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Phytopathology

EFFECT OF VITAMINS AND HORMONES ON THE GROWTH OF *PESTALOTIOPSIS FUNEREA* CAUSING LEAF SPOT OF *EUCALYPTUS GLOBULUS*

by R. K. UPADHYAY* *and* R. S. DWIVEDI

Department of Botany, Banaras Hindu University

Varanasi 221 005

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The effect of vitamins and hormones on the growth and sporulation of *Pestalotiopsis funerea* Desm., causing leaf spot of *Eucalyptus globulus* Labill., has been studied. The test pathogen responded well to the external supply of these two. Out of seven vitamins, best growth was obtained with 50 ppm of thiamine followed in order by 50 ppm of calcium pantothenate, 100 ppm of biotin, 100 ppm of vitamin B_{12} and 50 ppm of inositol. Growth was found to decline beyond their respective optimum concentrations. Ascorbic acid and nicotinic acid were not found to be beneficial. Different combinations of vitamins were also tried and proved to be more effective. Varying concentrations of hormones exhibiting maximum growth were 25 ppm of maleic hydrazide (MH), 10 ppm of indole-3-butyricum acetic acid (IBA), 5 ppm of indole-3 yl-acetic acid (IAA) and 5 ppm of 2,4. dichlorophenoxy acetic acid (2.4.D) in descending order. Serious depression in growth occurred beyond their respective optimum concentrations and ultimately reached to a level much lower than the control.

INTRODUCTION

Fungi require vitamins and hormones for their growth and reproduction. Some fungi have the ability to synthesize vitamins while others depend on their exogenous supply. Schopfer (1934) was first to report that *Phycomyces blackesleeanus* needed thiamine for its growth. Most of the known vitamins in the cell have a catalytic function as co-enzymes or constituent parts of co-enzymes. Bilgrami and Verma (1978) reviewed various literatures regarding the metabolic role of vitamins in fungal physiology. Differences in growth of fungi have been observed by addition of vitamins (Leonian & Lilly, 1942; Mathur *et al.*, 1950; Tandon & Bilgrami, 1957; Chatrath *et al.*, 1968; Bais *et al.*, 1970; Jamaluddin *et al.*, 1973; and Bahl & Grewal, 1975) and hormones (Singh, 1964; Sankhla *et al.*, 1965; Haware, 1969; Sankhla *et al.*, 1973 and Khan *et al.*, 1975). During our detailed study of *Pestalotiopsis funerea* Desm., causing leaf spot of *Eucalyptus globulus* Labill., it was observed that the addition of 0.05% yeast extract (containing vitamins, hormones and amino acids) in Czapek-Dox medium induced good mycelial growth and sporulation of the pathogen, as against poor growth on the medium devoid of it. Keeping it in view, present investigation was undertaken to ascertain the effect of certain vitamins and hormones on the growth and sporulation of *P. funerea*.

**Present address:* Department of Dravyaguna, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221 005.

MATERIAL AND METHODS

Single spore cultures of *P. funerea* isolated from the leaves of *E. globulus* were maintained on purified Czapek-Dox medium at 24±1°C for further study.

The basal Czapek-Dox medium was treated with activated powdered charcoal to remove any trace of vitamins if present as an impurity (Lilly & Barnett, 1951). Chemicals supplied by B.D.H. and E. Mercks were employed throughout the present investigation. All the glass-wares were thoroughly cleaned and rinsed with distilled water treated with activated charcoal (Vitamin free) and dried thereafter. Seven vitamins namely thiamine, vitamin B_{12}, niacin, biotin, ascorbic acid, inositol and calcium pantothenate were mixed individually in aseptic conditions with the medium in desired quantities so as to give the final concentrations of 25, 50, 100 and 200 ppm of each in their respective separate sets. Three sets of vitamins in different combinations were also prepared which are as follows:

i) thiamine + biotin + niacin + ascorbic acid

ii) thiamine + biotin + ascorbic acid + calcium pantothenate + inositol + niacin

iii) inositol + calcium pantothenate + niacin

Vitamins were added in such amounts so as to furnish 100 ppm of each in the mixture. *p*H of the media was adjusted to 5.8 before autoclaving which was found to be most favourable for the growth of the pathogen.

From the respective stock solutions, 50 ml medium was apportioned in each of 250 ml conical flask and subjected to fractional autoclaving for three successive days. Each flask was inoculated by agar disc method (Garrett, 1936) and incubated at an optimum temperature 24±1°C for 15 days. Inoculum disc was taken from a culture growing on vitamin free basal medium. Mycelial mats were harvested on weighed Whatman's filter paper (No. 42), dried in an electric oven at 60° for 48 hr and reweighed after cooling in a desiccator. Triplicate sets were used in each 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 (Upadhyay *et al.*, 1977). The results were statistically analysed and are recorded in the tables.

Four hormones namely maleic hydrazide (MH), 2.4. dichlorophenoxy acetic acid (2.4.D), indole-3-yl-acetic acid (IAA) and indal-3-butyricum acetic acid (IBA) were added individually to the basal medium in separate sets. To get different concentrations, the varying amounts of each were dissolved in 1 c.c. of acetone separately and added to the sterilized medium while it was still warm. The different concentrations of each were 1, 5, 10, 25, 50 and 100 ppm. The hormone solutions were always freshly prepared and comparison was made with two controls, one containing acetone and the other without acetone in the basal medium. The rest of the processes for hyphal dry weight, *p*H and sporulation were same as described above.

TABLE I

Average hyphal dry weight, final pH and sporulation of Pestalotiopsis funerea *on purified Czapek-Dox medium supplemented with different vitamins and their concentrations*

S. No.	Vitamins	Concentration in ppm											
		25			50			100			200		
		Hyphal dry wt pH (mg)	Final pH	Sporulation*	Hyphal dry wt (mg)	Final pH	Sporulation*	Hyphal dry wt (mg)	Final pH	Sporulation*	Hyphal dry wt (mg)	Final pH	Sporulation*
1.	Thiamine	611	6	E	643	6	E	478	6	G	475	6	G
2.	Vitamin B_{12}	560	6	G	585	6	G	600	6	E	530	6	G
3.	Niacin	568	6	G	567	6	G	541	6	G	501	6	G
4.	Biotin	561	6	G	611	6	G	614	6	E	557	6	G
5.	Ascorbic acid	560	6	G	565	6	G	546	6	G	533	6	E
6.	Inositol	565	6	G	611	6	E	574	6	G	549	6	G
7.	Calcium Pantothenate	603	6	E	631	6	E	607	6	E	570	6	E
8.	Control	560	6·5	F	560	6·5	F	560	6·5	F	560	6·5	F

* E, Excellent; G, Good; F, Fair

Statistical analysis : There is significant variation among the treatment of different vitamins and their concentrations indicated by the exceeding of the calculated value beyond the tabulated value.

Treatment (vitamins) = $3{\cdot}67 > 2{\cdot}57$ with 7 and 21 degrees of freedom at 5 %

Concentrations = $5{\cdot}407 > 3{\cdot}07$ with 3 and 21 degrees of freedom at 5 %

Results And Discussion

(i) *Effect of Vitamins*

It is evident from the Table I that mycelial growth of *P. funerea* responded well to the external supply of vitamins. As compared to control, better and maximum hyphal dry weight and sporulation were obtained when thiamine, vitamin B_{12}, biotin, inositol and calcium pantothenate were added individually in the vitamin free basal media at 50, 100, 100, 50 and 50 ppm of concentrations respectively. The hyphal dry weight increased with the increase in concentrations of vitamins reaching to a maximum and then declined. Niacin and ascorbic acid were not proved to be beneficial even at lower concentrations.

Best growth was obtained with thiamine followed in order by calcium pantothenate, biotine, inositol, vitamin B_{12}, ascorbic acid and niacin at their respective optimum concentrations. Increased growth with the former five vitamins exhibited the fact that medium was partially deficient for them. Good sporulation was recorded with all vitamins treated media as compared to fair with control.

Thiamine promoted best and highly significant growth and excellent sporulation up to a concentration of 50 ppm and above which declined suddenly. Lilly and Barnett (1951), Singh (1963), Sankhla and Mathur (1967) and Bais *et al.* (1970) reported it to be more beneficial for the growth of the fungi studied by them. Wahi (1967) showed that mycelial development decreased after a particular optimum concentration. This beneficial effect of thiamine might be due to its role in various metabolic activities as it performs the function of co-enzymes in several biochemical reations (Bilgrami & Verma, 1978).

Next best and maximum growth was recorded with the medium supplemented by calcium pantothenate at 50 ppm of concentration and above which decreased gradually. Sporulation was always excellent on lower as well as higher concentrations. Similar results were obtained by Satyavir and Grewal (1973) on *Fusarium caeruleum*. Induced growth with calcium pantothenate might be considered to be caused by (i) calcium itself which was found to promote growth (Upadhyay *et al.*, 1977), and (ii) Pantothenic acid which forms a fraction of the co-enzymes 'A' that catalyzes all the biological reaction concerning transfer of acetyl group and fatty acid metabolism in general (Bilgrami & Verma, 1978).

Biotin and vitamin B_{12} supported maximum and good growth at 100 ppm and beyond which proved to be depressive. Satyavir and Grewal (1973), Sankhla and Mathur (1967) and Bahl and Grewal (1975) have reported biotin deficiency for the fungi studied by them. Biotin has been found to be associated with reactions involving fixation of CO_2 into large organic molecules, phosphorylation, deamination of some amino acids, ornithine cycle as well as utilization of ammonia (Bilgrami & Verma, 1978; and Fries, 1943). Vitamin B_{12} was found to be essential for the reduction of l-carbon fragments e.g. reduction of formyl to methyl group.

Inositol stimulated growth and sporulation up to 50 ppm of concentrations only. Higher concentrations (more than 100 ppm) were found to be inhibitory for the growth. Singh (1963) and Bais *et al.* (1970) reported its deficiency for the fungi studied by them. Its partial deficiency for various yeasts was reported by Leonian

and Lilly (1942) and Burkholder (1943). Similarly Jamaluddin *et al.* (1973) obtained depression in growth at higher concentrations. The statements of Bilgrami and Verma (1978) also support the present observation as it does not have a catalytic role. Moreover, it comprises structural components of the cells rather than a functional unit.

Ascorbic acid and nicotinic acid were not found to be beneficial even at lower concentrations however, higher concentrations were detrimental. Similar results with ascorbic acid were also obtained by Misra and Mahmood (1961) and Bais *et al.* (1970). Shukla and Sarkar (1972) obtained minimum mycelial growth of *Botryodiplodia theobromae* Pat. with nicotinic acid. Nicotinic acid was found to be essential as a component of NAD and NADP, it reversibly oxidized and reduced and thereby, serve as oxidizing and/or reducing agents. Since pathogen does not response to its addition, it may be inferred that pathogen was auxoautotrophic for this vitamin.

TABLE II

Average hyphal dry weight, final pH *and sporulation of* Pestalotiopsis funerea *on purified Czapek-Dox medium supplemented with different combinations of vitamins*

S. No.	Mixture of vitamins	Hyphal dry wt (mg)	Final *p*H	Sporulation
1.	Thiamine + niacin + biotin + ascorbic acid	630	6·0	Excellent
2.	Thiamine + biotin + ascorbic acid + calcium pantothenate + inositol + niacin	638	6·0	Excellent
3.	Inositol + calcium pantothenate + niacin	632	6·0	Excellent
4.	Control	560	6·5	Fair

There is significant variation among the different combinations of vitamins as compared to control indicated by the exceeding of the calculated value beyond their tabulated value 746·33 > 4·07 (at 5 % level) with 3 and 8 degrees of freedom.

Table II shows that the various combinations of vitamins produced significantly better growth and excellent sporulation as compared to control. The individual inhibitory action of niacin and ascorbic acid in combination with others thus got rid of which might be due to modifications in their individual responses. A very slight change in *p*H (5.8–6.0) was noticed in all the vitamins amended culture filtrates however, it was comparatively more in control (5.8–6·5). Better stability in *p*H with vitamin amended media exhibited the fact that they influenced directly/indirectly to the ionic exchange and acid and bases formed or withdrawn from the growth media.

TABLE III

Average hyphal dry weight, final p*H and sporulation of* Pestalotiopsis funerea *on Czapek-Dox medium supplemented with different hormones and their concentrations*

S. No.	Concentration in ppm	** MH			** IBA			** IAA			** 2·4·D		
		Hyphal dry wt. (mg)	Final *p*H	Sporulation*	Hyphal dry wt. (mg)	Final *p*H	Sporulation*	Hyphal dry wt. (mg)	Final *p*H	Sporulation*	Hyphal dry wt. (mg)	Final *p*H	Sporulation*
1.	100	446	7·0	E	360	7·0	A	304	4·5	—	302	7·5	A
2.	50	557	7·0	E	500	7·0	A	362	4·0	G	350	7·0	A
3.	25	640	7·0	E	512	7·0	F	452	4·0	G	355	7·0	A
4.	10	637	7·0	E	565	7·0	G	535	4·0	G	528	7·0	A
5.	5	575	7·2	G	552	6·5	G	590	6·5	E	577	7·0	P
6.	1	565	7·2	G	548	6·5	G	590	6·5	E	551	7·0	P
7.	Control in acetone	546	7·0	F	546	7·0	F	546	7·0	F	546	7·0	F
8.	Control	552	6·2	F	552	6·2	F	552	6·2	F	552	6·2	F

* *E*, Excellent; *G*, Good; *F*, Fair; *P*, Poor; *A*, Absent.

** MH = Maleic hydrazide; IBA = indole-3-butyricum acetic acid; IAA = indole-3 yl-acetic acid 2·4·D = 2·4·dichlorophenoxy acetic acid.

Statistical analysis : There is significant variation among the treatment of different hormones and their concentrations indicated by the exceeding of the calculated value beyond the tabulated value.

Treatment (hormones) = 4·869 > 2·57 with 7 and 21 degrees of freedom at 5 %

Concentrations = 8·960 > 3·07 with 3 and 21 degrees of freedom at 5 %

(ii) *Effect of hormones*

It was observed that all the growth substances tried proved beneficial to some extent at lower concentrations only (Table III). The concentrations supporting maximum growth were 25 ppm of MH, 10 ppm of IBA, 5 ppm of IAA and 5 ppm of 2.4.D in descending order. The growth of the pathogen started declining on concentrations higher than the optimum reaching to a level much lower than the control. 2.4.D proved to be most toxic at higher concentrations. Similarly Misra and Mahmood (1961), Sankhla *et al.* (1965), Agnihotri and Prasad (1965) and Chatrath *et al.* (1968) obtained beneficial effects at lower concentrations however, higher concentrations were detrimental.

The test pathogen responded best to the MH. The hyphal dry weight increased with the increase in concentrations and reached a maxima at 25 ppm beyond which it started declining. Sankhla *et al.* (1965) obtained maximum growth of *Fusarium oxysporum* f. *cumini* Prasad and Patel with maleic hydrazide amended media. Sporulation was found to be excellent on higher concentrations.

IBA and IAA exhibited maximum growth at 10 and 5 ppm of concentrations respectively and above which proved to be depressive. Similar observations were made by Singh (1964) and Sankhla *et al.* (1970). No sporulation was recorded above 10 ppm of IBA, however, it was good even on highest concentration of IAA.

Maximum growth of the pathogen was recorded at 5 ppm concentration of 2.4.D beyond which serious depression in growth occurred. It was found to be detrimental to sporulation at all concentrations. Haware (1969) tested 2.4.D with *S. cepivorum* and *S. rolfsii* but failed to get induced growth.

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