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Effect of Different Nitrogen Sources on Forest Litter Colonizing Activity of Fungi

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ABSTRACT

Two dominant forest litter colonizing fungi *viz., Scopulariopsis constantini and Syncephalastrum racemosum* were able to utilize efficiently all of the three forms of nitrogen in common sources i.e., NO3, NH4, and organic nitrogen sources. The litter colonizing activity of fungi, utilizing different N sources was measured in two terms: (i) the weight of mycelium + colonized litter substrate and (ii) growth and sporulation. The order of preference for utilizing the nitrogen compound by both the test fungi was more or less the same as I. Sodium nitrate II. Asparagine III. Leucine IV. Urea, and V Ammonium nitrate.

Keywords: Nitrogen Source. *Scopulariopsis constantini. Syncephalastrum racemosum.* litter colonizing fungi.

I. INTRODUCTION

Fungi are essentially heterotrophs and one of the most active participants of decomposer saprobiota. However, they differ in their substrate hydrolyzing capability. The difference in the hydrolysis of forest litter by fungi may be due to (a) the difference in the amount of exoenzyme (s) produced by fungi, (b) the difference in the substrate specificity of the enzyme (s) that are synthesized by fungi, and (c) the change in the pH of the medium caused by fungal growth.

Nitrogen plays a vital role in the nutrition of fungi and can be utilized in various forms. All nitrogen sources are not equally suitable for different litter decomposing fungi and even these fungi may be quite specific in their choice of this element. Besides, no single pattern of nitrogen utilization can be described to apply to all fungi.

II. MATERIAL AND METHODS

Two dominant and frequently occurring species *viz., Scopulariopsis constantini* and *Syncephalastrum racemosum* were selected from the mycoflora associated with the litter of the Mala Forest, Pilibhit, U.P.
Five nitrogen sources were selected, each representing one of the following five different categories:

A. **Inorganic compounds:**

1. Nitrate - Sodium Nitrate ($NaNO_3$)
2. Both ammonia and nitrate - Ammonium Nitrate (NH_4NO_3)

B. **Organic compounds:**

3. Monoamino monocarboxylic acid – Leucine
   [$(CH_3)_2$ CH CH_2 CH NH_2 COOH]
4. Amide of carboxylic acid – Asparagine
   (H_2N $COCH_2$ $CHNH_2$ COOH)
5. Amide – Urea (NH_2 CO NH_2)

Freshly fallen leaf litter of *Tectona grandis* (teak) collected during the forest survey was used as the substrate. The litter substrate was cleaned, cut into pieces of $1cm^{2,}$ and washed with 0.1% $HgCl_2$ followed by successive washings with sterilized distilled water. These pieces were pressed between sterilized filter papers to get rid of excess water and then transferred to Petri dishes, which were placed in an oven at 65^oC overnight.

Czapek's Dox liquid medium, minus nitrogen compound ($NaNO_3$), was used as the basal medium. Based on their molecular formulae, the quantities of nitrogen sources were calculated to supply an equal amount of nitrogen, *i.e.*, 500 mg/litre in each case. The selected nitrogen compounds and 500 mg of oven-dried substrate were added to 3 ml of basal medium in each conical flask of 150 ml, singly in triplicates for both the test fungi. The substrate remained floating on the surface of the medium, the initial pH of the culture medium was adjusted to 6.5 and all the flasks along with two control sets were autoclaved at 15 lbs pressure for half an hour at 121^0C. Two control sets of three replicates each contained basal medium + substrate + inoculum but lacked any nitrogen source.

All the flasks were seeded with an inoculum disc of 5 mm obtained from the growing margins of 6-day-old fungal cultures. The flasks were kept in an incubator at 30 ± 2^0C for 20 days.

From all the flasks, the substrate along with the growing mycelium was taken out using Whatman no. 1 filter papers and dried as such at 75^0C in the oven for 24 hrs. After cooling them in a desiccator, the total dry weight was determined and compared with the weight of the substrate in the control sets sporulation was measured by the hemocytometer.

Statistical analysis- The significance of the difference in dry weight of each fungal species was tested using one-way analysis of variance (ANOVA) and the comparisons between the nitrogen sources within each fungus were made using Duncan's multiple range test. The standard error of means and coefficient of variation were also computed.

III. RESULTS AND DISCUSSION

The litter colonizing activity was determined in terms of oven-dried weights of substrate + mycelium and growth and sporulation (Table 1). The weights were measured to be higher in the treatment sets than in the control sets.

One-way analysis of variance (ANOVA) has resulted in a significant difference ($P<0.05$) between different nitrogen sources utilizing behaviors of the test fungi.
However, the variability within each nitrogen source's replicates was insignificant (CV ranged from 1.1 to 6.3%). The overall mean for *Scopulariopsis constantini* was 814.8 ± 28.5 mg and for *Syncephalastrum racemosum* 774.2 ± 9.5 mg. Overall CV for *S. constantini* was higher (14.8%) than less variable *S. racemosum* (5.2 % CV).

The test fungi showed the most active litter colonization in the case of Sodium Nitrate ($NaNO_3$). The mean dry weight under this inorganic compound was the highest in the case of both the fungi tested. Vijaya Kumar (1978) has also observed Sodium Nitrate to be the best source of nitrogen for several species of *Fusarium.*

Nitrate reduction in fungi is carried out by the activity of nitrate reductase in fungi which remains linked with cytochrome system (Bilgramy, 1975). According to Lilly and Barnett (1951), a fungus that utilizes nitrate nitrogen must be able to reduce it to the oxidation level of ammonia. In the present study, both the fungi utilized nitrogen in the nitrate as well as ammonia form. However, the growth and sporulation, and weights were minimal under ammonium nitrate i.e., 757.7± 22.3 mg in *S. constantini* and 755± 6.6 mg in *S. racemosum* (Table 1).

The test fungi also showed good colonization under Asparagine. The mean dry weights were 902 ± 13 mg in the case of *S. constantini* and 812.7 ±5 mg in *S. racemosum*. The compounds that readily yield ammonia such as amides of carboxylic acid i.e., asparagine, have been reported as excellent nitrogen sources by Brock (1951). Khasanov and Mirchodzhaev (1969) observed intensive cellulose decomposition in organic sources of N by *Penicillium, Humicola,* and *Torula* species. Jain (1983) has also found it to be a good source of N for the growth of all the test organisms of keratinophilic fungi.

Leucine and Urea also supported the growth of test organisms but moderately. Leucine was reported as a poor source of N for *Fusarium equisetti* by Vijaya Kumar (1978). Raizada (1958) found a mixture of amino acids suitable for the growth of some members of Mucorales. Fungal colonization was also poor in the control sets, without a nitrogen source. This poor growth is possible as the fungi must have utilized the nitrogenous compound in the litter pieces suspended in the medium.

On the perusal of the above results, the order of preference of nitrogen sources in terms of weight was found to be the same for both the test fungi as 1. Sodium Nitrate, 2. Asparagine, 3. Leucine, 4. Urea, and 5. Ammonium Nitrate. The preferential order in terms of growth and sporulation also paralleled that found by weight basis in the case of *S. constantini* (Table 1), but slightly differed in S. racemosum as 1. Sodium Nitrate (excellent), 2. Urea (good), 3. Asparagine (moderate), 4. Leucine (fair), and 5. Ammonium nitrate.

Thus, it was concluded that Sodium Nitrate was proved to be the best source of nitrogen to support the highest litter colonizing activity of both the test fungi. *Scopulariopsis constantini* and *Syncephalastrum racemosum* very well fit in Group II as classified by Robbins (1937), by utilizing three forms of nitrogen i.e., NO_3, NH_4, and organic nitrogen.

Table 1. Effect of different nitrogen sources on litter colonizing activity of *S. constantini* and *S. racemosum* (showing variability i.e., standard error of means and variation and one-way analysis of variance)

S.No.	Nitrogen source	*Scopulariopsis constantini* Substrate + Mycelium dry wt in mg** Mean*	±	SE	(CV%)	Growth	*Syncephalastrum racemosum* Substrate + Mycelium dry wt in mg** Mean*	±	SE	(CV%)	Growth
1.	Sodium Nitrate	948.6	±	27.1	5.0	+++++	821.0	±	10.0	2.1	+++++
2.	Ammonium Nitrate	757.7	±	22.3	5.1	++	755.0	±	6.6	1.5	++
3.	Leucine	887.7	±	32.7	6.4	+++	775.6	±	5.4	1.2	
4.	Asparagine	902.0	±	13.1	2.5	++++	812.7	±	5.0	1.1	
5.	Urea	781.0	±	28.5	6.3	+++	761.0	±	15.5	3.5	
6.	Control	612.0	±	6.4	1.8	+	720.0	±	25.2	6.0	
	Overall Mean	814.8	±	28.8	14.8		774.29.5	±	9.5	5.2	
	One way ANOVA Variance ratio 'F'	27.57					8.01				
		Significant difference at p<0.05					Significant difference at p<0.05				

*Mean of three replicates each. Within each group, Means with similar superscript do not differ significantly (P<0.05) by the Duncan's multiple range test.
Growth: Excellent +++++; Good ++++; Moderate +++; Fair ++; Poor +. ** Initial dry weight of substrate was 500mg.

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