QUANTITATIVE SIGNIFICANCE OF TRACE ELEMENTS IRON (Fe), MANGANESE (Mn) AND MOLYBDENUM (Mo) ON GROWTH AND SPORULATION OF *Pyricularia oryzae*

SANDEEP SHUKLA & RENUBALA SHARMA

Department of Botany, Govt. E.V.P.G. College, Korba C.G. – India

ABSTRACT

Pyricularia oryzae is one of the most primary causes to destroy Rice crop in all the Rice producing countries. It causes Blast disease in Rice, other species of *Pyricularia* also infect the different monocot plant. Humidity, temperature, Rainfall and nutrients are the major factors which control the growth and spread of the *Pyricularia oryzae*.

Some nutrients in very small quantities are required for the growth, development, and other physiological and metabolic activities of the fungi. In *P. oryzae* we can control the production of spores and growth by changing the quantity of these trace elements.

In the present study, we tried to understand the effect of three trace elements or micronutrients i.e. Iron (Fe), Manganese (Mn), and Molybdenum (Mo) on spore formation and growth efficiency of *P. oryzae*. 5 ppm (part per million) concentration of Fe is most suitable for optimum spore formation and growth in *Pyricularia oryzae* when we increased the concentration of Fe in the media it poses a negative effect. Mn is required in a little more quantity than Fe. As we increased Mn concentration growth and sporulation increased respectively. On other hand, controlled quantity of Mo is sufficient for growth and sporulation. The increase in a small quantity of Mo in the medium poses a negative effect on *Pyricularia oryzae* growth and spore production. So, by manipulating quantities of these trace elements in the food chain, we can control blast disease.

KEYWORDS: Trace element, *Pyricularia*, Blast disease, Micronutrients

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INTRODUCTION

Pyricularia oryzae is a specific pathogen for Blast disease of the rice. The sexual stage of the fungi is also known as *Magnaporthe oryzae*. Blast is a very common and widely distributed Rice disease, and cause of up to 30 % yield loss worldwide (Skamnioti et al .2009). *Pyricularia oryzae* is a host-specific fungus, many other species of *Pyricularia* infect other monocots.

Rice is the main food item for more than half the population of the world. Most Asian countries including India are the major producer of Rice. A decrease in the production of Rice poses a real threat to starvation.

The blast disease of rice caused by *P. oryzae* affects all aerial parts of the Rice plant. Most of the symptoms are visible on the leaves, a gray or white lesion on the panicles, which turn white and die before the grains are filled (S.C. Scardaci et al. 1997) ^[1]. For the Rice plant *Pyricularia oryzae* is highly specific. The blast disease was recorded more than three centuries ago in Asia and is now reported from more than eighty countries.

About 17 elements are required to meet the nutritional requirements of the fungi (Godbold and Hittermann 1985, Breckle 1991, Nies 1999) [2-4]. Some element which is present in very minute quantity is called trace element. The previous studies conducted by Sharma and Shukla (2020) [5], Foster (1939) [6], Allaway (1965) [7], Lily (1965) [8], Bowen (1966) [9]. Trace elements Zn, B, Mg, Fe, Mn, Cu, Mo, and Ca are essential for the proper development of almost all the fungus, revealed by Thind and Mira Madan (1977). [10]

The current study reveals the role of Fe, Mn, and Mo on the growth and spore development of *Pyriculariaoryzae*.

MATERIALS AND METHOD

Until washing in distilled water, the glassware was soaked in chromic acid for a few hours. For 2 hours the glassware was sterilized at 120°C. As micronutrients, the following chemicals were used (Table 1).

Table 1: Micronutrients and Chemical Salt

Micronutrients	Salt	Chemical formula
Iron (Fe)	Ferrous Sulphate	FeSO ₄ .7 H ₂ O
Manganese (Mn)	Manganese Chloride	MnCl ₂ .4 H ₂ O
Molybdenum (Mo)	Sodium Molybdenum	Na ₂ MoO ₄ . H ₂ O

The amount of the test compound nutrients (Fe, Mn, Mo) were dissolved in 1000ml. of double distilled water for preparation of stock solution. The quantity was determined by dividing the compound's molecular weight by the trace elements molecular weight. For each compound the stock solution was prepared (table 2). This stock solution formulated was concentrated at 1000 ppm.

Table 2: Stock Solution of Trace Elements at a Concentration of 1000.

Trace Elements	Amount Dissolved in 1000 ml. Distilled Water (mg)
FeSO ₄ .7 H ₂ O	497
MnCl ₂ .4 H ₂ O	360
Na ₂ MoO ₄ . H ₂ O	252

The amount of different trace elements was determined according to the standard formula given below-

$$C_1 \times V_1 = C_2 \times V_2$$

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.

Ph of the media was maintained to 7.0. Pre sterilized Cz-Fe medium containing various concentrations of the test micronutrients is poured into sterile petri-plates and each plate was incubated with 5 mm diam. after solidifying. For each concentration of trace element, five replications were made. The plates were incubated up to 96 hours at 27±1°C.

Rice plants infected with blast disease were collected from surrounding areas of Korba, Chhattisgarh. Small pieces from the infected stem were taken and sterilized with 0.5 % Sodium hypochlorite for 1 minute and then washed.

Pieces of infected stem are aseptically transferred into petri plates containing PDA medium. At 27±1°C for 96 hours, the plates were incubated. The pure culture was further transferred to Cz-Fe and incubated after the growth of the fungi. One week old culture was cut into 5 mm diameter disc and used for different experiments.

Same time, spore formation was also reported (few, good, very good in numbers). Five replication were made for each treatment and proper control i.e. without test trace element was maintained in each case.

RESULT AND DISCUSSION

(A) Effect of Iron (Ferrous sulphate) on growth and microsclerotia formation of *Pyricularia oryzae*

Table 3

Concentration (ppm)	Radial Growth (mm) *			Microsclerotia Formation at 96 hrs of Incubation
	Incubation Time (hour)			
	24	48	72	
0	25.6	73.2	80.5	++
2	27.0	74.0	82.6	++
5	28.4	75.2	83.5	++
10	20.8	68.4	78.4	+
20	19.4	61.4	75.2	+

*Average of 5 replication; one plate constitute one replication

+ Less than 5 sclerotia/objective

++ 6-10 sclerotia/objective

+++ More than 10 sclerotia/objective

Between macro and micro essential elements Fe seems to hold an intermediate position. When we supplied different concentrations of Fe into the media, radial growth is slightly increased up to 2 ppm. Maximum radial growth is observed in 5 ppm where radial growth is 28.4 mm in 24 hours, 75.2 mm in 48 hours, and 83.5 mm in 72 hours. After that radial growth is decreased as we increased concentration e.g. 10 and 20 ppm (table 3). Whereas, sporulation is concern it is constant from control to 5 ppm concentration. As we increased concentration of Fe sporulation is decreased. A 50 % reduction in sporulation has been seen when we double the concentration from 5 to 10 ppm. It indicates that very little amount of Fe e.g. 5 ppm is required for radial growth and spore production of *Pyricularia oryzae*. A higher concentration of Fe keeps a lethal effect on the fungus. Many other workers have demonstrated similar results in the case of *Alternaria tenuis*, *Fusarium*, *Colletotrichum capsici* and *Pyricularia oryzae* (Mishra and Mahmood, 1959; Saraswathi Devi 1958; Apparo et al. 1955) [11-13].

(B) Effect of Manganese (Manganese Chloride) on growth and microsclerotia formation of *Pyricularia oryzae*

Table 4

Concentration (ppm)	Radial Growth (mm) *			Microsclerotia Formation at 96 hrs of Incubation
	Incubation Time (hour)			
	24	48	72	
0	24.5	67.4	75.3	++
2	20.4	64.3	74.0	++
5	18.9	62.4	69.5	++
10	25.8	70.0	78.4	+++
20	28.9	72.4	80.5	+++

*Average of 5 replication; one plate constitute one replication

- + Less than 5 sclerotia/objective
- ++ 6-10 sclerotia/objective
- +++ More than 10 sclerotia/objective

The predetermined quantity of Mn as per the calculations mentioned in materials and method was added in the medium. When the concentration of Mn is 2 and 5 ppm, the radial growth is less than the controlled concentration. The lowest radial growth 18.9 mm is reported at 5 ppm (table 4). After this, the radial growth increased dramatically when the concentration was increased. Maximum growth was reported 28.9 in 24 hours, 72.4 in 48 hours, and 80.5 in 72 hours at 20 ppm. It seems that *Pyricularia oryzae* takes some time to get acclimatize to the Mn. After some time it gets adapted and probably produces some enzymes which help in the absorption of the Mn. Fall in the radial growth at 5 ppm is a matter of further investigation. A similar observation also appears in sporulation. As we increased Mn concentration sporulation increased and after 10 ppm it gets steady. Some report says that Mn is required in very small quantity in the medium i.e. 0.005 to 0.01 ppm whereas, in our study we found that *Pyricularia oryzae* required more quantity for its better growth and sporulation. Donald et al. (1952)^[14] and Sulochana and Lakshmanan (1968)^[15] have observed that Mn deficiency reduced 50 % growth of *A. niger*. Jinghua et al. (2004)^[16] observed that the application of Mn along with *Trichoderma* reduced the melon wilt caused by *Fusarium* sp.

(C) Effect of Molybdenum (Sodium Molybdenum) on growth and microsclerotia formation of *Pyricularia oryzae*

Table 5

Concentration (ppm)	Radial Growth (mm) *			Microsclerotia formation at 96 hrs of incubation
	Incubation time (hour)			
	24	48	72	
0	27.0	70.8	82.4	++
2	26.4	69.4	80.2	++
5	25.6	68.4	79.5	++
10	24.3	62.5	76.8	++
20	24.4	60.3	74.2	+

*Average of 5 replication; one plate constitute one replication

- + Less than 5 sclerotia/objective
- ++ 6-10 sclerotia/objective
- +++ More than 10 sclerotia/objective

Mo was added in the form of Sodium molybdate in the medium and radial growth and sclerotia formation were recorded. Maximum growth, as well as sporulation, is reported at controlled conditions i.e. 0 ppm (table 5). After that, as we increased the concentration of Mo radial growth is decreased respectively at all the incubation period. Sporulation is minimum at 20 ppm. It shows that a very minute quantity of the Mo is sufficient for the growth and spore development of the *Pyricularia oryzae*. A small change in concentration of the Mo poses a negative effect on growth and sporulation. Adding a small amount of Mo in the medium will minimize the growth of *Pyricularia oryzae*. There are reports that *A. Niger* and *Neurospora crassa* require more Mo otherwise, for most of the fungi its requirement is very minute (Steinberg, 1936)^[17].

CONCLUSIONS

The primary goal of the agriculture scientist and plant pathologist is to minimize the spread of various diseases caused by fungi. One of the most common cause of declining Rice crop productivity is *P. oryzae*. By manipulating the concentration of these trace elements, we can control the growth of *P. oryzae*. An increase in concentration of Fe and Mo is beneficial to minimize the spread of the disease. Whereas, Removal of the Mn from the food chain reduces the growth and spread of the blast disease. This is an elementary work which provides a platform for further research.

PHOTOGRAPH

Photograph showing blast disease on leaves of the Rice plants.

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