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EFFECT OF CERTAIN MACRO-MICRO NUTRIENTS ON *HELMINTHOSPORIUM ORYZAE* BREDA DE HAAN

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Abstract

This paper explained about the effect of certain macro-micro nutrients *viz.,* calcium sulphate, copper sulphate, ferrous sulphate, magnesium sulphate, manganese sulphate, potassium sulphate and zinc sulphate on the growth of *H. oryzae* was studied by incorporating the nutrients separately in Czapek's (Dox) medium. All the chemicals were tested at 1000, 2000 and 3000 ppm conc. The results revealed that Among the various macro-micro nutrients, $ZnSO_4$ @ 3000 ppm recorded the maximum inhibition of mycelial growth, mycelial dry weight and spore germination. Also, Silicon based nutrients Potassium Silicate, Calcium Silicate and Sodium Silicate, the silicon based macro- micro nutrient, potassium silicate @ 3000 ppm recorded the maximum inhibition of mycelial dry weight.

Key words : Macro-Micro nutrient, Silicon based nutrient, Mycelial growth and Mycelial dry weight

Introduction

Rice (*Oryza sativa* L.) is the second most cultivated crop worldwide and it has been estimated that half the world's population survives wholly or partially on this crop (Van Nguyen and Ferrero, 2006) and rice provides more calories per ha than any other cereal food grains. Rice crop is widely affected by a number of diseases caused by fungi, bacteria, viruses and mycoplasma which results in considerable yield losses (Ou, 1985). Among the various fungal diseases of rice, brown spot or sesame leaf spot incited by *Helminthosporium oryzae* (Breda de Haan) Subram. and Jain (Syn: *Bipolaris oryzae* (Breda de Haan) Shoemaker) is found to occur in most rice growing areas. The disease is also referred as fungal blight or Helminthosporiose. The fungus *H. oryzae* causes characteristic leaf spot on all the susceptible varieties and the pathogen is capable of infecting the rice plant at all stages of its growth. Spots are formed on the blade and sheath of the leaf. They vary in shape and size.

The pathogen affects the nodes of the culm and the base of the panicle also (Ou, 1985). On susceptible varieties the spots are much larger and may reach one cm or more in length. Sometimes numerous spots occur and as a result the leaf withers. Also, concentric lines or zones on the spot have been observed occasionally (Sato, 1965). Also this pathogen is responsible for the grain discolouration in rice (Savary *et al.,* 2000). The possible management practices of brown spot disease are the use of fungicides, growing resistant varieties, biological agents and resistance inducing chemicals.

The brown spot disease is very common in poorly managed soils and occurs in high proportions under stress conditions due to nutrients and water. Soil and foliar application of sulphur based nutrients has proved to increase resistance against a variety of fungal pathogens on different crops (Wang *et al*., 2003; Klikocka *et al*., 2005). Also, application of zinc fertilizer in Indian soil conditions increased the resistance to diseases, better seed viability and seedling vigour, abiotic stress tolerance and crop yield (Cakmak*,* 2009). Several authors have reported the use of zinc sulphate for the management of rice diseases (Ramabadran and Velazhagan, 1988; Reddy *et al.,* 2000; Singh *et al.,* 2009). Besides, a promising alternative for the control for many rice diseases, including brown spot, is the application of silicon (Si) to soils deficient in this element (Datnoff *et al*., 2007). In recent years, silicon (Si) is being used for the control of fungal diseases with promising results (Yanar *et al.,* 2011) and silicon accumulation has been reported to be one of the main factors responsible for enhanced resistance against various pathogens of rice (Junior *et al*., 2009). In this context balanced nutrition seems to be a promising alternative for the control of brown spot (Carvalho *et al.,* 2010). The paper deals with the certain macro-micro nutrients and silicon based nutrients on mycelial growth, dry weight of *Helminthosporium oryzae*.

Materials and Methods

In vitro studies - Effect of Certain macro- micro Nutrients on *H. oryzae*

Mycelial Dry Weight and Mycelial Growth

Effect of certain macro-micro nutrients *viz.,* calcium sulphate, copper sulphate, ferrous sulphate, magnesium sulphate, manganese sulphate, potassium sulphate and zinc sulphate on the growth of *H. oryzae* was studied by incorporating the nutrients separately in Czapek's (Dox) medium. All the chemicals were tested at 1000, 2000 and 3000 ppm conc. (Ragavan, 2003).

Fifty ml of the Czapek's (Dox) broth with respective conc. of macro-micro nutrients was dispensed in 250 ml Erlenmeyer flasks and autoclaved. Each flask was inoculated aseptically with nine mm fungal disc obtained from the actively growing region of a week old culture of *H. oryzae*. The flasks were incubated at room temp. (28±3^{o}C) for 15 days. After incubation the mycelial mats were filtered through a Buchner funnel under suction using a filter paper of known weight. The fungal growth retained on the filter paper was dried until constant weight and the dry weight of the mycelium was recorded as mg/50ml broth. With regard to mycelial growth, 15 ml of Czapek's agar medium with respective macro-micro nutrients was poured into sterile Petri plates. The medium without any amendment served as control. Each plate was inoculated aseptically with nine mm fungal disc obtained from the actively growing region of a week old culture of *H. oryzae*. The plates were incubated at room temperature (28±3^{o}C) and the diameter of the mycelial growth was recorded when the growth in the control plates touched the periphery and was expressed in mm.

Spore Germination

Effect of calcium sulphate, copper sulphate, ferrous sulphate, magnesium sulphate, potassium sulphate, manganese sulphate and zinc sulphate on the spore germination of *H. oryzae* was studied using cavity slide method. All the chemicals were tested at 1000, 2000 and 3000 ppm conc.

The spore suspension of the *H.oryzae* with adequate cfu *viz*., 50,000 spores/ml was prepared from seven days of old culture. The suspension containing spores and mycelium was centrifuged at 2100 rpm to remove the mycelium. 0.1 ml of spore suspension was added into cavity slide and 0.1 ml of suitable macro-micro nutrient solution at different conc. were added in cavity slide. Three replications were maintained for each treatments and the cavities without any nutrient served as control. Carbendazim 50 WP at 0.1% was used as the standard fungicide for comparison. The slides were incubated in Petri plates containing moist cotton at the bottom with to provide sufficient humidity. The spore germination was recorded after 24 h. of incubation.

Effect of Silicon Based macro-micro Nutrients on *H.oryzae*

Effect of silicon based nutrients *viz.,* potassium silicate, calcium silicate and sodium silicate on the growth of *H. oryzae* was studied by incorporating separately in Czapek's (Dox) broth. All the chemicals were tested at 1000, 2000 and 3000 ppm conc. The observation on mycelial dry weight was recorded as mentioned earlier.

Results and Discussion

Effect of Certain macro – micro Nutrients on *H. oryzae*

Mycelial Growth and Mycelial Dry Weight

The study revealed that all the nutrients tested significantly inhibited the growth and dry weight of the fungus at all conc. when compared to control. Also, enhanced inhibition was noticed with increase in conc. of the nutrients tested. Among them, zinc sulphate recorded maximum mycelial inhibition at 3000 ppm conc. with 83.3 per cent inhibition over control. It was followed by potassium sulphate, calcium sulphate, copper sulphate, ferrous sulphate, magnesium sulphate and manganese sulphate in the decreasing order of merit. With regard to mycelial dry weight, zinc sulphate recorded maximum growth inhibition (54 mg) at 3000 ppm conc. as against 335.0 mg recorded in control. It was followed by potassium sulphate which recorded 79.0 mg/ 50 ml of broth. There was great inhibitory effect while increasing conc. in both mycelial growth and dry weight of fungus. Complete inhibition of mycelial growth and dry weight of *H.oryzae* was observed with Carbendazim (0.1 %) (Table 1 and Table 2).

Addition of Ca, Mn, Cu, Zn, Fe, B and Mo at different conc. against rice pathogens showed reduced fungal growth (Madhiyazhagan, 1989). Inhibitory effect of calcium sulphate against rice pathogens under *in vitro* was also reported (Eswaran and Narayanasamy, 2000; Ragavan, 2003). As observed in the present study they also reported increased inhibition with increase in conc. of $ZnSO_4$.

Spore Germination

In general the results showed that an increase in the conc. of nutrients resulted in increased inhibition of spore germination percentage. However, the maximum per cent inhibition of spore germination was recorded at 3000 ppm conc. of zinc sulphate with 85.39 per cent reduction followed by 83.14, 79.77, 78.65, 76.40, 73.03

and 69.66 per cent with potassium sulphate, calcium sulphate, copper sulphate, ferrous sulphate, magnesium sulphate and manganese sulphate, respectively. Also, the comparison fungicide Carbendazim (0.1 %) recorded complete inhibition of spore germination of *H. oryzae* (Fig. 1).

The results of the present study revealed that certain micro nutrients had a significant effect on growth as well as sporulation of *H. oryzae* which tallies with the observations of Mass (1976) with *Phytophthora fregariae*. The stimulatory effect of "Ca and Mg" for sporangium production was also documented (Allen and Nandra, 1975). Iron, zinc, molybdenum and copper were inhibitory to growth as well as sporulation of fungal pathogens (Kennedy and Erwin, 1961; Ragavan, 2003; Vengadesh Kumar, 2005). These earlier reports corroborates with the present findings.

Effect of Silicon Based Nutrients on *H. oryzae*

Mycelial Dry Weight

The study revealed that all the silicon based nutrients significantly inhibited the dry weight of the fungus at all conc. when compared to control. Among the silicon based nutrients tested, potassium silicate recorded maximum mycelial dry weight with a value of 62 mg at 3000 ppm conc. with 82.08 per cent inhibition over control. It was followed by calcium silicate which recorded 86 mg as against 346.0 mg in control (Fig. 2). Complete inhibition of mycelial growth of *H. oryzae* was observed with test fungicide Carbendazim (0.1 %).

The *in vitro* efficacy of silicon based nutrients against various pathogens has been reported. Li *et al.* (2009) observed that silicate salts directly inhibited the *in vitro* spore germination and mycelial growth of *Fusarium sulphureum*. Bi *et al.* (2006), found that 100-mM sodium silicate completely inhibited the mycelial growth of *Alternaria alternata*, *F. semitectum* and *Trichothecium roseum*. Similar results were also observed with *Penicillium expansum* and *Monilinia fructicola* (Biggs *et al.,* 1997; Qin and Tian 2005), *Colletotrichum* spp. (Biggs, 1999) and *Botryosphaeria dothidea* (Biggs, 2004). The present findings are in agreement with these earlier reports.

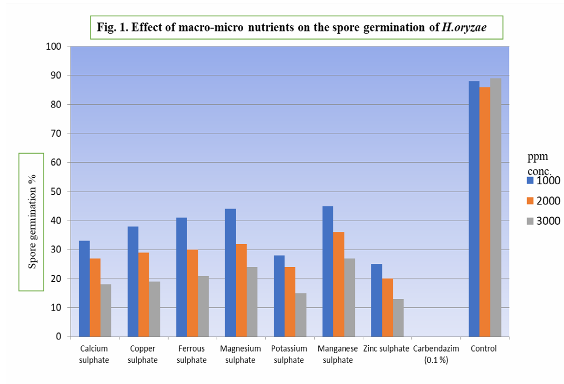

Fig. 1. Effect of macro-micro nutrients on the spore germination of *H.oryzae*

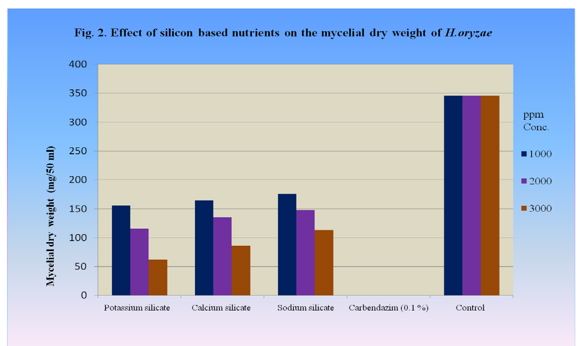

Fig. 2. Effect of silicon based nutrients on the mycelial dry weight of *H.oryzae*

Table 1 : Effect of Macro-micro nutrients on the mycelial growth of *H.oryzae*

Sl. No.	Macro-micro nutrients	Mycelial growth (mm)			Per cent inhibition (%)		
		1000 ppm	2000 ppm	3000 ppm	1000 ppm	2000 ppm	3000 ppm
1.	Calcium sulphate	51	39	26	43.3	56.6	71.1
2.	Copper sulphate	56	44	28	37.7	51.1	68.8
3.	Ferrous sulphate	59	47	31	34.4	47.7	65.5
4.	Magnesium sulphate	60	46	34	33.3	48.8	62.2
5.	Potassium sulphate	47	34	23	47.7	62.2	74.4
6.	Manganese sulphate	61	52	35	32.2	42.2	61.1
7.	Zinc sulphate	42	28	15	53.3	68.8	83.3
8.	Carbendazim (0.1 %)		0			100	
9.	Control	90	90	90	---	---	---
C.D. (p=0.05)		2.19	2.50	2.75	---	---	---

Table 2 : Effect of Macro-micro nutrients on the mycelial dry weight of *H.oryzae*

Sl. No.	Macro-micro nutrients	Mycelial dry weight(mg/50 ml)			Per cent inhibition (%)		
		1000 ppm	2000 ppm	3000 ppm	1000 ppm	2000 ppm	3000 ppm
1.	Calcium sulphate	161	125	94	51.94	62.68	72.53
2.	Copper sulphate	176	148	113	47.46	55.82	66.26
3.	Ferrous sulphate	193	160	129	42.38	52.23	61.49
4.	Magnesium sulphate	205	179	143	38.80	46.56	57.31
5.	Potassium sulphate	148	108	79	55.82	67.76	76.41
6.	Manganese sulphate	218	192	158	34.92	42.68	52.83
7.	Zinc sulphate	132	97	54	60.59	71.04	83.88
8.	Carbendazim (0.1 %)	0			100		
9.	Control	335	335	335	---	---	---
C.D. (p=0.05)		5.19	6.29	7.19	---	---	---

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