EFFECT OF VARIOUS TRACE ELEMENTS ON THE GROWTH AND SPORULATION OF *CLAVICEPS MICROCEPHALA* AND *MICROXYPHIELLA HIBISCIFOLIA*

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Abstract

Out of fifteen trace elements tested, Fe, Zn, Mn and Cu were found to be essential for the growth as well as sporulation of *Claviceps microcephala* and *Microxyphiella hibiscifolia*; Mo for *C. microcephala* but not for *M. hibiscifolia*; Ca for *M. hibiscifolia* but not for *C. microcephala*. No other trace element was found to be essential for either of these fungi. Optimum concentrations in ppm of essential trace elements were found to be as follows : *C. microcephala*, Fe 0·2, Zn 0·1, Mn 1·0, Cu 0·01 (0·01–0·1) and Mo 1·0; *M. hibiscifolia*, Fe 0·01 (0·01–0·1), Zn 10·0, Mn 1·0, Cu 10·0 and Ca 10·0. Concentrations higher than the optimum were progressively inhibitory to the respective fungi.

Introduction

Fe, Zn, Mn and Cu have been found to be essential for the growth of most of the fungi studied up-to-date, while Mo and Ca are known to be essential for the growth of some fungi. However, there are isolated reports for the essentiality of more trace elements for some fungi but these have not been adequately substantiated as yet by other workers. These include W and Cb for *Penicillium javanicum* (Lockwood *et. al.*, 1934), Ga for *Aspergillus niger* (Steinberg, 1938), V for *A. niger* (Bertrand, 1941), B for *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* (Yogeswari, 1948), Ur for *Alternaria tenuis* (Grewal, 1956), and Co for *Gloeosporium psidii* (Tandon, 1961).

The effect of various carbon and nitrogen sources on the growth and sporulation of representative isolates of *C. microcephala* and *M. hibiscifolia* are already reported by the authors (Thind and Madan, 1973 *a*, *b*). This paper deals with the trace elements requirements of the same isolates. No such study has been carried out previously on these pathogens.

Material and Methods

The study on trace elements requirements of *C. microcephala* and *M. hibiscifolia* was carried out with liquid basal medium of the following composition : dextrose 20 g, asparagine 3·740 g, KH_2PO_4 10 g, $MgSO_4 \cdot 7H_2O$ 0·250 g, and pure water* 1,000 ml. *C. microcephala* was grown at temperature 24° C, pH 6·0 and incubation period 20 days, while *M. hibiscifolia* at temperature 28° C, pH 4·0 and incubation period 14 days. These conditions were found to be optimum for these fungi as already reported by the authors (Thind and Madan, 1973 *a*, *b*).

All the chemicals used were of B.D.H. Analar or E. Merck G.R. grade. The following procedures were adopted to remove the trace elements impurities as far as possible from the glassware, water, chemicals and inoculum.

Pyrex glassware.—Rinsed out with hot acid dichromate solution, then steamed with dilute acid dichromate solution in the autoclave for half an hour, washed thoroughly with tap-water, rinsed out with hot boiling distilled water and boiling solution of disodium salt of EDTA (Ethylene diamine tetra acetic acid disodium salt 1 g dissolved per litre pure water), washed twice with pure cold water, then with hot boiling pure water and again with pure cold water and finally dried at 60° C in hot air oven before use.

Water.—Copper distilled water was passed through a column of ion-exchange resins (Amberlite CG 50, De-Acidite E, Zeo-Karb 226, De-Acidite FF, Zeo-Karb 225, Amberlite 1RA 400, Zeo-Karb 215 and Bio-Deminrolit resin) in an ascending manner and collected at the rate of 8–10 drops per minute and finally distilled thrice in an all Pyrex glass still containing 0·5 g/l w/v. EDTA. The water obtained by this method was referred to as *PURE WATER* which gave negative test with chloroform dithizone (Stout and Arnon, 1939).

Dextrose and asparagine.—Stock solutions of these were passed separately through a column of ion-exchange resins (Zeo-Karb 225, De-Acidite FF, Bio-Deminrolit and mixed bed of cation and anion resins) in an ascending manner at the rate of 8–10 drops per minute.

Rest of the basal medium.—Following the procedure of Steinberg (1935) rest of the components of the basal medium (KH_2PO_4, $MgSO_4.7H_2O$) were

* (Obtained by the procedure given under water).

dissolved in pure water and then autoclaved together with $CaCO_3$ (15 g/l) for half an hour to remove the trace elements impurities from them.

Mycelial inoculum.—The trace elements impurities from the inoculum were minimized by making 2–3 successive transfers of the mycelial growth into a liquid basal medium from which the trace elements were removed by the above procedures.

The rest of the material and methods were the same as for the carbon and nitrogen nutrition of these fungi already reported by the authors (Thind and Madan, 1973 *a*, *b*).

EXPERIMENTAL WORK AND RESULTS

Fifteen trace elements were tested to find out their essentiality for the growth and sporulation of *C. microcephala* and *M. hibiscifolia*. They were used in the form of following salts: $Fe(NO_3)_3 \cdot 9H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6 Mo_7 O_{24}.4H_2O$, $CaCl_2.2H_2O$, $(CH_3COO)_2 Pb.3H_2O$, KBr, KI, $K_2Cr_2O_7$, H_3BO_3, $HgCl_2$, $Na_2WO_4.2H_2O$, $Li_2SO_4.H_2O$, $3CdSO_4.8H_2O$. The amounts of various salts were so adjusted as to provide the following quantities (in mg) of the trace elements in one litre of the basal medium : Fe 0·2, Zn 0·1, Cu 0·04, Ca 1·0 and the remaining 11 trace elements 0·02 each.

The trace elements impurities were removed from the basal medium as discussed under Material and Methods. The basal medium without any trace elements was divided into 15 different lots and in each lot (three replicates) were added all the trace elements except one. Two controls were kept in each case, one with no trace element and second with all the trace elements in it. Each flask was inoculated with 1 ml standardized mycelial suspension (8–16 mycelial bits, mostly 150–200 μ long, per low power field of the compound microscope) in the case of each fungus. Rest of the procedure was as usual.

The data presented in Table I shows that *C. microcephala* made suppressed growth as well as sporulation with the omission of Fe, Zn, Mn, Cu and Mo, while *M. hibiscifolia* made suppressed growth as well as sporulation with the omission of Fe, Zn, Mn, Cu and Ca. The omission of remaining trace elements had no adverse effect on the growth as well as sporulation of these pathogens. There was no significant change in the final pH of the various media as a result of growth of each fungus.

TABLE I

Effect of omission of different trace elements, omitted singly, from the basal medium on the growth and sporulation of C. microcephala *and* M. hibiscifolia *at their respective optimum temperature, incubation period and initial pH*

Element omitted	*C. microcephala*			*M. hibiscifolia*		
	Dry wt in mg	Sporulation	Final pH	Dry wt in mg	Sporulation	Final pH
All	49	..	6·0	15	..	3·6
None	302	++++	6·4	185	++++	4·6
Fe	69	++	6·0	49	++	3·6
Zn	89	++	6·2	40	++	3·6
Mn	98	++	6·2	56	++	3·6
Cu	100	++	6·2	52	++	3·6
Mo	109	++	6·2	190	++++	4·6
Ca	301	++++	6·4	80	++	3·6
Pb	300	++++	6·4	181	++++	4·6
Br	308	++++	6·4	190	++++	4·6
I	304	++++	6·4	180	++++	4·6
Cr	304	++++	6·4	182	++++	4·6
B	300	++++	6·4	181	++++	4·6
Hg	300	++++	6·4	183	++++	4·6
W	307	++++	6·4	185	++++	4·6
Li	309	++++	6·4	185	++++	4·6
Cd	309	++++	6·4	180	++++	4·6

Effect of different concentrations of essential trace elements on the growth and sporulation of two fungi.—Six experiments were conducted to find out the optimum and toxic concentrations of trace elements found to be essential for the growth and sporulation of two fungi. Range of concentrations used

was : 0·0001 to 400 ppm of Fe, Zn, Mn, Cu and Mo; and 0·0001 to 1000 ppm of Ca. The optimum amount of an essential trace element as found out in the first or previous experiments was used in later experiments. In each experiment each lot of three flasks contained the varying amounts of one essential trace elements *plus* the optimum (or otherwise) amounts of other essential trace elements.

The data presented in Tables II–VII show that there is always increase in growth with an increase in concentrations of trace elements up to a certain

TABLE II

Effect of different concentrations of Fe on the growth and sporulation of C. microcephala *and* M. hibiscifolia *at their respective optimum temperature, incubation period and initial pH*

Fe concentration in ppm added to the basal medium	*C. microcephala*			*M. hibiscifolia*		
	Dry wt in mg	Sporulation	Final pH	Dry wt in mg	Sporulation	Final pH
000	63	++	6·0	48	++	3·6
0·0001	81	++	6·0	97	+++	4·2
0·001	121	++	6·1	127	++++	4·4
0·01	193	+++	6·2	195	++++	4·6
0·1	235	++++	6·4	189	++++	4·6
0·2	300	++++	6·4	180	++++	4·6
1·0	260	++++	6·3	163	++++	4·6
10·0	197	+++	6·1	150	++++	4·5
100·0	54	..	6·0	107	++	4·4
200·0	0	..	6·0	90	++	4·3
400·0	0	..	6·0	54	+	4·2

optimum level, which is different for two fungi, after which the growth falls progressively. Optimum concentrations in ppm of the essential trace elements for these fungi are as follows : Fe 0·2 for *C. microcephala*, 0·01 (0·01–

0·1) for *M. hibiscifolia* (Table II); Zn 0·1 for *C. microcephala*, 10·0 for *M. hibiscifolia* (Table III); Mn 1·0 for *C. microcephala* and *M. hibiscifolia* (Table IV); Cu 0·01 (0·01–0·1) for *C. microcephala*, 10·0 for *M. hibiscifolia* (Table V); Mo 1·0 for *C. microcephala* (Table VI); and Ca 10·0 for *M. hibiscifolia* (Table VII). Concentrations higher than the optimum are always progressively inhibitory to the growth of both the unugi. No marked change in final pH is observed at all concentrations of essential trace elements in case of both the fungi.

TABLE III

Effect of different concentrations of Zn on the growth and sporulation of C. microcephala *and* M. hibiscifolia *at their respective optimum temperature, incubation period and initial pH*

Zn concentration in ppm added to the basal medium	*C. microcephala*			*M. hibiscifolia*		
	Dry wt in mg	Sporulation	Final pH	Dry wt in mg	Sporulation	Final pH
000	89	++	6·2	40	++	3·6
0·0001	149	++	6·2	51	++	4·0
0·001	199	+++	6·2	64	++	4·0
0·01	248	++++	6·3	99	++	4·0
0·1	324	++++	6·4	160	++++	4·2
1·0	300	++++	6·4	189	++++	4·4
10·0	200	++++	6·3	220	++++	4·6
100·0	93	..	6·0	203	++++	4·6
200·0	0	..	6·0	59	..	4·2
400·0	0	..	6·0	0	..	4·0

DISCUSSION

Fe, Zn, Mn and Cu are found to be essential for the growth of *C. microcephala* and *M. hibiscifolia* studied here. These elements are reported to be essential for the growth of fungi, which have been critically studied so far,

TABLE IV

Effect of different concentrations of Mn on the growth and sporulation of C. microcephala *and* M. hibiscifolia *at their respective optimum temperature, incubation period and initial pH*

Mn concentration tin ppm added to he basal medium	*C. microcephala* Dry wt in mg	Sporulation	Final pH	*M. hibiscifolia* Dry wt in mg	Sporulation	Final pH
000	79	++	6·2	69	++	3·6
0·0001	151	+++	6·2	95	+++	4·0
0·001	206	++++	6·3	109	++++	4·1
0·01	280	++++	6·3	132	++++	4·4
0·1	308	++++	6·4	207	++++	4·6
1·0	336	++++	6·4	223	++++	4·6
10·0	286	++++	6·3	200	++++	4·6
100·0	202	+++	6·1	186	++++	4·5
200·0	158	++	6·0	150	+++	4·4
400·0	97	..	6·0	120	+++	4·0

TABLE V

Effect of different concentrations of Cu on the growth and sporulation of C. microcephala *and* M. hibiscifolia *at their respective optimum temperature, incubation period and initial pH*

Cu concentration in ppm added to the basal medium	*C. microcephala* Dry wt in mg	Sporulation	Final pH	*M. hibiscifolia* Dry wt in mg	Sporulation	Final pH
000	100	++	6·2	69	++	4·0
0·0001	200	++++	6·2	105	+++	4·1
0·001	280	++++	6·3	126	+++	4·2
0·01	330	++++	6·4	189	++++	4·4
0·1	320	++++	6·2	206	++++	4·6
1·0	300	++++	6·2	229	++++	4·8
10·0	290	++++	6·0	259	++++	4·8
100·0	210	++	6·0	239	++++	4·6
200·0	29	..	6·0	175	++++	4·6
400·0	0	..	6·0	130	+++	4·2

TABLE VI

Effect of different concentrations of Mo on the growth and sporulation of C. microcephala *at its optimum temperature, incubation period and initial pH*

Mo concentration in ppm added to the basal medium	Dry wt in mg	Sporulation	Final pH
000	100	++	6·0
0·0001	193	+++	6·2
0·001	215	+++	6·2
0·01	243	++++	6·2
0·1	326	++++	6·4
1·0	340	++++	6·4
10·0	301	++++	6·4
100·0	285	++++	6·1
200·0	270	+++	6·0
400·0	60	+	6·0

TABLE VII

Effect of different concentrations of Ca on the growth and sporulation of M. hibiscifolia *at its optimum temperature, incubation period and initial pH*

Ca concentration in ppm added to the basal medium	Dry wt in mg	Sporulation	Final pH
000	97	++	3·8
0·0001	131	++++	4·5
0·001	173	++++	4·5
0·01	203	++++	4·6
0·1	225	++++	4·8
1·0	252	++++	4·8
10·0	270	++++	4·8
100·0	230	++++	4·6
200·0	201	++++	4·5
400·0	178	++++	4·4
600·0	150	+++	4·2
800·0	106	++	4·0
1000·0	28	..	4·0

Mo is essential for the growth of *C. microcephala* and in this respect it resembles *A. niger* (Steinberg, 1937; Nicholas, 1952), *Fusarium oxysporum*, *Rhizoctonia solani*, *Cercospora nicotianae*, *Sclerotium rolfsii*, *Pythium irregulare* and *Thielaviopsis basicola* (Steinberg, 1950), *Helminthosporium sacchari* and *H. oryzae* (Thind and Rawla, 1967), *Monochaetia* sp. and *Cercospora crotalariae* (Thind and Mandahar, 1968) and *Pleospora indica* (Mandahar, 1971). However, Mo is not found to be essential for the growth of *M. hibiscifolia*. Similarly, it is reported not to be essential for 7 *Helminthosporium* spp. (Peterson and Katznelson, 1956; Thind and Rawla, 1967) and *Pestalotia theae*, *Cercospora withaniae* and *C. hibiscina* (Thind and Mandahar, 1968).

Ca is essential for the growth of *M. hibiscifolia* and in this respect it resembles *R. solani*, *S. rolfsii*, *C. nicotianae*, *P. irregulare* and *T. basicola* (Steinberg, 1948; 1950), *A. tenuis* (Grewal, 1956), *Cladochytrium replicatum* (Willoughby, 1962), *H. sacchari* and *H. oryzae* (Thind and Rawla, 1967). However, it is not found to be essential for the growth of *C. microcephala*. Similarly, Ca is reported not to be essential for 7 *Helminthosporium* spp. (Peterson and Katznelson, 1956; Thind and Rawla, 1967) and *Cercospora* spp., *Monochaetia* sp. and *P. theae* (Thind and Mandahar, 1968).

Fungi differ in their optimum requirements of essential trace elements for their maximum growth. *C. microcephala* requires 0·2 ppm Fe for its maximum growth and thus resembles *C. hibiscina* (Thind and Mandahar, 1968). *M. hibiscifolia* requires 0·01 ppm Fe for its maximum growth and, therefore, resembles *Helminthosporium teres* (Thind and Rawla, 1967) and *C. crotalariae* (Thind and Mandahar, 1968). However, these differ from *H. oryzae* which requires 0·001 ppm and *H. sacchari* which requires 1·0 ppm (Thind and Rawla, 1967) and *P. theae* which requires 10·0 ppm Fe (Thind and Mandahar, 1968) for their maximum growth.

C. microcephala requires 0·1 ppm Zn for its maximum growth and thus it resembles *Monochaetia* sp. and *P. theae* (Thind and Mandahar, 1968). *M. hibiscifolia* requires 10·0 ppm Zn for its maximum growth and thus resembles *Helminthosporium avenae*, *H. teres*, *H. oryzae* and *H. sacchari* (Thind and Rawla, 1967), *C. crotalariae* (Thind and Mandahar, 1968) and *Phymatotrichum omnivorum* (Blank, 1941). However, these fungi differ from *Helminthosporium sativum* which requires 0·0001 ppm (Thind and Rawla, 1967) and *C. withaniae* which requires 1·0 ppm Zn (Thind and Mandahar, 1968) for their maximum growth.

C. microcephala and *M. hibiscifolia* both require 1·0 ppm Mn for their maximum growth and thus resemble *Helminthosporium turcicum* (Thind and Rawla, 1967). However, these differ from *P. omnivorum* (Blank, 1941) and *C. withaniae* (Thind and Mandahar, 1968), which require 10·0 ppm M_n for their maximum growth.

C. microcephala requires 0·01 ppm Cu for its maximum growth and, therefore, resembles *H. sacchari* (Thind and Rawla, 1967) and *C. hibiscina* and *P. theae* (Thind and Mandahar, 1968). However, *M. hibiscifolia* requires 10·0 ppm Cu for its maximum growth. The optimum concentration of Cu for fungi in general lies below 1·0 ppm. *M. hibiscifolia*, therefore is rather unique among fungi because 10·0 ppm Cu is highly toxic for most of the fungi investigated so far.

C. microcephala requires 1·0 ppm Mo for its maximum growth and differs from other fungi in this respect, such as *H. sacchari* which requires 0·0001 ppm (Thind and Rawla, 1967), *Monochaetia* sp. which requires 0·01 ppm (Thind and Mandahar, 1968), *F. oxysporum* which requires 0·06 ppm, *R. solani*, *C. nicotianae* and *P. irregulare* which require 0·04 ppm and *T. basicola* which requires 0·02 ppm Mo (Steinberg, 1950) for their maximum growth.

M. hibiscifolia requires 10·0 ppm Ca for its maximum growth and differs from other fungi in this respect, such as *C. replicatum* which requires 20 ppm (Willoughby, 1962), *Phytophthora fragariae*, which requires 20–50 ppm (Devies, 1959) and *H. sacchari* which requires 250 ppm and *H. oryzae* which requires 750 ppm Ca (Thind and Rawla, 1967) for their maximum growth.

Concentrations of essential trace elements higher than the optimum have been found to be inhibitory for the growth of *C. microcephala* and *M. hibiscifolia*. This is true also of other fungi studied from this point of view (Thind and Rawla, 1967; Thind and Mandahar, 1968).

C. microcephala and *M. hibiscifolia* show excellent sporulation only when all the essential trace elements are present in the basal medium. Similarly, Tandon and Chandra (1962) reported that Fe, Zn, Cu and Mn are essential fort he normal sporulation of *Cercosporina ricinella*, *Colletotirchum gloeo sporioides* and *Curvularia penniseti*. Bhatnagar and Prasad (1968) also recorded that two isolates (F_1 and F_2) of *Fusarium solani* f. *aurantifoliae* show excellent sporulation when the medium is supplemented with a combination of Fe, Zn and Mn.

REFERENCES

*Bertrand, D. .. "Le Vanadium comme facteur de croissance pour L' *Aspergillus niger*," *Bull. Soc. Chim. Biol.*, 1941, **23**, 467–71.

Bhatnagar, G. C. and Prasad, N. "Effect of micro nutrients on the growth and sporulation of *Fusarium solani* f. *aurantifoliae* Bhat. and Prasad," *Proc. Indian Acad. Sci.*, 1968, **68 B**, 169–74.

Blank, L. M. .. "Response of *Phymatotrichum omnivorum* to certain trace elements," *Jour. Agric. Res.*, 1941, **62**, 129–59.

Davies, L. N. .. "The nutrition of *Phytophthora fragariae*," *Trans. Brit. Mycol. Soc.*, 1959, **42**, 193–200.

Grewal, J. S. .. "Effect of Trace Elements on Growth and Sporulation of *Alternaria tenuis*," *Lloydia*, 1956, **19**, 188–91.

*Lockwood, L. B., Ward, G. E., May, O. E., Herrick, H. T. and O'Neill, H. T. "The production of fat by *Penicillium javanicum* von Beijma," *Zentr. Bakt. Parasitenk Abstract* 11, 1934, **90**, 411–25.

Mandahar, C. L. .. "Micronutrient studies on *Pleospora indica*," *Indian Phytopath.*, 1971, **24**, 26–31.

Nicholas, D. J. D. .. "The use of fungi for determining trace metals in biological materials," *Analyst*, 1952, **77**, 629–41.

Peterson, E. A. and Katznelson, H. .. "The effect of trace elements on growth of *Helminthosporium sativum* and several related species," *Canad. Jour. Microbiol.*, 1956, **2**, 441–46.

Steinberg, R. A. .. "Nutrient solution purification for removal of heavy metals in deficiency investigations with *Aspergillus niger*," *Jour. Agric. Res.*, 1935, **51**, 413–24.

——— .. "Role of molybdenum in the utilisation of ammonium and nitrate-nitrogen of *Aspergillus niger*," *ibid.*, 1937, **55**, 891–902.

——— .. "The essentiality of gallium to growth and reproduction of *Aspergillus niger*," *Ibid.*, 1938, **57**, 569–74.

——— .. "Essentiality of calcium, in the nutrition of fungi," *Science*, 1948, **107**, 423.

——— .. "Growth on synthetic nutrient solutions of some fungi pathogenic to tobacco," *Amer. Jour. Bot.*, 1950, **37**, 711–14.

Stout, P. R. and Arnon, D. I. "Experimental methods for the study of the role of copper, manganese and zinc in the nutrition of higher plants," *Ibid.*, 1939, **26**, 144–49.

Tandon, R. N. .. "Physiological studies on some pathogenic fungi," *Uttar Pradesh Scientific Research Committee Monographs, Allahabad, India*, 1961.

Tandon, R. N. and Chandra, S. — "Trace Element Nutrition of some fungi causing Leaf Spot Disease," *Lloydia*, 1962, **25**, 130–36.

Thind, K. S. and Madan, M. .. "Effect of various carbon and nitrogen sources on the growth and sporulation of *Claviceps microcephala*," *Proc. Indian Acad. Sci.*, 1973 *a* (in press).

———— .. "Effect of various carbon and nitrogen sources on the growth and sporulation of *Microxyphiella hibiscifolia*," *Ibid.*, 1973 b (in press).

———— and Mandahar, C. L. .. "Trace element studies on some pathogenic fungi," *Ibid.*, 1966, **68 B**, 37–51.

———— ———— and Rawla, G. S. .. "Trace elements studies on six species of *Helminthosporium*," *Ibid.*, 1967, **66 B**, 250–65.

Willoughby, L. G. .. "The fruiting behaviour and nutrition of *Cladochytrium replicatum*," *Ann. Bot.*, 1962, **26**, 13–37.

Yogeswari, L. .. "Trace element nutrition of Fungi I. The effect of boron, zinc and manganese on *Fusarium* species," *Proc. Indian Acad. Sci.*, 1948, **28 B**, 177–201.

* Originals not consulted.