Evaluation of nutrient sources and *in vitro* efficacy of fungicides for the management of brown spot of rice

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Abstract

This study reports the colonization of brown spot caused by *Helminthosporium oryzae* with symptomatic samples of rice and its management with fungicides. Among all the tested samples, maximum infection (84%) of *H. oryzae* was observed in samples from Tando Muhammad Khan while the least fungal colonization (79%) was observed in rice samples from Sijawal area. In order to get the highest recovery of fungus, different nutrient sources were also evaluated. The results showed potato dextrose agar as the best nutrient media among all other tested nutrient sources. Similarly, starch proved effective carbon source and potassium nitrate remained the best nitrogen source for fungal recovery in amended Richards agar medium. *In vitro* efficacy of the five fungicides showed significant reduction in fungal growth over control. The fungicide, dimethomorph + mancozeb was very effective chemical against the fungal pathogen with 94% inhibition and difenoconazole showed the least inhibition (59%) through poison food method. The present study showed the prevalence of brown spot of rice in southern parts of Sindh. Good crop husbandry along with judicious application of fungicides can be handful to limit its further spread.

Keywords: Brown Spot, Fungicides, Management, Nutrient sources, Rice.

Introduction

Rice (*Oryza sativa* L.) is one of the leading dietary sources for masses. Being an export commodity, it is an important source of foreign exchange earnings for most of the rice producing countries. However, sustainable rice production is affected by various biotic and abiotic constraints. Among the biotic problems, emergence of new biotypes of plant pathogens has challenged its production and global food security. Farming community face substantial losses in yield due to various pathogens attacking their crops at various developmental stages (FAO, 2017). Rice crop is affected by a number of diseases (Javaid and Anjum, 2005; Javaid *et al.*, 2019). Brown spot of rice caused by *Helminthosporium oryzae* is one of the destructive diseases that impacted rice cultivation historically. It is prevalent in all rice growing tracts of the world and attack all parts of rice plant. Under severe conditions, it can cause significant yield losses (Chakrabarti, 2001; Sunder *et al.*, 2014). This disease is characterized by the presence of light reddish-brown lesions with yellowish haloes on leaves. Grain discoloration caused by this disease reduces market value of the produce and make them unfit for human consumption (Barnwal, 2013).

Rice is an important kharif crop in Pakistan. It is the second most important staple food crop and also have a key role in agro based industry of the country. It is mainly cultivated in irrigated plains of Punjab and Sindh province. During 2022-23, area under rice cultivation, in Pakistan, was 2976 thousand hectares with a total production of 7322 million tons. High input prices effected rice cultivation in the country as a result area and production of this commodity was significantly lower than the preceding years (Govt of Pakistan, 2023). Rice is an important source of livelihood for masses in Sindh. It is cultivated in the upper and lower parts of the province. Rice cultivation in the country is affected by various biotic and abiotic factors. Water scarcity, drought and nutritional imbalance are important abiotic constraints. Its severity is relatively higher in soils with low pH, less available potassium. Deficiency of major and trace elements also favor its incidence (Chakrabarti, 2001).

Among diseases, brown spot of rice is a major limiting factor in the poorly managed and less attended fields. Infected rice seeds, crop debris and collateral hosts serve as source of inoculum (Biswas *et al.*, 2008; Abrol *et al.*, 2022). Wind borne inoculum also favor its dissemination to the nearby fields (Sato *et al.*, 2008). Availability of suitable temperature and relative humidity also favor disease incidence (Minnatullah and Sattar 2002; Ismail *et al.*, 2014). In order to manage plant pathogens, host plant resistance is the most economical method. Similarly, use of botanical extracts and biocontrol agents for disease management have gained attention in farming community (Dahar *et al.*, 2017; Ismail *et al.*, 2021; Ferdosi *et al.*, 2023). However, application of chemical fungicides is a common and preferred practice to prevent and cure plant disease (Khan and

Javaid, 2015; Deresa and Diriba, 2023). Keeping in view the importance of brown spot of rice, disease sample were collected from lower parts of Sindh province to assess the colonization of the fungus. The study was designed to evaluate the effect of carbon and nitrogen sources on colony growth of *H. oryzae.* Similarly, various fungicides were also evaluated for the management of this disease in rice.

Materials and Methods

Collection of samples and isolation of pathogen

Symptomatic leaf samples from farmer fields in Thatta, Tando Muhammad Khan, Badin and Sijawal were collected. The samples were brought to the lab and washed thoroughly for further processing. These samples were sliced into small pieces along with some healthy portion and surface sterilized with 70% ethanol and 5% sodium hypochlorite (NaOCL) solution for two minutes. After blotter drying, the pieces were placed on PDA medium in a laminar flow and incubated at 27 °C for 15 days. The fungal growth in petri dishes was monitored on daily basis. The frequency of colonization was calculated as follows:

$$\text{Colonization } (\%) = \frac{\text{Number of pieces with fungal growth}}{\text{total number of pieces cultured}} \times 100$$

Initial growth of the test fungus was purified aseptically on fresh potato dextrose agar medium. Similarly, isolates were identified on the basis of phenotypic features under light microscope and confirmed with the help of illustrated genera of imperfect fungi.

Pathogenicity of isolated fungi

The isolated fungal cultures were tested for their pathogenicity on one-month old rice seedlings grown in pots. Inoculum of each of the test isolate was prepared and adjusted with haemocytometer. The leaf surface of the seedling was aberrated with carbrendum. Inoculum was sprayed on the aberrated surface of seedlings which were covered with polythene sheet. The seedlings sprayed with sterile distilled water served as control. The inoculated seedlings were sprayed with water on daily basis to establish high humid conditions for fungal infection. Observations were made on daily basis for symptom development on rice seedlings. Re-isolation of fungi was also carried out from the developed pustules on inoculated rice seedlings for confirmation. Controlled seedling remained healthy and symptomless.

Influence of nutrient media on colony growth of *H. oryzae*

In this experiment six nutrient sources including Potato Dextrose Agar (PDA), Richards Agar Medium (RAM), Sabouround agar medium (SAM), Capedox agar medium (CAM), water agar (WA) and nutrient agar (NA) were tested to assess the growth response and maximum mycelial growth of *H. oryzae*. The mycelial tip of the purified fungal colony was placed aseptically on petri plates containing aforementioned nutrient sources. The experiment was conducted with completely randomized design (CRD) with five replications. Petri dishes were incubated at 27 °C for fifteen days. Observations were made on daily basis to observe the mycelial growth on all replicates of each nutrient source.

Effect of carbon and nitrogen sources on mycelial growth of *H. oryzae*

Different carbon sources viz glucose, sucrose, starch, and nitrogen sources viz sodium nitrate, ammonium nitrate, peptone and potassium nitrate were tested against *H. oryzae*, for this purpose the Capedox agar medium was amended with these sources. The cultured petri plates containing each carbon and nitrogen source were incubated at 27 °C for fifteen days. Petri plates containing Capedox agar medium without any amendments served as control. This study was conducted under CRD with five replications. Difference in colony growth of test fungus was compared in amended Capedox agar medium for carbon and nitrogen sources with control plates.

In vitro* efficacy of fungicide against *H. oryzae

Efficacy of fungicides *viz.* fosetyl aluminium, carbendazim, difenoconazole, topsin-M and tebuconazole + trifloxystrobin was tested against *H. oyzae.* Different concentrations of selected fungicides *viz.* 10, 20, 50 and 100 ppm were applied using poison food technique. The fungicides were added to the sterilized molten potato dextrose agar media. A 5mm disc of actively growing colony of fungus was placed aseptically on the treated PDA. The petri plates without fungicides served as control in this experiment. All these plates were incubated for fifteen days at 27 °C. The observations regarding the colony growth of test fungus was recorded on daily basis till control plates were full of mycelial mat of test fungus. Inhibition percentage in the linear colony growth of fungus was calculated as follows:

$$\text{Inhibition } \% = \frac{\text{Fungal growth in control} - \text{Fungal growth in treatment}}{\text{Fungal growth in control}} \times 100$$

Results

Colonization and Pathogenicity of *H. oryzae*

All the infected samples cultured for colonization of *H. oryzae* produced growth on potato dextrose agar. The random samples from Thatta showed maximum infection (84%) which was followed by 83% fungal colonization from infected samples collected from farmer fields in T.M. Khan and Badin areas (Fig.1). Similarly, rice samples from Sijawal area showed comparatively low colonization

(79%) of *H. oryzae* as compared to other surveyed areas. These results show the prevalence of brown spot of rice in southern areas of Sindh province, however its incidence was variable in farmer fields depending upon application of judicious agronomic practices by farmers.

Preliminary growth from the infected samples from each locality was purified on fresh nutrient media. Growth pattern on PDA media, phenotypic features of fungal colony under microscope, spore shape and orientation confirmed fungus as *H. oryzae*, these parameters were validated with the help of illustrated genera of imperfect fungi. Spore suspension of seven days old fungal culture was prepared and adjusted with haemocytometer and inoculated in one-month old rice seedlings. The inoculated seedlings induced identical symptoms under controlled conditions of temperature and humidity. The fungus was re-isolated from the developed lesions and Koch postulates were fulfilled.

Influence of nutrient media on colony growth of *H. oryzae*

Present work was carried out to assess the effect of nutritional composition of various media on colony growth of *H. oryzae.* It was found that fungal growth on PDA was very profound as compared to the other tested nutrient sources (Table 1). Apart from PDA, fungal growth on CAM was also significant however RAM produced the least mycelial growth on same conditions of temperature and incubation. This variable growth pattern of the test fungus in different nutrient sources can be attributed to nutritional composition of these media.

Effect of carbon and nitrogen sources on mycelial growth of *H. oryzae.*

In this study three carbon sources were tested against *H. oryzae.* Among these sources starch gave highest fungal recovery followed by glucose and sucrose (Table 2). Starch and glucose are main ingredient of PDA medium which showed maximum fungal growth in this work also, while potassium nitrate proved effective nitrogen source for the tested pathogen as compared to the other nitrogen sources (Table 2).

In vitro* efficacy of fungicides against *H. oryzae

Difenoconazole, fosetyl aluminium, thiophenate methyl, tebuconazole +trifloxystrobin and dimethomorph + mancozeb were tested in lab conditions at 10, 20, 50 and 100 ppm concentrations to assess their effect on mycelial growth of *H. oryzae*. The results showed that each fungicide significantly inhibited mycelial development at all tested doses. However, inhibition increased at higher doses of fungicides (Table 3). In present results dimethomorph + mancozeb showed 94% inhibition of *H. oryzae* as compared to control which was followed by tebuconazole +trifloxystrobin with 85% and least reduction was observed in thiophenate methyl (Fig. 2). Among all the tested fungicides dimethomorph + mancozeb was effective at its lower doses *i.e.* 10 and 20 ppm which was followed by tebuconazole + trifloxystrobin. All other fungicides including difenoconazole thiophenate methyl and fosetyl aluminium were found effective against brown spot pathogen at their higher doses of 50 and 100 ppm.

Discussion

Rice crop in the surveyed areas was in good condition however brown spot of rice was prevalent in fields where proper crop husbandry was lacking. According to the previous reports brown spot is disease of poorly managed fields (Chakrabarti *et al.*, 1992; Barnwal, 2013). Cloudy weather favors its development. Optimum temperature for its development is 27–28.5 °C whereas, relative humidity of 90–99% favor its severity. The other environmental parameters like wind velocity and low to moderate rainfall are conducive for its dispersal (Ou, 1985; Ramakrishnan, 1997). Choudhry *et al.*, (2019) have correlated various climatic conditions and found that high relative humidity is a key factor in the onset of brown spot in rice. Their findings also reveal maximum incidence of brown spot in the month of October during 2014–2017 season. The occurrence of brown spot of rice is strongly related with water scarcity, drought, high dew and humid conditions. Rice crop grown in nutrient deficient soils is also prone to attack of this disease. Rice is an important crop of lower Sindh especially in Thatta, Badin, Tando Muhammad Khan, Sijawal and adjoining areas. The surveyed area in this study have a similar pattern of environmental conditions which are very conducive for its development.

Isolation of microbes especially fungal flora is dependent on many factors like nutrient sources, incubation period and availability of optimum temperature, humidity and light. In order to get the profound growth of test fungus under lab conditions, nutrient sources must contain necessary elements. The results of present studies indicate significantly higher fungal growth on PDA which suggest nutrient rich nature of this source. Shehu and Ibrahim (2014) have reported potato dextrose agar, Czapek-Dox agar and nutrient agar media as the best nutrient sources for the maximum growth of *H. fulvum*. Arshad *et al*. (2013) have also obtained maximum growth of *B. oryzae* on malt extract and PDA.

Yamaguchi and Mutsunobu (2010) evaluated nine carbon sources against *B. oryzae* and found all carbon sources suitable for optimal growth of test fungus except L-sorbose. Among nitrogen sources, potassium nitrate recovered maximum fungal growth as compared to the other two nitrogen sources i.e. peptone and ammonium nitrate (Table 2). Naza *et al.* (2012) reported potassium nitrate as the best source nitrogen source among other nitrogen sources for maximum radial growth of tested fungus. Prasher *et al.* (2014) evaluated organic nitrogen sources and found chlamydospores production in basal medium amended with ammonium acetate, ammonium chloride, ammonium nitrate, ammonium oxalate and ammonium sulphate as inorganic nitrogen compounds. The nutrient media amended with nitrogen sources supports conidial formation. In another study, Channakeshava and Pankaja (2018) evaluated various carbon and nitrogen sources against brown spot pathogen and found glucose and ammonium peptone as best carbon and nitrogen source for optimum growth of *H. oryzae*.

Fungicide application in plant disease management is the most preferred approach because of their frequent availability and quick action. A wide range of systemic and contact fungicides with different modes of action are being used to manage diseases in plants. However, it is necessary to use them judiciously (Ismail *et al.*, 2018; Soomro *et al.*, 2020; Karan *et al.*, 2021). Kumar *et al.*, 2017 evaluated four fungicides against brown spot of rice and found Propiconazole very effective with 97% inhibition in lab conditions. The studies conducted by Chouhan and Kumar (2022) also confirmed the efficacy of various fungicides to manage brown spot of rice. It is evident from our results that all fungicides are capable of reducing the fungal growth under lab condition, however the effect of dimethomorph + mancozeb on *H. oryzae* growth is very significant as compared to all other tested fungicide.

Conclusion

The results of the present work indicated prevalence of brown spot in rice growing areas of lower Sindh. The current study also reveals potato dextrose agar as a good nutrient source for maximum recovery of *H. oryzae*. In order to reduce its further spread in the nearby fields good crop husbandry along with application of suitable fungicides like dimethomorph + mancozeb, tebuconazole + trifloxystrobin and fosetyl aluminum can be very fruitful.

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Author’s contributions

FJ carried out lab work and collected data, MI conducted survey, collected infected samples, analyzed the data and wrote the initial draft, RMM and KHW reviewed the manuscript. All the authors read the manuscript and agreed to finalize it for publication.

Conflict of interests

There is no conflict of interest between authors.

Table 1: Influence of nutrient media on colony growth of *H. oryzae.*

Nutrient Media	Mycelial Growth (mm)
Potato Dextrose Agar	4.33a
Capedox Agar Medium	3.63b
Water Agar	2.84c
Sabouround Agar Medium	1.69d
Nutrient Agar	0.89e
Richards Agar Medium	0.67e
LSD	2.179

Values with different letters in a column show significant difference (P≤0.05).

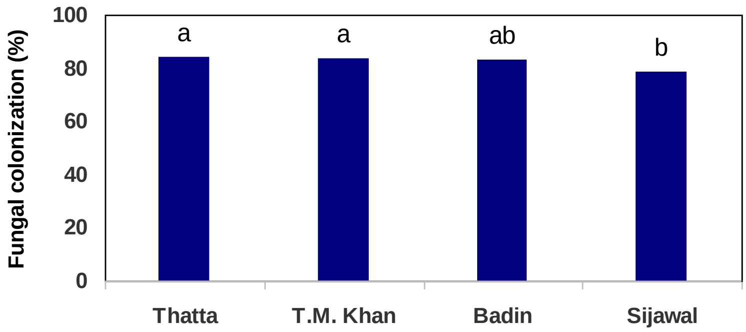

Fig. 1: Colonization percentage of *H. oryzae* with infected rice samples from different areas. Bars with different letters show significant difference (P≤0.05).

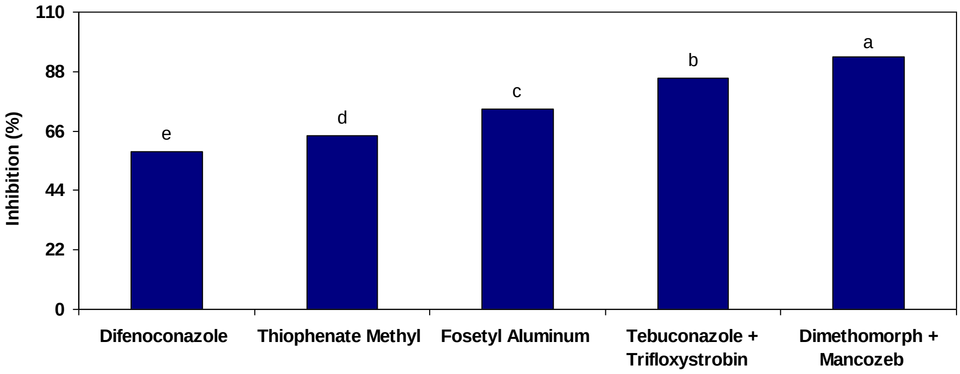

Fig. 2: Percent mycelial inhibition produced by various fungicides.

Table 2: Effect of carbon and nitrogen sources on mycelial growth of *H. oryzae*.

Carbon sources	Mycelial growth (mm)	Nitrogen sources	Mycelial growth (mm)
Starch	14.993a	Potassium nitrate	11.773a
Glucose	11.773b	Peptone	10.217ab
Sucrose	10.107c	Ammonium nitrate	8.8867b
SE	0.3743	SE	0.6494
LSD	0.9158	LSD	1.5890

Values with different letters in a column show significant difference (P≤0.05).

Table 3: *In vitro* efficacy of some fungicides against *H. oryzae*.

Fungicides	Concentration (ppm)				
	10	20	50	100	Mean
Difenoconazole	3.60b	2.30b	0.80b	0.13b	1.70b
Thiophenate methyl	3.00c	2.20b	0.38c	0.11c	1.42c
Fosetyl aluminum	2.40d	1.43 c	0.26cd	0.03d	1.03d
Tebuconazole + Trifloxystrobin	1.35e	0.96c	0.18d	0.00e	0.62e
Dimethomorph + Mancozeb	0.52f	0.40d	0.15d	0.00b	0.27f
Control	4.14a	4.14a	4.07a	4.00a	4.08a
LSD	0.267	0.485	0.162	0.013	0.124

Values with different letters in a column show significant difference (P≤0.05).

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