TRACE ELEMENT STUDIES ON SOME PATHOGENIC FUNGI

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Received March 19, 1968

Abstract

Trace element studies were carried out on five pathogenic fungi—*Cercospora hibiscina* Ellis and Everh., *C. withaniae* H. and P. Syd., *C. crotalariae* Sacc., *Monochaetia* sp., and *Pestalotia theae* Sawada. The trace element contaminants from glassware, basal medium, water, glucose and inoculum were removed by various usual means. In addition, EDTA was used for the removal of trace elements from glassware and water. Out of the 15 trace elements tested, Fe, Zn, Cu and Mn were found to be essential for the growth of these five fungi while Mo was found essential only for the growth of *C. crotalariae* and *Monochaetia* sp. No other trace element was found to be essential for any of these fungi. Optimum concentrations in ppm of essential trace elements for these fungi were found to be as follows: *C. hibiscina*: Fe 0·2, Zn 0·01-0·1, Cu 0·01 and Mn 1·0; *C. withaniae*: Fe 1·0, Zn 1·0, Cu 0·1 and Mn 10·0; *C. crotalariae*: Fe 0·01, Zn 10·0, Cu 0·001, Mn 0·1, and Mo 0·001; *Monochaetia* sp. : Fe 1·0, Zn 0·1, Cu 0·1, Mn 0·1 and Mo 0·01; and *Pestalotia theae* : Fe 10·0, Zn 0·1, Cu 0·01 and Mn 0·01. Concentrations higher than the optimum were inhibitory to the respective fungi.

Introduction

Raulin (1869) observed an increase in growth of *Aspergillus niger* on addition of Fe and Zn to basal medium, concluding thereby that these trace elements were indispensable to growth of the fungus. Later other workers considered these elements as mere stimulants rather than being essential (Pfeffer, 1895 ; Richards, 1899). However, Steinberg (1919), after removing trace element impurities from glassware, chemicals and inoculum, proved conclusively that Fe and Zn were essential trace elements for *A. niger* and that these did not act as stimulants. He also found out in later researches that Cu, Mn and Mo were also essential for this fungus (Steinberg, 1935, 1936, 1937). Similarly other workers found these five trace elements to be essential for the growth of other fungi (Niethammer, 1938; Rogers, 1938; Blank, 1941;

Bertrand, 1941; Robbins and Hervey, 1944, 1965 ; Ezekiel, 1945 ; Yoge wari, 1948 ; Perlman, 1948 ; Steinberg, 1948, 1950 ; Jarvis and Johnso 1950 ; Hofmann *et al.*, 1950 ; Machlis, 1953 ; Purdy and Grogan, 1954 Painter, 1954 ; Grimm and Allen, 1954 ; Sadasivan and Subramania 1954 ; Ingraham and Emerson, 1954 ; English and Barnard, 1955 ; Grewa 1956 ; Peterson and Katznelson, 1956 ; Saraswathi, 1958 ; Agarwa 1959 ; Davies, 1959 ; Tandon, 1961 ; Willoughby, 1962 ; Mathur a Sankhla, 1965 ; Barnett and Lilly, 1966 ; Daftari, 1966 ; Thind and Rawl 1967). But comparatively only fewer number of fungi have been invest gated so far with regard to their trace element requirements and the opt mum concentrations needed.

An extensive study has been initiated by the senior author in this lab ratory to investigate trace element requirements of a large number of fung As a result of this study, Thind and Rawla (1967), have already reporte the trace element requirements of six species of *Helminthosporium*. The have found out that Fe, Zn and Mn were essential for the growth of all t six species of *Helminthosporium*. However, Cu was reported to be esse tial only for the growth of *H. avenae*, *H. oryzae*, *H. turcicum* and *H. saccha* and Mo and Ca only for the growth of *H. oryzae* and *H. sacchari*. Th paper deals with the trace element requirements of five more pathogen fungi : *Cercospora hibiscina* Ellis and Everh. isolated from *Hibiscus cann binus* Linn., *C. withaniae* H. and P. Syd. from *Withania somnifera* Du *C. crotalariae* Sacc. from *Crotalaria juncea* Linn., *Monochaetia* sp. fro *Eriobotrya japonica* Lindl., and *Pestalotia theae* Swada from *Thea sinen* Linn. No such studies have been carried out on these fungi previous

MATERIAL AND METHOD

The above five pathogenic fungi were isolated from their respecti hosts from various localities of Punjab. Several monosporic isolates these pathogens were prepared on P.D.A. slants (pealed and sliced potato 200 g., dextrose 20 g., agar agar 20 g., and distilled water 1,000 ml.). A the isolates of these pathogens exhibited no morphological variability this agar medium. One representative monosporic isolate was select in each case for further investigations. The stock cultures of the isolates these fungi were maintained on P.D.A. and kept in the refrigerator at 0–4° The cultures were revived regularly after 6 months. The cultures of t different isolates have been deposited in the herbarium of the Panj University, Chandigarh, India.

The basal medium used for all the five fungi comprised of glucose 20 g., KNO_3 5·0 g., KH_2PO_4 5·0 g., $MgSO_4.7H_2O$ 0·5 g., and water to make 1,000 ml.

The optimum conditions for the growth of five fungi were as follows : temperature 28° C. for *C. hibiscina*, *C. withaniae*, and *P. theae* and 26° C. for *C. crotalariae* and *Monochaetia* sp. ; incubation period 24 days for *C. hibiscina*, *C. withaniae* and *Monochaetia* sp., 12 days for *C. crotalariae* and *P. theae* ; and pH 5 for *P. theae* and 6 for the rest of the four fungi. These conditions were found to be optimum for the growth of these fungi in previous studies (Thind and Mandahar, 1964 ; Mandahar, 1966).

The rest of the procedure regarding removal of trace element impurities from water, chemicals and inoculum were the same as already mentioned by Thind and Rawla (1967).

Twenty-five millilitres of the basal medium were poured into 125 ml. Erlenmeyer flask. The various media were sterilized at 15 lb./sq. inch steam pressure for 15 minutes. Each flask was seeded with 1 ml. of the standardized mycelial suspensions prepared in the same way as described by Thind and Rawla (1967) in the case of different fungi. Three replicates were taken in each case as well as in the case of controls. The flasks were then incubated at the corresponding optimum temperatures of these pathogens. The cultures were harvested after the corresponding optimum days of their incubation, filtered through a fine square meshed wire guaze (150–165 μ wide meshes) and transferred into gooch crucibles and then dried at 60° C. to a constant weight in a hot air oven for 24 hours. The data were recorded in terms of final pH and dry weight of the mycelium of each pathogen.

Experimental Procedure

Fifteen trace elements were tested to find out their essentiality for the growth of these fungi. The following salts of the trace elements were used : $Fe(NO_3)_3.9H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6Mo_7O_{24}$, $4H_2O$, $CaCl_2.2H_2O$, $(CH_3COO)_2Pb.3H_2O$, 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 elements used were Fe 0·2, Zn 0·1, Cu 0·04, Ca 5·0 and the rest of the trace elements 0·02 mg./l. of the basal medium each. The trace elements were removed from the basal medium which was then sterilized and its pH was adjusted to the corresponding optimum pH of each pathogen. Two controls were kept in each case. In one of these no trace elements were added and in the other all the trace elements were added. The rest of the medium

was divided into 15 lots and in each lot were added all the trace elements except one. The rest of the procedure was as usual. The data on dry weight and final pH are given in Table I.

RESULTS

Table I indicates that Fe, Zn, Cu and Mn are essential for growth of the five fungi while Mo is essential only for *C. crotalariae* and *Monochaetia* sp. None of the other trace elements appeared to be essential for these fungi. On the other hand, some of the trace elements used appeared to be slightly inhibitory such as B, Mo and W for *C. hibiscina* ; Ca, W and Hg for *C. withaniae* ; Ca, Br, Hg and Cd for *C. crotalariae* ; Br and B for *P. theae* ; and Cr, Li and Cd for *Monochaetia* sp.

Effect of different concentrations of essential trace elements on growth of the five fungi.—Five experiments were conducted to find out the optimum and toxic concentrations of trace elements found to be essential for the growth of five fungi. Ranges of concentrations used were : 0·0001 to 100 ppm of Fe ; 0·0001 to 200 of Zn, Mn and Mo ; and 0·00005 to 200 ppm of Cu. In subsequent experiments only the optimum amount of an essential trace element as found out in the first or previous experiments was used. Data obtained are given in Tables II to VI.

It would be observed from these tables that there is always an increase in growth of these fungi with an increase in concentrations of trace elements up to a certain optimum level, which is different for different fungi, after which their growth began to fall progressively. Optimum trace element concentrations in ppm for these are : Fe 0·01 for *C. crotalariae*, 0·2 for *C. hibiscina*, 1 for *C. withaniae* and *Monochaetia* sp. and 10 for *P. theae* (Table II) ; Zn 0·01 to 0·1 for *C. hibiscina*, 0·1 for *Monochaetia* sp. and *P. theae*, 1 for *C. withaniae* and 10 for *C. crotalariae* (Table III) ; Cu 0·001 for *C. crotalariae*, 0·01 for *C. hibiscina* and *P. theae* and 0·1 for *C. withaniae* and *Monochaetia* sp. (Table IV) ; Mn 0·01 for *P. theae*, 0·1 for *C. crotalariae* and *Monochaetia* sp., 1 for *C. hibiscina* and 10 for *C. withaniae* (Table V) ; and Mo 0·001 for *C. crotalariae* and 0·01 for *Monochaetia* sp. (Table VI). Concentrations of essential trace elements higher than the optimum were always progressively inhibitory to growth of the respective fungi.

DISCUSSION

Fe, Zn and Mn have been found to be essential for the growth of five fungi studied here. In this respect they resemble other fungi investigated

TABLE I

Effect of omission of different trace elements, omitted singly from the basal medium, upon the growth of Cercospora *spp.*, Monochaetia *sp. and* P. theae *at their respective optimum temperature, incubation period and initial pH*

Element omitted	*C. hibiscina*		*C. withaniae*		*C. crotalariae*		*Monochaetia* sp.		*P. theae*	
	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH
All ..	30	6·0	44	6·1	52	6·1	28	6·1	50	5·1
None ..	174	6·4	168	6·3	186	6·5	92	6·3	190	5·4
Fe ..	41	6·1	44	6·1	60	6·1	40	6·1	70	5·1
Zn ..	68	6·1	59	6·1	74	6·2	45	6·1	60	5·1
Mn ..	66	6·1	62	6·1	62	6·2	30	6·1	82	5·2
Mo ..	198	6·4	168	6·3	70	6·2	40	6·1	188	5·6
Cu ..	50	6·1	52	6·1	54	6·1	50	6·1	51	5·2
Ca ..	172	6·4	182	6·3	198	6·5	90	6·4	190	5·6
Pb ..	175	6·4	172	6·3	185	6·5	91	6·4	190	5.6
Br ..	172	6·4	168	6·3	200	6·5	93	6·4	202	5·6
I ..	174	6·4	166	6·3	185	6·5	92	6·4	188	5·6
Cr ..	168	6·5	170	6·3	186	6·5	102	6·4	191	5·6
B ..	190	6·4	170	6·3	198	6·5	92	6·3	206	5·6
W ..	186	6·4	184	6·4	184	6·5	92	6·3	190	5·6
Li ..	166	6·4	168	6·3	190	6·5	110	6·4	188	5·6
Hg ..	168	6·4	185	6·4	196	6·5	91	6·3	190	5·6
Cd ..	170	6·4	165	6·3	198	6·5	98	6·4	190	5·5

TABLE II

Effect of different concentrations of Fe on growth of Cercospora *spp.*, Monochaetia *sp. and* P. theae *at their respective optimum temperature, incubation period and initial pH*

Iron concentration in ppm added to basal medium	*C. hibiscina*		*C. withaniae*		*C. crotalariae*		*Monochaetia* sp.		*P. theae*	
	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH
Basal medium (without adding trace elements) ..	26	6·1	30	6·1	22	6·0	28	6·1	40	5·1
Basal medium (without adding iron) ..	48	6·1	52	6·1	56	6·1	36	6·1	50	5·1
0·0001 ..	72	6·2	66	6·1	98	6·2	40	6·1	60	5·1
0·001 ..	98	6·2	94	6·2	160	6·4	50	6·2	82	5·2
0·01 ..	126	6·3	106	6·4	190	6·5	66	6·2	103	5·3
0·1 ..	163	6·3	125	6·4	190	6·5	82	6·3	121	5·3
0·2 ..	180	6·4	142	6·4	182	6·4	90	6·3	146	5·3
1·0 ..	160	6·3	178	6·4	155	6·3	96	6·3	182	5·3
10·0 ..	154	6·3	140	6·4	86	6·1	96	6·3	200	5·3
100·0 ..	132	6·3	115	6·2	44	6·1	90	6·3	185	5·3

Zinc concentration in ppm added to basal medium	*C. hibiscina*		*C. withaniae*		*C. crotalariae*		*Monochaetia* sp.		*P. theae*	
	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH
Basal medium (without adding trace elements) ..	18	6·0	11	6·0	16	6·0	10	6·0	12	5·0
Basal medium (without adding zinc) ..	50	6·1	38	6·0	20	6·0	30	6·1	46	5·1
0·0001 ..	82	6·2	124	6·4	92	6·2	43	6·1	88	5·4
0·001 ..	125	6·3	150	6·5	124	6·4	75	6·2	144	5·4
0·01 ..	190	6·4	172	6·5	153	6·4	99	6·4	192	5·4
0·1 ..	190	6·4	190	6·5	180	6·4	110	6·4	200	5·4
1·0 ..	155	6·4	198	6·5	205	6·6	87	6·4	150	5·4
10·0 ..	110	6·4	130	6·4	220	6·6	55	6·3	123	5·4
100·0 ..	70	6·3	72	6·3	126	6·4	36	6·1	100	5·4
200·0 ..	30	6·2	48	6·1	22	6·1	15	6·1	88	5·4

TABLE IV

Effect of different concentrations of Cu on growth of Cercospora *spp.*, Monochaetia *sp. and* P. theae *at their respective optimum temperature, incubation period and initial pH*

Copper concentration in ppm added to the basal medium	*C. hibiscina*		*C. withaniae*		*C. crotalariae*		*Monochaetia* sp.		*P. theae*	
	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH
Basal medium (without adding trace elements) ..	6	6·0	10	6·0	20	6·1	10	6·0	12	5·0
Basal medium (without adding copper) ..	90	6·4	68	6·1	102	6·3	50	6·2	80	5·2
0·00005 ..	116	6·4	100	6·3	174	6·4	62	6·2	105	5·5
0·0001 ..	154	6·4	138	6·3	200	6·4	90	6·2	180	5·5
0·0005 ..	182	6·5	174	6·3	240	6·7	112	6·4	215	5·5
0·001 ..	206	6·5	204	6·5	262	6·7	130	6·4	260	5·5
0·01 ..	235	6·6	220	6·5	246	6·7	146	6·4	280	5·5
0·1 ..	210	6·5	240	6·5	213	6·5	155	6·4	270	5·5
1·0 ..	155	6·5	200	6·5	150	6·3	132	6·4	214	5·5
10·0 ..	106	6·4	162	6·3	94	6·1	84	6·2	162	5·4
50·0 ..	72	6·2	125	6·3	40	6·0	20	6·1	86	5·3
100·0 ..	20	6·2	44	6·1	0	6·0	0	6·0	16	5·0
200·0 ..	0	6·0	16	6·1	0	6·0	0	6·0	0	5·0

TABLE V

Effect of different concentrations of Mn on growth of Cercospora *spp.*, Monochaetia *sp. and* P. theae *at their respective optimum temperature, incubation period and initial pH*

Concentration of Mn in ppm added to basal medium	*C. hibiscina*		*C. withaniae*		*C. crotalariae*		*Monochaetia* sp.		*P. theae*	
	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH	Dry weight mg.	Final pH
Basal medium (without adding trace elements) ..	10	6·0	12	6·0	16	6·0	6	6·0	5	5·0
Basal medium (without adding manganese) ..	30	6·0	40	6·0	46	6·0	10	6·0	68	5·2
0·0001 ..	88	6·1	84	6·3	125	6·3	30	6·1	150	5·4
0·001 ..	146	6·4	120	6·3	180	6·3	86	6·1	220	5·5
0·01 ..	190	6·4	160	6·3	240	6·4	122	6·3	300	5·5
0·1 ..	230	6·4	188	6·5	284	6·4	175	6·3	250	5·5
1·0 ..	250	6·4	234	6·5	225	6·4	120	6·3	206	5·4
10·0 ..	204	6·4	270	6·5	160	6·3	66	6·1	160	5·4
100·0 ..	156	6·4	196	6·5	95	6·2	30	6·1	104	5·2
200·0 ..	60	6·1	44	6·1	40	6·1	0	6·0	62	5·1

so far, which, in general, require these trace elements for their growth. However, there are recent reports that Fe, Zn and Mn are not required for the growth of *Allomyces arbuscula* strain Burma ID*b* (Ingraham and Emerson, 1954), *Fusarium aqueductum* and *Geotrichum* sp. (Painter, 1954), *Penicillium javanicum* (Lockwood *et al.*, 1934) and *Cladochytrium replicatum* (Willoughby, 1962).

TABLE VI

Effect of different concentrations of Mo on growth of C. crotalariae *and* Monochaetia *sp.*

Concentration of Mo in ppm added to the basal medium	*C. crotalariae*		*Monochaetia* sp.	
	Dry weight mg.	Final pH	Dry weight mg.	Final pH
Basal medium (without adding trace elements) ..	6	6·0	5	6·0
Basal medium (without adding molybdenum) ..	140	6·3	42	6·1
0·0001 ..	250	6·4	84	6·3
0·001 ..	302	6·4	165	6·5
0·01 ..	300	6·5	190	6·5
0·1 ..	265	6·5	180	6·5
1·0 ..	220	6·4	144	6·5
10·0 ..	180	6·3	100	6·3
100·0 ..	96	6·3	40	6·1
200·0 ..	65	6·0	10	6·0

These five fungi require Cu for their growth and thus they resemble *Aspergillus flavus*, *A. fumigatus*, *A. cinamomeus* and *A. oryzae* (Roberg, 1928), *A. flavus*, *Rhizopus nigricans* and *Saccharomyces cerevisiae* (Mc Hargue and Calfee, 1931), *A. niger* (Steinberg, 1935), *Trichophyton interdigitale* (Mosher *et al.*, 1936), *Acaulium velatum* and a member of Dematiaceae (Starky and Waksman, 1943), *Phymatotrichum omnivorum* (Ezekiel

et al., 1945), *Sclerotium delphinii* (Perlman, 1948), *Fusarium oxysporum*, *Rhizoctonia solani*, *Cercospora nicotianae* and *Sclerotinia rolfsii* (Steinberg. 1950), *Sclerotium sclerotiorum* (Purdy and Grogan, 1954), *Sepedonium* sp. (Painter, 1954), *T. mentagrophytes* and *T. rubrum* (English and Barnard, 1955), *Fusarium* spp. (Saraswathi, 1958), and *Helminthosporium avenae*, *H. oryzae*, *H. turcicum* and *H. sacchari* (Thind and Rawla, 1967). However, all these fungi differ from *F. vasinfectum*, *F. udum* and *F. moniliforme* (Yogeswari, 1948), *Allomyces arbuscula* strain Burma ID *b* (Ingraham and Emerson, 1954), *F. aqueductum* and *Geotrichum* sp. (Painter, 1954), *H. sativum*, *H. biforme*, *H. setariae* and *H. halodes* (Peterson and Katznelson, 1956), *Cladochytrium replicatum* (Willoughby, 1962) and *H. sativum* and *H. teres* (Thind and Rawla, 1967), which do not require Cu for their growth. *Monochaetia* sp. and *C. crotalariae* require Mo for their growth. In this respect these fungi resemble *P. javanicum* (Lockwood *et al.*, 1934), *A. niger* (Steinberg, 1935 ; Mulder, 1948 ; Nicholas, 1952 ; Donald *et al.*, 1952), *F. oxysporum*, *R. solani*, *C. nicotianae* and *S. rolfsii* (Steinberg, 1950), *H. oryzae* and *H. sacchari* (Thind and Rawla, 1967). However, they differ from *F. oxysporum*, *F. udum* and *F. moniliforme* (Yogeswari, 1948), *Helminthosporium* spp. (Peterson and Katznelson, 1956), *P. theae*, *C. withaniae* and *C. hibiscina* (studied here) and *H. sativum*, *H. avenae*, *H. teres*, and *H. turcicum* (Thind and Rawla (1967), which do not require Mo for their growth.

Monochaetia sp. and *C. withaniae* resemble each other and *H. avenae* and *H. sacchari* (Thind and Rawla, 1967) in requiring 1 ppm Fe for their optimum growth. *C. crotalariae* resembles *H. oryzae* (Thind and Rawla, 1967), *C. hibiscina* resembles *A. niger* (Steinberg, 1935) and *P. theae* resembles *H. sativum* (Thind and Rawla, 1967), and *P. omnivorum* (Blank, 1941) in requiring 0·01, 0·2 and 10 ppm Fe, respectively, for their optimum growth. However, all the above fungi differ from *F. oxysporum* which requires 0·4, *S. rolfsii* and *T. basicola* which require 0·6 and *R. solani*, *P. irregulare* and *C. nicotianae* which require 0·8 ppm Fe (Steinberg, 1950) and *T. mentagrophytes* and *T. rubrum* which require 5·8 μg./50 ml. Fe (English and Barnard, 1955), for their optimum growth.

Monochaetia sp. and *P. theae* require 0·1 ppm Zn for their optimum growth and thus they resemble *A. niger* (Steinberg, 1935) and *H. turcicum* (Thind and Rawla, 1967) which also require the same optimum concentration of Zn for their growth. *C. hibiscina* requires 0·01–0·1 ppm Zn for its optimum growth and thus it resembles *F. vasinfectum* (Yogeswari, 1948). *C. withaniae* requires 1 ppm Zn for its optimum growth and thus it resembles

closely *F. oxysporum*, *R. solani*, *T. basicola*, *S. rolfsii*, *C. nicotianae* and *P. irregulare* (Steinberg, 1950). *C. crotalariae* requires 10 ppm Zn and thus it resembles *P. omnivorum* (Blank, 1941), *H. avenae*, *H. teres*, *H. oryzae* and *H. sacchari* (Thind and Rawla, 1967). However, all the above fungi differ from *Ustilago sphaerogena* which requires 0·001 ppm Zn (Grimm and Allen, 1954) and from *H. sativum* (Thind and Rawla, 1967) which requires 0·0001 ppm Zn for their optimum growth.

In requiring 0·1 ppm Mn for their optimum growth, *C. crotalariae* and *Monochaetia* sp. resemble *H. avenae* and *H. oryzae* (Thind and Rawla, 1967) and very closely *A. niger* (Steinberg, 1935), *F. vasinfectum*, *F. moniliforme* (Yogeswari, 1948), and *F. oxysporum*, *R. solani*, *T. basicola*, *S. rolfsii*, *C. nicotianae* and *P. irregulare* (Steinberg, 1950). *C. withaniae* requires 10 ppm Mn for its optimum growth and thus it resembles closely *Saccharomyces cerevisiae* (Mc Hargue and Calfee, 1931), and *P. omnivorum* (Blank, 1941). *C. hibiscina* requires 1 ppm Mn for its optimum growth and thus it resembles *H. turcicum* (Thind and Rawla, 1967). However, all the above fungi differ from *H. sativum* and *H. sacchari* (Thind and Rawla, 1967) which require as high as 100 ppm Mn and *A. oryzae* which requires as high as 400 ppm Mn (Hofmann *et al.*, 1950), and *H. teres* (Thind and Rawla, 1967) which requires as low as 0·0001 ppm In and from *P. theae* (studied here) which require 0·01 Mn for their optimum growth.

Monochaetia sp. and *C. withaniae* require 0·1 ppm Cu for their optimum growth and thus they resemble *A. niger* (Steinberg, 1935), *F. oxysporum*, *R. solani*, *S. rolfsii*, *C. nicotianae*, *P. irregulare* and *T. basicola* (Steinberg, 1950) and *H. turcicum*, *H. sacchari* and *H. avenae* (Thind and Rawla, 1967). However, they differ from *C. hibiscina* and *P. theae* (studied here) in requiring 0·01, *C. crotalariae* (studied here) in requiring 0·001 ppm Cu for their optimum growth and also from *P. omnivorum* (Blank, 1941) which requires 7·5 μg./50 ml. and *A. niger* (Steinberg, 1935) which requires 0·4 ppm Cu for their optimum growth.

In requiring 0·01 ppm Mo for its optimum growth *Monochaetia* sp. resembles *A. niger* (Steinberg, 1935), but it differs from *C. crotalariae* (studied here) which requires 0·001, *F. oxysporum* which requires 0·6, *R. solani*, *S. rolfsii*, *C. nicotianae* and *P. irregulare* which require 0·04 and *T. basicola* which requires 0·02 ppm Mo (Steinberg, 1950), *H. oryzae* and *H. sacchari* (Thind and Rawla, 1967) which require 0·0001 ppm Mo for their optimum growth.

Different concentrations of essential trace elements higher than the optimum have been found to be inhibitory for the growth of the five fungi studied here. In this respect they resemble the fungi studied by Thind and Rawla (1967).

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