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Studies on factors affecting *Rhizoctonia bataticola* : II. Micronutrients

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Out of seven micronutrients tested at two different concentrations against isolates of *Rhizoctonia bataticola* following poisoned food technique in Asthana and Hawker's medium, cobaltous chloride was most effective in affecting both mycelial growth and sclerotial characters followed by copper sulphate, ferrous sulphate, zinc sulphate, borax, manganese chloride and ammonium molybadate. At higher concentration the effect was more pronounced than lower. Different micronutrients showed different types of effects on mycelial growth and sclerotial characters. Deformed, very minute, poorly melanized and coalesced sclerotia were observed in the treatment of cobaltous chloride, manganese chloride and ammonium molybdate. Differential sensitivity of isolates was recorded in different micronutrients which indicates strain difference.

Key words : *Rhizoctonia bataticola*, micronutrients, growth, sclerotial morphology

INTRODUCTION

The importance of balanced nutrition (macro and micronutrients) to disease resistance in traditionally and generally accepted fact. Still it is often ignored in disease management. The micronutrients are considered as essential metallic elements both for plants and fungi due to their structural and functional role. It may be assumed that many of these metallic ions activate enzyme system, while others are integral part of enzymes and other essential organic compounds. Micronutrients in adequate amount not only enhance the growth of plant but also growth of fungi, but the doses of micronutrients which are favourable for plant growth and nutrition is generally unfavourable for fungal growth. Detailed studies with micronutrients have been carried out by Lilly and Barnett (1951), Graham and Webb (1991) and Nema and Sharma (1999). In the present investigation the effect of seven micronutrients on growth and sclerotial morphology of isolates of *Rhizoctonia bataticola* have been studied.

MATERIAL AND METHODS

The seven isolates of *R. bataticola* (Jha, 2004) were maintained on potato dextrose agar medium. Seven micronutrients viz., Cu (Copper sulphate pentahydrate $CuSO_45H_2O$), Fe (Ferrous sulphate heptahydrate : $FeSO_4$ $7H_2O$), Zn (Zinc sulphate heptahydrate : $ZnSO_47H_2O$), Co (Cobaltous chloride : $CoCl_2$ $6H_2O$), B (Borax : Na_2 B_4 O_7 $10H_2O$), Mo (Ammonium molybdate : $(NH_4)_6$ Mo_7 $O_{24}4H_2O$) and Mn (Manganese chloride : $MnCl_2$) were tested against isolates by employing poisoned food technique on Asthana and Hawker's medium. The salt of nutrients incorporated separately into the medium, so as to get 0.05 and 0.1 per cent concentrations for ferrous sulphate, cobaltous chloride, ammonium molybadate and manganese chloride, where as 0.025 and 0.1 ; 0.05 and 0.15 ; 0.1 and 0.15 per cent concentrations for cupric sulphate, zinc sulphate and borax, respectively. The medium without any micronutrients served as control. The amended medium was then poured into sterilized petri-plates in triplicate for each isolate. Poured petri-plates were inoculated with 8 mm discs from the margin of seven days old cultures of different isolates of *R. bataticola* and incubated at 29 ± 1°C temperature. Observations for growth and morphological parameters were recorded on 3rd and 5th day of incubation.

RESULTS AND DISCUSSION

The effects of different micronutrients on mycelial growth and sclerotial characters of different isolates were found different (Figs. 1 & 2 ; Table 1). All the micronutrients at some extent inhibited growth and other morphological characters at both the concentrations, but inhibition was observed more pronounced on higher concentration than lower concentration. Similar observations were reported

Table 1 : Effect of micronutrients on the morphological characters of different isolates of *R. bataticola*

Micro-nutrient	Isolate	Conc. (%)	Colony	Hyphae		Sclerotia		
			Pattern / Margin	Pattern / Colour	L × W (μ)	Size / Shape	Pattern / Initiation	Colour
Control	Rb1	Nil	Appr./Even	Dn/LB to B	107.99 × 92.08	Md/R to O	Dn / Ely	DB
	Rb2		Flocc./Wavy	Dn/LB to B	116.46 × 93.09	Md/R to O	Dn / Ely	DB
	Rb3		Flocc./Wavy	Dn/B	87.44 × 77.38	Small/R to O	Dn / Ely	DB
	Rb4		Appr./Even	Sp/B	142.35 × 114.73	Large/R to O	Dn / Ely	Bl
	Rb5		Flocc./Even	Dn/LB	94.41 × 77.51	Md/R to O	Dn / Ely	DB
	Rb6		Appr./Even	Dn/B	119.34 × 109.11	Md/Irre	Dn / Ely	DB
	Rb7		Flocc./Wavy	Dn/B	91.00 × 74.43	Md/R to O	Sp / Ely	DB
Copper sulphate ($CuSO_4.5H_2O$)	Rb1	0.025	Appr./Irre	Dn/B	83.43 × 71.25	Small/R to O	Dn / Dly	Bl
	Rb2		Flocc./Wavy	Dn/B	107.18 × 89.53	Md/R to O	Dn / Dly	DB
	Rb3		Flocc./Irre	Dn/B	76.25 × 64.06	Small/R to O	Sp / Ely	DB
	Rb4		Appr./Irre	Dn/B	109.38 × 86.25	Md/R to O	Dn / Ely	DB
	Rb5		Flocc./Even	Dn/B	55.90 × 46.90	Small/R to O	Dn / Dly	DB
	Rb6		Appr./Even	Dn/B	82.62 × 71.87	Small/Irre	Dn / Ely	DB
	Rb7		Flocc./Irre	Dn/B	91.87 × 65.31	Small/R to O	Sp / Dly	DB
	Rb1	0.1	—	—	—	—	—	—
	Rb2		—	—	—	—	—	—
	Rb3		Flocc./Irre	Dn/B	76.61 × 61.51	Small/R to O	Sp / Ely	DB
	Rb4		—	—	—	—	—	—
	Rb5		Flocc./Irre	Dn/B		No Sclerotia		
	Rb6		Appr./Even	Sp/B	73.43 × 65.62	Small/Irre	Dn / Ely	DB
	Rb7		Flocc./Irre	Dn/B	62.18 × 56.25	Small/Irre	Sp / Dly	DB
Ferrous sulphate ($FeSO_4.7H_2O$)	Rb1	0.05	Appr./Irre	Dn/LB	113.75 × 88.12	Md/R to O	Sp / Dly	B
	Rb2		Flocc./Even	Dn/LB	103.43 × 90.00	Md/R to O	Dn / Dly	DB
	Rb3		Flocc./Irre	Dn/B	58.75 × 49.38	Small/R to O	Sp / Ely	Bl
	Rb4		Appr./Abrupt	Sp/B	108.75 × 80.78	Md/E	Dn / Dly	Bl
	Rb5		Flocc./Irre	Dn/B	99.06 × 83.13	Md/R to O	Sp / Ely	Bl
	Rb6		Appr./Wavy	Sp/B	92.66 × 79.06	Md/R to O	Dn / Dly	DB
	Rb7		Flocc./Irre	Dn/H to LB	84.37 × 70.62	Small/Irre	Dn / Dly	B
	Rb1	0.1	—	—	—	—	—	—
	Rb2		Flocc./Irre	Dn/LB	104.37 × 84.23	Md/R to O	Dn / Dly	DB
	Rb3		Flocc./Irre	Dn/LB	58.12 × 52.81	Small/R to O	Sp / Dly	B
	Rb4		Appr./Abrupt	Sp/B	108.75 × 78.13	Md/E	Dn / Dly	DB
	Rb5		Flocc./Irre	Sp/LB	42.65 × 36.56	V. small/R to O	Sp / Ely	DB
	Rb6		Appr./Abrupt	Sp/B	93.18 × 74.68	Md/Irre	Dn / Dly	Bl
	Rb7		Flocc./Wavy	Dn/H to LB	84.37 × 70.62	Small/Irre	Sp / Dly	B
Zinc sulphate ($ZnSO_4.7H_2O$)	Rb1	0.05	Appr./Irre	Dn/LB	126.21 × 94.90	Large/Irre	Dn / Ely	DB
	Rb2		Appr./Irre	Dn/B	110.33 × 95.05	Md/R to O	Dn / Ely	DB
	Rb3		Flocc./Irre	Dn/LB	68.58 × 59.47	Small/R to O	Sp / Ely	B
	Rb4		Appr./Abrupt	Dn/B	156.32 × 92.62	Large/E	Dn / Ely	DB
	Rb5		Flocc./Irre	Dn/L Bl	69.88 × 63.05	Small/R to O	Sp / Ely	Bl
	Rb6		Appr./Irre	Dn/B	101.24 × 83.20	Md/Irre	Dn / Ely	DB
	Rb7		Flocc./Irre	Dn/B	86.13 × 76.38	Small/Irre	Dn / Ely	DB
	Rb1	0.15	Appr./Irre	Dn/B	146.52 × 96.20	Large/Irre	Dn / Dly	DB
	Rb2		Appr./Irre	Sp/B	114.73 × 92.62	Md/Irre	Dn / Dly	Bl
	Rb3		Flocc./Irre	Dn/B	56.88 × 52.81	Small/R to O	Sp / Ely	DB
	Rb4		Appr./Abrupt	Dn/B	133.90 × 93.60	Large/E	Dn / Ely	Bl
	Rb5		Flocc./Abrupt	Dn/B	80.92 × 72.15	Small/Irre	Dn / Ely	Bl
	Rb6		Appr./Abrupt	Dn/B	82.68 × 68.25	Small/Irre	Dn / Ely	DB
	Rb7		Flocc./Irre	Dn/B	92.62 × 76.37	Small/Irre	Dn / Ely	Bl

Cobaltous chloride ($CoCl_2.6H_2O$)	Rb1		Appr./Irre	Sp/B	66.16 × 51.46	Small/Irre	Dn(c)* / Ely	DB
	Rb2		Appr./Irre	Dn/B	67.32 × 54.16	Small/R to O	Sp / Dly	Bl
	Rb3		Flocc./Abrupt	Sp/H	69.12 × 54.22	Small/R to O	Sp / Dly	B
	Rb4	0.05	Appr./Abrupt	Sp/B	83.12 × 72.48	Small/Irre	Sp / Dly	Bl
	Rb5		—	—	—	—	Sp / Ely	—
	Rb6		Appr./Irre	Dn/B	72.47 × 60.74	Small/Irre	—	DB
	Rb7		—	—	—	—	Sp / Ely	—
	Rb1		—	—	—	—	Sp / Ely	—
	Rb2		Appr./Abrupt	Sp/H		No sclerotia		
	Rb3		—	—	—	—	—	—
	Rb4	0.1	Appr./Abrupt	Sp/B	78.84 × 66.82	Small/Irre	Dn(c)* / Ely	Bl
	Rb5			—	—	—	—	—
	Rb6			—	—	—	—	—
	Rb7			—	—	—	—	—
Borax ($Na_2B_4O_7.10H_2O$)	Rb1		Appr./Irre	Dn/B	84.29 × 74.14	Small/R to O	Dn / Ely	DB
	Rb2		Flocc./Wavy	Dn/B	100.54 × 85.65	Md/R to O	Dn / Ely	Bl
	Rb3		Flocc./Wavy	Sp/L Bl	51.18 × 44.68	Small/R to O	Sp / Ely	Bl
	Rb4	0.1	Appr./Irre	Dn/B	101.90 × 89.03	Md/Irre	Dn / Ely	DB
	Rb5		Flocc./Wavy	Dn/B	74.50 × 69.46	Small/R to O	Sp / Ely	Bl
	Rb6		Appr./Irre	Dn/B	71.43 × 67.37	Small/R to O	Dn / Ely	Bl
	Rb7		Flocc./Irre	Dn/LB	62.29 × 54.50	Small/R to O	Dn / Dly	DB
	Rb1		Flocc./Irre	Sp/B	90.59 × 81.65	Md/R to O	Dn / Ely	Bl
	Rb2		Flocc./Abrupt	Dn/H. to LB	85.87 × 70.28	Small/R to O	Sp / Dly	Bl
	Rb3		Flocc./Abrupt	Dn/LB	50.25 × 46.37	Small/R to O	Sp / Ely	Bl
	Rb4	0.15	Appr./Irre	Sp/B	113.30 × 97.09	Md/Irre	Dn / Ely	Bl
	Rb5		Flocc./Irre	Dn/H to LB	79.62 × 68.25	Small/R to O	Sp / Dly	Bl
	Rb6		Appr./Irre	Dn/B	75.46 × 68.29	Small/R to O	Dn / Ely	Bl
	Rb7		Flocc./Irre	Dn/H to LB	(c)	—	Dn(c) / Dly	Bl
Ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$]	Rb1		Appr./Irre	Sp/LB	61.14 × 53.01	Small/R to O	Dn / Ely	B
	Rb2		Appr./Irre	Dn/H to LB	53.39 × 46.51	Small/R to O	Dn / Ely	B
	Rb3		Flocc./Irre	Dn/B		No sclerotia		
	Rb4	0.05	Appr./Irre	Dn/LB	64.18 × 50.37	Small/D	Dn / Ely	B
	Rb5		Flocc./Irre	Sp/B	66.87 × 61.18	Small/R to O	Sp / Ely	Bl
	Rb6		Appr./Irre	Sp/B	49.56 × 42.25	V. Small/D	Dn / Ely	B
	Rb7		Flocc./Irre	Dn/LB	50.18 × 42.50	Small/Irre	Dn / Ely	DB
	Rb1		Appr./Abrupt	Dn/LB	49.56 × 45.50	V. small/R to O	Dn / Dly	LB
	Rb2		Appr./Irre	Sp/H to LB	52.17 × 41.90	Small/R to O	Dn / Ely	LB
	Rb3		Flocc./Irre	Dn/LB		No sclerotia		
	Rb4	0.1	Appr./Irre	Sp/H to LB	60.93 × 53.64	Small/D	Dn / Ely	B
	Rb5		Flocc./Irre	Sp/H to LB	49.15 × 43.87	V. small/R to O	Sp / Dly	Bl
	Rb6		Appr./Irre	Sp/H to LB	43.87 × 39.00	V. small/D	Dn / Ely	B
	Rb7		Flocc./Irre	Dn/H to LB	42.37 × 40.25	V. small/R to O	Sp / Dly	B
Manganese chloride ($MnCl_2$)	Rb1		Appr./Irre	Dn/B	85.71 × 71.50	Small/Irre	Dn(c)* / Ely	Bl
	Rb2		Flocc./Irre	Dn/B	104.00 × 83.68	Small/R to O	Dn / Ely	Bl
	Rb3		Flocc./Irre	Dn/LB	64.59 × 56.87	Small/R to O	Sp / Ely	DB
	Rb4	0.05	Appr./Irre	Sp/B	112.13 × 87.34	Md/Irre	Sp / Ely	Bl
	Rb5		Flocc./Irre	Dn/H to LB	80.43 × 66.82	Small/Irre	Sp / Ely	Bl
	Rb6		Appr./Even	Dn/B	83.68 × 67.84	Small/Irre	Dn / Ely	Bl
	Rb7		Flocc./Irre	Dn/B	73.93 × 68.25	Small/Irre	Sp / Ely	Bl
	Rb1		Appr./Abrupt	Sp/B	(c)	—	Dn(c) / Dly	Bl
	Rb2		Appr./Abrupt	Sp/B	(c)	—	Dn(c) / Ely	Bl
	Rb3		Flocc./Irre	Sp/H to LB	63.45 × 54.16	Small/R to O	Sp / Ely	DB
	Rb4	0.1	Appr./Irre	Sp/B	(c)	—	Dn(c) / Ely	Bl
	Rb5		Flocc./Irre	Dn/LB	67.32 × 53.39	V. small/R to O	Dn / Ely	Bl
	Rb6		Appr./Even	Dn/B	(c)	—	Dn(c) / Dly	Bl
	Rb7		Flocc./Irre	Dn/B	(c)	—	Dn(c) / Ely	Bl

Appr. — Appressed, Flocc. — Floccose, D — Deformed, Irre — Irregular, Dn — Dense, Sp — Sparse, B — Brown, DB — Dark Brown, Bl — Black, LB — Light Brown, LBl — Light Black, Md — Medium, R — Round, O — Oval, E — Elongated, Ely — Early, Dly — Delayed, H — Hyaline, V — Very, (C)* — Coalescence present : individual sclerotia has the identity, (c) — Several sclerotia coalesced : individual sclerotia lost the identity.

by Murugesan and Mahadevan (1987, 1988) and Gupta (1998, 1999). Cobaltous chloride was most inhibitory, it not only inhibited mycelial growth drastically but also caused greater sclerotial deformity at both the concentrations. At higher concentration of cobalt no sclerotia were observed in isolates Rb1, Rb3, Rb5, Rb6 and Rb7.

In isolates Rb1, Rb2 and Rb4 at 0.1 per cent concentration of copper sulphate and isolate Rb1 at 0.1 per cent concentration of ferrous sulphate, least mycelial growth and pronounced effect on sclerotial characters were observed.

Manganese chloride and ammonium molybadate have more drastic effect on sclerotial characters than mycelial growth. The decreasing order of effect of micronutrients on both of their concentrations on isolates were cobaltous chloride followed by copper sulphate, ferrous sulphate, zinc sulphate, borax, manganese chloride and ammonium molybadate. Differential sensitivity in different micronutrient was observed, it clearly indicates strain difference, which caused differential physiological actions in isolates. General reasons for mycelial growth inhibition and sclerotial characters differences may be direct toxicity of the micronutrients to the isolates because the requirement of micronutrients for fungus is much less [Zn (0.001 to 0.5 ppm), Cu (0.01-0.1 ppm), Fe (0.1-0.3 ppm), Mn (0.005-0.01 ppm) and Mo (0.1 ppb-10ppb)] than plants.

Higher concentration than the requirement of the fungus causes toxicity (Cochrane, 1965 ; Sankhla and Mathur, 1967 ; Daftri, 1966). Copper is a toxic heavy metal with great affinity for nitrogenous organic legands including proteins. Its ability to denature proteins may be fundamental to its direct toxicity to fungi. Due to interaction between

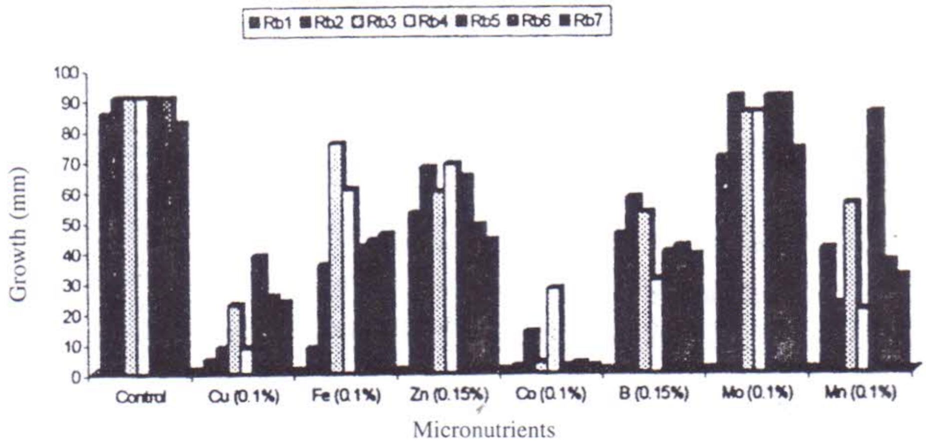

Fig. 1 : Effect of micronutrients on the growth of different isolates of *R. bataticola*

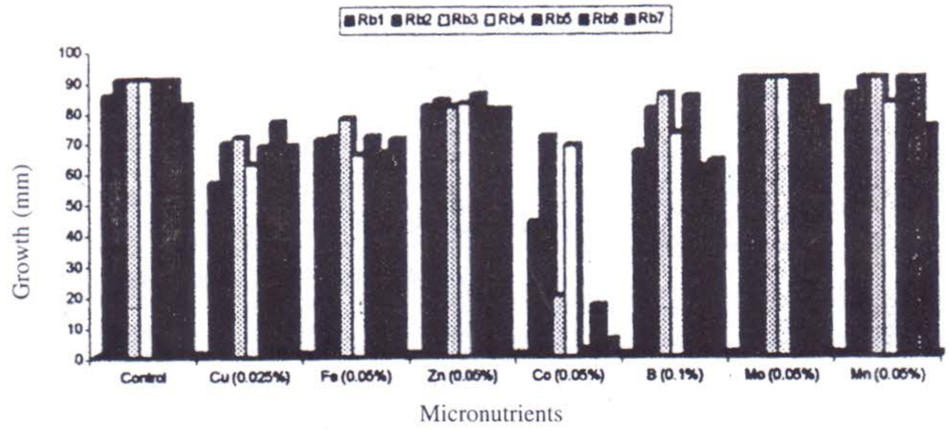

Fig. 2 : Effect of micronutrients on the growth of different isolates of *R. bataticola*

micronutrients by which one micronutrient depletes the amount of other and make it unavilable to the fungi, which may result in deficiency.

Toxic effect of micronutrients on fungus can be further supported with the following examples (Graham and Webb. 1991).

High soil Mn concentrations controlled the inoculum potential of *Verticillium albo-atrum* by inhibiting sclerotial production. *Fusarium* wilt of cotton decreased from 80 to 43% when the soil was pretreated with $ZnSO_4$. Infection of foot rot of soybean (*Sclerotium rolfsii*) was decreased at high rates (0.5%) of $ZnSO_4$ application. Boron has also been shown to reduce disease such as *Rhizoctonia solani* in mungbean, pea and cowpea and *R. bataticola* in groundnut. Molybdenum had a direct effect by reducing the production of the Roridin E, a toxin produced by *Myrothecium roridum* and also reported to suppress *Verticillium* wilt of tomato.

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