Proc. Indian natn. Sci. Acad., Vol. **44**, Part B, pp. 146-150 (1978)
Plant Pathology

UTILIZATION OF NITROGEN BY *PESTALOTIOPSIS FUNEREA* CAUSING LEAF SPOT OF *EUCALYPTUS GLOBULUS*

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(Received 13 *June*, 1977; *after revision* 17 *July*, 1978*)*

Effect of 19 different organic and inorganic nitrogen compounds was studied on the growth and sporulation of *Pestalotiopsis funerea* Desm. causing leaf spot disease of *Eucalyptus globulus* Labill. Peptone, L-cystine, urea, ammonium acetate and sodium nitrate supported best growth while sodium nitrite was least effective. The test pathogen could utilize ammonium, nitrate and organic sources of nitrogen but was unable to assimilate atmospheric nitrogen. Excellent sporulation was recorded on sodium nitrate, ammonium nitrate, and L(−) histidine, whereas complete inhibition in growth and sporulation was observed on diphenylamine. There was no correlation between changes in *p*H and sporulation.

INTRODUCTION

Nitrogen is an essential requirement of fungi for their functional as well as structural purposes. Pathogenic fungi usually exhibit certain degree of specificity in utilizing various sources of nitrogen. During last several years a number of workers including Fothergill (1955), Tandon and Bilgrami (1957), Vidyasekharan (1969), Bhargava (1970), Singh (1973) Pal and Grewal (1975) and Arora and Upadhyay (1978) studied the role of nitrogen compounds in the growth and development of the fungi selected by them. Present paper deals with the effect of different nitrogen sources on the growth and sporulation of *Pestalotiopsis funerea* Desm. causing leaf spot disease of *Eucalyptus globulus* Labill.

MATERIALS AND METHODS

Monosporic culture of *P. funerea* was prepared from infected leaves of *E. globulus* and maintained on Czapek-Dox medium at 24 ± 1°C for further study.

Czapek-Dox ($NaNO_3$, 2·0 g; K_2HPO_4, 1·0 g; $MgSO_4$, 0·5 g; KCl, 0·5 g; $FeSO_4$, 0·01 g; Sucrose, 30·0 g; 1000 ml distilled water) was used as the basal medium. For the study of utilization of nitrogen from different nitrogen compounds, they were singly substituted for sodium nitrate in the basal medium. The concentration of different nitrogen compounds was so adjusted as to contain an amount of nitrogen equivalent to that present in 2·0 g of sodium nitrate, however, the quantity of peptone was equal to that of sodium nitrate. The media were adjusted to *p*H 5·8 before autoclaving. Fifty ml of the medium was apportioned in 250 ml Erlenmeyer flask and autoclaved at 15 lb. p. for 15 min. Each flask was inoculated with two agar discs of equal size cut from the margin of a vigorously growing colony of the test pathogen and incubated for 15 days at

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24±1°C. After incubation, culture was filtered through dried and weighed Whatman filter paper No. 42 and *p*H of the filtrate was determined simultaneously. The mycelial mat was thoroughly washed with distilled water, dried to a constant weight in an electric oven at 60°C for 48 hr and reweighed after cooling in a desiccator.

Triplicate sets were used in each case and average dry weight was recorded. The growth in terms of hyphal dry weight (mg) was graded as Best growth : more than 635, Next to best : 555-635, Good : 455-554, Poor : 255-454, and absent : 0.

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 recorded in Table I.

TABLE I

Average dry weight, sporulation and final pH of Pestalotiopsis funerea *on different sources of nirtrogen*

Nitrogen compounds*	Dry weight (in mg)	Final *p*H	Sporulation**
1. Sodium nitrate	569	6·7	E
2. Ammonium nitrate	473	5·2	E
3. Di-ammonium hydrogen orthophosphate	498	2·0	P
4. Ammonium sulphate	521	2·0	A
5. Ammonium chloride	506	1·3	A
6. Urea	591	4·0	F
7. Peptone	681	4·0	P
8. Potassium nitrate	491	5·8	A
9. Calcium nitrate	415	3·7	A
10. Ammonium acetate	622	5·8	P
11. Ammonium dihydrogen orthophosphate	540	2·2	F
12. Sodium nitrite	327	5·0	A
13. Diphenylamine	0	0	A
14. L-asparagine	426	3·6	P
15. L-cystine	640	2·7	A
16. L (—) histidine	519	3·7	A
17. L-leucine	567	4·0	F
18. L (+) alanine	427	4·2	P
19. Arginine	566	3·0	P
20. No nitrogen*	000	5·8	A **

* There is highly significant variation among the treatments indieated by exceeding of the calculated value beyond the tabulated value (175·59 > 2·37) (at 0·01 level) with 19 and 40 degree of freedom. C.D. = 18·628 at 00·1 level

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

Results and Discussion

The test pathogen exhibited no growth when nitrogen was excluded from the culture medium, thereby showing that it was incapable of assimilating atmospheric nitrogen for the growth.

Peptone and L-cystine supported best growth. Peptone was reported to be a good source for a number of fungi studied by Lilly and Barnett (1951) and Misra and Mahmood (1960), possibly due to the fact that a mixture of peptides and amino acids are liberated by it in the medium. Tandon (1950) and Tandon and Mitra (1963) made similar observations for *Pestalotia malorum* and *Pestalotiopsis versicolor* respectively. L-cystine was also found to be a good source by Bais *et al.* (1970) and Bhargava (1970). According to Cochrane (1958) effect of L-cystine was more related to sulphur than nitrogen metabolism.

Sodium nitrate, urea, ammonium acetate, L-leucin and L (+) arginine were next best sources for the growth of the test pathogen. Vidyasekharan (1969), Bhargava (1970), Bais *et al.* (1970) and Arora and Upadhyay (1978) also found the first three substances to be good sources for the growth and sporulation. Stockdale (1953) reported L-leucine as a good source for *Trychophyton persicolor.*

Good growth of the test pathogen was recorded with ammonium nitrate, diammonium hydrogen orthophosphate, ammonium sulphate, ammonium chloride, potassium nitrate, ammonium dihydrogen orthophosphate and L (−) histidine. Similar results were obtained by Tandon and Mitra (1963), Vidyasekharan (1969), Bhargava (1970), Weststeijn (1973) and Pal and Grewal (1975) for the fungi studied by them. Leal *et al.* (1971) reported L (−) histidine to be a good source for the growth of *Phytophthora cactorum.*

Poor growth was obtained on calcium nitrate, sodium nitrite, L-asparagine and L (+) alanine. Calcium nitrate as a poor source was reported by Vidyasekharan (1969). Sodium nitrite was also found to be a poor source by Bhargava (1970) and Singh (1973). Similarly Tandon and Mitra (1963) obtained poor growth of *Pestalotiopsis versicolor* on it. Lilly and Barnett (1951) reported it to be toxic in acidic range of *p*H as it was generally present in the form of undissociated nitrous acid causing destruction of proteins and amonoacids. In a separate experiment, when nitrite was tested at higher *p*H (=8), it was found that the test pathogen exhibited better mycelial yield and sporulation. Similar results were also reviewed by Bilgrami and Verma (1978). Thus it may be inferred that utilization of nitrite is influenced by the initial *p*H of the medium. Fothergill (1955) found L-asparagine as a poor source of nitrogen.

Growth and sporulation of the test pathogen were completely inhibited when diphenylamine was supplied as sole source of nitrogen in the medium. It was in conformity with the findings of Stockdale (1953) for *Trichophyton* sp.

The final *p*H of all culture media showed acidic reactions except with sodium nitrate in which case it drifted towards neutral side, however, it remained unaltered with potassium nitrate and ammonium acetate as nitrogen sources. These variations in *p*H might be explained on the basis of utilization of either

nitrate ions (as anion) or ammonium ions (as cation) developing alkalanity or acidity in the medium respectively (Lilly & Barnett, 1951).

A critical study of culture Medium incorporated with ammonium nitrate showed that *p*H value decreased (*p*H 3·8) during first five days and thereafter increased (*p*H 5·2) in the last ten days of incubation. This variation in *p*H was presumptive evidence for rapid and preferential utilization of ammonium ions followed by a slight assimilation of nitrate ions (Morton *et al.*, 1954). Foster (1949) stated that at every low *p*H ammonium utilization was reduced and at least some nitrate was assimilated which strengthened the above explanation.

Constant *p*H of the culture medium incorporated with ammonium acetate might be explained as both the cation and anion of this salt were utilizable as nitrogen and carbon sources respectively.

A perusal of Table I depicts that sodium nitrate, ammonium nitrate and L(−) histidine supported excellent sporulation. Shreemali (1973) found that the former two compounds were best sources for the sporulation of different isolates of *Botryodiplodia theobromae*. Fair sporulation was recorded with urea, ammonium dihydrogen orthophosphate and L-leucine. Purohit (1972) reported urea as a satisfactory source for the sporulation of some species of *Pestalotia*. Sporulation was poor on diammonium acetate, diammonium hydrogen orthophosphate, peptone, L-asparagine, L(+) alanine and L(+) arginine. However, L-cystine, sodium nitrite, potassium nitrate, ammonium chloride, ammonium sulphate and diphenylamine induced no sporulation. Ammonium sulphate, L-cystine and ammonium chloride were also reported to be poor sources by Misra and Mahmood (1960), Tandon and Bhargava (1961) and Purohit (1972). Bais *et. al* (1970) observed that sporulation was fair and nil with calcium nitrate and L-cystine respectively.

Sporulation seemed to be unrelated directly with the alteration in *p*H as it was recorded excellent on *p*H 2·7 and 6·7 with L(−) histidine and sodium nitrate respectively.

The results of the present study depict that the test pathogen could utilize nitrate, ammonium and organic sources of nitrogen but was incapable of assimilating atmospheric nitrogen. It may, therefore, be placed in group II of the classification proposed by Robbins (1977).

ACKNOWLEDGEMENTS

Thanks are due to the Head, Department of Botany, Banaras Hindu University for providing lab facilities and to the C.S.I.R., New Delhi, for providing financial assistance to one of the authors (RKU).

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