International Journal of

Life Sciences

(A peer reviewed International Journal)

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International Journal of Life Sciences, Vol. 1 No. 2. pp. 16-18   2277-193x. 2012

ISSN *2277 – 193x*

Article type *Full Length Research Article*

Submission date *15 February 2012*

Acceptance date *30 March 2012*

Publication date   *15 April 2012*

Article URL *http://www.crdeep.org/category/ijls*

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International Journal of Life Sciences Vol.1 No.2. 2012. Pp. 16-18

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Effect of Carbon and Nitrogen Sources on the Growth of *Rhizoctonia solani* Isolated from *Rhynchostylis retusa* Blume

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ABSTRACT
The growth of *Rhizoctonia solani* in different carbon and nitrogen sources were studied. Growth rate of fungus was measured by taking the dry mycelial weight produced in the medium at particular time intervals. Initial and final pH of the medium was also recorded. Maximum growth of fungi was supported by glucose (carbon source) and mannitol (nitrogen source).

Key words: *Rhizoctonia solani, Carbon, Nitrogen, pH.*

INTRODUCTION
Rhizoctonia solani is a common symbiont of the members of Orchidaceae. It is an endophytic fungus occurring in the roots of orchids in the form of pelotons, which is the distinctive characteristic of orchid mycorrhiza (Kaushik & Pal, 2011). In nature mycorrhizal fungus obtain nutrients from dead organic matter in the case of saprophytic terrestrial orchids and from dead bark in case of epiphytic orchids Kaushik (1983). By using radioisotopes Melin and co-workers c.f. Bakshi (1974) demonstrated that fungal symbionts are able to transfer carbon (C^{14}), nitrogen (N^{15}), phosphorus (P^{32}), sodium (Na^{22}) and calcium (Ca^{45}) from nutrient solution into the plant in considerably larger quantities as compared to non-mycorrhizal roots. Thus, present investigation was done to study the carbon and nitrogen utilization by orchid mycorrhizal fungus.

MATERIALS AND METHODS
The fungus used in the present study was *Rhizoctonia solani* isolated from *Rhynchostylis retusa* an epiphytic orchid**.** To study the growth of mycorrhizal fungus it was grown in PD broth, in which different carbon sources *i.e.* glucose, glycerine, maltose, starch and sucrose; and nitrogen sources like ammonium chloride, peptone, potassium nitrate, sodium nitrate and yeast extract were added and a control was maintained. The concentration of carbon sources taken was 16 g/L, starch was added 20 g/L and the various nitrogen sources were added at the rate of 0.3 g/L. The pH of the medium was adjusted at the respective pH optimum for the fungus by using 0.1 N HCl and 0.1 N NaOH. A volume of 25 ml each of the aliquots was dispensed in 100 ml Erlenmeyer flasks and sterilized at 1.05 Kg/cm^3 pressure for 20 min. Inoculated the flasks by adding 7 mm mycelium disc of the fungus. The inoculated flasks were incubated at 28 ±2°C for 15 d as shake culture. The fungal mat was collected on previously weighed filter paper and dried at 60-80˚C to a constant weight. Three replicates were used for each treatment. The final pH of respective culture filtrate was also noted.

RESULTS AND DISCUSSION
Growth response of mycorrhizal fungus to different six carbon sources *i.e.* glucose, glycerine, maltose, mannitol, starch and sucrose were recorded. The data thus obtained were presented in **(Fig. 1, table 1)**. The data revealed that glucose supported the maximum growth 660 ± 0.83 mg of fungus followed by maltose 620±1.01 mg and sucrose 615±1.17 mg while growth was least on glycerine with only 139 ± 1.07 mg.

Utilization of nitrogen by fungus was studied with five different nitrogen sources *i.e.* ammonium chloride, peptone, potassium nitrate, sodium nitrate and yeast extract. The effect on growth was estimated in terms of biomass production **(Fig. 2, table 2)**. The fungus showed the maximum growth in yeast extract 284±1.30 mg followed by peptone 267± 1.01 mg and sodium nitrate 240±1.28 mg. Lowest growth was observed with ammonium chloride 175±1.0 mg.

Tolba and Salama (1960), Beever and Bollard (1970), Midgley et al. (2006) observed that fungus can grow *in vitro* on PD broth and it supports the good growth of fungus. The factors influencing the growth of fungus that have been studied here were utilization of carbon and nitrogen sources which was in conformity with earlier reports (Israel & Ali, 1964; Bakshi, 1974; Smith and Read, 1997; Midgley et al., 2006).

Bakshi (1974) extensively studied the mycorrhiza of forest plants; he studied the effect of various carbon and nitrogen sources like glucose, sucrose, maltose and glycerine. The nitrogen sources like ammonium nitrate, ammonium sulphate, potassium nitrate, ammonium tartarate, glycine, urea, asparagin, tryptophan and casein hydrolysate also have been worked out successfully on mycorrhizal fungi. Results showed that the growth was maximum in media containing glucose, sucrose and maltose, unsatisfactory in glycerine while in nitrogenous source yeast extract supports the best growth results, similar observations were made by Stephens and Fung (1971a, b), Hijner and Arditti (1973). They concluded that yeast extract is known to have a requirement for thiamine, p-amino benzoic acid and certain amino acids. Perombelon and Hadley (1965) studied the five *Rhizoctonia* isolates had similar growth patterns on glucose and pectin C sources, with a higher mycelial dry weight on glucose. All produced endopolymethyl galacturonase and protopectin on pectin media.

Table 1. Growth of *Rhizoctonia solani* on different carbon sources

Carbon sources	Initial pH	pH after 15 d	Mycelial weight (mg)
Glucose	6.20	6.93	**660 ± 0.83**
Glycerine	7.67	7.23	139 ± 1.07
Maltose	7.62	7.03	620 ± 1.01
Mannitol	9.10	7.00	145 ± 1.38
Starch	6.62	6.93	220 ± 0.70
Sucrose	7.23	7.12	615 ± 1.17
Control	6.10	6.50	107 ± 1.20

Each value is expressed as mean ± S.E. (n=3)

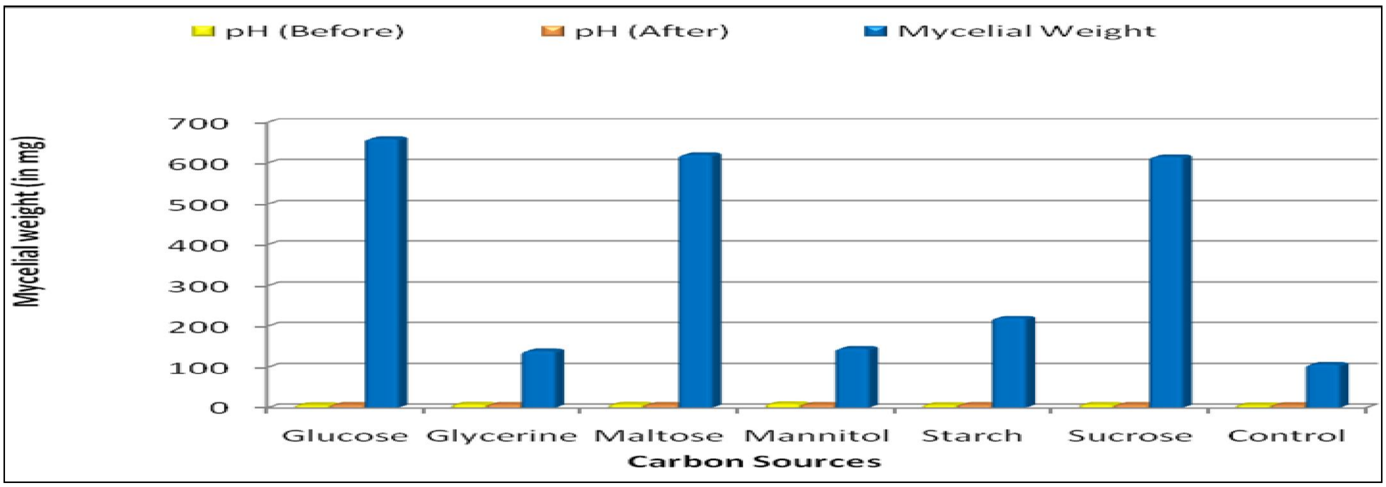

Figure 1. The mycelial growth of *Rhizoctonia solani* on different carbon sources

Table 2. Utilization of complex organic and inorganic nitrogen sources by mycorrhizal *Rhizoctonia solani*

Nitrogen sources	Initial pH	pH after 15 d	Mycelial weight (mg)
Ammonium chloride	9.16	6.97	175 ± 1.00
Peptone	8.29	7.26	267 ± 1.01
Potassium nitrate	9.29	6.67	225 ± 1.30
Sodium nitrate	9.29	7.62	240 ± 1.28
Yeast extract	9.22	7.46	**284 ± 1.30**
Control	6.10	7.60	65 ± 1.20

Each value is expressed as mean ± S.E. (n=3)

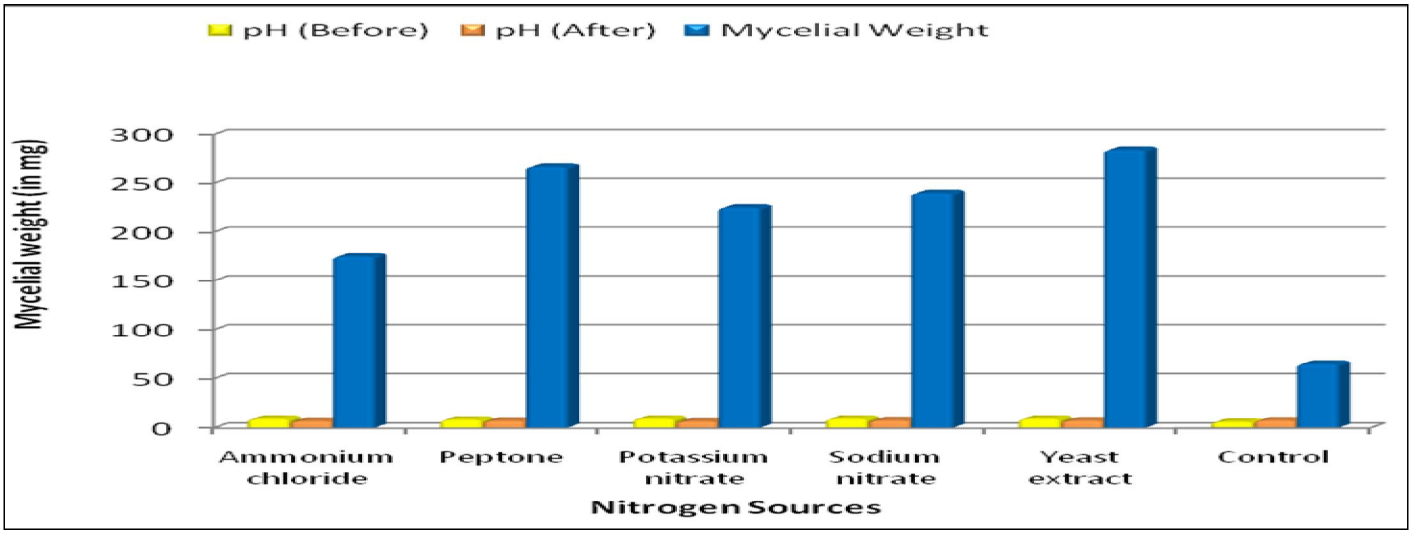

Figure 2. The mycelial growth of *Rhizoctonia solani* on different nitrogen sources

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