J. Indian bot. Soc., 61 : 47—50, 1982.

TRACE ELEMENT REQUIREMENT FOR THE GROWTH AND SCLEROTIA PRODUCTION OF *RHIZOCTONIA BATATICOLA* (TAUB) BUTLER, CAUSING ROOT ROT OF GRAM (*CICER ARIETINUM* L.)[1]

M. K. GOYAL[2] AND R. S. MEHROTRA

Department of Botany, Kurukshetra University, Kurukshetra

ABSTRACT

The trace element requirements of *Rhizoctonia bataticola*, causal organism of root rot of gram (*Cicer arietinum* L.) was studied. Out of fifteen elements, viz., Fe, Zn, Mn, Cu, Mo, Ca, B, Co, W, Li, Hg, Ni, Cd, I and Br tried, the essentiality of only five could be demonstrated. It was found that Iron, Zinc, Manganese, Copper and Calcium are required for the growth of this fungus at their optimum concentrations as follows : Fe : 0.1—1.0 ppm, Zn : 1.0—10.0 ppm ; Mn : 1 ppm ; Cu : 0.01 ppm and Ca : 0.01—0.1 ppm. The concentrations higher than the optimum of these essential trace-elements were found to inhibit the growth of the fungus progressively.

INTRODUCTION

The fungi usually exhibit degree of specificity in utilizing various nutritional substances for their growth and sporulation. The history of investigation on the mineral nutrition of fungi has been reviewed by Perlman (1949), Foster (1939), Steinberg (1939) and Nicholas (1963). Micro nutrients or trace elements are required in very small amount for the growth of fungi. They are important in the nutrition of fungi because they are indispensable for fungal growth and reproduction. A review of literature reveals that Fe, Zn, Mn and Cu are essential for the growth of majority of fungi investigated so far and Mo and Ca for the growth of a few. There are some reports of essentiality of a few trace elements such as Vanadium for the growth of *Aspergillus niger* (Bertrand ,1947) ; Boron for the growth of *Fusarium* spp. (Yogeshwari, 1948), *Alternaria burnsii* (Sankhla *et al.*, 1970), *Aschochyta caulicola* (Chahal, 1977) ; Cobalt for the growth of *Ramulispora sacchari* (Rawla and Chahal, 1975) and Scandium for the growth of *Aspergillus niger* (Steinberg, 1939a) and *Cercospora granati* (Chahal and Rawla, 1977). Present investigations were undertaken to establish the essentiality of various trace-elements for the growth of *Rhizoctonia bataticola* causal organism of root rot of gram (*Cicer arietinum* L.).

MATERIALS AND METHODS

Gram plants suffering from the root rot disease caused by *R. bataticola* were collected from Kurukshetra and its adjoining areas. The pathogen was isolated by the usual micro-biological techniques and its pathogenicity was established.

1. Accepted for publication on February 28, 1981.

2. Present address : Biology Division, Forensic Science Lab., Madhuban (Haryana).

Most virulent isolate was employed for further studies.

Glassware of Pyrex or Corning brand and chemicals of Analytical Reagent grade were used. The contamination of trace elements from different sources (such as glassware, polyethylene ware, water, chemicals and inoculum) were removed by the method described by Thind and Rawla (1967). The basal medium consisted of glucose 20 g, KNO_3 2.5 g, KH_2PO_4 5 g., $MgSO_4.7H_2O$ 0.5 g and distilled water 1000 ml. Medium was sterilized at 10 lb/sq. inch of steam pressure for 20 minutes and was adjusted to an optimum pH of 5.5 after sterilization. The salts of fifteen trace elements were tested to know their essentiality and the optimum concentrations of essential elements. Fifty ml of the basal medium were added to each 250 ml Erlenmeyer conical flask. Three replicates were taken for each treatment. Stock solution of each micro-nutrient was made in pure water. Micro elements were added to the purified basal medium eliminating one micro-element from each combination. The concentrations employed of these elements were 0.1 ppm each of Fe, Zn, Mn, Cu and 0.01 ppm each of all the rest trace elements. Flasks were inoculated with a 4 mm disc of *R. bataticola* (grown on trace-element deficient medium). Two controls were kept (i) with no trace elements added and (ii) with all trace elements added. Flasks were incubated at 30°C for 12 days and filtered through previously weighed Whatman filter Paper No. 1, and dried at 60°C in an hot air oven to a constant weight. Average dry weight of three replicates along with final pH of the culture filtrate are recorded in Tables I and II.

TABLE I

ESSENTIALITY OF TRACE ELEMENTS FOR THE GROWTH AND SCLEROTIAL PRODUCTION OF *Rhizoctonia bataticola*

Trace elements omitted from basal medium	Salts	Concentration used (ppm)	Average dry wt.*	Sclerotial production	Final pH	Essentiality
All (control I)	..		32	—	4.0	..
None (control II)	..	..	180	++++	8.2	..
Fe	$FeSO_4.7H_2O$	0.1	46	—	4.0	+
Zn	$ZnSO_4.7H_2O$	0.1	57	+	5.1	+
Mn	$MnCl_2.4H_2O$	0.1	64	—	4.9	+
Cu	$CuSO_4.5H_2O$..	0.1	96	+	5.0	+
Mo	$(NH_4)_6MoO_{24}.4H_2O$	0.1	200	++	8.2	—
Ca	$CaCl_2.2H_2O$	0.01	120	++++	7.8	+
B	H_3BO_3 ..	0.01	193	++++	8.0	—
Co	$CoCl_2.6H_2O$	0.01	190	++	7.2	—
W	$Na_2WO_42H_2O$	0.01	179	++++	8.1	—
Li	Li_2SO_4 ..	0.01	192	+++	7.9	—
Hg	$HgCl_2$ ..	0.01	188	+++	7.2	—
Ni	$NiSO_4.H_2O$	0.01	182	++	7.8	—
Cd	$CdSO_4.8H_2O$..	0.01	181	++++	7.7	—
I	KI ..	0.01	189	++++	8.1	—
Br	KBr ..	0.01	176	++++	7.5	—

*Average of three replicates.
+=Poor, ++=fair, +++=Good, ++++=Excellent.

TABLE II

AVERAGE DRY WEIGHT* (MG) OF *Rhizoctonia bataticola* WITH DIFFERENT CONCENTRATIONS OF ESSENTIAL ELEMENTS AT 30°C, pH 5.5 AND 12 DAYS OF INCUBATION

Different trace element concentration (ppm)	Fe	Zn	Mn	Cu	Ca
No trace element	32	32	32	32	32
0.0001	75	62	42	54	40
0.0001	96	68	53	67	58
0.001	105	103	95	72	93,
0.01	140	142	131	177	180
0.1	187	155	166	105	185
1.0	186	191	188	85	152
10.0	167	189	165	46	136
100.0	130	124	134	24	110

*Average of three replicates.

RESULTS AND DISCUSSION

Study of Table I reveals that Fe, Zn, Mn, Cu and Ca were found to be essential for the growth of *R. bataticola*, whereas the requirement for the rest of the trace elements could not be demonstrated. It is further evident from Table II that the optimum concentrations of essential elements for the growth of the fungus were as follows : Fe : 0.1-1.0 ppm ; Zn : 1.0-10.0 ppm ; Mn : 1.0 ppm ; Cu : 0.01 ppm and Ca : 0.01-0.1 ppm. The concentrations higher than the optimum of these essential elements inhibited the growth progressively. Elimination of Fe and Mn did not support the formation of sclerotia at all while poor production was observed with the elimination of Zn and Cu.

The requirement of Fe, Zn, Mn and Cu for its growth is similar to majority of fungi investigated so far. These elements being integral parts of various metalloenzymes, play an important role in the metabolism of fungi (Nicholas, 1963). In addition *R. bataticola* requires Calcium also for its growth. In this respect it resembles *Rhizoctonia solani* (Young and Bernett, 1922). Copper is required in exceedingly small amounts for the growth of fungi and higher concentrations reduced the growth. *R. bataticola* requires 0.01 ppm concentration of Copper while higher concentrations reduced its growth and sclerotial proeuction. Similar results with Cu were also obtained by Daftari (1966) with *R. bataticola* and *R. solani*.

REFERENCES

BERTRAND, D. 1941. Le Vanadium Comme facteur de Croissance pour, *Aspergillus niger*. *Bull. Soc. Chem. Biol.* **23** : 467.

CHAHAL, S. S. 1977. Trace elements for the growth of *Ascochyta caulicola*. *Indian Phytopath.* **30**: 344-346.

CHAHAL, S. S. AND G. S. RAWLA 1977. Trace element requirement for the growth of *Cercospora granati*. *Indian. Phytopath.* **30** : 47-50.

DAFTARI, L. N. 1966. Effect of trace elements on RHIZOCTONIA spp. *Indian. Phytopath.* **19**: 118-119.

FOSTER, J. W. 1939. The heavy metal nutrition of fungi. *Bot. Rev.* **5** : 207-239.

LILLY, V. G. AND L. H. LEONIAN 1945. The inter-

relationship of iron and certain factors in the growth of *Rhizobium trifolii* strain 205. *J. Bact.* **50** : 383-395.

NICHOLAS, D. J. D. 1963. Inorganic nutrient nutrition of micro-organisms, pp. 363-447 in F. C. Steward (Ed.) *Plant Physiol* Vol. *III. Inorganic Nutrition of Plants.* Acad. Press, New York and London.

Perlman, D. 1949. Effects of minor elements on the physiology of fungi. *Bot. Rev.* **15** : 195-200.

RAWLA, G.S. AND S. S. CHAHAL 1975. Comparative trace-element and organic growth factor requirements of *Ramulispora sacchari* and *R. sorghi. Trans. Brit. mycol. Soc.* **64** : 532-536.

Steinberg, R. A. 1939a. Growth of fungi in synthetic nutrient solution. *Bot. Rev.* **5** : 327-350.

Steinberg, R. A. 1939b. Growth of fungi in synthetic nutrient solution. *Bot. Rev.* **5** : 327-350.

Steinberg, R. A. 1939c. Relation of carbon nutrition to trace element and accessory requirements of *Aspergillus niger. J. agric. Res.* **59** : 749.

THIND, K. S. AND G. S. RAWLA 1967. Trace element studies on six species of *Helminthosporium. Proc. Ind. Acad. Sci.* **66** : 250-265.

YOGESHWARI, L. 1948. Trace element nutrition of fungi I: The effect of boron, zinc and managanese on *Fusarium* species, *Proc. Ind. Acad. Sci.* **28** : 177.

YOUNG, H. C. AND C. W. BERNETT 1922. Growth of some parasitic fungi in synthetic culture media. *Amer. J. Bot.* **9** : 459-469.