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TRACE ELEMENT STUDIES ON *PESTALOTIOPSIS FUNEREA* CAUSING LEAF SPOT OF *EUCALYPTUS GLOBULUS*

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Present investigation was undertaken on the effect of six trace elements (Fe, Zn, Mo, Mn, Cu and Ca), singly and in all possible combinations on the growth and sporulation of *Pestalotiopsis funerea* Desm. causing leaf spot of *Eucalyptus globulus* Labill. Significantly maximum growth of the test pathogen was recorded at 0.01, 0.1, 50, 1.0, 100 and 0.1 ppm of Fe, Zn, Mo, Mn, Cu and Ca respectively. Growth in Mn, Mo, and Cu was directly related with the increase of concentration while reverse happened with Fe, Zn and Ca. Zn and Mo in combination supported best growth. Next best combination for the growth and sporulation was Fe+Zn, Fe+Ca, Cu+Ca, Mn+Cu, Mn+Ca and Zn+Cu. The combination of Zn+Ca and Mo+Cu exhibited toxic effect. Calcium failed to induce sporulation and this effect was totally removed by addition of either Mn or Fe to it.

INTRODUCTION

The essentiality of micronutrients in nutrition of fungi is well established as some of them activate enzyme system (e.g. Zn to enolase, dipeptidase; Mn to yeast arginase) while others constitute integral parts of enzymes (e.g. Fe to catalase, the cytochromes and cytochromes oxidase; Cu to trysinase; Zn to carbonic anhydrase), Co-enzymes and other different essential organic compounds (Steinberg, 1919, 1935; Foster & Waksman, 1939 ; Lilly & Leonion, 1945; Lilly & Barnett, 1951; Tandon, 1961; Singh & Prasad, 1967; Singh & Garraway, 1975). The concentration of various essential ions also influences the formation of pigments, synthesis of vitamins, dissimilation of carbohydrates and other products. As no work on the trace element nutrition of *Pestalotiopsis funerea* Desm. causing leaf spot of *Eucalyptus globulus* Labill. has so far been reported, the present investigations were undertaken.

MATERIALS AND METHODS

Monosporic cultures of *P. funerea* isolated from the leaves of *E. globulus* were maintained on purified Czapek-Dox medium for further study.

The basal medium containing sucrose 30.0 g; $NaNO_3$ 2.0 g; K_2HPO_4 1.0 g; $MgSO_4$ 0.5 g; KCl 0.5 g; and double distilled water, 1000 ml; was employed. Only A. R. grade chemicals, double Pyrex glass distilled water and thoroughly cleaned and rinsed glass apparatus were used throughout these studies. The trace elements Fe, Zn, Cu, Mn, Mo and Ca were used in the form of their sulphates.

To get rid of traces of heavy metal contaminants, medium was purified with Steinberg (1935) method. Fifteen grams of $CaCO_3$ were added per litre of medium which was autoclaved at 10 lb. pressure for 20 minutes. The mixture was allowed to stand overnight and the supernatant was decanted off and filtered through Whatman filter paper (No. 42). This process was repeated until the medium responded to the colorimetric test (Sandell, 1959) of trace elements. Trace elements were added singly to the purified medium so as to provide 0.01, 0.1, 1, 10, 25, 50 and 100 ppm of each in different stock solutions. To study the combination effect, they were added to the purified medium in different possible paired combinations (Table II) so as to furnish 1 ppm of each in stock solution. Medium without any trace element was kept as control. An optimum *p*H 5.8 was adjusted for all the media before autoclaving.

From stock solution, 50 ml was apportioned in each of 250 ml conical flask and autoclaved at 10 lb pressure for 20 minutes. Each flask was inoculated by agar disc method (Garrett, 1936) and incubated at an optimum range of temperature 24 $\pm$ 1°C for 15 days. Mycelial mat was harvested on weighed Whatman's filter paper (No. 42) dried in an electric oven at 60°C for 48 hours and reweighed after cooling in a desiccator. Triplicate sets were used in every case and average dry weight was recorded. The final *p*H of the culture filtrates was also determined. The degree of sporulation was classified into five categories, viz., excellent, good, fair, poor and absent (nil) on the basis of visual observations. Surface area of hyphal mats covered by black spores and their colour intensity were made as criteria for visual observations which provided a clear-cut comparison among different grades of sporulation. Spore count as a means of sporulation was not found to be more appropriate. The results were statistically analysed and are recorded in the Tables.

Results and Discussion

Effect of Different Trace Elements and their Concentrations

The results obtained have been summarised in Table I.

It was observed that all the trace-elements tried proved beneficial and increased the mycelial growth at their optimum concentration. Significantly maximum growth of the *P. funerea* was recorded at 0.01, 0.1, 50, 1, 100 and 0.1 ppm of Fe, Zn, Mo, Mn, Cu and Ca respectively. Growth was found to be better in Mo, Mn, Cu and Ca as compared to Fe and Zn at their optimum. Growth in Mn, Mo and Cu was directly related to the increase in concentration while reverse happened with Fe, Zn and Ca.

It is evident from Table I that only lower concentration of Fe (0.01) and Zn (0.1) supported maximum significant growth. Similar results were obtained by Tandon (1961) and Sankhla *et al.*, (1970). Concentration of Fe and Zn higher than the optimum was found to inhibit the growth and sporulation progressively as had also been reported by Steinberg (1920); Yogeshwari (1948); Thind and Rawla (1967); Rawla (1969); Singh and Garraway (1975) with the fungi investigated by them. Singh and Prasad (1967) also suggested that the isolates of *Colletotrichum gloeosporioides* were deficient for Fe, Zn and Mn. The test pathogen, thus differed from the observations of Wahi (1967), that as the concentration of Fe in the medium increased, the sporulation of *Phytopthora colocasiae* changed from poor to fair to good or even excellent.

TABLE I

Average hyphal dry weight, final pH *and sporulation of* Pestalotiopsis funerea

Trace elements	Concentra-								
	0			0.01			0.1		
	Dry wt in mg	Sporula- tion	Final *p*H	Dry wt in mg	Sporula- tion	Final *p*H	Dry wt in mg	Sporula- tion	Final *p*H
Iron				580	F	5.5	553	F	6.0
Zinc				560	F	3.5	604	F	3.5
Molybdenum				566	P	3.5	633	P	4.5
Mangenese				570	F	6.5	559	F	7.2
Copper				579	F	5.8	613	F	5.0
Calcium				565	A	5.2	712	A	5.0
Control	552	F	4.0	—	—				—

There is significant variation among the treatment of different trace elements and their concentrations.
Treatment = 3.029 and 2.42 with 6 & 30 degree of freedom at 5%
Concentrations = 5.068 and 2.53 with 5 & 30 degree of freedom at 50%
E = Excellent, G = Good, F = Fair, P = Poor, A = Absent (Nil).

Molebdenum promoted mycelial growth upto a concentration of 50 ppm and above that there was a little fall in the level of the growth of the test pathogen. Steinberg (1936b) and Nicholas (1952) reported it to be an essential micro-element for the growth. Molebdenum showed excellent sporulation at 100 ppm while it was either absent or poor below this concentration.

Present investigation showed that there was much variation in hyphal dry weight and sporulation of the test pathogen in copper deficient and supplemented medium. Both dry weight and sporulation were found to be directly proportional to the variation in concentration. Tandon and Chandra (1962) found Cu indispensible for the sporulation of *Cercospora ricinella*, *Colletotrichum gloeosporioides* and *Curvularia penniseti*. The maxima for the growth in Cu was found at 100 ppm, although Sankhla *et al*, (1970) reported it to be toxic even at 50 ppm. Starkey and Waksman (1943) reported two fungi capable of making visible growth even in saturated solution of Copper sulphate, Thind and Rawla (1964) and Wahi (1967) found Cu essential for growth of the fungi studied by them.

Manganese supported maximum growth at 1 ppm and above which it declined gradually. Higher concentrations were of course toxic to the growth. Sporulation was found to increase with the increase of concentration. Yogeshwari (1948), with three species of *Fusarium* found that Mn not only increased the growth but also induced good sporulation. Thind and Rawla (1964) found Mn, essential for the growth of the six species of *Helminthosporium*.

on Czapek Dox medium supplemented with different trace elements and their concentrations

tion in ppm

1.0			25			50			100		
Dry wt in mg	Sporulation	Final *p*H	Dry wt in mg	Sporulation	Final *p*H	Dry wt in mg	Sporulation	Final *p*H	Dry wt in mg	Sporulation	Final *p*H
447	P	4.0	413	A	5.8	403	A	4.0	60	A	4.0
525	P	5.8	516	P	4.4	331	P	4.0	0	A	5.8
640	A	3.5	650	A	4.0	666	A	5.8	658	E	4.5
668	F	7.2	610	G	7.0	532	E	6.5	0	—	5.8
632	G	4.0	656	E	3.5	690	E	5.5	708	E	5.8
617	A	4.5	431	A	5.0	317	A	4.0	0	A	5.8
	—			—			—			—	

indicated by the exceeding of the calculated value beyond the tabulated value :

P. funerea was found to respond to added calcium by significant increase in dry weight over the control, 0.1 ppm of the concentration of Ca was an optima for its maximum growth and above which there was sudden fall in the level of growth of the test pathogen. Calcium induced no sporulation at all. It was also found to be essential for the vegetative growth of *Phytophthora fragariae* (Davies, 1959), *Phytophthora colocasiae* (Wahi, 1967) and *Helminthosporium sacchari* & *H. oryzae* (Thind & Tawla, 1964). Lampreht (1957) found it to be essential for the growth of the fungi.

(*ii*) *Effect of different combinations of the micro nutrients* :

The data on hyphal dry weight, sporulation and final *p*H have been illustrated in Table II.

As compared to the response of single trace elements, the combination of trace elements at 1.0 ppm proved much more effective (Agrawal, 1959). Zinc and Fe alone did not produce as much mycelium as when both were combined. This synergestic response of the element was also observed by earlier workers (Steinberg, 1919; Agrawal, 1959 ; Lilly & Barnett, 1951; Singh & Prasad, 1967; Singh & Garray, (1975). Singh & Garraway (1975) assumed that Zn and Fe somehow activated the production of certain enzymes and or essential organic compounds which help in proper utilization of carbohydrates, resulting in good growth of the fungus. McHan & Johnson (1970), however, had indicated the major Zn effect might be involved in regulatory mechanism controlling interrelationships between carbohydrate and nitrogen metabolism. Maximum hyphal dry weight was obtained where Zn and Mn were added. The next best

TABLE II

Showing average hyphal dry weight, final pH and sporulation of P. funerea *on Czapek Dox medium supplemented with different combination of the trace elements*

Paired combinations of the trace-elements	Hyphal dry weight in mg	Sporulation	Final *p*H
Fe+Zn	689	G	5.8
Fe+Mo	625	F	5.8
Fe+Mn	543	A	4.0
Fe+Cu	628	G	3.5
Fe+Ca	640	G	4.0
Zn+Mo	769	F	5.8
Zn+Mn	582	A	3.0
Zn+Cu	670	G	3 5
Zn+Co	103	G	5.8
Mo+Mn	596	F	4.5
Mo+Cu	203	A	3.0
Mo+Ca	623	P	4.5
Mn+Cu	695	G	7.0
Mn+Ca	655	E	4.2
Cu+Ca	646	G	5.0
Control	552	F	4.0

There is highly significant variation among the different paired combinations of micronutrients indicated by the exceeding of the calculated value beyond the tabulated value 5.303 2.01 (at 5% level) with 15 and 47 degree of freedom.

combination for the growth and sporulation was Fe+Ca, Cu+Ca, Mn+Ca and Zn+Cu. However, calcium, individually was found to inhibit sporulation at all concentrations. This effect of calcium was totally reversed by addition of either Mn or Fe to it. These beneficial effects might be due to modification in response of individual element in presence of the other. Gortener (1949) suggested that the relative concentration of various metallic ions might regulate the process of adsorption.

Combinations like Zn+Ca, Zn+Mn and Mo+Cu showed toxic effect on fungal growth and sporulation. This indicated that beneficial effect of a metal could be retarded by the presence of another incompatible metal. Singh & Garraway (1975) also observed the toxic effect of Zn and Mn in combination. Similarly Singh & Prasad (1967) while working with different isolates of *C. gloeosporioides* observed that combination of Mn and Fe with Zn had no appreciable effect on these isolates when compared to Zn alone. These ionic incompatibilities were also realised by Lilly & Barnt (1951) when they proposed a term 'ion antagonism'.

A critical study of Table I indicated that final *p*H of all the culture filtrates showed acidic reaction except Mn supplemented media where it was alkaline. These changes in *p*H might be due to changes in the relative amounts of acids and bases formed or withdrawn and to the ionization constant of these compounds (Lilly & Barnett 1951). The utilization of anions or cations from the medium drifted the *p*H either towards acid side or alkaline side respectively (Lilly & Barnett, 1951).

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