TRACE ELEMENT STUDIES ON SIX SPECIES OF HELMINTHOSPORIUM

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Abstract

Trace element studies were carried out on six species of *Helminthosporium*—*H. sativum* P. K. B., *H. avenae* Eidam, *H. teres* Sacc., *H. oryzae* Br. de Haan, *H. turcicum* Pass., and *H. sacchari* (Br. de Haan) Butler. The trace element contaminants from glassware, water, basal medium and inoculum were removed by various usual means. In addition, disodium salt of Ethylene diamine tetra acetic acid (EDTA) was used to remove trace elements from glassware and water. Out of the 16 trace elements tested, Fe, Zn and Mn were found essential for the growth of all these species of *Helminthosporium*; Cu for the growth of *H. avenae*, *H. oryzae*, *H. turcicum* and *H. sacchari* but not for the growth of *H. sativum* and *H. teres*; Mo and Ca for the growth of *H. oryzae* and *H. sacchari* but not for the growth of *H. sativum*, *H. avenae*, *H. teres* and *H. turcicum*. No other trace element was found essential for the growth of any of these fungi. Optimum concentrations in ppm of the essential trace elements for these fungi were as follows: *H. sativum*: Fe 10·0, Zn 0·0001, Mn 100·0, *H. avenae*: Fe 1·0; Zn 10·0; Mn 0·1, Cu 0·1; *H. teres*: Fe 0·01, Zn 10·0, Mn 0·01; *H. oryzae*: Fe 0·001, Zn 10·0, Mn 0·1, Cu 0·0001, Mo 0·0001, Ca 750·0; *H. turcicum*: Fe 0·1, Zn 0·1, Mn 1·0, Cu 0·1; *H. sacchari*: Fe 1·0, Zn 10·0, Mn 100·0, Cu 0·01, Mo 0·0001, Ca 250·0. Concentrations higher than the optimum were inhibitory to the respective fungi.

Introduction

The studies on the trace element nutrition of fungi are helpful to establish the essentiality and the role of trace elements for the growth of fungi. Such studies are highly important for the advancement of industrial microbiology because certain trace elements have been shown to be required for some of the enzyme-catalyzed processes in the production of citric acid, alcohol,

antibiotics, etc., by fungi. The studies on the trace element nutrition of fungi have yielded *Aspergillus niger* and *Penicillium glaucum* as the test organisms for some of the trace elements. These test organisms are useful for the determination of very small amounts of trace elements which cannot be estimated by even most sensitive chemical tests. Thus the studies on the trace elment nutrition of fungi indirectly find a valuable place in the field of analytical chemistry. *Aspergillus niger* has been employed for the estimation of trace elements available to plants for growth in soil. This has also been correlated with the symptoms and control of such deficiency diseases of crop plants by supplying the deficient element in soil. Thus such studies on the trace element nutrition of fungi are of much importance in the field of agronomy and plant pathology.

Few fungi have been investigated with regard to the essentiality of the trace elements. Only about 5 dozen species of fungi have so far been investigated with respect to the essentiality of the trace elements for their growth (Wolff and Emmerie, 1930; Porges, 1932; Lockwood *et al.*, 1934; Steinberg, 1935, 1936, 1948, 1950; Niethammer, 1938; Rogers, 1938; Blank, 1941; Bertrand, 1941; Robbins and Hervey, 1944, 1965; Ezekiel, 1945; Yogeswari, 1948; Perlman, 1948; Jarvis and Johnson, 1950; Hofmann *et al.*, 1950; Machlis, 1953; Purdy and Grogan, 1954; Painter, 1954; Grimm and Allen, 1954; Sadasivan and Subramanian, 1954; Ingraham and Emerson, 1954; English and Barnard, 1955; Grewal, 1956; Peterson and Katznelson, 1956; Saraswathi, 1958; Agarwal, 1959; Davies, 1959; Tandon, 1961; Willoughby, 1962; Mathur and Sankhla, 1965; Barnett and Lilly, 1966; Daftari, 1966). So far, Fe, Zn, Mn and Cu have been found essential for the growth of majority of the fungi studied up-to-date. However, Mo and Ca are known to be essential for the gowth of a few fungi only. There are isolated reports for the essentiality of the other trace elements for some fungi but these have not been adequately substantiated as yet by other workers. These include V for the growth of *A. niger* (Bertrand, 1941); W and Cb for the growth of *P. javanicum* (Lockwood *et al.*, 1934); B for the growth of *Fusarium vasinfectum*, *F. udum* and *F. moniliforme* (Yogeswari, 1948); Ur for the growth of *Alternaria tenuis* (Grewal, 1956); Co for the growth of *Gloeosporium psidii* (Tandon, 1961).

This paper deals with the studies on the trace element nutrition of six species of *Helminthosporium*. Such studies have not been previously recorded with these species.

MATERIAL AND METHOD

The following six species of *Helminthosporium* were collected on the crops mentioned against each from various localities of the Panjab, India.

1. *Helminthosporium sativum* P. K. B. on *Triticum vulgare* L.
2. *H. avenae* Eidam on *Avena sativa* L.
3. *H. teres* Sacc. on *Hordeum vulgare* L.
4. *H. oryzae* Br. de Haan on *Oryza sativa* L.
5. *H. turcicum* Pass. on *Zea mays* L.
6. *H. sacchari* (Br. de Haan) Butler on *Saccharum officinarum* L.

Several monosporic isolates of the above pathogens were prepared on P.D.A. slants (peeled and sliced potatoes 200 g., dextrose 20 g., agar agar 20 g. and distilled water 1,000 ml.). All the isolates of these pathogens exhibited no morphological variability on this medium as well as on other media tested. *Helminthosporium avenae*, *H. teres* and *H. sacchari* gave abundant growth but no sporulation on P.D.A., oat meal agar, and malt agar, while *H. sativum*, *H. oryzae* and *H. turcicum* yielded abundant growth and sporulation on P.D.A., but poor sporulation on malt agar and oat meal agar. In all these fungi only one of the representative monosporic isolates was selected for further investigations. The stock cultures of the isolates of these fungi were maintained on P.D.A. and kept in the refrigerator at 0–4° C. The culture tubes were sealed by fixing aluminium caps with cellotape. They were revived regularly after 6 months. The cultures of the different isolates have been deposited in the herbarium of the Panjab University, Chandigarh, India.

Mostly Pyrex glassware have been employed but occasionally Sigcol glassware and Polyethyleneware have also been employed. The glassware were at first cleaned and washed with tap-water, rinsed out with hot acid chromate solution, then steamed in the autoclave for ½ an hour containing dilute acid chromate solution, again washed thoroughly with tap-water, rinsed out with hot distilled water and boiling solution of disodium salt of EDTA (1·0 g./l. pure water), washed twice with pure cold water and finally dried at 50° C. in a hot air oven before use. The glassware prepared by the above procedure did not give test for the trace elements by dithizone chloroform (0·01% w./v.) even up to 20 days. The polyethyleneware were washed thoroughly at first with tap-water and distilled water, respectively, and then rinsed out with hot solution of disodium salt of EDTA (1·0 g./l. pure water) and finally washed thrice with pure water before use. The trace elements

were removed from distilled water obtained from copper or aluminium still by at first passing through a column of ion-exchange resins (Amberlite IRA-400 at the base and Amberlite CG-50 above) in an ascending manner and collecting at the rate of 8–10 drops/min. and finally distilling twice in an all-glass Pyrex still containing 0·5 g./l. w./v. EDTA. Resultant water was designated as pure water and did not give test for the trace elements with the usual dithizone chloroform test.

All the chemicals used were of B.D.H. AnalaR or Emerck G.R. except for ferric nitrate and sodium tungstate which were of Riedle ordinary. The trace elements were removed from a solution of sucrose and asparagin by passing these solutions separately through a column of ion-exchange resins (De Acidite packed below and Zeokarb-215 above) in an ascending manner at the rate of 8–10 drops/min. Rest of the components of the basal medium (KNO_3, KH_2PO_4, $MgSO_4 \cdot 7H_2O$) were dissolved in pure water and then autoclaved together with $CaCO_3$ (15 g./l.) for ½ an hour to remove the trace elements from them. The trace elements from the inoculum were minimized by making 2 successive transfers of the mycelium into a liquid basal medium from which the trace elements were removed.

The basal medium containing sucrose 20 g., KNO_3 2·50 g., KH_2PO_4 5·0 g., $MgSO_4.7H_2O$ 0·25 g., pure water 1000 ml. was employed for the growth of *H. sativum*, *H. oryzae* and *H. sacchari* but in place of KNO_3 asparagin (1·85 g./l.) was used in the above basal medium for the growth of *H. avenae*, *H. teres* and *H. turcicum*. This basal medium was found to give good growth with these fungi. The initial pH of the basal medium was adjusted to 4·5 for the growth of *H. oryzae* and to 7·0 for the growth of the rest of the fungi. *H. sativum*, *H. avenae*, *H. teres* were grown at 25° C., for 8 days in the case of *H. avenae* and *H. teres* but for 10 days in the case of *H. sativum*. However, *H. oryzae*, *H. turcicum*, *H. sacchari* were grown at 28° C., for 10 days in the case of *H. turcicum* but for 12 days in the case of *H. oryzae* and *H. sacchari*. The above conditions for the growth of these fungi were found optimum in the preliminry experiments on their growth.

Only mycelial suspension of different fungi was employed as inoculum throughout these studies. The creamy submerged mycelium obtained after two successive transfers of the mycelium in a liquid basal medium from which trace elements were removed was collected aseptically with sterilized spathulate platinum needle and transferred into a mincing and crushing device. This device consists of a flask (250 ml. Erlenmeyer) and a glass rod with one of its ends flattened. A muslin cloth containing a thin pad of cotton in its folds is wrapped round the glass rod in the centre, which serves as a plug for

the flask. The glass rod is free to move around its axis as well as up and down. A separate such like assembly was used for each of the pathogens. These assemblies containing few drops of pure water were sterilized at 15 lb./sq. inch steam pressure for 15 minutes. Mycelial inoculum was then transferred separately in each flask and minced thoroughly with the glass rod. In this way, the mycelial suspensions of the different fungi were prepared separately. More sterilized pure water was then added with the help of sterilized pipette so as to get a standard load of 20–25 mycelial bits (mostly 150–200 μ long) per low power field of the compound microscope. Twenty-five milliliters of the basal medium were poured into 250 ml. Erlenmeyer flask. The various media were sterilized at 15 lb./sq. inch steam pressure for 15 minutes. Each flask was seeded with 0·5 ml. of the standardized mycelial suspension in the case of different fungi. Four replicates were taken in each case as well as in the case of two 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-gauze (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 WORK AND RESULT

Effect of different trace elements on the growth of six species of Helminthosporium.—Sixteen 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, RbCl, $HgCl_2$, $Na_2WO_4.2H_2O$, $Li_2SO_4.H_2O$, $3CdSO_4.8H_2O$. The amounts of the various elements used were: Fe 0·2, Zn 0·1, Cu 0·04, Ca 5·0 and rest of the trace elements 0·02 mg./l. of the basal medium. These amounts of the trace elements were within the optimum range of concentrations which have been reported so far for the growth of different fungi by other workers.

In the case of each pathogen, the basal medium after removing the trace elements was 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 basal medium was divided into 16 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.

TABLE I

Effect of the omission of different trace elements omitted singly from the basal medium on the growth of six species of Helminthosporium *at their respective optimum temperature, incubation period and intial pH*

Elements omitted from basal medium	*H. sativum*		*H. avenae*		*H. teres*		*H. oryzae*		*H. turcicum*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
None	74	8·0	82	7·0	100	7·0	95	7·4	85	8·0	98	8·0
All	44	7·0	78	7·0	50	7·0	40	6·2	50	7·3	40	7·3
Fe	46	5·1	37	5·6	61	7·0	46	6·5	66	7·0	44	8·0
Zn	84	8·0	70	7·0	71	7·0	48	6·7	76	7·0	45	8·0
Mn	69	8·0	73	7·0	73	7·0	76	6·7	77	8·4	48	8·0
Mo	79	8·0	95	7·0	100	7·0	73	6·7	92	7·0	85	8·0
Cu	75	8·0	76	7·0	100	7·0	64	6·7	66	8·0	52	8·0
Ca	85	8·0	100	7·0	103	7·0	68	6·2	85	8·0	59	8·0
Pb	73	8·0	88	7·0	103	7·0	113	7·4	87	8·0	110	8·0
Br	78	8·0	97	7·0	102	7·0	95	7·5	87	8·0	100	8·0
I	78	8·0	97	7·0	102	7·0	96	7·2	84	8·0	102	8·0
Cr	80	8·0	82	7·0	98	7·0	95	7·4	100	8·0	99	8·0
B	76	8·0	100	7·0	105	7·0	95	7·4	87	8·0	98	8·0
W	88	8·0	100	7·0	112	7·0	95	7·4	84	8·0	100	8·0
Rb	78	8·0	108	7·0	102	7·0	95	7·1	90	8·0	101	8·0
Li	78	8·0	100	7·0	100	7·0	96	7·4	95	8·0	102	8·0
Cd	78	8·0	99	7·0	102	7·0	99	7·4	88	8·0	100	8·0
Hg	78	8·0	102	7·0	110	7·0	98	7·4	88	8·0	108	8·0

The study of Table I reveals that Fe and Mn are essential for the growth of all these species of *Helminthosporium*; Zn for the growth of *H. avenae*, *H. teres*, *H. oryzae*, *H. turcicum* and *H. sacchari*; Cu for the growth of *H. avenae*, *H. oryzae*, *H. turcicum* and *H. sacchari*, but not for the growth of *H. sativum* and *H. teres*; Mo and Ca for the growth of *H. oryzae* and *H. sacchari*, but not for the growth of *H. sativum*, *H. avenae*, *H. teres* and *H. turcicum*. No other trace elements are found to be essential for the growth of any of these fungi. The pH is not changed with the growth of *H. avenae* and *H. teres* in any medium, except in the medium in which Fe was not added in the case of *H. avenae*. It is also not changed with the growth of *H. turcicum* in the media in which Fe, Zn, Mo and no trace elements were added and with the growth of *H. sacchari* and *H. sativum* in the medium in which no trace elements were added. The pH increases slightly with the growth of *H. sativum* and *H. sacchari* in all the media except in the medium in which no trace elements were added and with the growth of *H. turcicum* in all the media except in the media in which Fe, Zn, Mo and no trace elements were added. However, the pH increases considerably with the growth of *H. oryzae* in all the media and it decreases with the growth of *H. sativum* and *H. avenae* in the medium in which Fe was not added.

The effect of different concentrations of the six above-noted essential trace elements was studied on the growth of these fungi. In one experiment the effect of different concentrations of only one of the essential trace elements on the growth of all these pathogens was studied. The concentrations of an element observed to be optimum for different pathogens in an experiment were substituted in all the subsequent experiments. Two controls were kept. In one of these no trace elements were added and in the other all the essential trace elements were added except for one the effect of concentrations of which was studied. The ranges of concentrations in ppm of these elements used were: Fe: 0·0001–100; Zn, Mn, Cu and Mo: 0·0001–200; Ca: 1–1000. The rest of the procedure was as usual. The data in terms of dry weight and final pH are given in Tables II–VII.

The study of Tables II–VII reveals 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 the growth falls progressively. Optimum concentrations in ppm of the essential trace elements for these fungi are as follows: Fe: 0·001 for *H. oryzae*; 0·01 for *H. teres*; 0·1 for *H. turcicum*; 1·0 for *H. avenae* and *H. sacchari*; 10·0 for *H. sativum* (Table II). Zn: 0·0001 for *H. sativum*; 0·1 for *H. turcicum*; 10·0 for *H. avenae*, *H. teres*, *H. sacchari* and *H. oryzae*

TABLE II

Effect of different concentrations of Fe on the growth of six species of Helminthosporium *at their respective optimum temperature, incubation period and intial pH*

Iron concentrations ppm added to the basal medium	*H. sativum*		*H. avenae*		*H. teres*		*H. oryzae*		*H. turcicum*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
Basal medium (without adding trace elements)	22	7·0	35	7·0	42	7·0	15	5·2	15	7·0	25	7·0
Basal medium (without adding iron)	62	7·0	35	7·0	45	7·0	65	4·5	15	7·0	96	7·0
0·0001	73	7·0	45	7·0	130	7·0	180	6·0	36	7·0	176	7·0
0·001	73	7·0	50	7·0	133	6·5	193	6·0	36	7·0	186	7·0
0·01	87	7·0	60	7·0	150	6·5	190	6·0	45	7·0	190	7·0
0·1	125	7·0	105	7·0	145	6·5	183	6·8	83	7·0	198	7·0
0·2	170	7·0	130	7·0	130	6·5	183	6·8	80	7·0	240	7·0
1·0	176	7·5	170	7·0	125	6·5	183	6·8	76	7·0	254	7·0
10·0	180	7·5	115	7·0	125	6·5	183	6·8	56	7·0	223	7·0
100·0	160	7·5	80	7·0	120	7·0	10	4·8	40	7·0	83	7·0

(Table III). Mn: 0·01 for *H. teres*; 0·1 for *H. avenae* and *H. oryzae*; 1·0 for *H. turcicum*; 100·0 for *H. sativum* and *H. sacchari* (Table IV). Cu: 0·0001 for *H. oryzae*; 0·01 for *H. sacchari*; and 0·1 for *H. turcicum* and *H. avenae* (Table V). Mo: 0·0001 for *H. oryzae* and *H. sacchari* (Table VI). Ca: 250 for *H. sacchari*; 750 for *H. oryzae* (Table VII). In all these pathogens no marked change in pH is observed with all the different concentrations of the essential trace elements except that in the case of *H. oryzae* the pH shifts towards neutrality with different concentrations of Fe, Zn, Mn, Mo and Ca but not with different concentrations of Cu.

TABLE III

Effect of different concentrations of Zn on the growth of six species of Helminthosporium *at their respective optimum temperature, incbubation period and initial pH*

Zinc concentration ppm added to the basal	*H. sativum*		*H. avenae*		*H. teres*		*H. oryzae*		*H. turcicum*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
Basal medium (without adding trace elements)	14	7·0	40	7·0	10	7·0	11	5·0	5	5·8	12	7·0
Basal medium (without adding zinc)	98	7·0	45	7·0	12	7·0	25	5·2	25	6·8	38	7·0
0·0001	180	7·0	120	7·0	40	7·0	50	5·6	105	6·8	42	7·0
0·001	175	7·0	150	6·8	50	7·0	80	5·8	108	6·8	62	7·0
0·01	168	7·0	175	6·8	55	7·0	110	6·0	120	6·8	92	7·0
0·1	160	7·0	216	6·5	105	7·0	140	7·0	198	6·8	120	7·0
1·0	106	7·0	255	6·5	115	7·0	190	7·5	150	6·5	190	7·0
10·0	66	7·0	265	6·5	187	7·0	190	7·0	150	6·5	250	7·0
100·0	37	7·0	21?	6·5	105	7·0	70	6·0	146	6·5	180	7·0
200·0	32	7·0	20	6·5	105	7·0	45	4·5	98	6·5	136	7·0

DISCUSSION

Ion-exchange resins have been used to remove trace elements from sucrose, asparagin in this study. This technique has also been used to remove trace elements from sucrose and glucose by Perlman (1948). Ethylene diamine tetra acetic acid (EDTA) disodium salt has been employed to remove trace elements from glassware which has also been employed by Nicholas, 1952, for this purpose. Due to the formation of strong water-soluble non-ionic chelate complexes with nearly 40 elements (Charles *et al.*, 1958), EDTA has been successfully employed for the first time for further removal of trace

TABLE IV

Effect of different concentrations of Mn on the growth of six species of Helminthosporium *at their respective optimum temperature, incubation period and initial pH*

Manganese concentrations ppm added to the basal medium	*H. sativum*		*H. avenae*		*H. teres*		*H. oryzae*		*H. turcicum*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH.	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
Basal medium (without adding trace elements)	4	7·0	20	7·0	12	7·0	0	4·5	5	6·8	10	7·0
Basal medium (without adding manganese)	48	7·0	80	7·0	21	7·0	86	6·8	92	6·8	10	7·0
0·0001	100	7·0	120	7·0	63	6·8	100	7·0	152	6·5	10	7·0
0·001	108	7·0	215	7·0	132	6·5	120	7·0	168	6·5	12	7·0
0·01	122	7·0	230	7·0	186	6·5	163	7·0	200	6·5	22	7·0
0·1	130	7·0	240	7·0	110	6·0	195	7·0	220	6·5	150	7·0
1·0	133	7·0	150	7·0	88	6·0	160	7·0	237	6·5	175	7·0
10·0	178	7·0	100	7·0	86	6·0	65	7·0	230	6·5	228	7·0
100·0	217	7·0	95	7·0	70	6·0	0	4·5	170	6·5	287	7·0
200·0	160	7·0	40	7·0	50	6·0	0	4·5	98	6·5	196	7·0

elements from water after passing through ion-exchange resins in this study. Highly pure water of the specific conductance of 1·05 mhos has been obtained by this procedure. Calcium carbonate has been employed here to remove trace elements from rest of the components of the basal medium. Its efficiency of removal of trace elements has been shown by Steinberg, 1935; Donald *et al.*, 1952; Nicholas, 1952. The trace elements are minimized in the inoculum by growing it on a basal medium from which trace elements were removed for two successive subcultures before seeding it in the flasks.

TABLE V

Effect of different concentrations of Cu on the growth of six species of Helminthosporium *at their respective optimum temperature, incubation period and initial pH*

Copper concentrations ppm added to the basal medium	*H. sativum*		*H. avenae*		*H. teres*		*H. oryzae*		*H. turcicum*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
Basal medium (without adding trace elements)	5	7·0	30	7·0	12	7·0	0	4·5	12	7·0	10	7·0
Basal medium (without adding copper)	220	7·0	222	7·0	198	7·0	137	4·5	75	7·0	97	7·0
0·0001	185	7·0	222	7·0	162	7·0	195	4·5	108	7·0	185	7·0
0·001	175	7·0	222	7·0	135	7·0	168	4·5	183	7·0	220	7·0
0·01	150	7·0	222	7·0	120	7·0	157	4·5	210	7·0	276	7·0
0·1	100	7·0	240	7·0	105	7·0	105	4·5	240	7·0	245	7·0
1·0	62	7·0	210	7·0	52	7·0	42	4·5	160	7·0	130	7·0
10·0	0	7·0	130	7·0	0	7·0	20	4·5	110	7·0	85	7·0
100·0	0	7·0	93	7·0	0	7·0	0	4·5	90	7·0	13	6·5
200·0	0	7·0	90	7·0	0	7·0	0	4·6	14	7·0	5	6·0

All the *Helminthosporium* species included in this study resemble one another as well as *H. sativum, H. biforme, H. halodes* and *H. setariae* (Peterson and Katznelson, 1956) in requiring Fe, Zn and Mn for their growth. It may be mentioned here that Fe, Zn and Mn are known to be essential for the growth of fungi in general. However, there are a few fungi for the growth of which these elements have not been known to be essential. Thus, Fe, Zn and Mn are not essential for the growth of *Fusarium aqueductum* and *Geotrichum* sp. (Painter, 1954), *Penicillium javanicum* (Lockwood *et al.*, 1934), *Allomyces arbuscula* strain Burma IDb (Ingraham and Emerson, 1954), *Cladochytrium replicatum* (Willoughby, 1962). *H. avenae, H. oryzae,*

TABLE VI

Effect of different concentrations of Mo on the growth of Helminthosporium sacchari *and* H. oryzae *at their respective optimum temperature, incubation period and initial pH*

Molybdenum concentrations ppm added to the basal medium	*H. oryzae*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
Basal medium (without adding trace elements)	8	4·5	2	7·0
Basal medium (without adding molybdenum)	90	6·8	300	7·0
0·0001	188	7·0	325	7·0
0·001	105	7·0	310	7·0
0·01	90	7·0	300	7·0
0·1	86	7·0	300	7·0
1·0	83	7·0	300	7·0
10·0	75	7·0	290	7·0
100·0	10	4·5	290	7·0
200·0	0	4·5	270	7·0

H. turcicum and *H. sacchari* resemble one another in requiring Cu for their growth but differ from *H. sativum*, *H. teres* studied here and also from *H. sativum*, *H. biforme*, *H. halodes* and *H. setariae* (Peterson and Katznelson, 1956) which do not require Cu for their growth. *H. oryzae* and *H. sacchari* resemble each other in requiring Mo and Ca for their growth but differ from *H. sativum*, *H. teres*, *H. avenae* and *H. turcicum* studied here as well as *H. sativum*, *H. biforme*, *H. halodes* and *H. setariae* (Peterson and Katznelson, 1956) which do not require Mo and Ca for their growth.

TABLE VII

Effect of different concentrations of Ca on the growth of Helminthosporium sacchari *and* H. oryzae *at their respective optimum temperature, incubation period and initial pH*

Calcium concentrations ppm added to the basal medium	*H. oryzae*		*H. sacchari*	
	Dry wt. mg.	Final pH	Dry wt. mg.	Final pH
Basal medium (without adding trace elements)	0	4·8	10	7·0
Basal medium (without adding calcium)	0	5·0	20	7·0
1	25	5·0	35	7·0
5	35	5·0	35	7·0
25	45	5·5	65	7·0
50	45	5·5	102	7·0
75	83	5·9	145	7·0
100	83	5·9	198	7·0
250	115	5·6	285	7·0
500	155	5·6	160	7·0
750	190	5·6	150	7·0
1000	115	5·2	86	7·0

Different *Helminthosporium* species have been found to require different concentrations of the essential trace elements for their optimum growth as has also been observed in the case of different fungi studied by Blank, 1941; Yogeswari, 1948; Robbins and Hervey, 1944; English and Barnard, 1955; Peterson and Katznelson, 1956; Steinberg, 1920, 1935, 1950.

H. avenae and *H. sacchari* resemble each other in requiring 1·0 ppm Fe for their optimum growth but differ from *H. sativum*, *H. teres*, *H. oryzae* and *H. turcicum* studied here as well as *H. sativum* (Peterson and Katznelson, 1956) which require 10·0, 0·01, 0·001, 0·1 ppm Fe and 0·4–25 mg. $FeSO_4$/l. for their optimum growth, respectively.

H. avenae, *H. teres*, *H. sacchari* and *H. oryzae* resemble one another in requiring 10·0 ppm Zn for their optimum growth, but differ from *H. turcicum* and *H. sativum* studied here as well as *H. sativum* (Peterson and Katznelson, 1956) which require 0·1, 0·0001 ppm Zn and 0·4–25 mg. $ZnSO_4.7H_2O$/l., respectively, for their optimum growth.

H. sativum and *H. sacchari* resemble each other in requiring 100 ppm Mn for their optmum growth, but differ from *H. avenae*, *H. oryzae* which require 0·1 ppm Mn for their optimum growth, from *H. teres* and *H. turcicum* which require 0·0001 and 1·0 ppm Mn, respectively, for their optimum growth and from H. *sativum* (Peterson and Katznelson, 1956) which requires 0·1–25 mg. $MnCl_2.4H_2O$/l. for its optimum growth.

H. turcicum, *H. sacchari* and *H. avenae* resemble one another in requiring 0·1 ppm Cu for their optimum growth but differ from *H. oryzae* which requires 0·0001 ppm Cu for its optimum growth.

H. oryzae and *H. sacchari* resemble each other in requiring 0·0001 ppm Mo for their optimum growth, but they differ from each other in the optimum requirements of Ca for their growth. *H. oryzae* requires 250 ppm while *H. sacchari* requires 750 ppm Ca for their optimum growth. From the studies carried so far on Ca requirements for the growth of fungi, such high concentrations of Ca have not been reported for the optimum growth of any fungus. It would be very interesting to investigate the role of this element in the growth of fungi.

Different concentrations of essential trace elements higher than the optimum have been found to be inhibitory for the growth of different species of *Helminthosporium*.

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