was found to be the least effective. *In vivo* results were also consistent with the benefit-cost ratio also. The test fungicides also have curative effects even after the initiation of the disease and the occurrence of the symptoms. This experiment can be further exploited for multi-location trials to obtain the best results, and the best fungicides can be suggested to the end users to have higher yields.

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