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Emergence and Management of Southern Rust (*Puccinia polysora*) in Maize: An Epidemiological and Fungicide Efficacy Study in Manipur, India

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Maize is a major cereal crop in the state of Manipur, India, grown across all its districts. Southern rust, also known as polysora, is a sporadic and troublesome foliar disease of maize caused by the fungus *Puccinia polysora* Underw. Though the disease was not prevalent in Manipur before, it is currently emerging due to climatic alterations and posing fresh challenges in production. To analyze and manage the disease's impacts on crop loss, both *in vitro* and *in vivo* studies were carried out. Our epidemiological studies found changing correlations between meteorological factors and disease severity/progression in 2021-2022, with temperature and precipitation displaying varying relationships; interactions between variables proved more consistent than individual ones, emphasizing disease dynamics complexity. Among all the nine fungicides (six systemic and three non-systemic) tested in vitro, significantly lowest LC 50/LD 50 value was observed in Mancozeb 75% WP (LD50 = 0.005), followed by Hexaconazole 5% SC (LC50 = 0.006) and Zineb 75% WP (LD50 = 0.008). The least effective fungicide was Tebuconazole 25.9% EC (LC50 = 0.021). The results of fungicide treatment in vivo showed that *Mancozeb* 75% WP at 0.20% was most effective where lowest PDI (22.03%), maximum yield (66.22 q ha-1) and maximum profit (B:C ratio 2.58) were observed as compared to the control plot. Whereas, Tebuconazole 25.9% EC at 1 ml l^{-1} spray was found to be the least effective, showing highest PDI (34.04%) with lowest grain yield (50.8 q ha-1) and comparatively less amount of profit (B:C ratio 1.75).

Keywords : Polysora rust, uredospore, germination percentage, fungicides, management

INTRODUCTION

Maize is a vital cereal crop grown in the hill and valley districts of Manipur. According to the 2019–20 statistical data, the state recorded a maize cultivation area of 2.22 thousand hectares, an average yield of 2228.35 kg per hectare, and a total production of 4.94 thousand metric tons (ESM, 2020). Polysora rust, also known as southern rust, is an occasional but significant disease of maize caused by *Puccinia polysora* Underw. This disease is restricted to tropical and subtropical regions worldwide due to the pathogen's temperature sensitivity.

In India, it has been reported in the states of Andhra Pradesh, Karnataka, and Tamil Nadu. High incidences of this disease have also been observed in Manipur during the late *Kharif* season, leading to substantial yield reductions. Among the three rusts that occur worldwide, polysora rust of maize has been reported as the most troublesome among all, where 42 to 53% yield losses were observed in northern china (Chen *et al*. 2004). A major epidemic of southern maize rust has been reported to have caused a yield loss of up to 45% in Florida (Jackson-Ziems, 2014). 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. Breeding and release of resistant hybrids is a time taking process and therefore, only option of managing this emerging disease by integrating available disease management methods, like the use of available cultural practices and fungicidal sprays

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in the short term for ensuring potential yield of maize in north- eastern India. Several fungicides, *viz*., Bavistin, Dithane M-45, Benlate, and Kitazin, were proven to be effective against *P. sorghi*, causing common rust in maize.. 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(s) against the disease, the current study was lined up to test various chemical fungicides to develop an effective control strategy against this 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.

MATERIALS AND METHODS

In vitro examinations were carried out in the laboratory of Plant Pathology, College of Agriculture, CAU, Imphal, Manipur, India. *In vivo* experiments were conducted at Andro Research Farm (CAU), Imphal East, Manipur. In the context of the *in vivo* experiment, the study was carried out over two successive years, specifically in the *kharif* season of 2021 and 2022.

Collection of spores and preparation of inoculum

Naturally infected samples showing the typical characteristics of polysora rust were collected during November 2020 at regular intervals. A standard protocol was followed for the collection and storage of the uredospores (AICMIP, 1983). The spores were collected from infected leaf areas using a vacuum pump and subsequently stored for the next season in sealed glass vials under vacuum at a low temperature of -20°C. 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 carried out in the latter half of the day using a uredospore suspension at a concentration of 1×10^6 spores/ml, targeting plants at the 6–8 leaf stage.

Epidemiological studies

The effect of meteorological factors such as minimum and maximum temperature (^{0}C), maximum and minimum wind speed, precipitation (mm), and relative humidity percentage (RH %) on initiation and subsequent development of disease incited by *Puccinia polysora* under field conditions were recorded. Disease data were recorded at fortnight intervals and disease severity (PDI) and disease progression were calculated, starting 1st week of June 2021 to fourth week of September 2021 and again from 1st week of June 2022 to 4th week of September 2022. The PDI was correlated with meteorological factors collected from NASA Power single point data access. The effect of "weather parameters" on polysora rust of maize was correlated with the help of Statistical Package for Social Sciences (SPSS) version 23.

In vitro evaluation of fungicides against P. polysora uredospore

Eight fungicides, consisting of two non-systemic fungicides, namely Mancozeb 75% WP, and Zineb 75% WP, and six systemic fungicides, namely Hexaconazole 5% SC, Kresoxim methyl 44.3% SC, Tebuconazole 25.9% EC, Propiconazole 25% EC, Azoxystrobin 18.2 % + Difenoconazole 11.4 % SC, and Pyraclostrobin 13.3% + Epoxyconazole 5%, were evaluated against the germination of *P. polysora* uredospores in the laboratory (CIB & RC, 2020). Five different concentrations, *viz*., 0.005, 0.01, 0.015, 0.02, and 0.025%, were tried for each fungicide. A tenfold dilution of the recommended dose for each fungicide was used. 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). Following that, the germination percentage of the uredospore was noted after 24 hours. Subsequently, the LD50/LC50 value was calculated based on the degree of germination inhibition. Each concentration was replicated three times and for each treatment hundred spores were observed.

In vivo evaluation of different fungicides for control of polysora rust disease of maize

All eight fungicides tested for the *in vitro* study were also tested in the *in vivo* experiment. 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 crushed between the palms and soaked in a bucket of water until the water turned brown. The resulting uredospores were stored in a freezer at a temperature of 5-7°C and later transported to the research field for inoculation. A knapsack sprayer was used to inoculate the plants in the field. The spore suspension was applied during the latter part of the day when the sunlight was less intense. During spraying, the nozzle of the sprayer was moved over the plant's whorl, ensuring all the leaves were evenly covered. The spore suspension was continuously stirred throughout the spraying process to prevent the lightweight spores from clustering on the water's surface. Inoculation was repeated twice to get the best result following the standard procedure (Hooda *et al*. 2018).

For each fungicide, two sprays were given: the first one two days after inoculation of the pathogen and the second one eight days after inoculation. Plots without any fungicide and only water-sprayed were used as control plots. The experiment was conducted twice in 2021 and 2022 by following the above procedure. Several parameters of disease scoring, *viz*., disease incidence (PDI), per cent disease control (PDC), grain yield (q/ha), and per cent increase in yield, were taken during this experiment. Disease scoring data was calculated as per the recommended scale (Lübberstedt *et al*. 1998). Per cent disease incidence (PDI) was calculated using the standard protocol (McKinney, 1923).

Statistical analysis

A two-factorial randomized block design (2FRBD) with three replications was employed to assess the impact of various fungicides on uredospore germination under *in vitro* conditions. For the *in vivo* study, a randomized block design (RBD) was used, with each plot measuring 3.6 x 2 m^2. Six rows of 2 m each were made in each plot with spacing of 60 x 20 cm^2. Three replications were kept for each treatment. Rank analysis was conducted to determine the most effective treatment.

Economics

For the purpose of calculating the experiment's economics, all inputs and outputs were considered. The average Net Benefit-cost ratio from 2021 and 2022 was calculated.

RESULTS AND DISCUSSION

Correlation analysis of weather parameters with polysora rust disease severity and progress rate

The daily weather data of Andro Research Farm (CAU), Imphal East, Manipur collected from NASA power database through the latitude ($24^0$07' N) and longitude ($94^0$05' E) of that location.

In the study it was observed that the temperature difference (Tmax-Tmin) exhibited a negative correlation with disease severity in the year 2021(Table 1). However, in the subsequent year of 2022, this correlation reversed, manifesting as a positive correlation with disease severity. Conversely, precipitation (mm) displayed a consistent negative correlation with disease severity across both 2021 and 2022. Furthermore, an examination of the interaction between precipitation and temperature difference revealed a consistent negative correlation with disease severity in both years under study. Regarding Tmax, a negative correlation was identified with disease severity in 2021, whereas a positive correlation emerged in 2022. Additionally, the correlation with precipitation and Tmax together remained consistent across both years, demonstrating a negative association with disease severity.

Regarding disease progress rate, variables including Tmax. Tmin, temperature difference (Tmax-Tmin), Daily temperature, precipitation (mm), Precipitation*Tmax, Precipitation* Temp difference and Tmax*RH were found to be positively correlated with disease progress rate in both years. Moreover, RH (%) consistently displayed a negative correlation with disease progress across both years. Variability was observed in Max. wind speed and Min. wind speed. Specifically, in 2021, a negative correlation was noted, whereas in 2022, a positive correlation was observed with the disease progress rate.

Overall, this study found that interactions between weather factors were more consistent predictors of maize disease severity and progression than individual variables, highlighting the complexity of disease epidemiology and the importance of multiple weather factors in disease establishment.

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

The efficacy of six systemic and two non-systemic fungicides was evaluated against uredospore germination of *P. polysora*. Among all the eight fungicides, the significantly lowest LC 50/LD 50 value was observed in Mancozeb 75% WP (LD 50=0.005), followed by Hexaconazole 5% SC (LC 50 = 0.006), Zineb 75% WP (LD 50=0.008), Kresoxim methyl 44.3% SC (LC 50 = 0.011), Azoxystrobin 18.2 % w/w + Difenoconazole 11.4% w/w SC (LC 50 = 0.011), Pyraclostrobin 133g/l + Epoxiconazole 50g/l SC (LC 50 = 0.014), Propiconazole 25% EC (LC 50 = 0.019) and Tebuconazole 25.9% EC (LC 50 = 0.021). Non-systemic fungicide Mancozeb 75% WP was found to be the best in the inhibition of spore germination at all five different concentrations and systemic fungicide Tebuconazole 25.9% EC 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 probit analysis of different fungicides is illustrated in Fig.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 the control of polysora rust disease of maize was observed also. *In vivo* results were found slightly different from in vitro results which may be attributed to interactions between the host and fungicide, or potentially influenced by environmental factors. Here, Mancozeb 75% WP (2 g l^{-1}) was found to significantly be the most effective treatment. The lowest disease score (2.2), lowest PDI (22.03%) were observed as compared to 3.9 disease score, 42.95 % PDI in the control plot. Also, the highest disease control efficacy (48.68%), highest per cent increase in yield (44.32%), highest profitability (2.58 B:C ratio) and maximum yield (66.22 q ha^{-1}) were observed in Mancozeb treatment (T-9). Zineb 75% WP (2g l^{-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 2.5, 24.85 %, 42.82%, 1.96, 62.17 q ha-1, and 31.24 % respectively. Among all fungicide treatments, Tebuconazole 25.9% EC (1ml l^{-1}) spray was found to be least effective in combating polysora rust disease with the highest disease score (3.4) and PDI (34.04 %), lowest PDC (20.75 %) and a one of the lower B:C ratio (1.75). Effect of fungicides on disease incidence, yield etc. are presented in Table 3 and the economics are presented in Table 4.

Epidemiological study highlighted shifting correlations between meteorological variables and disease severity and progress rate across 2021 and 2022. While temperature difference and Tmax displayed reversals in correlation with disease severity, precipitation consistently correlated negatively. Interactions between variables were more consistent than individual variables, emphasizing the complexity of disease dynamics. Additionally, variables including Tmax, Tmin, mean daily temperature, precipitation, and interactions were positively correlated with disease progress rate, while RH (%) consistently correlated negatively. The shift from negative to less negative correlation between weather variables and disease severity from 2021 to 2022 in Manipur can be explained by several factors. Variations in temperature, humidity, and other weather events in the region's hilly topography, along with notable climatic changes over the two years, likely affected disease patterns differently. Additionally, changes in disease outbreak cycles could have influenced the impact of weather on disease progression, explaining the sporadic nature of the disease. Similarly, in Brazil, the epidemic rate of southern corn rust showed a positive correlation with the daily temperature but a negative correlation with sunshine duration when the relative humidity exceeded 90% (Godoy,

Table 1 : Correlation analysis of weather variables with disease severity and progress rate

Weather Variables	Polysora Rust			
	Disease severity (PDI)		Disease progress rate	
	2021	2022	2021	2022
Tmax	-0.617	0.319	0.221	0.351
Tmin	-0.468	0.113	0.643	0.590
Temp. Difference	-0.561	0.433	0.055	0.025
Mean Daily Temp.	-0.504	0.209	0.376	0.455
Max. Wind Speed	-0.781*	0.146	-0.206	0.119
Min. Wind Speed	-0.588	0.247	-0.216	0.156
RH (%)	0.500	-0.148	-0.013	-0.075
Precipitation (mm)	-0.226	-0.687	0.541	0.072
Precipitation*Tmax	-0.322	-0.699	0.606	0.092
Precipitation*Temp difference	-0.533	-0.688	0.681	0.073
Tmax* RH	-0.346	0.412	0.548	0.486

2003). The infectivity and germinability of *Puccinia polysora* uredospores were found to be consistently reduced by high storage relative humidity, regardless of storage temperature. Even after only 1 day of storage at temperatures above 24^{0}C, 65 and 95% RH reduced germination and infection more than 15 and 35% RH. In our study, variability was observed in wind speed correlations with disease progress rate between the two years.

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 Mancozeb 75% WP was found to be the best in inhibition of spore germination at all five different concentrations, followed by Hexaconazole 5% SC and Zineb 75% WP, and systemic fungicide Tebuconazole 25.9% EC 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 four non-systemic, eight systemic, and two combi-product fungicides against uredospore germination of *Puccinia sorghi* (Dey *et al.* 2013). Among the non-systemic fungicides, Mancozeb at 0.25% (12.38% germination) was found to be the most effective one followed by Zineb at 0.25% (13.01% germination). 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% inhibition), followed by chlorothalonil (40.89% inhibition) (Chaudhary *et al.* 2015).

In vivo results of 2021 and 2022 indicated that Mancozeb 75% WP (2 ml l-1) was found to be

Table 2: LC50/LD50 Values and Regression Analysis for Various Fungicides

Fungicides	LC 50 / LD 50	Equation	R^2	Upper bound	Lower bound
Hexaconazole 5% SC	0.006	y = 0.875x + 1.925	0.811	0.020	0.000
Tebuconazole 25.9% EC	0.021	y = 1.5625x + 2.6875	0.981	0.034	0.007
Propiconazole 25% EC	0.019	y = 1.5x + 2.7	0.956	0.033	0.006
Azoxystrobin 18.2 w/w + Difenoconazole 11.4% w/w SC	0.011	y = 1.25x + 2.55	0.793	0.025	0.001
Pyraclostrobin 133g/l + Epoxiconazole 50g/l SC	0.014	y = 1x + 1.8	0.992	0.032	0.001
Zineb 75% WP	0.008	y = 0.875x + 2.025	0.869	0.019	0.000
Kresoxim methyl 44.3% SC	0.011	y = 1.5x + 2.9	0.962	0.024	0.001
Mancozeb 75% WP	0.005	y = 0.875x + 2.025	0.967	0.022	0.000

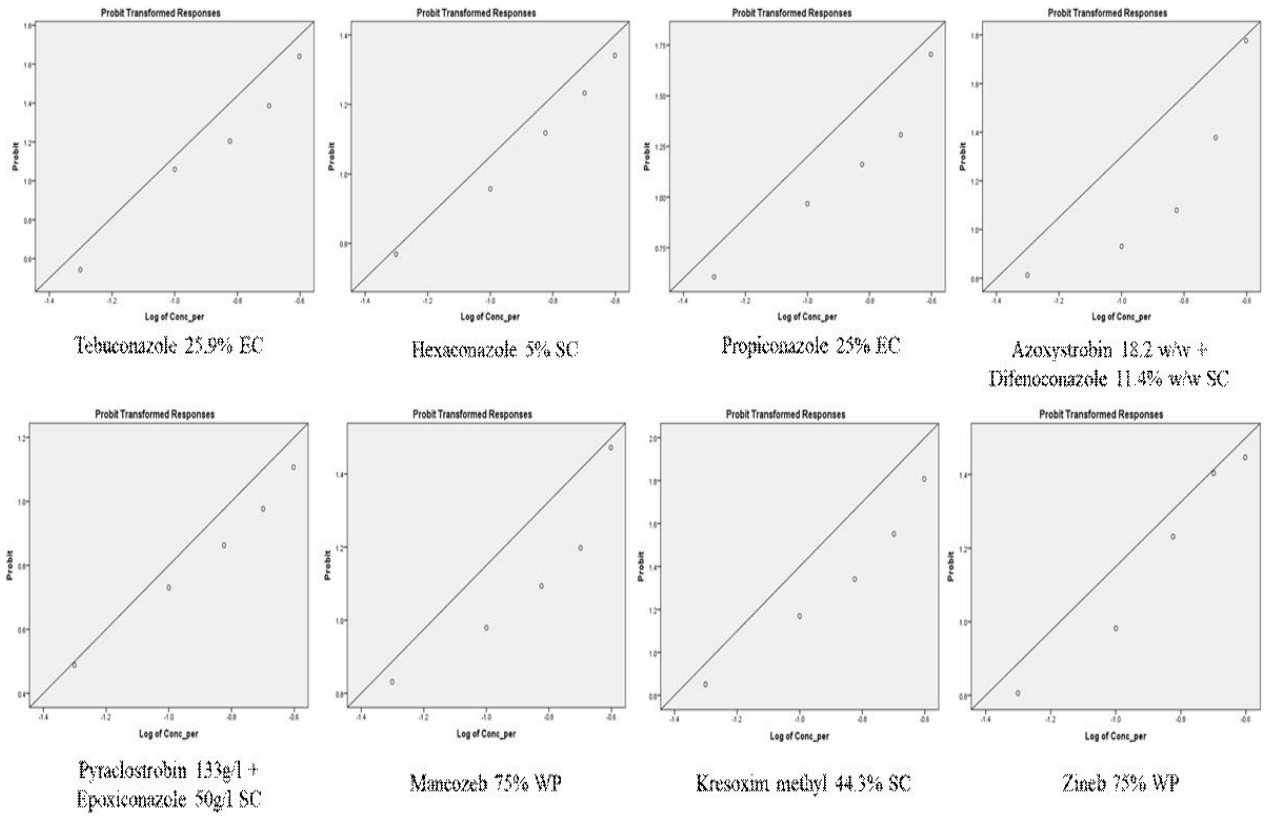

Fig. 1. Probit analysis of different fungicides against *P. polysora*

Table 3. Effect of different fungicides on disease incidence, disease control, and grain yield in maize (2021-2022)

Treatments	Disease Score			Percent Disease Incidence (PDI)			Percent disease control (PDC)			Grain Yield (q ha^{-1})			Percent increase in yield		
	2021	2022	Pooled Mean	2021	2022	Pooled Mean	2021	2022	Pooled Mean	2021	2022	Pooled Mean	2021	2022	Pooled Mean
Hexaconazole 5% SC @ 0.10%	2.5	2.8	2.7^{de}	28.97	30.95	29.96^{bc}	32.51	27.96	30.23	61.80	60.23	61.02^{abc}	33.77	32.19	32.98
Tebuconazole 25.9% EC @ 0.10%	3.3	3.5	3.4^{abc}	32.83	35.24	34.04^{b}	23.53	17.97	20.75	51.20	50.40	50.80^{bcd}	10.82	10.61	10.71
Propiconazole 25% EC @ 0.10%	2.9	3.3	3.1^{bcd}	30.03	33.46	31.75^{b}	25.39	19.46	22.43	55.07	54.1	54.58^{abcd}	19.19	18.73	18.96
Azoxystrobin 18.2 w/w + Difenoconazole 11.4% w/w SC @ 0.10%	2.8	2.9	2.9^{cde}	23.63	26.16	24.90^{cd}	44.96	39.11	42.04	63.57	61.21	62.39^{ab}	37.59	34.32	35.96
Pyraclostrobin 133g/l + Epoxiconazole 50g/l SC @ 0.15%	3.2	3.4	3.3^{abc}	32.20	34.80	33.50^{b}	23.70	18.83	21.27	53.33	50.30	51.82^{bcd}	15.44	10.39	12.91
Zineb 75% WP @ 0.20%	2.5	2.5	2.5^{de}	24.38	25.33	24.85^{cd}	43.00	42.64	42.82	62.53	61.80	62.17^{ab}	30.74	31.75	31.24
Kresoxim methyl 44.3% SC @ 0.10%	2.8	3.1	3.0^{bcd}	31.13	32.03	31.58^{bc}	27.49	25.44	26.46	60.4	60.03	60.22^{abc}	35.35	35.63	35.49
Mancozeb 75% WP @ 0.20%	2.2	2.2	2.2^{e}	21.87	22.20	22.03^{d}	49.05	48.32	48.68	66.37	66.07	66.22^{a}	43.65	44.99	44.32
Untreated control (water spray)	3.8	3.9	3.9^{a}	42.93	42.96	42.95^{a}	-	-	-	46.20	45.57	45.88^{d}	-	-	-
SE.m (±)	0.22	0.20	0.14	2.31	2.14	1.57	-	-	-	3.82	4.04	2.78	-	-	-
C.D. @ 0.05	0.66	0.60	0.42	6.87	6.35	4.52	-	-	-	11.34	12.02	7.98	-	-	-
C.V. %	12.79	10.86	11.68	12.98	11.49	12.23	-	-	-	11.57	12.54	12.06	-	-	-

Table 4. Benefit-Cost analysis of various fungicides for controlling polysora rust disease in maize

Treatments	Cost of Cultivation (INR/ha)	Gross returns (INR/ha)	Net returns (INR/ha)	Net B:C
Hexaconazole 5% SC (T1)	46305	147718.9^{abc}	101413.9^{b}	2.19^{ab}
Tebuconazole 25.9% EC (T2)	46809	128712.7^{bcde}	81903.7^{bcd}	1.75^{bcd}
Propiconazole 25% EC (T3)	46524	126818.8^{cde}	80294.8^{bcd}	1.73^{cd}
Azoxystrobin 18.2 w/w + Difenoconazole (T5)	51563	148905.9^{ab}	97342.9^{bc}	1.89^{bcd}
Pyraclostrobin 133g l^{-1} + Epoxiconazole 50g l^{-1} (T6)	49314	133526.7^{bcde}	84212.7^{bcd}	1.71^{cd}
Zineb 75% WP (T7)	48499	143534.1^{bcd}	95035.1^{bc}	2.04^{bc}
Kresoxim methyl 44.3% SC (T8)	49839	147529.3^{abc}	97690.3^{bc}	1.96^{bcd}
Mancozeb 75% WP (T9)	46929	167866.5^{a}	120937.5^{a}	2.58^{a}
Untreated Control (T10)	45339	116244.5^{e}	70905.5^{d}	1.56^{d}
C.V. %	-	8.03	12.26	12.31

significantly the most effective in controlling polysora rust disease, and Tebuconazole 25.9% EC (1 ml l-1) spray was found to be the least effective. Similar findings were noticed that the fungicides Carbendazim, Triadimefon, and Mancozeb are most effective against maize rust. . Several protectant chemicals, such as HOE 6052, HOE 2873, HOE 6053, sulfur, and zineb, have been tested experimentally for controlling *P. polysora* infection.. These chemicals effectively delayed rust progression and significantly boosted grain yields under conditions conducive to rust development. Similarly, another study found that fungicide applications led to the highest yield gains when *Puccinia sorghi* disease severity was high.. The findings suggested that under favorable conditions for common rust development, such as cool and wet weather, foliar sprays of mancozeb, chlorothalonil, or propiconazole effectively reduce yield losses caused by foliar diseases in hybrid maize 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 systemic fungicides. The more direct surface action of non-systemic fungicides may have provided quicker and more effective inhibition of spore germination. 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.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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