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EFFECT OF CARBON AND NITROGEN ON THE GROWTH OF LIGNICOLOUS FUNGI FROM RATHANMAHAL WILDLIFE SANCTUARY, GUJARAT, INDIA

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ABSTRACT
The lignicolous fungi like *Lenzites sterioides, Tremates pini, Hexagonia apiaria* and *Navisporus floccosa* were studied for the effect of carbon and nitrogen on the growth. The effect of different carbon nitrogen sours on the growth of *N. floccosa* was studied for the first time. *H. apiaria* showed the maximum growth of mycelium in D – arabinose and D-xylose. *L. sterioides* showed maximum growth in D-xylose, sucrose, and malt extract, in *T. pini* maximum growth of mycelium was observed in case of teak wood sawdust supplemented in the medium as carbon source. All the four test fungi faild to consume D-xylose completely up to 15 days of incubation. Sucrose was slowly breakdown into monosaccharides, it remained present up to 10 days in case of *T. pini* and *N. floccosa* while it was present up to 8 days in *L. sterioides* and 12 days in *H. apiaria*. Maltose was utilized by the present fungi through a hydrolytic pathway. The effect of five different nitrogen sources was observed in case of four wood rotting fungi. The results indicate that potassium nitrite showed better growth for *T. pini* and *N. floccosa,* the sodium nitrate showed better growth in *L. sterioides*, and the ammonium nitrate as sole nitrogen source produced better growth in *H. apiaria.* Based upon growth supporting ability the inorganic nitrogen compounds are grouped as calcium nitrate >Sodium nitrate >Potassium nitrate > Potassium nitrite > ammonium nitrate (35%) for *L. sterioides*.

KEY WORDS: Carbon, Nitrogen, Growth, Lignicolous fungi, *Navisporus floccose*

INTRODUCTION

On land, lignicolous fungi are important decomposers and major recyclers of nutrients. In forest ecosystems the decomposition of forest litter is essential to nutrient recycling. So lignicolous fungi are able to decompose material like wood in forest and reduce it to a soft almost paper-like substance. Lignicolous fungi able to use wood as a carbon source form a relatively small and specialized group that may be subdivided into brown-rot, white-rot or soft-rot organisms according to the type of decay they cause. Brown-rot fungi degrade only the polysaccharide fraction of wood, whereas the white-rot and soft-rot organisms utilize both the lignin and the polysaccharide fractions (King 1966). Wood-decaying basidiomycetes are able to develop very large colonies with wood, a carbon-rich, but nitrogen poor material, as their sole source of nutrients. Their growth presumably requires sensitive control of their nitrogen economy, involving regulation of proteinase activity both for the extracellular digestion of the protein in wood, and for the intracellular turnover and spatial reallocation of nitrogen from mycelial protein (Wadekar *et al*., 1995). Very few studies have been made of the relative rates of removal of the structural components of wood during decay by white rot fungi, and brown rot fungi (Kirk, 1973) the quantitative determination of changes in the individual types of structural sugar polymers (glucan, mannan and xylan) during the decay of conifer woods by either white rot or brown rot fungi have not been made. For heart woods such detailed analyses seem to have been done only by Cowling (1961). The quantitative changes in lignin, glucan, mannan and xylan during decay of five conifer woods by three white rot and three brown rot fungi were determined by Kirk and Highly (1973). Studies on the relative rates of utilization of the structural components of wood by white and brown rot fungi are quite frequent (Santra and Nandi, 1981). Cowling (1961) has reported preferential removal of mannan by brown rot fungi from hardwoods, while neither the major hemicellulose xylan nor the mannan is consistently removed before the glucan by the white rot fungi. Seifert (1968) has reported depletion of cellulose and xylan from pine almost simultaneously by *Cpniophora cerebella* a brown rot fungus. Nitrogen nutrition is a critical factor in the growth of wood-decay fungi. Growth on woody substrates makes special demands on nitrogen metabolism, because wood is a poor source of nitrogen. Wood contains only 0.03% to 0.1% nitrogen, mostly in organic forms (Laidlaw and Smith, 1965). The carbon: nitrogen ratio of wood can easily reach 1250:1 (Merrill and Cowling, 1966). Wood-decay basidiomycetes have evolved extremely efficient mechanisms for assimilating the nitrogen available in wood

and soil and then recycling the nitrogen by autolysis into new, actively growing portions of mycelium (Levi *et al*., 1968). Nitrogen derived from wood is accumulated in the mycelium to levels well above those in surrounding wood, for example Serpzlla lacymans mycelium utilizing wood as a sole nutrient source contains 3.7% nitrogen compared with 0.07 % in the wood substrate (Watkinson *et al*., 1981). Fermor and Wood (1981) showed that a number of fungi could degrade the walls of heat killed *Bacillus subtilis* cells and use these as a sole of carbon for growth. They suggest that microbial nitrogen might be particularly valuable to fungi such as wood decay basidiomycetes, which grow in low nitrogen substrates. Grant et al., (1986) added *Phanerochaete Chrysosporium* and three unidentified basidiomycetes to the list of decay fungi degrading heat killed *Bacillus* cell walls. In the present paper the effect of different carbon and nitrogen sources on growth of *L. sterioides, T. pini, H. apiaria* and *N. floccose* was studied.

MATERIALS & METHODS

Effect of carbon sources on timber degrading fungi

For the study of the effect of carbon the amount of individual substance in the basal medium was calculated, and a quantity equivalent to that was singly substituted in the basal medium by replacing the original corresponding substance *viz,* Sucrose. The amount of polysaccharides was similar to the amount of sucrose present in the basal medium. The medium devoid of sucrose served as control for carbon.

To study the effect of carbon sources on growth of timber degrading fungi, the Xylose, Arabinose, Maltose sugars were used. These sugars will acts as carbon sources which are supplemented in Czapak Dox medium. Wood degrading fungi like *L. sterioides, T. pini, H. apiaria* and *N. floccosa* were grown on basal medium containing 1% Malt extract as carbon source. After inoculation with test fungi the flasks were incubated in dark for 21 days. After completion of incubation period the fungi were filtered with Whatman filter paper No.1 and dried for 48 h at 60 ºC in oven. The dried filter papers were weighed to calculate the growth of each test fungi. The filtrate was used to determine the final pH.

Effect of Nitrogen on growth of wood decay fungi

It was used for the growth of test fungi *i.e. L. sterioides, T. pini, H. apiaria* and *N. floccosa.* The basal medium supplemented with four nitrogen sources was used for growth of these fungi. Flasks containing 25 ml of basal medium were autoclaved at 121ºC temperature for 20 min, inoculated with test fungi and incubated for 5, 10, and 15days. After completion of incubation period, each test fungus was filtered by using Watman filter paper no 1. The filtrate was used to determine final pH. The filter papers were dried in oven and weighed to calculate the growth of wood decay fungi.

Utilization of the sugars by wood decay fungi

Utilization of different mono-oligo, and poly sachharides as well as the hydrolytic products of di- and tri sachharides was studies. Paper chromatography was used for this purpose. The quantity of various sugars was similar to that used in experiment dealing with carbon requirements. Dry weight of mycelial mat and pH of the medium was recorded after incubation period of 5, 10 and 15 days and filtrates were analyzed daily to detect the presence of various sugars. Drops of known volume (0.05 ml) were taken from the filtrates every day and were placed on the chromatogram by micropipette at a position located for this purpose. The running solvent was n-butonol-acetic acid- water (4:1:5) v/v). In order to separate glucose and galactose the running solvent was n-butonol-pyridine-water (6:4:3 v/v). a mixture of 5 vol of 4% aniline, 5 vol of 4% diphenylamine and 1 vol of orthophosphoric acid (Buchan and Savage, 1952) was used as spraying reagent for the detection of sugars. Chromatograms were developed after drying at room temperature by heating in an electric oven at 100^{o}C for 90 sec. the Rf values were calculated by the following formula:

$$Rf = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

RESULT & DISCUSSION

Effect of different carbon sources

Frie and Mcloughlin (2000) reported that mycelial growth of *Agraicus bisporus* was enhanced by malt extract a key component in PMP medium. Among the different carbon sources, mannitol and sorbitol stimulated the best mycelial growth of 110.15 and 100.45 mg/30 cm3, respectively in *S. commune* isolates (Adejoye *et al.* 2007). While studying *S. commune* found glucose as best source followed by fructose, xylose and mannose. Sugar alcohols like manitol also produced good growth of *S. commune* (Adejoye *et al.,* 2007). Sugar alcohols and polysaccharides get hydrolyzed to monosaccharide before they will enter into respective pathways (Mahier and Cordes 1971). Bealing (1953) observed that invertase preparations catalyzed the transfer of fructofuranosyl groups not only to water, but also to various alcohols and sugars by transglucosidation Glucose and manintol have been reported as good substrates for vegetative growth (Hammond, 1978). Scientists have tried to study the decomposition of *Sphagnum fuscum* plants and spruce wood chips *in vitro*. It was found that most taxa degraded cellulose and starch via the synthesis of cellulases and amylase, respectively (Thormann *et al*., 2002). In order to study the effect of fungi on different wood block, it was thought desirable to use teak sawdust as one of the carbon substance.

In the medium containing D – arabinose as carbon source the wood rotting basidiomycetes member *H. apiaria* showed the maximum growth of mycelium, whereas, least growth was observed in case of *L. sterioides* and *T. pini* (Table 1). In the medium containing D-xylose as carbon sources the maximum growth was shown by *H. apiaria* and *L. sterioides,* whereas, lowest mycelial growth was shown by *T. pini*. In the medium containing sucrose as the carbon source the maximum growth of mycelium was observed in case of *L. sterioides* whereas, lowest growth of mycelium was observed in *N. floccosa*. The final pH of the medium

varied from 3.31 to 5.93. In the medium containing malt extract as carbon source the maximum growth of mycelium was observed in *L. sterioides* whereas, lowest growth was shown by *N. floccosa*. In the medium containing teak wood sawdust as carbon source the maximum growth of mycelium was observed in case of *T. pini* whereas, the lowest growth of mycelium was shown by *N. floccosa*. The final pH of the medium varied from 2.54 to 5.24.

TABLE 1: Effect of Different Carbon source on mycelial dry weight and change in pH of four wood decay fungi

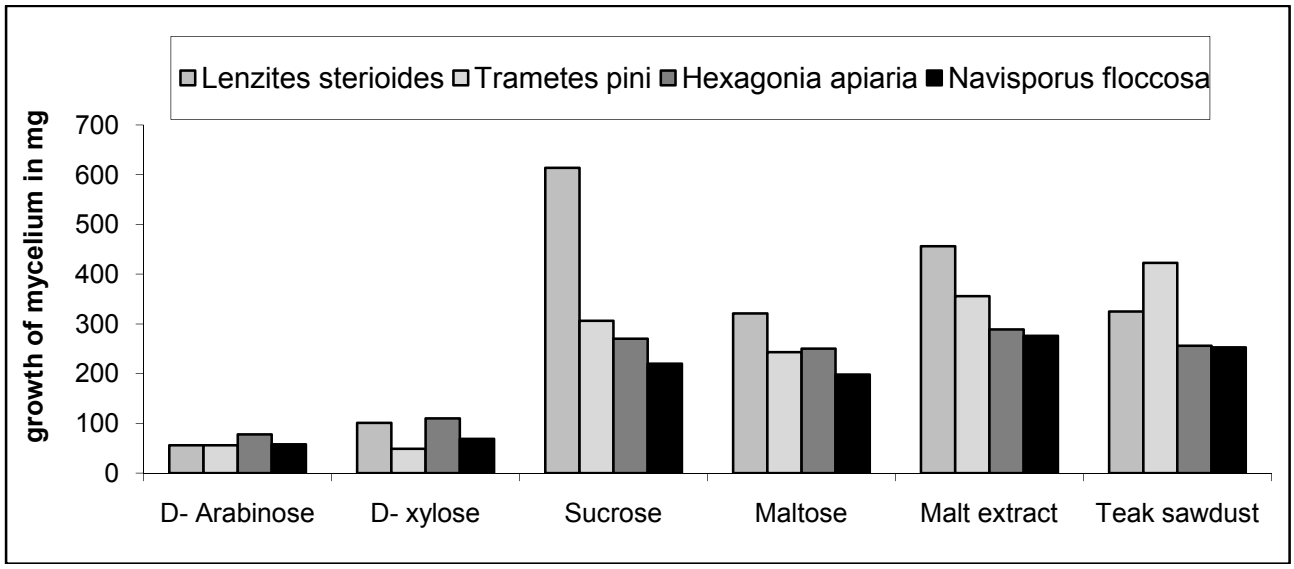
		Lenzites sterioides		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
Treatment No	Carbon source	Dry wt*	Final pH	Dry wt*	Final pH	Dry wt*	Final pH	Dry wt*	Final pH
1	D- Arabinose	56±2.8	3.80	56±1.8	3.96	78±1.8	3.98	58±1.4	4.43
2	D- xylose	101±2.5	3.80	49±1.5	3.98	110±1.5	3.59	69±2.6	4.80
3	Sucrose	614±1.8	5.93	306±1.2	3.31	270±2.6	4.98	220±2.8	5.45
4	Maltose	321±1.5	4.26	243±1.8	2.92	250±2.8	5.52	198±3.4	5.85
5	Malt extract	456±1.0	6.24	356±1.4	2.13	289±2.4	4.63	276±3.8	5.34
6	Teak sawdust	325±2.8	4.67	423±2.5	2.54	256±1.7	4.21	253±2.3	5.24

∗ indicates each component values are based on the three replicates.
± Results were significant at $P < .05$ level by one way ANOVA.

The maximum mycelial dry weight of *L. sterioides* was obtained (614 mg) when sucrose was used as carbon sources, whereas, the lowest growth was 56 mg when D - arabinose was used as sole carbon source. The maximum dry weight of *T. pini* was 423 mg when teak sawdust was used as carbon sources whereas D-xylose produced lowest dry weight. The maximum growth of *H. apiaria* was obtained on malt extract, Malt extract as a complex source was found suitable for 2 strains of *Stereum hirsutum* (Jonathan *et al.* 2009) and the lowest on D-arabinose. The maximum growth of *N. floccosa* was obtained on the malt extract as a carbon source while it was lowest on the D-arabinose.

The mycelial mass increased till 60th day after incubation and then it declined. The reason might be in deficiency of nutrients after two months of fungal feeding and consequently appearance of phenomena of autolytic degradation of hyphae. The similar process may happen in the wood as well. Swift (1978) found that mycelial mass increased till the weight loss of inoculated wood of some 40% but declines in later stages of decay, which was realized by using Hexosamine test methods. In the present study also after reaching to the maximum mycelial growth of wood rotting fungi, the Hyphae undergoing autolytic degradation. In the present study the best mycelial growth in *L. strioides, T. pini, H. apiaria* and *N. floccosa* were observed in case of sucrose, teak sawdust, and malt extract were used as carbon source in the medium.

In the present study the different carbon sources were used to see the maximum mycelial growth of the wood rotting fungi. As maximum growth of the mycelium is observed in all wood rotting fungi, they have the capacity to secret lignocellulolytic enzymes. The best growth of *H. apiarai* and *N. floccosa* was obtained on malt extract while it was maximum for *L. sterioides* and *T. pini* on sucrose and teak sawdust respectively (Histogram 1).



HISTOGRAM 1: Effect of different carbon sources on growth of four fungi

		Lenzites sterioides				*Trametes pini*				*Hexagonia apiaria*				*Navisporus floccosa*			
Mono-saccharides	**Days**	**Dry wt* (mg)**	**Rate of growth**	**Final pH**	**Presence (days)**	**Dry wt* (mg)**	**Rate of growth**	**Final pH**	**Presence (days)**	**Dry wt* (mg)**	**Rate of growth**	**Final pH**	**Presence (days)**	**Dry* wt (mg)**	**Rate of growth**	**Final pH**	**Presence (days)**
D- arabinose	5	37±1.3	37	3.50		22±1.8	22	4.00		28±2.6	28	4.21		25±1.0	25	4.50	
	10	48±1.8	11	3.82		35±1.5	13	4.03		54±2.8	26	4.24		42±1.5	17	4.45	
	15	56±1.5	12	3.80	15	56±1.2	21	3.96	15	78±3.2	24	3.98	15	58±2.5	16	4.43	15
D-xylose	5	38±2.5	38	3.50		29±2.3	29	3.50		46±3.5	46	3.85		30±2.3	30	4.20	
	10	45±2.9	13	3.84		38±2.5	9	3.95		67±2.8	21	3.76		51±2.6	21	4.39	
	15	101±1.0	56	3.80	15	49±1.3	11	3.98	15	110±1.5	43	3.59	15	69±2.8	18	4.80	15

Utilization of various sugars by four wood degrading fungi

Utilization of monosaccharides

Research findings have revealed that disaccharides get hydrolyzed to maonosaccharides before they enter into different pathways. The range of carbon source utilized for mycelial growth of different fungi is very wide. Monosaccharides, disaccharides and polysaccharides can be used as suitable carbon sources. Monosacharides (such as fructose, glucose *etc*.) or maltose among the disaccharide are the most suitable carbon sources for *A. auricula* **(**Luna et al 2004**).** Monosaccharides play an important role in the carbohydrate metabolism of fungi. Complex carbohydrates usually first split-up into monosaccharide units or their derivatives, which subsequently enter into the various metabolic pathways. Apart from occurring freely in various parts of the plants, these sugars are also present as component units of oligosaccharides and different polysaccharides. Monosaccharide also takes part in the synthesis of reserve carbohydrates of fungal mycelium and as such, various fungal polysaccharides are there which consist of monosaccharide units like glucose, mannose and galactose etc. In the present study daily chromatographic analysis was undertaken to determine the presence of various sugars in the culture medium. The results of mycelial growth, drift in pH and utilization of monosaccharides by different wood rotting fungi under study have been summarized in Tabel 2.

D-Xylose (Rf 0.62)

This is aldopentose occurs in nature in the form of xylans and as a constituent of polysaccharides of cell wall i.e., in hemicellulose and plant gums. It is evident from the table 2 that D- xylose was present in the culture filtrates of all the four test fungi up to 15 days of incubation. The growth rate increased in first 5 days and later on decreased. The fungal growth rate was increased up to first 5 days and later on decreased up to 10 days however a slight increase was observed up to 15 days in *L. sterioides* and *H. apiaria*. The final pH of the medium was acidic in *L. sterioides, T. pini* and *H. apiaria* and *N. floccosa* respectively.

D – arabinose (Rf 0.70)

This aldopentose occurs in nature in the form of arabans as common constituents of plant polysaccharides and various gums especially gum arabica. Chromatographic analysis of the medium showed that none of the wood rotting fungi under the study could assimilate this sugar completely within 15 days of incubation. The growth rate was increased in first 5 days in *L. sterioides* and *N. floccosa*, and decreasing later days of incubation. The growth rate is constantly going in *H. apiaria*. The growth rate was increased in first 5 days, later decreased up to 10 days and then increase up to 15 days in *T. pini*. The growth of mycelium was increasing as the incubation period is increasing. The final pH of the medium is acidic in *L. sterioides, T. pini* and *H. apiaria* and slightly acidic in *N. floccosa* respectively.

Both isomers of arabinose were employed in this nutritional investigation of *Calvatia* species but neither of them promoted satisfactory growth. According to Lilly and Barnett (1956), from the distribution of the isomers of arabinose in organisms, it would be expected that L-arabinose would be utilized readily by more fungi than D-arabinose. In the case of Calvatia species, two of the strains, 1019 B and 766, grew better on L-arabinose, and two strains, 1018 F and 1020, were superior on D - arabinose (Sedlmayr *et al.,* 1961). In the present study the D - arbinose showed 56mg, 78mg and 58mg of mycellial growth for *L. sterioides*, and *T. pini, H apiaraia*, and *N. floccosa* respectively. A study has been made of the carbon nutrition of *Coriolopsis occidentalis* using carbohydrates in liquid growth medium. Of the simple carbohydrates tested, the oligosaccharides supported growth best followed by xylose. (Fawole, 1973). In the present study the oligosaccharides showed better growth than the monosaccharides

Utilization of oligosaccharides

The oligosaccharides are complex sugars composed of two or more monosacharides units linked together by glycosidic bonds. They occur freely in nature or as the units of polysaccharides. These water soluble compounds yield monosaccharide components on hydrolysis.

The present studies were conducted in order to ascertain the pathway of utilization of different oligosaccharides and probable effect of their hydrolytic products on the growth of the wood rotting fungi under study. The rate if assimilation of the component sugars by the four organisms was detected chromatographically. The details of the results have been summarizes in table 3.

TABLE 3: Utilization of Sucrose and Maltose sugars by different wood decay fungi

	Average dry wt. (mg)*			Final pH			Presence of sugars (days)		
organism	5	10	15	5	10	15	Sucrose / Maltose	Glucose "	Fructose
Lenzites sterioides	170±1.8	197±2.8	614±1.8	6.15	6.36	5.93	8	1-5	1-10
Trametes pini	130±1.0	162±2.2	306±1.0	4.15	3.50	3.31	10	2-8	2-11
Hexagonia apiaria	148±2.5	185±1.8	270±1.5	5.35	5.10	4.98	12	1-8	1-14
Navisporus floccosa	70±3.5	100±3.6	220±1.2	6.13	5.93	5.45	10	1-8	1-12
Lenzites sterioides	150±3.0	187±2.7	321±2.5	4.30	4.70	4.26	6	6 -12	--
Trametes pini	120±3.8	159±1.6	243±2.8	3.58	3.13	2.92	15	4 -15	--
Hexagonia apiaria	138±2.4	174±2.5	250±1.7	5.80	5.74	5.52	10	6 -10	--
Navisporus floccosa	112±2.8	145±2.8	198±2.5	6.25	6.14	5.85	15	6-15	--

∗ indicates each component values are based on the three replicates.

± Results were significant at $P < .05$ level by one way ANOVA.

Disaccharides

Sucrose (Rf 0.43)

This disaccharide is of common occurrence in plants. A large number of workers have shown that most of the fungi are able to hydrolyse sucrose into glucose and fructose and thus it is assimilated through a hydrolytic pathway. Table 3 indicates that all the wood rotting fungi under the study utilized sucrose after hydrolysis, which indicates that they were capable of producing sucrase or trans –fructosidase enzyme in sufficient amount. It yields maximum mycelial yield of wood rotting fungi *i.e., L. sterioides, T. pini, H. apiaria* and *N. floccosa* after 15 d. As the incubation period is increased the growth of the wood rotting fungi also increased. It is evident from table 3 that sucrose was slowly breakdown into monosaccharides, it remained present up to 10 days in case of *T. pini* and *N. floccosa* while it was present up to 8 days in *L. sterioides* and 12 days in *H. apiaria*. This shows that hexagonia was able to utilize this disaccharide with much slower rate.

Maltose (Rf 0.40)

It does not usually occur in the free form in chlorophyllus plants but this disaccharide is obtained as an intermediate product during the digestion of starch to glucose. It consists of two glucose units which are held together by œ – 1, 4 glucoside linkage. Maltose is utilized by a majority of fungi through a hydrolytic pathway. It yields two molecules of glucose when hydolysis is accomplished by the enzyme œ – glucosidase.

Maltose was utilized by the present fungi through a hydrolytic pathway. Its presence was detected upto 6, 15, 10,15 days respectively in *L. sterioides, T. pini, H. apiaria*, and *N. floccosa*. Its hydrolytic products were detected in wood rotting fungi i.e., *L. sterioides, T. pini, H. apiaria*, and *N. floccosa*. Between 6 and 12, 4 and 15, 6 and 10, and 6 and 15 days respectively. The dry weight of all the wood rotting organisms was maximum on this sugar after15th days. The better yield may be due to hydrolysis of maltose by α – glucosidase enzyme which yields two glucose units. The glucose units were used efficiently by all the wood rotting organisms. The better growth of mycelium is also due to the slow and steady growth of all wood rotting fungi. The final pH of the medium was slightly acidic in case of *L. sterioides*. The pH of the medium is acidic in case of *T. pini*. The final pH of the medium was slightly acidic in case of *H. apiaria*. The pH of the medium shifted towards neutral side in case of *N. floccosa*.

Out of 21 carbon sources, *Lobivia lateritia* strains exhibited maximum mycelial growth on maltose followed by raffinose, starch and lactose with variable preference of different strains (Jana and Purkayastha 1987). Swartz (1933) was the only one who reported on the carbon requirement of *Calvatia* species. He found maltose the best sugar for producing mycelium of *Calvatia saccata, C. caelata* and *C. gigantean.* In the present study the maltose showed good growth of mycelium in wood rotting fungi i.e., *L. sterioides, T. pini, H apiaraia*, and *N. floccosa* respectively.

The mycelial growth of *Cystoderma amianthinum* was checked in the media supplemented with 11 different carbon sources. Fructose was found best screened as carbon source for the mycelial growth of *C. amianthinum* (Shim *et al.* 2005). But in the present study It was completely utilized in 13 days. Shim *et al*. (1997) reported that glucose, one of monosaccharides was exceedingly good for promoting a mycelial growth of *Grifola umbellata*. However, it was observed that glucose was unsuitable for promoting the mycelial growth of *C. amianthinum* (Shim *et al.* 2005) submerged culture of Nigerial mushroom *Pleurotus florida* grow well on glucose containing medium (Gbolagade *et al*. 2006). In the present study the wood rotting fungi under study showed fair growth of mycelium when maltose was broken down to two glucose molecules, it was utilized with in 4 to15 days of incubation. Chi *et al*. (1996) reported that though each of some monosaccharides was supplemented in the basal medium to check a mycelial growth of *Phellinus linteus*, its mycelial growth was dissimilar among monosaccharides. In the present study also the growth of mycelium depend on the nature of the wood rotting fungi under study.

Sucrose was not a good carbon source for *Calvatia* species. It could be that the organisms produced the hydrolyzing enzyme very slowly or in a small quantity, as both components of this disaccharide (glucose and fructose) produced a satisfactory growth when used separately. Apparently the glucose to fructose linkage was not easily broken (Sedlmayr *et al*., 1961). In the present study the sucrose yielded the glucose and fructose which were utilized in 8 to 10 days by wood rotting fungi under study.

Effect of different nitrogen sources

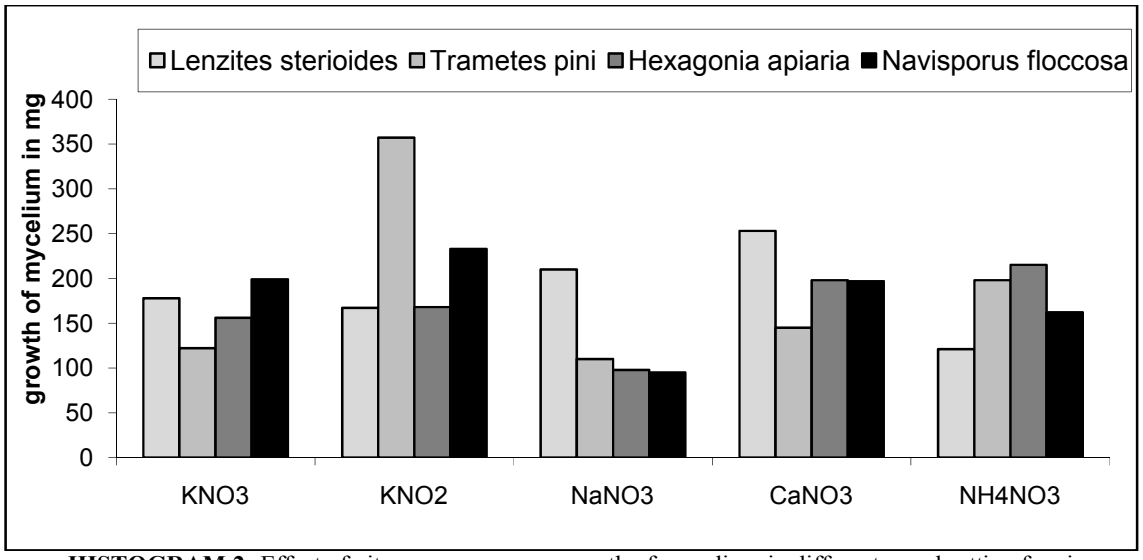
This essential element is used by fungi for functional as well as structural purposes. Chitin, the chief component of cell wall in most of the fungi, is a linear polymer of D-glucoseamine. Similarly proteins, the basis of protoplasm are composed of nitrogenous substance. Purines, pyrimidines, some vitamins and other essential metabolites are also nitrogen containing compounds. In nature both the organic and inorganic forms of nitrogen are available to fungi but as far as their utilization is concerned they fundamentally differ from each other in their metabolic potentialities. A few utilize atmospheric nitrogen, many utilize nitrate nitrogen and a still greater number utilize ammonium nitrogen. All species are able to utilize some form of organic nitrogen. Owing to the specific response of various fungi towards different nitrogenous substances numerous investigators have classified them into different groups on the basis of their abilities to utilize these sources.

For the few wood rot fungi previously studied both qualitative and quantitative difference have been found in the utilization of known organic and inorganic nitrogen compounds including certain amino acids (Hacskaylo *et al.* 1954 and Yusef 1953). Generally ammonium nitrogen is assimilated (Fries 1950, Lilly and Barnett 1951, Yusef 1953). A few wood rot fungi utilize nitrate nitrogen slowly in stationary culture (Lilly and Barnett 1951, Hacskaylo *et al.,* 1954). Discussing the possible pathway of protein synthesis Lilly and Barnett (1951) have mentioned, "with the exception of certain amino acids (primary amino acids) and ammonia, most nitrogen sources undergo modifications before entering the synthetic metabolic

pathways. Nitrates, nitrites and hydroxylamine are presumably reduced to ammonia before assimilation. Those amino acids (secondary amino acids) which do not enter directly into the metabolic pathways leading to the synthesis of protein are probably deaminated."

In urea there was substantial increase in the average amount of growth from the third to ninth serial subculture, with both the brown rot and the white rots. Presumably this increase reflected some degree of adaptation of certain organisms to the nutrients. *M. americanus* showed a marked increase in growth in casein hydrolysate and in ammonium sulfate and *F. fomentarius* in ammonium carbonate from the third to the ninth serial transfer. The negative data for growth in ammonium chloride, Potassium nitrate and Potassium nitrite were not included, since *T. serialis* was the only culture which could utilize even one of the compounds under the conditions used (Jennison *et al.,* 1955).

The effect of 5 different nitrogen sources was observed in case of 4 wood rotting fungi, the results are depicted in Table 4. The potassium nitrite showed better growth for *T. pini* and *N. floccosa*. The final pH of the medium changed from acidic to slightly neutral nature. The sodium nitrate showed better growth in *L. sterioides* and lowest growth was shown by *N. floccosa*. The calcium nitrate as sole nitrogen source showed better growth in *L. sterioides*, as compared to other fungi. The ammonium nitrate as sole nitrogen source showed better growth in *H. apiaria* and lowest growth in *L. sterioides* respectively (Histogram 2). Based upon growth supporting ability the inorganic nitrogen compounds are grouped as calcium nitrate > Sodium nitrate > Potassium nitrate > Potassium nitrite > ammonium nitrate for *L. sterioides*.



HISTOGRAM 2: Effect of nitrogen sources on growth of mycelium in different wood rotting fungi

TABLE 4: Effect of different Nitrogen sources on growth of wood decay fungi

		Lenzites sterioides		*Trametes pini*		*Hexagonia apiaria*		*Navisporus floccosa*	
Treatment No	Nitrogen source	Dry wt*	Final pH	Dry wt*	Final pH	Dry wt*	Final pH	Dry wt*	Final pH
1	KNO3	178±2.8	4.53	122±2.2	4.65	156±1.8	5.70	199±1.5	5.54
2	KNO2	167±3.4	6.30	357±1.0	4.70	168±1.2	6.76	233±2.5	7.12
3	NaNO3	210±1.5	7.10	110±1.5	6.86	98±1.8	7.26	95±2.8	7.60
4	CaNO3	253±2.5	6.20	145±2.5	5.10	198±1.9	5.23	197±3.4	6.18
5	NH4NO3	121±2.6	3.58	198±1.7	4.50	215±2.4	6.50	162±3.9	5.18

*indicates each component values are based on the three replicates.
± Results were significant at P <.05 level by one way ANOVA

The brown rot fungi as a group and for the white rot species, there was a consistent decrease in average growth-supporting ability for ammonium nitrate (Jennison *et al*., 1955). In the present study the wood rotting fungi showed the good mycelial growth in *H. apiaria* and lowest growth in *L. sterioides* for ammonium nitrate as sole nitrogen sources. The Basidiomyctes with the exception *Polyporus distortus*, grew very slowly on nitrate nitrogen. The species of basidiomycetes tested utilize nitrate nitrogen slowly or not at all, with the time of incubation (Hacskaylo et al., 1954). In the present study the wood rotting fungi showed good growth in nitrate nitrogen. The phenomenon of slow utilization of nitrate nitrogen appears to an exaggerated degree in some of the Basidiomycetes tested (Hacskaylo *et al*., 1954). In the present study the nitrate nitrogen shown better growth in 15 days of incubation as it take less time for stabilization

and the wood rotting fungi *i.e., L. sterioides, T. pini, H. apiaria* and *N. floccosa* showed good growth of mycelial mass in nitrate, nitrite, ammonium nitrogen sources which were incubated for short time. On the other hand none of these strains could grow on Sodium nitrite. All strains

responded moderately to the ammonium compounds studied. However the nitrates were found to support moderate to poor for growth of all strains (Singh and Verma, 1996). In the present study the sodium nitrite showed better growth in *T. pini* and *N. floccosa* and good growth in *L. sterioides* and also in *H. apiaria*.. Similarly sodium nitrite has been shown as a non available nitrogen source to several fungi including *Agaricus bisporus* (Hsu and Hu 1967) and *Lentinus edodes* (Tokimoto and Kumatsu 1979). In the present study *L. sterioides, T. pini, H. apiaria* and *N. floccosa* showed better to good growth of mycilial mass when sodium nitrite was used as sole nitrogen source. In the series of complex and inorganic nitrogen sources, it was observed that inorganic compounds supported moderate biomass production. The best biomass yield was found with ammonium nitrate closely followed by potassium nitrate. This result is contrary to that obtained by (Jonathan and Fasidi, 2001) for *P. atroumbonata* where ammonium nitrate supported insignificant mycelial yield. (Gbolagade *et al*., 2006). In the present study also the ammonium nitrate showed good growth for *H. apiaria* and *T. pini* to moderate growth in *N. floccosa* and *L. sterioides*. So ammonium nitrate is good source of nitrogen. As the incubation period increases the growth mycelium also increased in *Sterium hirsutum* on Ammonium sulphate and Potassium nitrate as sole nitrogen source (Minc 2005). The potassium nitrate also showed good growth in *N. floccosa* and *L. sterioides* and moderate growth in *T. pini* and *H. apiaria*. The differences in nitrogen sources requirements may suggest that biomass production in different fungi may be influenced by different nutritional requirements. It was also observed that very low concentration of nitrogen compounds generally supported little biomass yield while low concentration and above were supportive to high biomass yield (Yajie and Zhong, 2002). In the present study also the wood rotting fungi showed good growth in higher concentration of nitrogen as the free nitrogen was available to the fungi. The mycelial growth of *Cystoderma amianthinum* in Calcium nitrate was 5.6, Potassium nitrate was 16.4, and Sodium nitrate was 30.6 mg (Shim *et al.,* 2005). In the present study the mycelial mass of the *L. sterioides* in calcium nitrate was 25 3mg, potassium nitrate was 178 mg and sodium nitrate was 210mg. Nitrogen sources $(NH_4)_2HPO_4$, NH_4Cl, NH_4NO_3 $(NH_4)_2S0_4$ were good source of ammonium nitrogen sources whereas the KNO_3 and KNO_2 are poor source of nitrate nitrogen (Niederipruem, *et al.,* 1964). In the present study the ammonium nitrogen sources showed good to moderate growth whereas the inorganic nitrogen sources *i.e.* KNO_3 and KNO_2 showed better to good growth in all wood rotting fungi under study. These fungi, *Polyporus adustrzs* and *Liberfella befulincr,* grew better on ammonium nitrate. Utilization of nitrate led to an increase of pH in the medium. The changes in pH of media in which *Polyporrrs adusfus* had grown on ammonium nitrate indicates that the ammonium ion was taken up before the nitrate ion (Henningsson, 1967). In the present study also the pH of the medium is decreasing as incubation period is increasing. This decrease may be due to the release of cations and organic acids into the medium.

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