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Role of Trace Elements on the Growth and Sporulation of *Alternaria chartarum* and *A. solani*

MIRA MADAN and K S THIND*, FNA
Department of Biology, Guru Nanak Dev University, Amritsar 143 005

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Trace element studies were carried out on two species of *Alternaria* namely, *A. chartarum* Pruess and *A. solani* (Ellis and Mart.) Jones and Grout. Out of 20 trace elements tested Fe, Zn, Mn, Cu and Mo were found to be essential for the growth and sporulation of both the fungi; Co for *A. chartarum* and Ca for *A. solani*. 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: *A. chartarum* Fe 1.0–10.0, Zn 10.0, Mn 0.1, Cu 0.01, Mo 1.0 and Co 0.01, *A. solani* Fe 10.0, Zn 10.0, Mn 1.0, Cu 0.1, Mo 0.1 and Ca 10–100. Concentration higher than the optimum were progressively inhibitory to the respective fungi.

Key Words: *Alternaria* **spp., Trace element studies**

Introduction

Majority of the fungi investigated so far require Fe, Zn, Mn, Cu and Mo for their growth and a few need Ca as well. 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), Sc for *A. niger* (Steinberg 1939), V for *A. niger* (Bertrand 1941), B for three *Fusarium* spp. (Yogeswari 1948), *Alternaria burnsii* (Sankhla et al. 1970), U for *Alternaria tenuis* (Grewal 1956), Co for *Gloeosporium psidii* (Tandon 1961). These reports need elaboration with respect to many other fungi with refined and specific methodology. The present paper deals with the trace element requirements of *Alternaria chartarum* Pruess isolated from *Psidium guajava* L. (fruits) and *A. solani* (Ellis and Mart.) Jones and Grout isolated from *Solanum tuberosum* L. (leaves). Such

*Present address: Department of Botany, Panjab University, Chandigarh 160014

studies have not been recorded previously with these species.

Materials and Methods

For procedure of removal of traceelement impurities from water, glassware, chemicals and inoculum, the reader is referred to earlier publication of Thind and Madan (1977).

The basal medium containing glucose 20g, KNO_3 5g, KH_2PO_4 10g, $MgSO_4 \cdot 7H_2O$ 0.250g, pure water 1,000 ml was employed for the two species of *Alternaria*. *A. chartarum* was grown at 28°C, *p*H 5.0 and incubation period 14 days and *A. solani* at temperature 28°C, *p*H 6.0 and incubation period 12 days. These conditions were found to be optimum in the preliminary studies.

The salts of 20 trace elements tested were as follows: $Fe(NO_3)_3.9H_2O$, $(NH_4)_2$, $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, $CuSO_4.5H_2O$, $(NH_4)_6Mo_7O_{24}.4H_2O$, $CaCl_2.2H_2O$, $(CH_3COO)_2Pb.3H_2O$, KBr, KI, $K_2Cr_2O_7$, H_3BO_3, $HgCl_2$, $Na_2WO_4.2H_2O$, $Li_2SO_4.H_2O$, $3CdSO_4.8H_2O$, $RbCl$,

Table 1 *Effect of omission of different trace elements omitted singly on the growth and sporulation of* A. chartarum *and* A. solani *at their respective optimum temperature, incubation period and initial* p*H*

Element omitted	*A. chartarum*		*A. solani*	
	Dry wt. (in mg)	Sporulation	Dry wt. (in mg)	Sporulation
All	30	—	39	—
None	209	++++	239	++++
Fe	49	+	63	+
Zn	55	+	48	+
Mn	80	++	68	+
Cu	93	+	95	+
Mo	95	++	83	++
Ca	208	++++	118	++++
Pb	220	++++	249	++++
Br	220	++++	248	++++
I	206	++++	245	++++
Cr	202	++++	240	++++
B	218	++++	239	++++
Hg	215	++++	238	++++
W	217	++++	229	++++
Li	205	++++	230	++++
Cd	210	++++	224	++++
Rb	209	++++	229	++++
Co	115	++	232	++++
Cs	200	++++	239	++++
Ga	205	++++	249	++++
Sc	208	++++	232	++++

—Nil, +Poor, ++Fair, +++Good, ++++Excellent

Table 2 *Effect of trace elements on the growth and sporulation of two species of*

Trace element concentration in ppm added to the basal medium	*A. chartarum*													
	Fe		Zn		Mn		Cu		Mo		Co		Ca	
	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation
000	56	++	63	++	89	++	89	+++	115	+++	158	+++	268	++++
0.0001	91	++++	90	+++	110	+++	135	++++	135	++++	227	++++	269	++++
0.001	125	++++	135	++++	135	++++	183	++++	153	++++	268	++++	260	++++
0.01	139	++++	193	++++	189	++++	253	++++	212	++++	256	++++	250	++++
0.1	158	++++	225	++++	240	++++	240	++++	248	++++	238	++++	246	++++
1.0	238	++++	230	++++	228	++++	200	++++	265	++++	135	++	240	++++
10.0	236	++++	249	++++	200	++++	158	++++	208	++++	25	—	220	++++
100.0	129	+++	139	+++	173	++++	46	+	170	++++	0	—	200	++++
200.0	89	++	78	+++	138	++++	0	—	130	+++	0	—	180	+++ +
400.0	0	—	0	—	96	++	0	—	45	—	0	—	159	+++ +

—Nil, +Poor, ++Fair, +++Good, ++++Excellent

$CoCl_2.6H_2O$, CsCl, $Ga(NO_3)_3.8H_2O$ and Sc_2O_3. The amounts of various salts were so adjusted so as to provide the following quantities (in mg) of the trace elements in one litre of the medium: Fe 0.2, Zn 0.1, Cu 0.04, Ca 1.0 and the remaining 16 trace elements 0.02 each.

The basal medium without trace elements was divided into twenty 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 case of each fungus. Rest of the procedure was as usual.

Results and Discussion

The data presented in table 1 show that *A. chartarum* showed suppressed growth as well as sporulation with the omission of Fe, Zn, Mn, Cu, Mo and Co and *A. solani* with the omission of Fe, Zn, Mn, Cu, Mo and Ca. The omission of remaining trace elements had no adverse effect on the growth and sporulation of both the fungi. Since there was no significant change in final *p*H of various media, it is not discussed here.

Seven experiments were conducted to find out the optimum and toxic concentrations of trace elements found to be essential for the growth and sporulation of *Alternaria* spp. Range of concentration used was 0.0001 to 400 ppm of Fe, Zn, Mn, Cu, Mo, Co and Ca. The optimum amount of essential element

Alternaria *at their respective optimum temperature, incubation period and* pH

A. solani

Fe		Zn		Mn		Cu		Mo		Co		Ca	
Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation	Dry wt. in mg	Sporulation
42	++	59	++	100	+++	112	+++	130	+++	272	++++	140	+++
98	+++	75	++	123	++++	125	++++	170	++++	275	++++	156	+++
112	+++	139	+++	158	++++	173	++++	204	++++	260	++++	173	++++
120	+++	180	+++	200	++++	210	++++	250	++++	225	++++	190	++++
169	++++	202	++++	223	++++	263	++++	280	++++	200	++++	208	++++
232	++++	243	++++	250	++++	240	++++	263	++++	120	+++	223	++++
245	++++	260	++++	234	++++	205	+++	223	++++	20	—	290	++++
149	+++	175	++++	200	++++	70	+	153	++++	0	—	295	++++
73	++	112	+++	162	++++	0	—	112	+++	0	—	270	++++
0	—	58	++	112	+++	0	—	10	—	0	—	200	++++

as found out in the first or previous experiments was used in the later experiments. In each experiment each lot of three replicates contained varying amounts of one essential trace element plus the optimum (or otherwise) amounts of other essential trace elements.

The data presented in table 2 show that there was always increase in growth with increase in concentration of trace element up to a certain optimum level, which was different for the two fungi, after which the growth falls progressively. Optimum concentration in ppm of the essential trace elements were as follows: *A. chartarum*, Fe 1.0–10.0, Zn 10.0, Mn 0.1, Cu 0.1, Mo 1.0 and Co 0.01, *A. solani*, Fe 10.0, Zn 10.0, Mn 1.0, Cu 0.1. Mo 0.1 and Ca 10–100. Concentrations higher than the optimum were always inhibitory to the growth of both the fungi.

The two species of *Alternaria* included in this study resembled each other in requiring Fe, Zn, Mn, Cu and Mo for their growth. All these elements are reported to be essential for the growth of large majority of fungi which have been studied critically. *A. chartarum* also required Co for its growth and sporulation. The essentiality of Co has only been reported for *Gloeosporium psidii* (Tandon 1961). *A. solani* required Ca for its growth and sporulation and thus resembled fungi studied by Steinberg (1948, 1950) and Thind and Madan (1973).

A. chartarum and A. solani showed excellent sporulation when all the essential trace elements were present in the basal medium. Similarly, Thind and Madan (1973) reported that *Claviceps microcephala* and *Microxyphiella hibiscifolia* showed excellent sporulation when the basal medium had Fe, Zn,

Mn, Cu and Mo and Fe, Zn, Mn, Cu and Ca, respectively.

The two species of *Alternaria* studied here required different concentrations of essential trace elements for their optimum growth as had also been observed in the case of different fungi (Blank 1941, Steinberg 1950, Yogeswari 1948; English & Bernard 1955, Peterson and Katznelson 1956, Thind and Madan 1973, 1977).

Under similar conditions for removal of trace elements Co has been shown to be required by *A. chartarum* but not by *A. solani*, Ca for *A. solani* but not for *A. chartarum*. This indicates that thorough screening of larger number of fungi against trace elements is required before commenting on the essentiality of these elements.

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