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Efficacy of fungicides and bioproducts against Turcicum leaf blight disease of maize

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Abstract
An *in vivo* experiment was studied to check the efficacy of two fungicides and five bioproducts against Turcicum leaf blight disease in the HQPM-1 cultivar under field conditions during Kharif 2018, 2019, and 2020. The pooled results revealed significant differences among treatments with respect to disease severity, disease control percentage, Grain yield and increase in yield percentage. The seed treatment and foliar application @ 35 and 45 DAS with Azoxystrobin 18.2 w/w + Difenoconozole 11.4% w/w SC @ 0.1% recorded the lowest pooled PDI (32.00), highest disease control (51.18%), and maximum grain yield (54.85 q/ha) with a 30.89% yield increase over the unprotected check. Mancozeb 75 WP at 0.25% also proved effective, with a pooled PDI of 39.44 and a 26.77% yield increase. Among botanicals, *Allium sativum* (Garlic) @ 10% was superior to *Azadirachta indica* (Neem). The results indicate that integrated use of systemic fungicide and seed treatment is highly effective in managing TLB and enhancing maize productivity.

Keywords: Maize, turcicum leaf blight, *Exserohilum turcicum*, fungicides, bioproducts, disease management

Introduction
Maize (Zea mays L.) is considered the third-most-important crop after wheat and rice in India. It is known as the 'Queen of cereal' because of its wide application in a variety of products. It can be grown in various agro-climatic conditions in many parts of India. In India, it occupies the 5th rank in area and 3rd in production and productivity among cereal crops. It is cultivated in an area of 10.04 million ha, with a total annual production of 33.62 million tonnes, giving an average yield of 3.19 tonnes/ha (Agristat, 2022) [22].

The growing of maize is totally rainfed in the North-east region of India for its food security. It is used for direct consumption, and used as feed, fodder for livestock and for industrial purposes also. In the NER region, the production and production is very low as compared to other states of India; it needs expansion in area and an increase in production, as well as adoption of new hybrids which is resistant or tolerant to pests and diseases. Diseases are one of the major constraints for the reduction in yield. Amongst the diseases reported, such as Turcicum Leaf blight, Maydis Leaf blight, Banded Leaf and Sheath Blight, Common rust, Turcicum Leaf Blight of Maize, which is also known as Northern corn leaf blight caused by *Exserohilum turcicum*, is one of the major diseases of maize that affects the production and productivity of maize (Nongmaithem *et al*.). If it is infected by this disease, it can reduce the yield up to 90% under conducive environmental conditions. Therefore, a study was taken up for three consecutive years, 2018, 2019, and 2020, to evaluate the efficacy of Fungicides and Bioproducts against Turcicum Leaf Blight disease of Maize.

Materials and Methods
Isolation and purification of the pathogen
For isolation, infected leaf samples were collected from the TLB-infected Maize field showing spindle-shaped lesions, broadened at the centre and pointed at the tips; the lesions were straw-coloured. The infected lesions were brought to the laboratory. Wash in running tap water, then dry under the fan. Cut into small pieces that contain both healthy and infected-looking tissue. Surface sterilized with 1% sodium hypochlorite and washed in three

changes of sterile distilled water, and placed in sterile filter paper to absorb excess water. After that, it was placed in sterile Petri plates that contained 20 ml of solidified PDA agar medium. And kept in BOD at 28±1°C for 3 to 5 days. After 5 days, a bit of mycelium is taken from the periphery of and transferred to fresh PDA medium to get the pure culture. After getting the pure it was transferred to PDA slants and kept on BOD at 28±1 °C °C for full growth of the pathogen. After obtaining the full growth, it was maintained and kept it on a refrigerator at 4 °C for further study.

Mass Multiplication of Pathogens

The pathogen, i.e., *Exserohilum turcicum*, is mass multiplied using the sorghum grain culture method. The sorghum grains, weighing 200 g, were washed under tap water after removing all stubble and dust, then soaked in sterile water in a 500 ml conical flask for 6 hours. After soaking, the excess water was removed from the impregnated sorghum grains, which were then autoclaved at 120 °C for 20 minutes. The sterilized sorghum grains were then inoculated with a fresh, 12-day-old culture of *Exserohilum turcicum*. Following inoculation, these grains were kept in a BOD chamber at 25±1°C for 20 days, with intermittent shaking every other day to prevent clump formation at the bottom of the flask. When full pathogen growth and heavy sporulation were observed after 20 days, the sorghum grains were removed from the conical flask using a spatula and placed in a tray. The spore suspension was prepared by mixing the spores with sterile distilled water to maintain a concentration of $1 x 10^5$ spores/ml. It was then artificially inoculated by uniformly spraying onto maize plants at the three- to four-leaf stage, twice at 30 and 40 days after sowing (DAS), using a hand-held atomizer during the evening hours to facilitate infection. Immediately after inoculation, a fine water mist was applied to maintain adequate humidity and promote pathogen establishment.

Disease Assessment

Seven days after artificial inoculation, the disease symptoms start appearing as small, round, oval, elliptical or spindle-shaped, and they start appearing from the lower leaves and progress upward. The PDI was recorded at the dough stage of the maize plant with the help of a 1-9 disease rating scale developed by Chung *et al*. (2010) [5]; Mitiku *et al*. (2014) [13]. The disease severity was recorded twice at 10-day intervals, once at the dough stage and other at 10 days after the dough stage. According to the disease rating scale, it was categorised as resistant, moderately resistant, susceptible, moderately susceptible and susceptible. The parameters recorded were PDI, disease control%, grain yield and increase in grain yield%.

Efficacy of Fungicides and Bioproducts Against Turcicum Leaf Blight Disease of Maize

The field experiment was conducted at the Andro Research Farm of the College of Agriculture, Iroisemba, CAU, Imphal, Manipur (24.763793° N, 94.052882° E; 755.02 ± 2.5 m above mean sea level) over three consecutive *kharif* seasons (2018-2020). The experiment was laid out in randomized block design with three replications. There are 9 treatments, including two chemical fungicides and 7 bioproducts, as given in Table 1.

Table1: Different treatments were used in the experiments at 35 and 45 DAS

Sl. No.	Treatment
1.	Foliar application of Mancozeb 75 WP @ 0.25% @ 35 and 45 after sowing
2.	Seed treatment and foliar application of Azoxystrobin 18.2% + Difenoconazole 11.4% @ 0.10% @ 35 and 45 after sowing
3.	Foliar application of Azadirachta indica @ 10% @ 35 and 45 after sowing
4.	Allium sativum @ 10% @ 35 and 45 after sowing
5.	Lantana camara @ 10% @ 35 and 45 after sowing
6.	Cow urine @ 20% @ 35 and 45 after sowing
7.	Trichoderma asperillium @ 1% @ 35 and 45 after sowing
8.	Unprotected inorganic check (only application of NPK @120:60:60)
9.	Unprotected organic check (only application of FYM @10 tonnes/ ha)

Before sowing, the experimental plot was applied with the recommended dose of NPK fertilizers, i.e 120:60:60, where Nitrogen is applied in split doses. One-third of the nitrogen is applied as a basal dose at the time of soil preparation; the rest is applied as top dressing when the plants are at knee knee-high stage and at the flowering stage. The maize variety HQPM 1, which is susceptible to TLB disease of maize, were selected for study and sown on June 23, for the years 2018, 2019 and 2020 with a plot size of $3.6m^2$ with spacing of 60 cm x 20cm. The disease was inoculated when the plants were 30 days old, and the fungicides and bioproducts were sprayed after 3 days of inoculation by preparing aqueous extracts at 10% concentration, following the method of Mohana and Raveesha (2007) [23].

Table 2: Disease scoring according to Chung *et al*. (2010) [5]; Mitiku *et al*. (2014) [13]

Rating scale	Degree of infection (% DLA*)	PDI**	Disease Reaction
1.0	Nil to very slight infection (≤10%).	≤ 11.11	Resistant (Score: ≤ 3.0) (PDI: ≤ 33.33)
2.0	Slight infection, a few lesions scattered on two lower leaves (10.1-20%).	22.22	
3.0	Light infection, moderate number of lesions on four lower leaves (20.1-30%).	33.33	
4.0	Light infection, moderate number of lesions scattered on lower leaves, a few lesions scattered on middle leaves below the cob (30.1-40%).	44.44	Moderately Resistant (Score: 3.1-5.0) (PDI: 33.34-
5.0	Moderate infection, an abundant number of lesions scattered on lower leaves, a moderate number of lesions scattered on middle leaves below the cob (40.1-50%).	55.55	

			55.55)
6.0	Heavy infection, an abundant number of lesions scattered on lower leaves, moderate infection on middle leaves, and a few lesions on two leaves above the cob (50.1-60%).	66.66	Moderately susceptible (Score: 5.1-7.0) (PDI: 55.56-77.77)
7.0	Heavy infection, an abundant number of lesions scattered on lower and middle leaves, and a moderate number of lesions on two to four leaves above the cob (60.1-70%).	77.77	
8.0	Very heavy infection, lesions are abundantly scattered on the lower and middle leaves and spreading up to the flag leaf (70.1-80%).	88.88	Susceptible (Score: > 7.0) (PDI: > 77.77)
9.0	Very heavy infection, lesions abundantly scattered on almost all leaves, plants prematurely dried or killed (>80%).	99.99	

*DLA- Diseased leaf area; **Percent disease index (PDI)

Results and Discussion

Efficacy of Fungicides and Bioproducts on Turcicum Leaf Blight Incidence

The result of the field evaluation of the two best chemical fungicides and four bioproducts shows a significant difference among the treatments tested for their efficacy in reducing the PDI, increasing the grain yield, as shown in Table 3. The pooled data over three years (2018, 2019 and 2020) indicated that all treatments considerably reduced TLB severity compared with the unprotected checks.

Seed treatment of Azoxystrobin 18.2 w/w + Difenoconazole 11.4% w/w SC at 1g/kg of seed and foliar application at 35 and 45 days after sowing at 1ml/litre of water recorded the lowest pooled PDI (32.00), highest disease control (51.18%). This treatment also produced the maximum grain yield (54.85 q/ ha) with a 30.89% yield increase over the unprotected organic check. The dual mode of action of Azoxystrobin (a QoI fungicide) disrupts mitochondrial respiration by inhibiting electron transfer, whereas Difenoconazole (a DMI fungicide) interferes with ergosterol biosynthesis in fungal cell membranes, resulting in both preventive and curative protection against *Exserohilum turcicum*.

The next most effective treatment was Mancozeb 75 WP @ 0.25%, which achieved a pooled PDI of 39.44, disease control of 39.45%, and a grain yield of 53.10 q ha^{-1}, representing a 26.77% yield increase over the check. Mancozeb, being a multi-site contact fungicide, provides broad-spectrum protection by inhibiting multiple enzymatic activities in fungal spores, and its prophylactic sprays effectively suppressed the spread of TLB lesions under favourable weather conditions.

Performance of Botanicals and Bio-Products

Among the botanicals tested, *Allium sativum* (garlic extract @ 10%) showed better disease suppression (PDI = 49.45) and higher yield (52.31 q ha^{-1}) than *Azadirachta indica* (neem extract @ 10%), which recorded a PDI of 51.92 and yield of 51.34 q ha^{-1}. The antifungal properties of garlic are attributed to allicin and related organosulfur compounds that inhibit spore germination and mycelial growth of *E. turcicum*, while neem extract exerts a mild fungistatic effect through azadirachtin and nimbin.

Lantana camara @ 10% and cow urine @ 20% were comparatively less effective, with pooled disease control values of 10.09% and 16.81%, respectively. The bioagent *Trichoderma asperellum* @ 1% provided moderate control (PDI = 55.38) and a yield of 46.43 q ha^{-1}, showing a 10.96% yield increase. The moderate efficacy of *Trichoderma* may be due to its indirect antagonistic mechanisms by competition, mycoparasitism, and induction of host resistance, which generally require a longer period for establishment under field conditions.

Both untreated checks exhibited the highest disease intensity, confirming the epidemic potential of *E. turcicum* under favourable conditions. The unprotected inorganic check recorded a PDI of 61.18 with a grain yield of 45.39 q ha^{-1}, while the unprotected organic check showed the maximum disease incidence (PDI = 65.53) and the minimum grain yield of 41.88 q ha^{-1}. These results validate the necessity of integrated management strategies for effective TLB suppression.

The present findings corroborate earlier reports by Patil *et al*. (2018) [15] and Kaur *et al*. (2020) [11], who demonstrated the superior efficacy of strobilurin + triazole fungicide combinations against foliar blights of maize. The synergistic interaction of systemic and contact fungicides provides durable protection by targeting different biochemical pathways in the pathogen. These results are also similar to Manu *et al*. (2017) [19], who have reported that the efficacy of chemical fungicides and bioproducts for TLB management under humid and subtropical conditions

Table 2: Efficacy of fungicides and bioproducts on the incidence of TLB (Pooled mean of 2018-2020)

Treatment	PDI (Pooled)	Disease Control (%)	Grain Yield (q/ha)	Yield Increase (%)
Mancozeb 75 WP @ 0.25%	39.44	39.45	53.10	26.77
Azoxystrobin 18.2% + Difenoconazole 11.4% @ 0.10%	32.00	51.18	54.85	30.89
Azadirachta indica @ 10%	51.92	20.73	51.34	22.56
Allium sativum @ 10%	49.45	24.45	52.31	24.90
Lantana camara @ 10%	59.09	10.09	48.90	16.87
Cow urine @ 20%	54.66	16.81	47.99	14.67
Trichoderma asperellum @ 1%	55.38	15.43	46.43	10.96
Unprotected inorganic check	61.18	6.89	45.39	8.44
Unprotected organic check	65.53	-	41.88	-
Sem ±	2.39		4.61	
CD(P=0.05)	7.12		13.69	
CV%	8.10		16.20	

Conclusion

It can be concluded from the experiments conducted over three years, the pooled data of 2018, 2019 and 2020 showed that seed treatment at 1ml/kg of seed and foliar application of Azoxystrobin 18.2% + Difenoconazole 11.4% @ 1 ml/litre of water were most effective for the management of Turcicum Leaf Blight disease of maize in managing the disease as well as in increasing the grain yield. Among the organic and bioproduct treatments, *Allium sativum* at 10% showed promising results. Hence, the seed treatment followed by foliar spray of Azoxystrobin 18.2% + Difenoconazole 11.4% @ 0.1% is concluded for effective and sustainable management of TLB in maize under field conditions.

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