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EFFECTS OF TRACE ELEMENTS AND ANTIMYCOTICS TO MANAGE RICE BLAST DISEASE CAUSED BY *PYRICULARIA ORYZAE*

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ABSTRACT

Rice blast disease caused by *Pyricularia oryzae* causes significant yield losses. Sixteen commercial cultivars of fine rice from Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan were assessed for genomic resistance years as well as the efficacy of trace elements and antimycotics for two consecutive years (2021-22). Two varieties (Super Basmati and Basmati-370) were found highly susceptible, while KSK 133 was observed resistant, RC-8, CHECK, RC-7, PKBR21-8 and PK11876-1-2 were moderately resistant, KSK-282, RC-6, PKBR21-12,PKBR2-1 and PK11661-2-3 were moderately susceptible and the variety C1,C2 and C3 were susceptible against the disease. In management of the disease, combination of thiophanate methyl, tebuconazole + trifloxystrobin, and fosetyle-aluminium was more effective with minimum disease severity of 11.89% and 11.69%. In a moderatley susceptible vareity the application of thiophanate methyl and fosetyle-aluminium also effectively suppressed the disease severity 17.83% and 16.90% respectively. Tebuconazole + trifloxystrobin combination was less effective against the disease (36.67% and 36.51%). The combination of copper, zinc, and boron suppressed the disease to the maximum with disease severity of 9.681% and 9.62%. Overall, respective combinations of both fungicides and trace elements were found to be the most effective in controlling rice blast disease.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the extensively cultivated crops around the globe and serves as a staple and primary dietary source. It is widely consumed in Asia which is home to approximately 50 % of the world population (Bao *et al.*, 2014; Thapa and Bhusal, 2020). Its palatability makes it popular among the masses of Pakistan. It is a major cash crop whose exports fetches Pakistan highest foreign exchange after cotton exports (Rehman *et al.*, 2017). Rice grains are a major source of carbohydrate as well as several other nutrients especially, in developing countries (Huang *et al.*, 2015).

Both biotic and abiotic factors contribute to low yield of rice in the world (Acharya *et al.*, 2019). However, seed borne diseases are exclusively responsible for low paddy yield. Pathogens account for 30% annual yield loss of the rice crop (Junjie *et al.*, 2021). From the last twenty years in Pakistan, rice blast disease has been evolved to an existential threat to the rice crop in the rice growing areas of Punjab province (Arshad *et al.*, 2008).

Rice blast disease is caused by the fungus *Pyricularia oryzae* which is one of the most destructive pathogens of *Oryzae sativa* L. across the world. The annual destruction of 10-30% of rice yield is due to the attack of blast pathogen (Fernandez and Orth, 2018). Rice blast pathogen belongs to the filamentous ascomycetes that can reproduce and survive both in teleomorphic and anamorphic stage. The conidia are bi-septate, colorless, and darkened at the base (Sester *et al.*, 2014). More than eighty Poaceae family plants are affected by the rice blast pathogen that causes yield losses and deterioration of grain quality (Zeng *et al.*, 2009). Rice blast symptoms may be different on leaves, nodes and panicles due to environmental factors, resistance or susceptibility and age of the host plant (De-xi *et al.*, 2019). The early symptoms appear as white to brown followed by nodal or neck blast, which can cause necrosis and commonly blackening of the host panicles (Shahriar *et al.*, 2020). Unfavorable conditions that causes stresses involved drought, chilling, heat, nutritional dearth, light, pH, and the excess of toxic elements like cadmium, ozone, arsenate and aluminium (Hayasaka *et al.*, 2013) in the soil make the rice vulnerable to the blast. Moreover, susceptibility of hybrid rice causes high yield losses (Xing *et al.*, 2019).

Different management approaches considering the principles of plant disease management such as avoidance, exclusion, eradication and therapy has been tried by various researchers i.e., several approaches such as cultural, biological, chemical and host plant resistance alone or in combination under field conditions (Mohiddin *et al.*, 2021; Yadav *et al.*, 2019). Application of chemicals increase the resistance against blast on both moderately resistant and susceptible rice varieties, but the proper mechanism of these nutrients is not properly understood. Rice blast disease can be managed by using fungicides, but they indeed have some harmful effects on the soil, paddy grains and environment (Pati *et al.*, 2016). Extensive usage of chemicals for the control of disease has an adverse effect on the soil leaving some residues in the paddy grains (Kumar and Mukherjee, 2013). Using fungicides and trace elements with different mode of actions alternatively or in combination ensures the enhanced management of rice blast disease and minimizes the possibility of the emergence of fungicide-resistant strains.

MATERIALS AND METHODS

Collection of Infected Samples

Infected plants with characteristic symptoms i.e., Lesions on leaves, neck constriction, damaged panicle, and node cracking, were collected. Samples were carried to the laboratory in separate brown paper bags with appropriate labels. Samples were stored in refrigerator at 4 °C.

Isolation of Rice Blast Pathogen

Diseased tissue along with healthy part was cut into small pieces measuring 4-6 mm^2. Samples were washed with sterilized water, and the surface was sterilized with 1% sodium hypochlorite (NaOCl) for one minute. After that, the samples were rinsed several times with distilled water and placed on filter paper. The samples were transferred to the PDA (Potato Dextrose Agar) culture plates and incubated at 25 °C for four days and subsequent purification of single spore culture of the concerned pathogen was carried out.

Morphological Characterization

Characteristics of colony such as shape, size, growth pattern and color were observed. It was ascertained by microscopic dimensions like shape of conidia, hyphal characteristics as well as hyphal size.

Inoculum Preparation and Inoculation

The mature culture plates were used to prepare spore suspension. 10ml distilled water was added into the culture plate followed by scrapping with the sterile inoculated loop to detach the conidia. Spores were harvested and filtered through a muslin cloth and spore concentration was adjusted to 1×10^6 conidia/ml before inoculation.

Pathogenicity Test

Rice seedlings were grown in earthen pots filled with sandy loam soil along with farmyard manure and placed in green house. Five pots were designated for inoculum application and five without inoculum. Disease symptoms were examined, and re-isolation of pathogen was carried out at seedling stage from the inoculated diseased plants.

Screening of Rice Germplasm

Sixteen available rice cultivars (KSK 133, RC-8, CHECK, RC-7, PKBR 21-8, PK11876-1-2, KSK 282, RC-6, PKBR21-12, PKBR21-1, PK11661-2-3, Basmati 370, Super Basmati, Chenab basmati (C1), KSK 515 (C2) and C-622 (C3) was collected from Rice Research Institute (RRI), Kala Shah Kaku. The nursery was established and transplanted to the experimental field area of Plant Pathology, University of Agriculture Faisalabad for screening against rice blast disease. The screening trial followed an augmented design, with a row-to-row distance of 22 cm and plant to plant distance of 20 cm. Fertilizer and irrigation were applied according to the crop nutrients and water requirements. Rice plants were inoculated at the appropriate growth stage i.e., seedling stage, by spraying the spore suspension onto the foliage. Disease advancement and severity were evaluated at regular intervals after inoculation. Visual assessment involved scoring disease symptoms, such as leaf lesions, lesion size, and lesion number, based on a disease rating scale (Table 1) developed by IRRI (1996). All the data was collected with one-week interval after the appearance of the first disease symptom. The screening trial was repeated with a similar procedure during next growing season.

Table 1. 0-9 disease rating scale for screening of blast nursery (IRRI, 1996).

Grade	Disease severity	Host response
0	No lesion observed	Highly resistant
1	Small brown specks of pinpoint size	Resistant
2	Small roundish to slightly elongated lesions	Moderately Resistant
3	Significant number of lesions on the upper side of the leaves	Moderately Resistant
4	Typical susceptible blast lesions, 4% of leaf area	Moderately Susceptible
5	Typical susceptible blast lesions, 4 to 10% of leaf area	Moderately Susceptible
6	Typical susceptible blast lesions, 11-25% of leaf area	Susceptible
7	Typical susceptible blast lesions, 26-50% of leaf area	Susceptible
8	Typical susceptible blast lesions, 51-75% of leaf are many leaves are dead	Highly susceptible
9	Typical susceptible blast lesions, more than 75% leaf area affected	Highly susceptible

In-vitro Assessment of Fungicides

Thiophanate methyl (Topsin-M), Tebuconazole + Trifloxystrobin (Nativo), Fosetyle-aluminium (Aliette), Copper oxychloride, Difenoconazole (Score) and Cymoxanil + Mancozeb (Curzate M8) were evaluated with following concentrations i.e., 200, 400 and 600 ppm against *Pyrycularia oryzae* using the poisoned food technique in the lab. The concentrations of chemicals were adjusted in PDA and subsequently poured in petri plates under laminar flow chamber. The trail was carried out under a complete randomized design (CRD). The 5mm bits of the cultured fungus, carved out using cork-borer, were transferred to the treated culture plates and incubated at 25±2 °C. The control having no fungicide added was also maintained. Mycelial growth was noted down and compared with the mycelial growth in the control.

In-Vivo Evaluation of Effective Fungicides

A moderately susceptible variety was used out for *in-vivo* evaluation. Three fungicides (Topsin-M, Aliette and Nativo), which were effective to control mycelial growth in-vitro experiment, were evaluated against rice blast disease. The trial was conducted following the randomized complete block design (RCBD) with three replications during the rice growing season of 2021 and 2022 in the research area of Department of Plant Pathology, University of Agriculture Faisalabad. Disease severity data was recorded after one-week interval for the whole crop season.

Give formula of disease severity:

$$\text{Disease severity} = \frac{\text{Number of disease parts of plants}}{\text{Total plant parts observed}} \times 100$$

In-Vivo Assessment of Trace Elements

Trace elements (boron, zinc and Sulphur) with following combinations (boron + zinc; zinc + boron; boron + copper, zinc+ copper & boron + zinc + copper) were assessed against the pathogen of rice blast disease. The trial was carried out by following the above-mentioned procedure.

RESULTS

Germplasm Screening against Rice Blast Disease

Screening results involved two consecutive growing seasons i.e., 2021-22. During the first year i.e., 2021 among sixteen varieties, only one variety KSK133 exhibited resistance against blast disease. Five varieties namely, RC-8, CHECK, RC-7, PKBR21-8, and PK11876-1-2, were found to be moderately resistant against blast disease. Five varieties i.e., KSK 282, RC-6, PKBR21-12, PKBR21-1, and PK11661-2-3, were categorized as moderately susceptible to rice blast disease. Three varieties namely, Chenab Basmati (C1), KSK-515 (C2), and C-622 (C3), were found susceptible to the disease. Two varieties i.e., Basmati 370 and Super Basmati, showed high susceptibility towards blast disease (Table 2). Next year (2022) screening outlook was slightly different from the previous one, however, most of the screened varieties exhibited similar response. KSK 133 kept its resistant status maintained. RC-8 and CHECK slipped towards moderately susceptibility. Moreover, PK11661-2-3 showed susceptibility in contrast to moderate susceptibility against the blast disease (Table 2).

Table 2. Response of rice germplasm to the rice blast disease caused by *Pyricularia oryzae* during year 2021-2022.

Sr. No.	Varieties	Disease Severity (2021)	Disease Severity (2022)	Score	Host Response (2021)	Host Response (2022)
1	KSK 133	0.913 F	1.113 g	1	R	R
2	RC-8	2.400 f	4.320 fg	3	MR	MS
3	CHECK	2.133 f	8.127 ef	2	MR	MR
4	RC-7	1.693 f	2.357 fg	2	MR	MR
5	PKBR21-8	1.733 f	2.230fg	2	MR	MR
6	PK11876-1-2	2.367 f	2.673 fg	3	MR	MR
7	KSK 282	3.995 f	2.673 fg	4	MS	MS
8	RC-6	4.000 f	5.00 fg	5	MS	MS
9	PKBR21-12	6.697 ef	7.557 f	5	MS	MS
10	PKBR21-1	3.157 f	3.810 fg	4	MS	MS
11	PK11661-2-3	7.099 ef	13.843 de	5	MS	S
12	C1	16.000 d	16.000 d	6	S	S
13	C3	35.443 c	36.020 c	7	S	S
14	C2	12.803 de	14.240 de	6	S	S
15	Super Basmati	60.473 b	61.213 b	8	HS	HS
16	Basmati 370	72.867 a	76.607 a	9	HS	HS

R= Resistant, MR= Moderately Resistant, MS= moderately susceptible, S= Susceptible, HS= Highly Susceptible

These screenings aimed to identify rice germplasm with resistance to the blast disease, providing valuable information for breeding programs and disease management strategies.

In-Vitro* Assessment of Fungicide against *Pyricularia oryzae

The mycelial growth of *P. oryzae* was significantly different in response to three concentrations of each fungicide. Among the six tested fungicides, only Thiophanate Methyl (Topsin-M) appeared as a highly effective fungicide against *Pyricularia oryzae* (Figure 1). Concentration at 600ppm of Topsin-M restricted the maximum growth of *P. oryzae*. All other fungicides failed to suppress the mycelial growth to the extent as Topsin-M level (Figure 2).

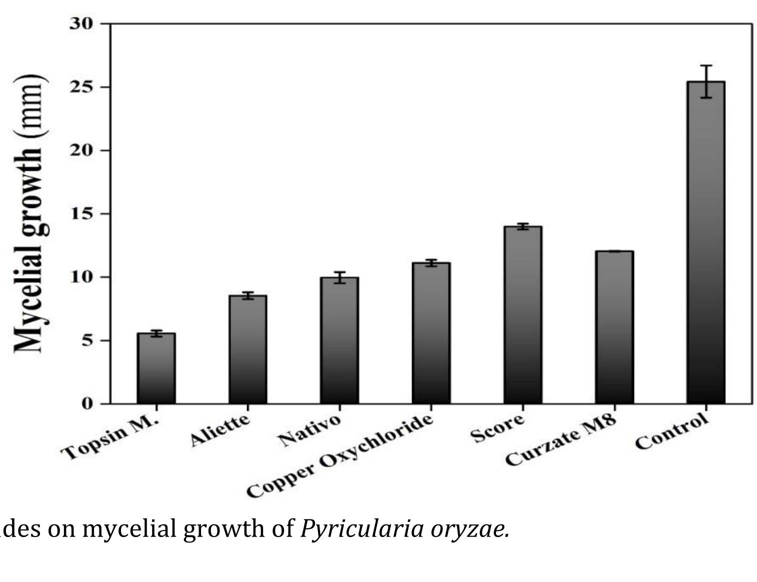

Figure 1. Effect of fungicides on mycelial growth of *Pyricularia oryzae.*

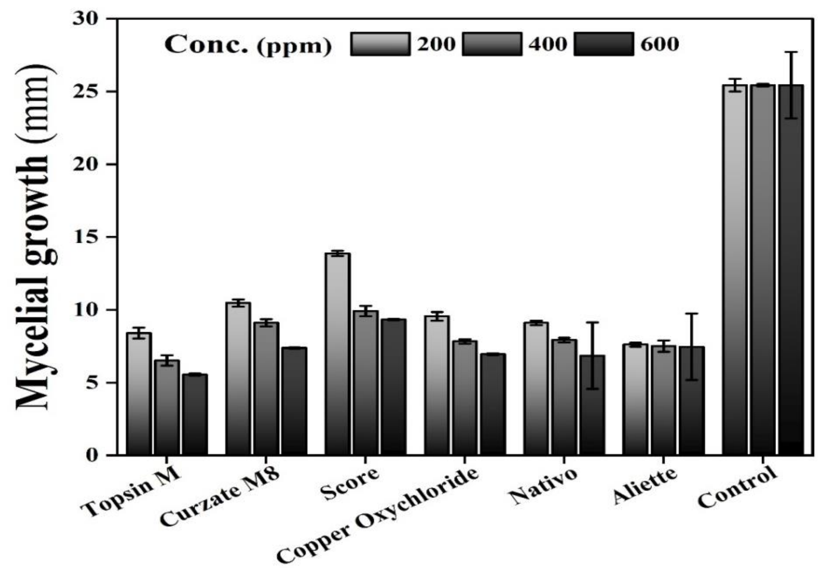

Figure 2. Effect of various concentrations of fungicides on mycelial growth of *Pyricularia oryzae.*

At high concentration (600ppm) the repression of mycelial growth was as follows; Topsin M (5.55mm), Aliette (7.46mm), Nativo (6.85mm), Copper oxychloride (6.95mm), Score (9.33mm), Curzate M8 (7.38mm) (Figure 3).

In-Vivo Assessment of Fungicides against Rice Blast Disease

In-vivo evaluation comprised two years data i.e., 2021-22. Overall, the combination of Topsin-M, Aliette, and Nativo was found to be the most effective in disease suppression with reported disease severity (DS) stood at 11.89% and 11.69 % for the first and the second year respectively (Figure 4). The combination of Topsin M and Aliette was effective only second to the combination of the three with disease severity of 17.83% and 16.90 % respectively. Nativo (Tebuconazole + Trifloxystobin) proved to be least effective in restricting disease progression with reported 36.67% and 36.51% respectively. Individual application of Topsin M suppressed the disease moderately as reflected by the DS i.e., 23.83% and 23.02% respectively.

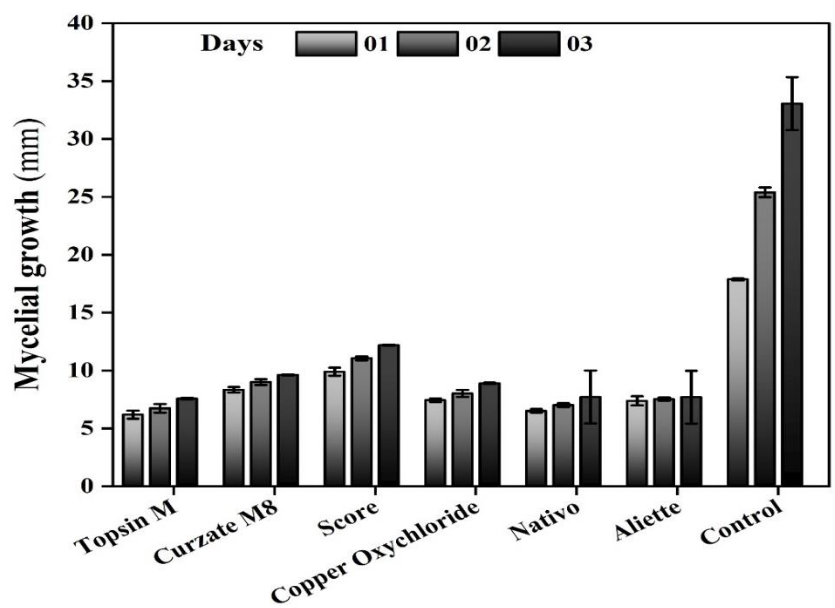

Figure 3. Effect of different fungicides on mycelial growth of *Pyricularia oryzae* with respect to days.

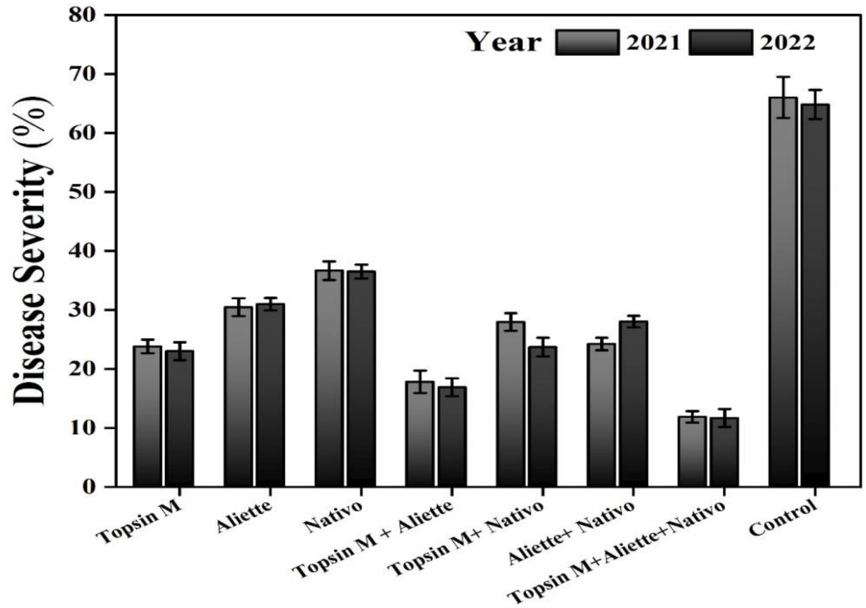

Figure 4. Disease severity of *Pyricularia oryzae* as measured against different fungicides during 2021 and 2022.

Efficacy of fungicides to suppress the disease increases with the time lapse. As results after the first week application of the combination of Topsin-M, Aliette, and Nativo reflected 15.16% and 15.55 % DS respectively. After the passage of the second and the third week DS recorded was 11.76 % and 8.73 % for the first year and 11.57 % and 8.73% for the second year respectively (Figure 5, 6).

In-Vivo Assessment of Trace Elements against Rice Blast Disease

Trace elements were equally effective to control the blast disease. The combination of copper (Cu) + zinc (Zn) + boron (B) was highly effective against blast disease as represented by DS i.e., 9.68 % and 9.12% for the 2021 and 2022 years respectively (Figure 7).

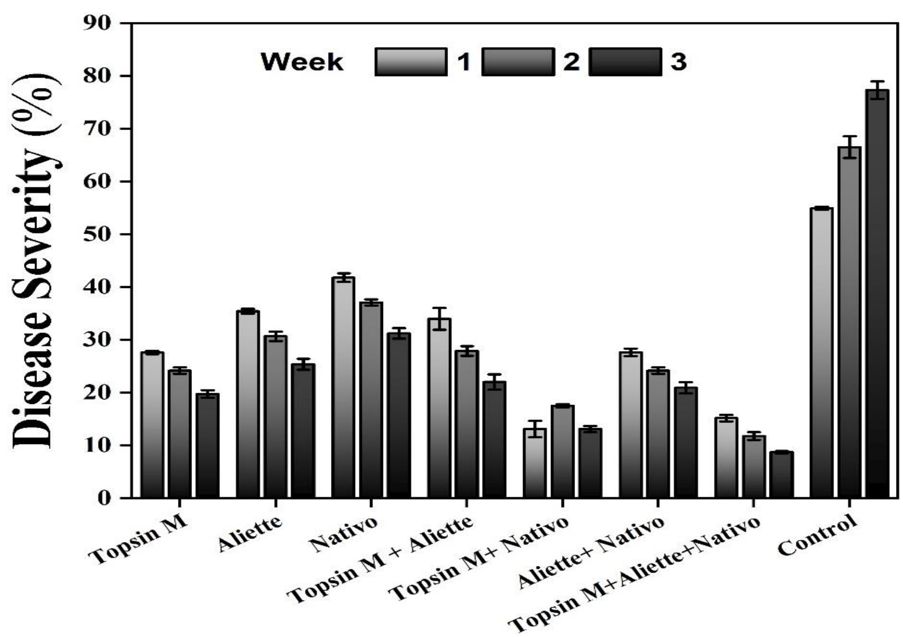

Figure 5. Weekly Disease severity of *Pyricularia oryzae* as measured against different fungicides during 2021.

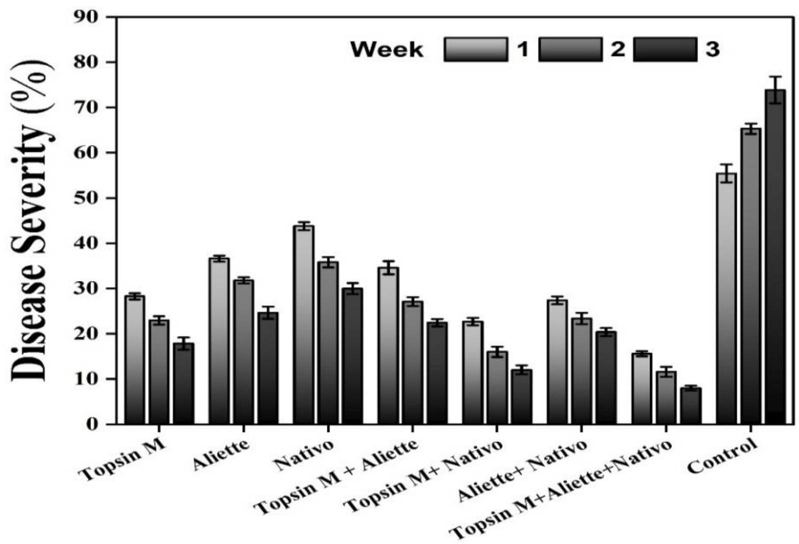

Figure 6. Weekly Disease severity of *Pyricularia oryzae* as measured against different fungicides during 2022.

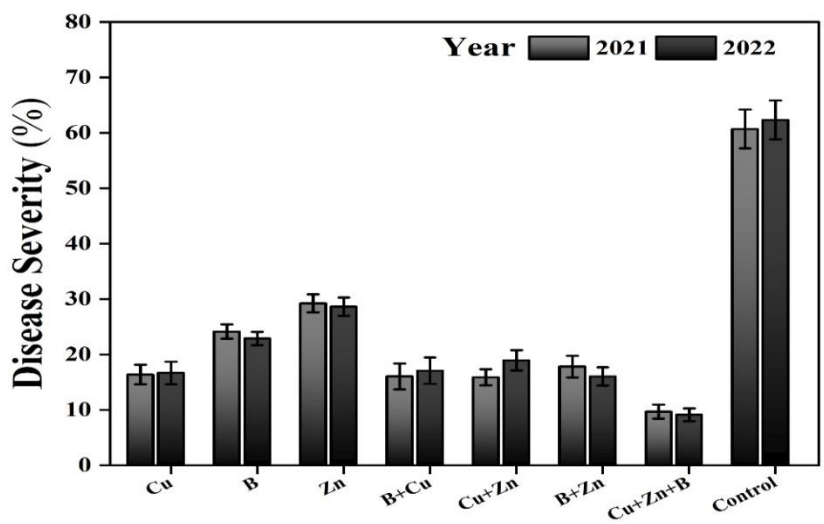

Figure 7. Disease severity of *Pyricularia oryzae* as measured against trace elements during 2021 and 2022.

Among individual application Cu has been found to be the most effective with reported DS of 16.37 % and 16.67 % respectively. Zn was the least effective among all tested treatments of trace elements with DS stood at 29.22% and 28.63% respectively. With the passage of time efficacy of trace elements also increases as DS reported for the combination of Cu + Zn + B for the first, the second and the third weeks were as follows; 14.09% & 13.18%, 9.10% and 8.32%, 5.84 % and 5.88% respectively (Figure 8, 9).

DISCUSSION

In the current study, blast disease was recorded in the rice field against the pathogen *P. oryzae,* and management practice was done by using different fungicides and nutirents. The disease was identified based on marphological characterization and pathogenicity test. The pathogen *P. oryzae* accounts for the massive yield losses to the rice crop (Junjie *et al.*, 2021). Developing resistant varieties by incorporating new genes through breeding techniques remains the most economically viable, ecologically sustainable and environmentally friendly technique. However, evolving pathogen continues to pose serious threat to the crop production (Xiao *et al.*, 2017).

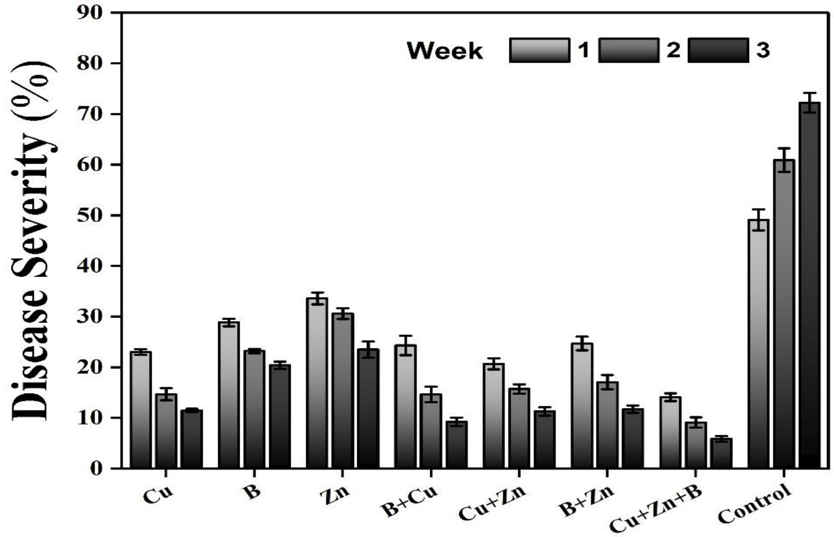

Figure 8. Weekly Disease severity of *Pyricularia oryzae* as measured against trace elements during 2021.

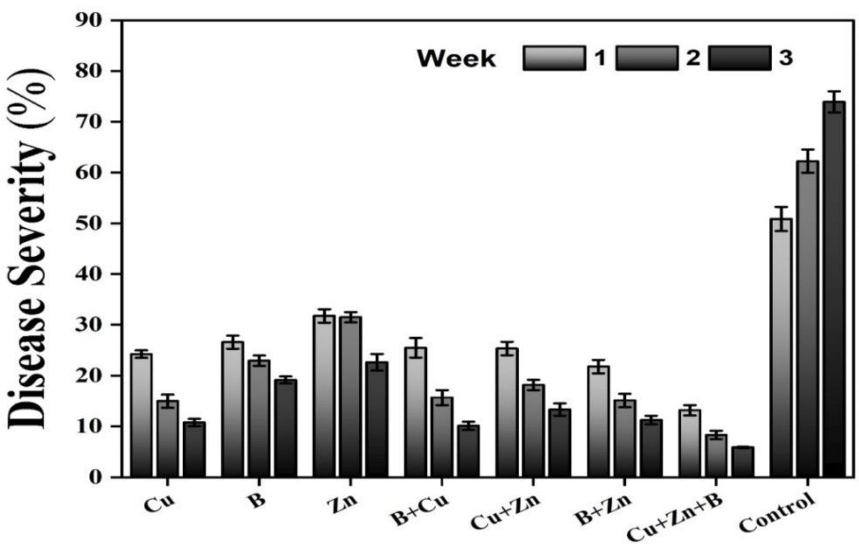

Figure 9. Weekly Disease severity of *Pyricularia oryzae* as measured against trace elements during 2022.

Among sixteen screened varieties only one variety KSK133 has shown resistance against the *P. oryzae*. During the first year RC-7, RC-8, CHECK, PKBR21-8, and PK11876-1-2 shown moderate resistant, however, the next year RC-8 and CHECK showed slightly susceptibility. It could be the increase in the virulence of the pathogen subsequently overcoming the resistance by *P. oryzaeq* or breakdown of horizontal resistant due to slippage of some genes. Synthetic fungicides are the most economically workable solution. Stroblurin, azoxystrobin along with fungicides belonging to triazole group i.e., tebuconazole + fluopyram and propiconazole + difenoconazole have been reported to suppress the disease in fields, however, mancozeb proved to be least effective (Kongcharoen *et al.*, 2020).

Another research has been found the floxystrobin + tebuconazole, propiconazole and difenoconazole + azoxystrobin to restrict the disease by 53.3%, 60.3% and 55.1% respectively (Raj and Pannu, 2017). Epoxiconazole and tricyclazole have been happened to reduce the rice blast by 75-77% and 73-76% respectively. A research conducted in Australia carried out at seedling stage showed azoxystrobin more effective in contrast to propiconazole (Pak *et al.*, 2017).

Tricyclazole has been reported to be repressed the disease by 89.43% while the least effectiveness was reflected by mancozeb + carbendazim i.e., 58.66% (Mohiddin *et al.*, 2021). This research has found combinations of fungicides more effective than their single use as combination of thiophanate methyl, Fosetyl- Aluminium and tebuconazole + Trifloxystrobin have been found to reduce the disease severity up to 11.6 %. The results of triazole group fungicides i.e., tebuconazole + trifloxystrobin are in consistent with the study of Raj and Pannu (2017).

Similar results regarding the efficacy of differnent fungicides that was used in research has been reported throughout the world against rice diseases like Variar *et al.* (1993) used eight different fungicides for management of rice blast disease and treated seeds with tricyclazole @ 4kg/kg that proved to be effective aftter 40 days of sowing.

Systemic fungicides can help to cope with the blast disease problems, but their efficacy largely depends upon their appropriate absorption in the foliar parts and subsequent circulation in the whole plant. Prabhu *et al., 2003* reported that the application of fungicides tends to increase the yield of rice. Mechanism behind their action remains open to debate, however, combinations have been proved more effective than their individual use. It could be the simultaneous suppression of the pathogen by synchronizing effect of the combination.

Novel fungicides and new combinations must be evaluated in order to keep check on the pathogen as the continuous stress evokes resistant response in the pathogen. However, their residual effects are serious hazards for the living organisms and environment.

Mechanism of boron, zinc and copper behind suppression of *P. oryzae* remains ambiguous, however, it has been thought that they might interfere with the spore production or metabolism (Nadeem *et al.*, 2018). Concentration of boron, zinc, copper and magnesium at 2 ppm promoted growth and sporulation of *P. oryzae,* however at greater concentration the growth and sporulation found to be decreased (Sharma and Shukla, 2020). The results of this study endorse the results of Nadeem *et al.* (2018). In this study combination of copper, zinc and boron have been found to be suppress the disease up to 9 %. Though trace elements are essential for plant growth and essentially enhances production capacity, however, their excess in the soil induces toxicity and negatively impact crop production (Gupta and Gupta, 1998).

AUTHOR CONTRIBUTIONS

Fatima Maira wrote the manuscript; Shahbaz Talib Sahi supervised the study; Safdar Ali helped in statistical analysis; Muhammad Ahsan Khan reviewed and edited the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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