



Effect of trace elements Zn, B, Mg and Cu on the growth and sporulation of *Pyricularia oryzae*, the causal organism of blast disease of rice

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ABSTRACT

Blast is one of the most common disease of the Rice crop caused by *Pyricularia oryzae*. Blast of Rice is a recurrent problem of Rice producing countries declines productivity drastically. Mycelium growth and sporulation of *P. oryzae* is depend upon many factors i.e. humidity, temperature, availability of nutrients etc. Like other fungi *P. oryzae* also requires some nutrients in very minute quantity for their physiological and metabolic activities. Regulating these micronutrients or trace elements we can control the growth and spore production in *P. oryzae*. In this paper, we studied effect of four trace elements i.e. Zinc (Zn), Boron (B), Magnesium (Mg) and Copper (Cu) on growth and sporulation of *P. oryzae*. Zinc, Boron and Copper are most effective and promote growth and sporulation at 2 ppm (parts per million) concentration when we increased concentration of these elements in the medium, growth and sporulation decreased. On the other hand less growth and sporulation reported in the absence of Magnesium. Minute quantity of Magnesium is required for optimum growth i.e. 2 ppm. after this increasing concentration of Magnesium is not significant.

KEYWORDS: Trace element, Pyricularia, rice blast, fungal sporulation

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INTRODUCTION

Roughly more than half of the world population depends on rice for their food. The major rice-producing countries are China, India, Japan, Bangladesh, Indonesia, Thailand and Myanmar (Burma). The United States Department of Agriculture (USDA) estimates that the World Rice Production 2019-2020 will be 499.31 million metric tons. Since most of the world's population dependent on rice for their food, any kind of decrease on production of the crop gives rise to a serious problem. A crop failure for any reason poses a real threat of the starvation. There are many pathogenic and environmental factors which cause different diseases on rice plant. One of the most common as well as important disease of rice is blast, caused by *Pyricularia oryzae*. The rice blast disease caused by *Pyricularia oryzae* strike all aerial part of the plant. Most infections occur on the leaves, causing diamond shape lesions with a gray or white center to appear, or on the panicles, which turn white and die before being filled with grains [1]. *Pyricularia oryzae* is highly specific to the rice plants. Blast disease was first reported in Asia more than three centuries ago and is now present in over 85 countries. It is highly adaptable to the environment. When *Pyricularia oryzae* infects rice plants

and produces neck rot or panicle blast, it will either kill the host plant or prevent seed development respectively. Fungi need about 17 elements to meet their nutritional requirements [2-4]. Apart from these elements few elements are present in very small quantities, known as trace elements. The previous studies conducted by Allaway [5], Lilly [6], Bowen [7], Thind and Mira Madan [8] revealed the trace elements Zn, Fe, Mn, Cu, Mo and Ca are necessary for the growth of the almost all the fungus. There is not much literature available in the subject but some workers have worked on role of these metals on pathogenicity of different fungus. Metals Zn, Cu, Fe etc. play an important role in development of fungal disease [9] More than 400 yeast gene are involved in growth under Zn limitation [10]. The present paper deals with the requirements of Zn, B, Mg, and Cu on the growth and sporulation of *Pyricularia oryzae* isolated from the diseased rice plant.

MATERIALS AND METHOD

Glassware

Glassware soaked in a cleaning solution (chromic acid) for a few hours and washed in tap water. They were rinsed thoroughly in

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distilled water and dried before use. Glassware used for culture work was sterilized in a hot air oven at 120°C for two hours.

Chemicals

All chemicals used were of analytical grade and dehydrated medium of Hi-media grade. Distilled water was used throughout the study.

Trace Elements

The following chemicals were used as micronutrients (Table 1).

Preparation of Stock Solution

For the preparation of the stock solution [8], the amount of the compound of test nutrients (Zn, B, Mg, Cu) dissolved in 1 liter of double distilled water was estimated by dividing the molecular weight of compound with the molecular weight of the trace elements. One hundred ml. The stock solution of each compound was prepared (Table 2). This stock solution so prepared was of 1000 ppm concentration.

Amount of Trace Elements

The amount of various trace elements were calculated as per standard formula given below:

$$C1 \times V1 = C2 \times V2$$

Where, C1 = Required concentration of test trace elements.

V1 = Required volume of medium.

C2 = Concentration of the trace elements stock solution (1000 ppm).

V2 = Required volume of test trace elements.

Preparation of Nutrient Containing Media

Two hundred ml Czapek Dox devoid of Fe (Cz-Fe) was prepared for each trace elements. The volume of each trace elements to be added was calculated by above formula. The pH of the media was adjusted to 7.0 and then poured in 250 ml Erlenmeyer flask and sterilized.

Table 1: Trace elements and chemical salt

Trace elements	Salt Used	Chemical formula
Zinc (Zn)	Zinc Sulphate	Zn SO ₄ . 7 H ₂ O
Boron (B)	Boric Acid	H ₃ BO ₃
Magnesium (Mg)	Magnesium Sulphate	MgSO ₃ .7 H ₂ O
Copper (Cu)	Cupric Sulphate	CuSO ₄ .5 H ₂ O

Table 2: Stock solution of trace elements of 1000 ppm concentration.

Trace elements	Amount dissolved in 1000 ml. Distilled water (mg)
Zn SO ₄ . 7 H ₂ O	252
H ₃ BO ₃	571
MgSO ₃ .7 H ₂ O	1014
CuSO ₄ .5 H ₂ O	251

Inoculation of Media (Czapek Media)

Pre-sterilized Cz-Fe medium containing different concentration of the test trace elements were poured in sterile Petri-plates and after solidifying each plate was incubated with 5 mm diam. Five replication were made for each concentration of the test trace elements. The plates were incubated up to 96 hours at 27 ± 1°C.

Sterilization

The medium/ distilled water dispensed into Erlenmeyer flask/ test tubes as per requirement and plugged with non-absorbent cotton wool and sterilized under steam in an autoclave at 121°C (15 lb pressure / square inch) for 20 minutes.

The Pathogen

Pyricularia oryzae the causal organism of blast disease of rice isolated from diseased rice plants were used. Sample of blast disease infected plants collected from the Rice field of Korba, Chhattisgarh. Infected portion of stems were cut with a sterile blade into small piece and sterilize with 0.5 % Sodium hypochlorite for 1 minute and then the pieces were washed with sterile distilled water. Two to three washing were done with sterile distilled water to remove excess Sodium hypochlorite and then pieces were transferred aseptically into Petri plates containing PDA medium. The plates were incubated at 27 ± 1°C for 96 hours. After growth of the fungi the pure culture was further transferred to Cz-Fe and incubated as described earlier. Seven days old culture was cut into 5 mm diameter disc with the help of sterile cork borer and used for different experiments.

Storage of Culture

Pyricularia oryzae culture was maintained on Cz-Fe slopes. The tubes were incubated at 27 ± 1°C until they attained full growth. After full growth these cultures were stored in a refrigerator. Transfers were made as and when required by taking a loopful of growth, inoculating a fresh PDA medium.

Observation

Radial growth of the fungus was observed at 24,48 and 72 hours of incubation Simultaneously spore/sclerotia formation and its frequency (few, good, very good in numbers) were also recorded. Five replicates were made for each treatment and proper control i.e. without test trace element was maintained in each case.

RESULTS AND DISCUSSION

Effect of Zinc (Zinc sulphate) on growth and sclerotia formation of *Pyricularia oryzae*

Pre determined quantity of Zinc was supplied in the medium as described in the method 0 (control) 2,5,10,20 ppm along suitable control was maintained. Table 3 shows that maximum

radial growth occurs at 2 ppm concentration which is 24.5, 70.5 and 82.0 mm at 24, 48 and 72 hours of incubation respectively. In other concentrations i.e. 5, 10 and 20 ppm radial growth was less than control. It indicates that Zn is required in very minute quantity i.e. 2 ppm for the optimum growth of the fungus and keeps an adverse effect on growth when provided in more than 2 ppm. Many workers have found that Zn is an essential nutrient required in 0.5 to 1.0 ppm in the medium as an activator for most of the enzymes for many fungi [11-18].

Effect of Boron (Boric Acid) on growth and sclerotia formation of *Pyricularia oryzae*

Pre determined quantity of Zinc was supplied in the medium as described in the method 0 (control) 2,5,10,20 ppm . Table 4 shows that maximum radial growth occurs at 2 ppm concentration which is 34.5, 78.5 and 80.4 at 24, 48 and 72 hours of incubation respectively. Maximum sporulation is also recorded at 2 ppm concentration of Boron. As we increase the concentration of Boron hyphal growth and sporulation both decrease. High concentration i.e. 20 ppm has a more adverse effect of growth and sporulation (Table 4).

Cresswell *et al.* [19] have reported that Boron was more inhibitory than Cu to mycelia growth of *Agaricus biosporus*. The radial growth of pathogenic fungi *Pyricularia oryzae*, *Fusarium oxysporum* and *Aspergillus niger* was reduced by the activity of Boron [20]. Chowdhury [21] observed that the use of Boron reduced the growth of *Macrophomina phaseolina*. Many other workers have also reported that by increasing concentration of Boron we can control the growth of many fungi.

Effect of Magnesium (Magnesium Sulphate) on growth and sclerotia formation of *Pyricularia oryzae*

Table 5 shows how Mg is effecting on the growth of *Pyricularia oryzae*. As we increase the concentration of Mg radial growth of fungus increasing slightly on every observation i.e. 24, 48 and 72 hours. Maximum growth reported on 20 ppm concentration but that is not very much significant than control (0 ppm). However Mg is essential for the proper growth of the fungus. There is a significant increase in radial growth from 24 hours to 48 hours but after that growth is not very much significant. On the other hand sporulation is minimum when no Mg provided, after adding Mg in medium sporulation is increased. Mg is critical for spore production in the fungus. After adding Mg at every concentration i.e. 2,5,10 and 20 sporulation is stable this indicates that Mg in small quantity is essential for high sporulation. Once minute requirement of Mg is fulfilled than increasing Mg concentration is not significant (Table 5).

Hasija [22] also reported that the addition of Magnesium sulphate in the medium produced best growth and sporulation in *Curvularia pallescens*, *Alternaria citri* and *A. tenuis*. Totani *et al.* [23] observed that a small amount of Magnesium sulphate was critical for the growth of some fungi.

Effect of Copper (Cupric Sulphate) on growth and sclerotia formation of *Pyricularia oryzae*

Cu is more effective at the concentration of 2 ppm. When we increased the amount of Cu from control to 2 ppm radial growth and sporulation increased. After that increasing, the concentration of Cu decreases the growth as well as sporulation (Table 6). Cu is needed in very minute quantity, adding

Table 3: Effect of Zinc

Concentration (ppm)	Radial Growth (mm) *			Sclerotia formation at 96 hrs of incubation
	Incubation time (hour)			
	24	48	72	
0	20.4	65.4	75.2	++
2	24.5	70.5	82.0	+++
5	18.5	63.4	70.5	++
10	17.6	60.5	68.4	+
20	16.4	58.0	64.5	+

*Average of 5 replication; one plate constitute one replication, + Less than 5 sclerotia/objective, ++ 6-10 sclerotia/objective, +++ More than 10 sclerotia/objective

Table 4: Effect of Boron

Concentration (ppm)	Radial Growth (mm) *			Sclerotia formation at 96 hrs of incubation
	Incubation time (hour)			
	24	48	72	
0	30.4	75.4	79.4	++
2	34.5	78.5	80.4	+++
5	28.4	70.6	76.5	++
10	27.4	68.2	73.5	++
20	25.4	67.6	70.4	+

*Average of 5 replication; one plate constitute one replication, + Less than 5 sclerotia/objective, ++ 6-10 sclerotia/objective, +++ More than 10 sclerotia/objective

Table 5: Effect of Magnesium

Concentration (ppm)	Radial Growth (mm) *			Sclerotia formation at 96 hrs of incubation
	Incubation time (hour)			
	24	48	72	
0	26.4	74.0	79.0	++
2	27.5	75.4	80.1	+++
5	28.4	76.5	80.2	+++
10	30.2	77.8	80.3	+++
20	32.4	79.4	80.4	+++

*Average of 5 replication; one plate constitute one replication, + Less than 5 sclerotia/objective, ++ 6-10 sclerotia/objective, +++ More than 10 sclerotia/objective

Table 6: Effect of Copper

Concentration (ppm)	Radial Growth (mm) *			Sclerotia formation at 96 hrs of incubation
	Incubation time (hour)			
	24	48	72	
0	22.0	68.5	79.8	++
2	24.8	71.0	81.4	+++
5	21.7	64.5	78.5	++
10	19.2	62.6	75.0	+
20	18.4	58.0	67.6	+

*Average of 5 replication; one plate constitute one replication, + Less than 5 sclerotia/objective, ++ 6-10 sclerotia/objective, +++ More than 10 sclerotia/objective

more Cu we can control the growth of the fungus. Similar to our study, Steinberg [24,25] reported that lack of Cu in the medium reduced the growth of a number of fungi, including *A. niger*, *F. oxysporum*, *Cercospora nicotianae*, *Sclerotium rolfsii*, *Theilaviopsis basicola* and *Pythium irregulare*.

CONCLUSIONS

P. oryzae is one of the most common cause of declining productivity of Rice crop. How to limit the growth of various pathogenic fungi has always been the primary object of agriculture scientists and plant pathologists. The current study provides the idea that we can regulate the growth of the *P. oryzae* by manipulating the concentration of the trace elements. Zn, B, and Cu are effective on very less quantity i.e. 2 ppm. and inhibit growth in higher concentration. Adding a few more quantity of these elements is helpful to reduce the spread of the disease. Whereas, the presence of Mg is sufficient for luxuriant growth and spore production. So, if we completely remove Mg from the supply chain of the nutrients is more beneficial to reduce the spread of blast. It is an elementary study and provide a platform for the future study.

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