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EFFECT OF VARIOUS TRACE ELEMENTS ON THE GROWTH AND SPORULATION OF FOUR FUNGI

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Out of fifteen trace elements tested Fe, Zn and Mn were found essential for the growth and sporulation of *Cephalothecium roseum*, *Fusarium moniliforme*, *Colletotrichum gloeosporioides* and *Penicillium expansum*; Cu for *C. rosenm* and *C. gloeosporioides*; and Mo for *C. roseum* alone. No other trace element was found to be essential for these fungi. Optimum concentrations (in ppm) of essential trace elements were found to be as follows : *C. roseum*: Fe 0.1, Zn 1.0, Mn 1.0; Cu 0.01, Mo 1.0; *F. moniliforme*: Fe 0.2, Zn 1.0, Mn 1.0; *C. gloeosporioides*: Fe 0.2, Zn 1.0, Mn 1.0, Cu 1.0; *P. expansum*: Fe 1.0, Zn 1.0 and Mn 0 1.

INTRODUCTION

Some excellent reviews on trace element nutrition of fungi are given by Foster (1939, 1949), Hawker (1950), Steinberg (1950), Lilly and Barnett (1951), Cochrane (1958), Stiles (1961), Tandon (1961) Nicholas (1963), Allaway (1965) Lilly (1965), and Bowen (1966). The study of these reviews has revealed that Fe, Zn, Mn, Cu, Mo, and Ca are essential for the growth of majority of the fungi investigated. However, there are isolated reports of essentiality of other trace elements such as W and Cb for *Penicillium javanicum* (Lockwood *et al.*, 1934); Ga for *Aspergillus niger* (Steinberg, 1938); V for *A. nigar* (Bertrand, 1941); B for *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* (Yogeswari, 1948), *Alternaria burnsii* (Sankhla *et al.*, 1970); Ur for *Alternaria tenuis* (Grewal, 1956); Co for *Gloeosporium psidii* (Tandon, 1961).

The present paper deals with the trace elements requirements of *C. roseum* Corda isolated from *Prunus amygdalus* (Tourn) L., *F. moniliforme* Sheldon isolated from *Zea mays* L., *C. gloeosporioides* Penzig isolated from *Punica granatum* L., and *P. expansum* Link isolated from *Malus sylvestris* Mill. No such studies have been carried out on these fungi previously.

MATERIALS AND METHODS

The basal medium containing glucose, 20g; KNO_3, 5g; KH_2PO_4 10 g; $MgSO_4.7H_2O$, 250 g; pure water, 1000 ml and was employed for the growth of *C. roseum*, *F. moniliforme* and *P. expansum*, but in place of KNO_3, asparagine (3.740 g/l) was used in the basal medium for the growth of *C. gloeosporioides*. This basal medium was found to give

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good growth with these fungi. *C. roseum* was grown at temperature 28°C, *p*H 6.0 and incubation period 20 days; *F. moniliforme* at temperature 28°C, *p*H 6.0 and incubation period 10 days; *C. gloeosporioides* at temperature 32°C, *p*H 5.0 and incubation period 14 days; while *P. expansum* at temperature 24°C, *p*H 4.0 and incubation period 10 days. These conditions were found optimum for the growth of these fungi in preliminary experiments.

All the chemicals used were of BDH 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; 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 IRA 400, Zeo-Karb 215 and Bio-Deminrolit resin) in an ascending manner and collected at the rate of 8-16 drops per minute and finally distilled thrice in 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 & Arnon, 1939).

Dextrose and asparagine: The 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/min.

Rest of the basal medium: Following the procedure of Steinberg (1935), rest of the components of the basal medium (KNO_3, KH_2PO_4, $MgSO_4.7H_2O$) were dissolved in pure water and then autoclaved together with $CaCO_3$ (15g/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.

Experimental Work and Results

Fifteen trace elements were tested to find out their essentiality for the growth and sporulation of four fungi. They were used in the form of following salts: $Fe(NO_3)_3.9H_2O$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6Mo_7O_{24}.H_2O$, $CaCl_2.2H_2O$ $(CH_3COO)_2$ Pb. $3H_2O$, KBr, KI, $K_2Cr_2O_7$, H_3BO_3, $Na_2MO_4.2H_2O$, $Li_2SO_4.H_2O$, $3CdSO_4.8H_2O$, $HgCl_2$. The amounts of various salts were adjusted so 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 element impurities were removed from the basal medium as discussed under Materials and Methods. The basal medium without any trace element 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 150-200μ 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 show that *C. roseum* made supressed growth as well as sporulation with the omission of Fe, Zn, Mn, Cu and Mo; *F. moniliforme* and *P. expansum* with the omission of Fe, Zn, and Mn; and *C. gloeosporioides* with the omission of Fe, Zn, Mn, and Cu. The omission of remaining trace elements had no adverse effect on the growth as well as sporulation of these fungi. There was no significant change in the final *p*H of the various media as a result of growth of each fungus.

Effect of Different Concentrations of Essential Trace Elements on the Growth and Sporulation of Four Fungi

Five experiments were conducted to find out the optimum and toxic concentrations of trace elements found to be essential for the growth and sporulation of four fungi. Range of concentrations used was 0.0001 to 400 ppm of Fe, Zn, Mn, Cu and Mo. 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 varying amounts of one essential trace element plus the optimum (or otherwise) amounts of other essential trace elements.

The data presented in Tables II-VI show that there is always increase in growth with an increase in concentration of trace elements up to certain optimum level, which is different for the four fungi, after which the growth falls progressively. Optimum concentrations (in ppm) of essential trace elements of these fungi are as follows: Fe 0.1 for *C. roseum*, 0.2 for *F. moniliforme*, 0.1 for *C. gloeosporioides* and 1.0 for *P. expansum* (Table II); Zn 1.0 for all the four fungi (Table III); Mn 0.1 for *P. expansum* and 1.0 for rest of the three fungi (Table IV); Cu 0.01 for *C. roseum* and 1.0 for *C. gloeosporioides* (Table V); and Mo 1.0 for *C. roseum* (Table VI).

DISCUSSION

Fe, Zn and Mn were found to be essential for the growth of four fungi studied here. These elements are reported to be essential for the growth of all fungi, which have been studied critically Cu which was essential for the growth of C. *roseum* and *C. gloeosporioides* is also known to be essential for the fungi studied by various workers. However, it was not found to be essential for the growth of *F. moniliforme* and *P. expansum*. It is possible that Cu is needed by these fungi in extremely minute quantities, which may be still present in the inoculum and chemicals used in the study. Mo was found to be essential for the growth of *C roseum* and in this respect it resembles *A. niger* (Steinberg, 1937; Nicholas, 1952), *Pleospora indica* (Mandahar, 1971) and *Claviceps microcephala* (Thind & Madan, 1973). However, Mo was not found to be essential for the growth of *C. gloeosporioides*, *F. moniliforme* and *P. expansum*. Similarly, it is reported not to be essential for 7 *Helminthosporium* spp. (Peterson & Katznelson, 1956; Thind & Rawla, 1967), *Pestalotia theae*, *Cercospora withanae* and *C. hibiscina* (Thind & Mandahar, 1968) and *Microxyphiella hibiscifolia* (Thind & Madan, 1973).

TABLE I

Effect of omission of different trace elements, omitted singly, from the basal medium on the growth and sporulation of four fungi at their optimum temperature, incubation period and initial pH

Element omitted	*C. roseum*			*F. moniliforme*			*C. gloeosporioides*			*P. expansum*		
	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H
All	26	—	6.0	20	—	6.0	48	—	5.0	12	—	4.0
None	173	++++	6.3	165	++++	6.7	200	++++	6.0	168	++++	4.6
Fe	61	++	6.0	45	++	6.2	54	—	5.1	34	++	4.2
Zn	53	++	6.0	46	++	6.2	69	—	5.2	30	—	4.2
Mn	59	++	6.0	49	++	6.2	79	—	5.2	50	++	4.2
Cu	74	++	6.4	169	++++	6.7	79	—	5.1	170	++++	4.6
Mo	100	++	6.4	160	++++	6.6	200	++++	6.0	162	++++	4.6
Ca	180	++++	6.2	167	++++	6.7	209	++++	6.0	161	++++	4.6
Pb	178	++++	6.4	162	++++	6.6	207	++++	6.1	162	++++	4.7
Br	175	++++	6 4	170	++++	6.7	200	++++	6.0	173	++++	4.6
I	172	++++	6.4	170	++++	6.7	198	++++	6.0	175	++++	4.7
Cr	172	++++	6.4	170	++++	6 7	209	++++	6.1	168	++++	4.7
B	172	++++	6.4	170	++++	6.7	210	++++	6.1	168	++++	4 6
W	170	++++	6.4	165	++++	6.7	200	++++	6.0	162	++++	4.6
Li	180	++++	6.4	160	++++	6.6	200	++++	6 0	162	++++	4.6
Cd	180	++++	6.4	162	++++	6.6	205	++++	6.0	160	++++	4.6
Hg	178	++++	6.3	160	++++	6.6	201	++++	6.0	162	++++	4.6

TABLE II

Effect of different concentrations of Fe on the growth and sporulation of four fungi at their respective optimum temperature, incubation period and initial pH

Fe concentration (in ppm) added to the basal medium	*C. roseum*			*F. moniliforme*			*C. gloeosporioides*			*P. expansum*		
	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H
000	61	++	6.0	48	++	6.0	59	—	5.1	34	++	4.2
0.0001	89	+++	6.1	96	++++	6 2	139	++	5.6	81	++++	4.6
0.0010	120	++++	6.2	113	++++	6.2	179	+++	6 0	109	++++	4.6
0.0100	147	++++	6.2	125	++++	6.4	190	++++	6.0	123	++++	4.8
0.1000	180	++++	6.5	139	++++	6.4	209	+++++	6.0	159	++++	4.8
0.2000	170	++++	6.4	155	++++	6.7	186	++++	6.0	167	++++	4.8
1.0000	123	++++	6.2	140	++++	6.4	169	+++	6.0	182	++++	4.8
10.0000	90	+++	6.0	102	++++	6.4	151	+++	6.0	145	++++	4.8
100.0000	0	—	6.0	29	—	6.2	125	—	6.0	73	++	4 8
200.0000	0	—	6.0	17	—	6.0	100	—	5.9	57	+	4.7
400.0000	0	—	6.0	11	—	6.0	76	—	5.8	25	—	4.7

TABLE III

Effect of different concentrations of Zn on the growth and sporulation of four fungi at their respective optimum temperature, incubation period and initial pH

Zn concentration (in ppm) added to the basal medium	*C. roseum*			*F. moniliforme*			*C. gloeosporioides*			*P. expansum*		
	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H
0	49	++	6.0	46	++	6.2	65	—	5.2	32	—	4.2
0.0001	69	++	6.0	69	++	6.2	94	+	5.6	49	—	4.6
0.0010	105	+++	6.0	83	++	6.2	134	++	5.9	69	—	4.6
0.0100	128	++++	6.2	119	+++	6.4	159	++++	6.0	89	++	4.6
0.1000	189	++++	6.5	148	++++	6.6	196	++++	6.2	169	++++	4.8
1.0000	220	++++	6 5	172	++++	6.6	226	++++	6.2	220	++++	5.6
10.0000	208	++++	6.4	160	++++	6.6	192	++++	5.8	205	++++	5.6
100.0000	173	+++	6.4	137	++++	6.4	102	++	5.2	66	++	4.2
200.0000	126	+++	6.3	121	+++	6.1	40	—	5.0	0	—	4.0
400.0000	85	++	6.2	90	++	6.0	0	—	5.0	0	—	4.0

TABLE IV

Effect of different concentrations of Mn on the growth and sporulation of four fungi at their optimum temperature, incubation period and initial pH

Mn concentration (in ppm) added to the basal medium	*C. roseum*			*F. moniliforme*			*C. gloeosporioides*			*P. expansum*		
	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H	Dry wt (mg)	Sporulation	Final *p*H
0	85	++	6.2	69	+++	6.2	89	++	5.2	92	++	4.4
0.0001	105	+++	6.2	109	++++	6.3	129	+++	5.0	112	++	4.6
0.0010	128	+++	6.2	130	++++	6.3	157	+++	6.0	159	+++	4.6
0.0100	168	++++	6.3	179	++++	6.4	189	++++	6.0	199	++++	4.7
0.1000	189	++++	6.5	192	++++	6.5	200	++++	6.1	230	++++	5.2
1.0000	230	++++	6.5	200	++++	6.7	222	++++	6.1	215	++++	5.0
10.0000	198	++++	6.5	170	++++	6.6	202	++++	6.0	194	++++	4.6
100.0000	170	++++	6.4	150	++++	6.4	183	+++	6.0	172	++++	4.4
200.0000	152	++++	6.0	123	++++	6.3	145	++	6.0	155	+++	4.2
400.0000	130	+++	6.0	97	+++	6.2	99	++	6.0	100	++	4.0

TABLE V

Effect of different concentrations of Cu on the growth and sporulation of C. roseum and C gloeosporioides *at their respective optimum temperature, incubation period and inisial pH*

Cu concentration (in ppm) added to the basal medium	*C. roseum*			*C. gloeosporioides*		
	Dry wt (mg)	Sporulation	Final *pH*	Dry wt (mg)	Sporulation	Final *pH*
0	140	++	6.2	108	++	6.2
0.0001	175	++	6.2	142	+++	6.0
0.0010	200	++++	6.4	172	++++	6.0
0.0100	230	++++	6.5	200	++++	6.0
0.1000	200	++++	6 5	205	++++	6.2
1.0000	189	++++	6 0	236	++++	6.2
10.0000	172	++++	6.0	195	+++	6.0
100 0000	0	—	6.0	120	++	5.6
200.0000	0	—	6.0	0	—	5.0
400.0000	0	—	6.0	0	—	5.0

TABLE VI

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

Mo concentration (in ppm) added to the basal medium	Dry wt (mg)	Sporulation	Final *pH*
0	139	+++	6.2
0 0001	158	+++	6 2
0.0010	192	+++	6.2
0.0100	200	++++	6.3
0.1000	218	++++	6.4
1.0000	239	++++	6.4
10.0000	208	++++	6.4
100.0000	170	+++	6.3
200.0000	143	——	6.0
400.0000	29	—	6.0

The four fungi studied here, have been found to require different concentrations of essential trace elements for their optimum growth, as has also been observed in the case of different fungi studied by Steinberg (1920, 1935, 1950), Blank (1941), Robbins and Hervey (1944), Yogeswari (1948), English and Barnard (1955), Peterson and Katznelson (1956), Thind and Rawla (1967), Thind and Mandahar (1968), Thind and Madan (1973).

C. roseum and *C. gloeosporioides* resemble each other in requiring 0.1 ppm Fe for their maximum growth. On the other hand, *F. moniliforme* and *P. expansum* studied here, require 0.2 ppm Fe for their maximum growth. These four fungi differ from *Fusarium oxysporum* which requires 0.4 ppm, *Sclerotinia rolfsii* and *Thielaviopsis basicola* which require 0.6 ppm and *Rhizoctonia solani*, *Pythium irregulare* and *Cercospora nicotianae* which require 0.8 ppm Fe (Steinberg, 1950), *Trichophyton mentagrophytes* and *T. ruburum* which require 5.8 μg/50 ml Fe (English & Barnard, 1955) and *M. hibiscifolia* which requires 0.01 ppm Fe (Thind & Madan, 1973) for their maximum growth.

The fungi studied here resemble one another in requiring 1.0 ppm Zn for their maximum growth. However, these differ from *Ustilago sphaerogena* which requires 0.001 ppm Zn (Grimm & Allen, 1957) and *C. microcephala* which requires 0.1 ppm Zn (Thind & Madan, 1973) for their maximum growth.

C. roseum requires 0.01 ppm Cu for its maximum growth and, therefore, it resembles *C. hibiscina* and *P. theae* (Thind & Mandahar, 1968) and *C. microcephala* (Thind & Madan, 1973). On the other hand, *C. gloeosporioides* requires 1.0 ppm Cu for its maximum growth. The optimum concentration of Cu for the growth of fungi, in general, lies below 1.0 ppm.

C. roseum requires 1.0 ppm Mo for its maximum growth and thus resembles *C. microcephala* (Thind & Madan, 1973). However, these fungi differ from *Monochaetia* sp. which requires 0.01 ppm (Thind & Mandahar, 1968), *F. oxysporum* which requires 0.06 ppm, *R. solani*, *C. nicotianae* and *P. irregulare* which require 0.04 ppm and *Thielaviopsis basicola* which requires 0.02 ppm Mo (Steinberg, 1950) for their maximum growth.

Concentrations higher than the optimum have been found to be inhibitory for the growth of *C. roseum*, *F. moniliforme*, *C. gloeosporioides* and *P. expansum*. This is true also of other fungi studied from this point of view (Thind & Rawla, 1967; Thind & Mandahar, 1968; Thind & Madan, 1973).

The present fungi show excellent sporulation when all the essential trace elements are present in the basal medium, Similarly, Thind and Madan (1973) reported that *C. microcephala* and *M. hibiscifolia* show excellent sporulation when the medium has Fe, Zn, Mn, Cu and Mo and Fe, Zn, Mn, Cu and Ca, respectively. Bhatnagar and Prasad (1968) also recorded that two isolates (F_1 and F_2) of *Fusarium solani* f. *aurantifoliae* show excellent sporulation when the basal medium is supplemented with combination of Fe, Zn and Mn.

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