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SULPHUR AND PHOSPHORUS REQUIREMENTS OF *PESTALOTIOPSIS FUNEREA* CAUSING LEAF SPOT OF *EUCALYPTUS GLOBULUS*

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Effect of different sulphur and phosphorus compounds on the growth and sporulation of *Pestalotiopsis funerea* Desm. causing leaf spot of *Eucalyptus globulus* Labill. was studied. Both were found to be essential as only traces of growth occurred in the medium devoid of either of them. Out of eleven sulphur compounds, magnesium sulphate supported best growth and excellent sporulation. Sulphite and sulphate of sodium yielded poor growth. Thiourea was found to be toxic to the growth and sporulation. Optimum concentration of magnesium sulphate was also determined and it was found that the maxima for growth and sporulation were at 2.0 g/lit and 1.0 g/lit of concentrations respectively. Six phosphorus compounds were tested. Growth and sporulation were best in dipotassium hydrogen orthophosphate and potassium dihydrogen orthophosphate respectively. Diammonium hydrogen orthophosphate supported poor growth and sporulation.

INTRODUCTION

Requirements of sulphur and phosphorus for the growth of various fungi are indispensable (Agrawal, 1958; Tandon & Bilgrami, 1958; Bhargava & Tandon, 1963; Jaurihar & Mehta, 1972; Jamaluddin, Tandon & Tandon, 1975). Sulphur constitutes proteins and has metabolic significance as prosthetic groups (-SH) of some enzymes and co-enzymes. Phosphorus plays an important role in the carbohydrate metabolism and as constituent of nucleic acids, nucleotides, phospholipids and other metabolic intermediates. Cockefair (1931) evidently showed that nitrates could not be reduced to amino acids without intervention of phosphorus adequately.

Present investigation was undertaken to study the effect of various sulphur and phosphorus compounds on the growth and sporulation of *Pestalotiopsis funerea* Desm. causing leaf spot of *Eucalyptus globulus* Labill.

MATERIALS AND METHODS

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

Czapek-Dox containing $NaNO_3$, 2.0 g; K_2HPO_4, 1.0 g; MgS0, 0.5 g, KC1, 0.5 g; Sucrose, 30.0 g and double distilled water, 1000 ml was used as the basal medium. The

various sulphur and phosphorus compounds were substituted in equivalent amounts for magnesium sulphate and dipotassium hydrogen orthophosphate respectively. The *p*H of the medium was adjusted to 5 8 before autoclaving. Chemicals supplied by E. Mercks and B.D.H. were used. Fifty ml of the medium was apportioned in 250 ml conical flask and autoclaved at 15 lb pressure for 15 minutes. Each flask was inoculated agar-disc method Garrett, (1936) and incubated for 15 days at 24±1°C. Thereafter by cultures were filtered on already weighed Whatman filter paper (no. 42), dried in an electric oven at 60±1°C and reweighed after cooling in a desiccator. Triplicate sets were used to determine average hyphal dry weight and final *p*H of the culture filtrates.

Growth in terms of hyphal dry weight (mg) has been graded into the following categories, viz. Best growth — 500 mg, Good — 450 to 499 mg, Fair — 300 to 449 mg, Poor — 229 mg or below it, and Absent — 0.

The degree of sporulation was classified into five categories, viz., Excellent, Good, Fair, Poor and Absent (nil) on the basis of the visual observations. Results were statistically analysed, and are presented in Tables I-III.

Results and Discussion

(*i*) *Effect of Different Sulphur Compounds*

Tables I and II present data on hyphal dry weight, sporulation and final *p*H of the culture filtrate of *P. funerea* with different sources and concentrations of sulphur compounds.

Table I

The average hyphal dry weight, final pH and sporulation of Pestalotiopsis funerea *on different sulphur sources*

Sulphur compounds	Hyphal dry wt (in mg)	Final *p*H	Sporulation
Sodium sulphate	253	5.8	Poor
Sodium thiosulphate 5 hydrate	425	5 8	Poor
Potassium sulphate	346	5.8	Fair
Sodium disulphite	470	4.0	Fair
Sodium sulphite	310	5.0	Absent
Potassium peroxydisulphate	384	5.8	Good
Methionine	405	4.0	Fair
Thiourea	0	5.8	Absent
Magnesium sulphate	549	5.5	Excellent
Ammonium sulphate	471	4.0	Good
L-cystin	488	4.0	Fair
No sulphur (control)	43	5.8	Absent

There is highly significant variation among the treatments indicated by the excess of the calculated value beyond the tabulated value 392.37 > 3.1 (at 0 01 level) with 11 and 24 degree of freedom.
C.D. = 15.865 at 0.01 level

Tables I indicates that there was very little growth of the test pathogen on medium without any sulphur compounds and in this respect it was similar to *Pythium* spp. (Saksena, Jain & Jafri, 1952), *Curvularia penniseti* (Agrawal, 1958), *Fusarium solani*, *Botryodiplodia annassae* and *Macrophomina phaseoli* (Bhargava& Tandon, 1963), and *Fusarium moniliforme* (Jaurihar & Mehta, 1973). It appears that it might have happened due to the traces of sulphur (0.02%, 0.01% and 0.01%) present as an impurities in the form of sulphates in sucrose, potassium chloride and sodium nitrate respectively.

Thiourea failed to induce growth and sporulation of the test pathogen. Similar results were obtained by Agrawal (1957) and Jamaluddin, Tandon & Tandon (1975). According to Sumner and Sommers (1947) as well as Lardy *et al.* (1949) it is known to be an enzyme inhibitor.

Among all the different sulphur compounds used here, magnesium sulphate was found to be the best source and therefore an attempt was made to determine the most suitable concentration of it for the growth and sporulation. Table II depicts that dry weight increased with increase in amount of magnesium sulphate up to 2.0 g/lit., while sporulation declined after 1.0 g/lit. of concentration. Agrawal (1958), Tandon (1961), Bhargava and Tandon (1963), Jaurihar and Mehta (1973), and Jamaluddin, Tandon and Tandon (1975) have also obtained similar results.

TABLE II

The hyphal dry weight results, final pH and sporulation of Pestalotiopsis funerea *at different concentrations of magnesium sulphate*

Magnesium sulphate (in g/lit.)	Hyphal dry wt (in mg)	Final pH	Sporulation
2.0	562.33	5	Good
1.0	520	5.8	Excellent
0.5	464	5.0	Good
0.25	430	5.0	Good
0.125	471	6.5	Good
0.0	43	5.8	Poor

There is highly significant variation among the treatments indicated by the excess of the calculated value beyond the tabulated value 190.5 > 5.06 (0.01 level) with 5 and 12 degree of freedom.

C.D = 39.99 at 0.01 level

Good growth was recorded on sodium disulphite, ammonium sulphate and L-cystin. The latter two compounds have been reported to be good sources by Bhargava and Tandon (1963), and Saksena and Kumar (1962) respectively. Similar results with sodium disulphite were also reported by Saksena, Jain and Jafri (1952), and Agrawal (1958).

Potassium peroxidisulphate, Potassium sulphate, sodium thiosulphate and DL-methionine supported fair growth of the test pathogen and in this respect the latter three compounds resembled with the findings of Bhargava and Tandon (1963), and

Jamaluddin, Tandon and Tandon (1975). Saksena, Jain and Jafri (1952), Agrawal (1958), and Jaurihar and Mehta (1972) have also obtained similar results with potassium sulphate and sodium thiosulphate.

Sodium sulphite and sodium sulphate supported poor growth and sporulation. Agrawal (1958) obtained similar results with *Curvularia penniseti.* Sodium sulphite has been reported to be toxic to the growth of the fungi studied by Steinberg (1941), Bhargava (1945) and Saksena and Kumar (1962).

Sporulation of the test pathogen was excellent on magnesium sulphate; good on potassium peroxydisulphate and ammonium sulphate; fair on potassium sulphate, sodium disulphite, DL-methionine and L-cystin and poor on sodium sulphate and sodium thiosulphate 5 hydrate. No sporulation was observed on sodium sulphite, thiourea and a medium devoid of sulphur. It is evident from the table that there was no correlation between growth and sporulation.

(ii) Effect of Different Phosphorus Compounds

The data on average dry weight, sporulation and final *p*H are presented in Table III.

TABLE III

The average hyphal dry weight, final pH and sporulation of Pestalotiopsis funerea *on different phosphorus compounds*

Different phosphorue compounds	Hyphal dry wt (in mg)	Final *p*H	Sporulation
Sodium dihydrogen orthophosphate	459	6.0	Good
Sodium phosphate dibasic	499	5.5	Excellent
Ammonium dihydrogen orthophosphate	463	4.0	Absent
Diammonium hydrogen orthophosphate	379	3.5	Poor
Dipotassium hydrogen orthophoshate	554	6.0	Good
Potassium dihydrogen orthophosphate	475	6.0	Excellent
No phosphorus (control)	51	4.0	Poor

There is highly significant variation among the treatments indicated by the excess of the calculated value beyond the tabulated value $314.12 > 4.46$ (0.01 level) with 6 and 14 degree of freedom.

C.D. = 24.76 at 0.01 level

Phosphorus was found to be essential for the test pathogen as only traces of growth occurred in its complete absence. This result was in accordance with the findings of Srivastava (1951), Agrawal (1957), and Tandon and Bhargava (1960).

Best growth and good sporulation were obtained in the medium supplemented with dipotassium hydrogen orthophosphate. The observations of Bhargava and Tandon (1963), and Jaurihar and Mehta (1973) are also in consistency with our findings. All the other sources were, however equally important as significant growth occurred in them over the control devoid of any phosphorus.

Sporulation was excellent on potassium dihydrogen orthophosphate and sodium phosphate — dibasic, good on dipotassium hydrogen orthophosphate and sodium

dihydrogen orthophosphate, and poor on diammonium hydrogen orthophosphate and on medium devoid of any phosphorus. Ammonium dihydrogen orthophosphote induced no sporulation. Bhargava and Tandon (1963) also reported similar results with phosphates of sodium, potassium and ammonium.

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