

NUTRITIONAL REQUIREMENTS OF TRACE ELEMENTS, VITAMINS AND GROWTH HORMONES FOR GROWTH AND SPORULATION OF *FUSARIUM GRAMINEARUM* SCH.

By

B. Pal, N. C. Chattopadhyay and B. Nandi
Department of Botany, Burdwan University,
Burdwan, West Bengal

The requirements of trace elements, vitamins and growth hormones for growth and sporulation of *F. graminearum* were studied in culture. Among five trace elements tested zinc exhibited maximum vegetative growth and sporulation at 0.01 ppm and 10 ppm respectively. In presence of different sources of vitamins, the test-fungus was found to be strongly deficient for biotin and partially deficient for thiamine and inositol. GA proved to be the best source among the externally supplied growth hormones tested for the fungus and was followed in descending order of promoting effects by IBA, NAA and IAA.

INTRODUCTION

The necessity of different trace elements, vitamins and growth hormones on the nutritional physiology of fungi has been extensively studied as is evident from the reviews (Nicholas, 1952; Steinberg, 1950; Fries, 1961, 1965; Gruen, 1959). Among the trace elements, iron, zinc, copper, manganese and molybdenum are known to be essential and indispensable for growth and development of most fungi whereas calcium is essential for some. Vitamin-requirements of fungi (Tandon *et al*, 1957; Cochrane, 1958) reveal that auxoautotrophic forms are able to grow in vitamin free medium indicating their ability to synthesize the substance while auxoheterotrophic forms show varied degree of deficiencies for

one or more vitamins. Most of the known vitamins have catalytic functions in cells and are known to be precursor of coenzymes or constituent parts of the enzymes. As such, the deficiency of a vitamin prevents normal metabolic activities of the organism (Tanber, 1939). Reports on growth response of fungi to growth hormones in different concentrations are conflicting (Gruen, 1959) showing inhibition at higher concentrations and often even strong promotion at lower concentrations (Leonian and Lilly, 1937).

MATERIALS AND METHODS

The test-fungus, *Fusarium graminearum* was isolated from malformed young vegetative tissues of Gandharaj (*Gardenia jasminoides*) plants collected from Hooghly in West Bengal in 1973. The disease was found to be very frequently occurring in several gardens in Hooghly District causing extensive malformation of young branches of Gandharaj plants showing abnormally reduced internodes and very small leaves. The identification of the organism was confirmed by the Division of Mycology and Plant Pathology, IARI, New Delhi.

The present investigation was undertaken to study the effect of different trace elements, vitamins and growth hormones on the growth and sporulation of *F. graminearum* in culture. The compounds were used as 0.01, 0.1, 1.0, 10.0 and 100.0 ppm.

Richard's synthetic solution was taken as basal medium for the present study. The medium was purified following Steinberg (1950). The pH of the medium was adjusted to 6.6 which was found to be optimum for the growth of the fungus in an earlier experiment. For the experiment with trace elements, zinc, copper, iron and manganese in the form of sulphate and molybdenum as ammonium molybdate were used. Vitamins, viz. biotin, thiamine, inositol, pyridoxine and growth hormones, viz., indole-3- γ 1-acetic acid (IAA), indole-3-butyric acid (IBA), gibberelic acid (GA), ϵ -naphthol acetic acid (NAA), maleic hydrazide (MH) were used in separate experiments to study their effects on growth and

sporulation. Calculated amount of these substances were added separately to 100 ml medium to get the desired concentrations.

The media (25 ml) for each series of experiment with trace elements, vitamins and growth hormones were taken in each Erlenmeyer flasks, plugged, sterilized and finally inoculated with uniform discs (5mm diam.) of inocula. The inocula were obtained from actively growing cultures of the fungus on trace element of vitamin or growth hormone free Richard's agar medium. Several flasks containing medium without trace element or vitamin or growth hormone were also inoculated as controls. Three replicates were prepared for each concentration of the substances as well as for controls. All the inoculated flasks were incubated at $28 \pm 1^\circ\text{C}$ in darkness. After seven days the mycelia were harvested, washed and dried at 60°C to a constant weight. For counting the spores the cultured medium was shaken thoroughly and one ml of this was taken in haemocytometer of 0.1 mm depth. The number was calculated per ml of the original medium. The results are presented in Tables 1, 2 and 3 respectively.

RESULTS

All the trace elements tried exhibited stimulatory effect with the exception of molybdenum which slightly inhibited growth (Table 1). Maximum growth was recorded in zinc (0.1 ppm) followed by copper and manganese (0.01 ppm). In zinc and iron optimum growth was found in 0.1 ppm below and above of which growth decreased. Growth in copper, manganese and molybdenum was maximum at the lowest level which decreased gradually with the increase in concentrations. Sporulation of the fungus was also greatly promoted by the trace elements excepting in copper and molybdenum showing distinct inhibition.

Biotin was the most effective among the vitamins for growth of fungus even at the lowest concentration (0.01 ppb) whereas at the same concentration, the rest of vitamins did not show any significant stimulatory effect as compared to control (Table 2). Following biotin promoting effect was observed in decreasing

Table 1. Effect of different concentrations of trace elements on growth and sporulation of *F. graminearum* in culture

Trace elements	Dry wt. of mycelium (mg)* / Sporulation (million/ml)*	Concentrations in ppm				
		0.01	0.1	1.0	10.0	100.0
Zn	Mycelial wt.	240.00	313.50	225.50	215.00	202.50
	Sporulation	0.45	0.45	0.59	0.67	0.52
Fe	Mycelial wt.	164.00	220.30	188.00	168.00	147.00
	Sporulation	0.25	0.12	0.00	0.37	0.16
Cu	Mycelial wt.	305.00	272.00	233.00	201.00	172.50
	Sporulation	0.12	0.25	0.60	0.00	0.00
Mn	Mycelial wt.	300.00	254.50	210.00	188.20	165.20
	Sporulation	0.50	0.45	0.37	0.25	0.12
Mo	Mycelial wt.	175.50	164.00	159.20	151.30	142.30
	Sporulation	0.25	0.12	0.00	0.00	0.00
Control	Mycelial wt.	198.00				
	Sporulation	0.45				

* Average of three replicates

Table 2. Effect of different concentrations of vitamins on growth and sporulation of *F. graminearum* in culture

Vitamins	Dry wt. of mycelium (mg)* / Sporulation (million/ml)*	Concentrations in ppm				
		0.01	0.1	1.0	10.0	100.0
Biotin	Mycelial wt.	378.00	374.00	353.50	230.00	210.00
	Sporulation	0.16	0.33	0.50	0.41	0.25
Thiamine	Mycelial wt.	153.00	190.00	200.50	252.50	183.30
	Sporulation	0.25	0.54	0.45	0.41	0.30
Inositol	Mycelial wt.	159.60	222.30	200.60	186.60	166.30
	Sporulation	0.25	0.25	0.33	0.37	0.62
Pyridoxine	Mycelial wt.	150.00	164.00	167.00	192.00	202.00
	Sporulation	0.25	0.33	0.37	0.65	0.45
Control	Mycelial wt.	152.00				
	Sporulation	0.12				

* Average of three replicates

Table 3. Effect of different concentrations of growth hormones on growth and sporulation of *F. graminearum* in culture

Growth hormones	Dry wt. of mycelium (mg) */ Sporulation (million/mi) *	Concentrations in ppm				
		0.01	0.1	1.0	10.0	100.0
IAA	Mycelial wt.	301.00	285.50	276.20	249.00	96.00
	Sporulation	0.16	0.12	0.08	0.08	0.04
GA	Mycelial wt.	433.50	485.30	425.00	415.20	388.00
	Sporulation	0.35	0.66	0.25	0.25	0.12
MH	Mycelial wt.	226.00	290.50	205.00	174.60	150.00
	Sporulation	0.08	0.25	0.12	0.00	0.00
NAA	Mycelial wt.	343.00	377.20	385.50	253.20	172.30
	Sporulation	0.04	0.08	0.12	0.00	0.00
IBA	Mycelial wt.	418.00	444.20	420.00	328.00	214.00
	Sporulation	0.04	0.08	0.08	0.04	0.04
Control	Mycelial wt.	272.00				
	Sporulation	0.25				

* Average of three replicates

order by thiamine, inositol and pyridoxine. However, the same concentration did not prove to be the optimum for all the vitamins. With increase in concentrations of the vitamins, growth increased upto a certain level and then decreased excepting in case of pyridoxine where maximum growth was observed at the highest concentration used (100 ppm). Sporulation of the fungus was also affected by the addition of vitamins. Pyridoxine which showed only slight promotion in vegetative growth exhibited maximum sporulation (10 ppm) followed by inositol (100 ppm). Biotin which was most effective for vegetative growth exhibited only moderate sporulation.

All the tested growth hormones showed stimulatory effects even at lowest concentration (0.01 ppm) excepting in MH where slight stimulatory effect was noted only at 0.1 ppm (Table 3). Among growth hormones showing stimulation

in growth, GA was most effective at 0.1 ppm. The second best was IBA (0.1 ppm) followed by NAA (1 ppm). Regarding sporulation GA was the most effective one and exhibited maximum sporulation at 0.1 ppm. Other growth hormones showed no significant effect in sporulation.

DISCUSSION

From the results with trace elements it can be concluded that not all are equally effective for the growth and sporulation of the test fungus. Zinc offers the maximum stimulatory effect in growth and sporulation followed by copper and manganese. Vir and Grewal (1973), Bhatnagar and Prasad (1968) also reported similar growth promoting effects of zinc in other species of *Fusarium*.

Results of experiments with vitamins reveal that the fungus is partial auxoheterotroph for the vitamins tested. However, the enormous increase in externally supplied biotin even at the lowest concentration proves that the fungus synthesises biotin in quantity much below the level of what it requires for normal growth and development. The stimulatory effect of biotin has also been reported by Vir and Grewal (1973) in *Fusarium caeruleum* and Chattopadhyay (1976) in other species of *Fusarium*.

GA stimulated maximum growth as well as sporulation of the fungus. This stimulatory effect of GA was previously studied by Chattopadhyay (1976) in *Fusarium moniliforme* var. *subglutinans*. It is evident that lower concentrations of all hormones excepting MH have promoting effect on the test fungus while higher concentrations except GA showed inhibitory effect. This is in full agreement with the results of Richard (1949) and Agnihotri *et al.* (1966).

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