

STUDIES ON THE PHYSIOLOGY OF HIGHER FUNGI :
VII. EFFECT OF DIFFERENT ESSENTIAL ELEMENTS
ON THE VEGETATIVE GROWTH OF SOME
SPECIES OF *FOMES* AND *GANODERMA*

BY

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The present investigation have been undertaken to provide some informations on the role of different essential elements on the vegetative growth of six species of basidiomycetes, viz., *Fomes senex* Nees & Mont., *F. fastuosus* Lev., *F. durissimus* Lloyd., *Ganoderma applanatum* (Pers.) Pat., *G. lucidum* (Leyss.) Karst., and *G. colossus* (Fr.) Bres.. The following elements have been selected and they have been purified according to the methods of Steinberg (1940) and Nicholas (1952): Fe, Zn, Cu, Mn, Mo, Ca, K, Mg, S, and P. The above mentioned elements have been used as salts. Cu has been found to have maximum stimulatory effect in case of *G. applanatum*, *Fomes senex*, and *F. fastuosus*, while Zn has best effect upon growth of *G. lucidum*, and *G. colossus*. In case *F. durissimus*, the maximum stimulatory effect has been shown by Fe. A possible explanation of this diversity in the metabolism of different mineral elements by the test-fungi have been discussed.

INTRODUCTION

The necessity of essential mineral elements in the nutrition of fungi has long been recognised since the cultivation of filamentous fungi on synthetic media.

Investigation on the mineral nutrition of fungi have started with the classical researches of Raulin. There is a voluminous literature on the subject including reviews by Steinberg (1939), Foster (1939, 1949), Perlman (1949), Lavollay (1955), Lilly and Barnett (1951), Hawker (1950), Cochrane (1958), and Lilly (1965).

Though considerable amount of work has already been done on mineral nutrition of some basidiomycetes yet no data are practically available on different species of *Fomes* and *Ganoderma*. Fries (1938) studied the mineral nutrition of *Polyporus abietinus* and *Polyporus adustus* and concluded that the addition of Cu and Mn salts to a glucose salts (including Fe) medium had no effect on vegetative growth of these fungi. Bertrand's researches (1943) showed that *Ammanita muscaria* contained 61 to 156 mg. of vanadium per kg. of the mycelia. Lindeberg (1944) demonstrated a synergistic effect between magnesium and calcium on the growth of various species of *Marasmius*. It was found that *Polyporus vaporarius* produced enough oxalic acid to counteract the growth of inhibiting effect of CuSO_4 present in

impregnated timber (Kind, 1945). Ingleman, (1948, 1949) and Malmgren (1952) reported that no depolymerization of phosphorus compounds take place by extracts from basidiocarps of *Collybia velutipes*, *Marasmius* spp., *Merulius domesticus*, *Polyporus betulinus*, and *Tricholoma equeata*. Grim and Allen (1945) found that 0.001 PPM of zinc concentration to be sufficient for optimal growth of *Ustilago sphaerogena*. Mizumoto (1956) reported that the lowest concentration of sodium-penta-chlorophenolate require to check the growth of *Lenzites abietina*, *L. subferruginea*, *L. trabea*, and *L. sepiaria* were 0.0006%, 0.0007%, 0.0008 % and 0.0007% respectively as against 0.002 % for *Polystictus sanguineus*. He also reported that the lower concentration of CuSO_4 would be necessary for inhibition of growth of *Lenzites abietina* is 0.3%, 0.4% for *L. subferruginea* and *L. sepiaria* and 0.5% for *L. trabea* in comparison with those of 0.7% for *Poria vaporaria* and 0.5% for *Polystictus sanguineus*. Bose and Maury (1959) mentioned that 100/ μc /2 cm. of P^{32} had inhibitory effect on growth of *Ganoderma applanatum*. While in India, Banerjee and Mukhopadhyay (1962) showed that 0.8% of ZnCl_2 and CuSO_4 inhibit the growth of *Merulius similis* in culture.

The present investigation is a further contribution on the series of Physiology of Higher Fungi, The previous contributions are on the effect of different physical and chemical environment on the vegetative growth (Samajpati, 1967). While the present one deals with the effect of different essential elements on the vegetative growth of six species of basidiomycetes.

MATERIALS AND METHODS

The test-fungi have been collected from Calcutta and suburbs. The basidiocarps of all the species of *Fomes* are found to grow on *Swietenia mahagoni* L., while those of the *Ganoderma* on *Polyalthia longifolia* Benth. & Hook. f. They have been found to appear during the months of July to September, every year.

The experiment has been conducted with the tissue cultures from the basidiocarps of the test-fungi, following the method proposed by Dugger et al. (1917). Glucose-caesin hydrolysate basal medium (Leonian and Lilly, 1945) has been selected for this investigation as it has been recommended as well balanced medium. The composition of the medium is as follows : Glucose-25 gms., Casein hydrolysate-2 gm., $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.5 gm., KH_2PO_4 1.0 gm., Fumaric acid-1.32 gm., Na_2CO_3 -1.12 gm., Fe-as sulphate-0.2 mg., Zn-as sulphate-0.2 mg., Mn-as sulphate-0.1 mg., and distilled water to make 1000 ml.

The following method has been adopted in determining the essential nature of each of the selected elements in the present investigation. In each experiment the basal medium has been made free of the element in question. The essentiality of each element of the test-fungi has been finally measured by the rate of the growth of the test fungi in response to the known concentration of the some soluble compound containing the element. The standard basal medium has been first prepared but without any source of the selected elements. The medium and the compounds containing the elements have been purified according to the method of Steinberg (1940) and Nicholas (1952).

After purification of both the medium and the compound containing the elements, the source of the mineral elements has been added as 0.005 PPM to the medium in several flasks. The different sources are compounds of sulphur, phosphorous, iron, zinc, copper, manganese, molybdenum, calcium, potassium, and magnesium,. Several flasks with the medium but without any mineral elements have been kept as controls. The media have been sterilized at 15 lb pressure for 15 minutes, and inoculated separately by the test-fungi following the method of Lindeberg (1944) modified by Norkrans (1953).

Pyrex Erlenmeyer flasks (250 ml.) have been used as culture vessels. In each of the required numbers of flasks, 25 ml. of the medium has been taken. They have been properly plugged before sterilization. After inoculation all the flasks have been incubated (stationary) at 30°C. in darkness. Sufficient flasks have been inoculated and incubated to provide five replicates for each treatments for 20 days. The length of the incubation period necessary for this experimental optimum growth is determined by running a series of experiments. Every fourth-day, five replicates of each treatments have been harvested five times and the contents have been filtered through a tared filter paper (Whatman No.1), using a Buchner funnel. The residual mycelia have been washed with distilled water to remove any trace of the medium and then dried at 60 C. in an oven for 24 hours. After drying they have been kept in a closed vacuum dessicator, cooled and later weighed in a chemical balance (Sartorius).

The quantitative data thus obtained on individual test nutrients have been based on the average dry weights of mycelia produced in the test medium of five flasks. In all the experiments, the pH of the medium for growth of each fungus has been maintained optimum by adding N/15 phosphate buffer.

RESULTS

The results obtained during the experimental periods are given in Tables 1-3.

Form the Tables 1-3, it is evident that the test -fungi differ widely among themselves in relation to their utilization of mineral elements. Phosphorous has maximum stimulatory effect on the vegetative growth of all the test-fungi. Sulphur, on the other hand, has inhibitory effect on the growth of all the three species of *Ganoderma*, while stimulatory in case of *Fomes*. Potassium has a inhibiting tendency on the growth of *G. applanatum*, *G. lucidum*, and *F. fastuosus*, and stimulatory effect in case of *G. colossus* and *F. senex*, while it has almost no effect on the growth of *F. durissimus*. Molybdenum has inhibitory effect on the growth of *G. applanatum* and *G. lucidum*, almost no effect on the *F. durissimus*, while stimulatory in case of other test-fungi. Copper has stimualtory effect on the growth of *G. applanatum*, *F. senex*, and *F. fastuosus*, but next to phosphorus, and followed by others. Zinc has stimulatory effect on the growth of *G. colossus* and *G. lucidum* while Iron for *F. durissimus*, but all of them next to phosphorus and followed by others. All the other elements have stimulatory effect on the growth of the test-fungi.

Table 1. Data (mean) showing the effect of different essential elements on the vegetative growth (mg./25 ml.) of different species of *Fomes* and *Ganoderma*

Fungi Essential elements	Fungi (F) X Essential elements (T)						T-means
	G. applanatum	G. lucidum	G. colossus	F. senex	F. fastuosus	F. durissimus	
Control	298.5	299.8	301.6	105.5	112.6	119.6	206.3
Cu	300.6	306.2	309.6	107.0	138.9	124.9	211.3
Fe	303.2	307.2	307.7	109.4	117.6	126.3	211.9
Zn	304.8	306.5	309.0	109.6	116.6	124.2	211.8
Mn	295.6	303.5	305.1	106.0	113.6	121.2	207.5
K	294.5	301.5	303.4	106.3	113.2	120.2	206.5
Ca	300.3	304.9	306.1	106.9	115.3	121.7	209.4
Mo	300.7	304.0	304.7	106.4	112.5	119.9	207.8
Mg	302.8	304.6	303.1	106.3	113.2	122.1	208.7
S	269.6	274.5	297.9	105.5	114.2	120.1	197.0
P	303.6	306.2	306.6	109.1	116.3	154.5	211.1
F-means	297.6	301.6	304.9	107.0	116.7	122.3	

S. Em for F = ± 0.00020

S. Em for T = ± 0.00024

S. Em for F X T = ± 0.00063

C. D. at 5% of P of F = 0.000556

C. D. at 5% of P of T = 0.000667

C. D. at 5% of P of F X T = 0.001752

Table 2. Data (mean) showing the effect of incubation periods on the vegetative growth (mg./ 25 ml.) of different species of *Fomes* and *Ganoderma* in the presence of different essential elements.

Fungi (F) X Incubation periods (I)

Fungi Incubation period	G. applanatum	G. lucidum	G. colossus	F. senex	F. fastuosus	F. durissimus	I-means
4 days	277.9	283.6	284.9	99.4	106.0	110.8	193.2
8 days	293.1	251.6	290.5	104.1	110.6	118.1	201.4
12 days	300.6	303.9	305.2	106.9	116.4	122.8	208.3
16 days	309.3	314.3	315.9	110.1	119.3	117.5	215.0
20 days	320.7	324.1	326.4	114.7	123.6	132.5	211.8
F-means	297.6	301.6	304.9	107.0	116.7	122.3	

S. Em for I = ± 0.00019

S. Em for F X I = ± 0.00046

C. D. at 5% of P for I = 0.000528

C. D. at 5% of P of F X I = 0.001279

Table 3. Data (mean) showing the effect of interactions of different essential elements on the vegetative growth (mg./25ml.) of different species of *Fomes* and *Ganoderma*

Incubation periods (I) X Essential elements

Incubation period Essential elements	4 days	8 days	12 days	16 days	20 days	T-means
Control	191.3	199.4	206.5	213.5	220.7	206.3
Cu	195.5	204.4	211.5	218.3	226.8	211.3
Fe	196.3	204.8	211.8	219.1	227.3	211.9
Zn	196.7	204.2	211.5	218.9	226.5	211.8
Mn	191.8	200.5	207.9	215.0	222.4	207.5
K	191.8	199.8	206.6	213.6	220.8	206.5
Ca	193.8	202.2	209.7	216.4	224.8	209.4
Mo	191.9	201.6	208.6	214.9	216.0	207.8
Mg	193.2	202.0	209.1	216.0	223.2	208.7
S	186.8	192.2	197.6	201.9	206.4	197.0
P	196.3	204.3	211.1	218.2	225.5	211.1
I-means	193.2	201.4	208.3	215.0	221.8	

S.Em. for I X T = 'F' test not significant

DISCUSSION

After evaluating the role of different mineral elements on the vegetative growth of the test-fungi, it has been found that phosphorus has the maximum stimulatory effect on growth of them, though Ingleman (1948, 1949) and Malmgren (1952) have reported that several basidiomycetes have failed to depolymerize the phosphorus compounds. Bose and Maury (1959) have stated that phosphorus has inhibitory effect on growth of *Ganoderma applanatum*, which does not tally with the present findings but in the utilization of other mineral elements by all the test-fungi have been found to be different. The inhibitory effect of sulfur on the growth of all the species of *Ganoderma* reveals that these species might lack the specific enzymes of the oxidation pathway of sulfur compounds.

Phosphorus is probably absorbed directly during the early stages of growth and is converted into organic compounds in the cell (Fries, 1956). The maximum stimulatory effect of phosphorus is obvious due to its manifold role in metabolism and specially in energy transfer. The stimulatory effect of sulfur on the growth of all the three species of *Fomes* shows that these species are able to use sulfur by oxidation and sulfur possibly is utilised in the synthesis of enzymes, proteins, amino acids, thiamine and biotin for the metabolism (Lilly, 1965). The essentiality of iron in the metabolism of fungi is now well established. Iron is directly utilised by fungi, (Cochrane, 1958) and is required as a constituents of various enzymes e. g., cytochrome, cytochrome oxidase, catalase, and also in nitrate and hyponitrite synthesis. The stimulating effect of the iron on the growth of the test-fungi reveals that iron must have been utilized in the same pathway by the test-fungi. The stimulatory effect of iron, copper, molybdenum, and magnesium also reveals that possibly all these elements are required by the test-fungi for nitrogen assimilation (Medina and Nicholas, 1957). The importance of zinc for the growth of fungi has been reported several times (Cochrane and Hawley, 1956). The metabolic effects of zinc on the growth of fungi have been discussed by Foster (1939, 1949) and Perlman (1949). Zinc is known to activate few enzymes specifically and other non-specifically. Copper plays an essential part in the formation of certain enzymes including tyrosinase, though it is used as a constituent of fungicides. The concentration of copper, therefore, has a vital role in this respect. Manganese has been shown to be the natural activator of arginase and other enzymes. Potassium plays an important role in the synthesis of pyruvic acid in the absence of thiamine (Nakamigawa and Okuda, 1960). The role of calcium is still controversial.

On the basis of present study, it can be said that the role of mineral elements on the rate of vegetative growth of the test-fungi are almost significant which have already been discussed. But to characterise the above

statements, extensive studies are essential for exploring the the inner mechanisms of metabolism of these mineral elements by the test-fungi. Researches in this line are already in progress, but nothing is at hand now to mention here.

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