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NITROGEN ENRICHMENT OF STRAW COMPOST ASSOCIATED WITH GROWTH OF *PLEUROTUS* MUSHROOM

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ABSTRACT:

Pleurotus is one edible wood rotting fungus artificially cultivated in cereal straw for production of protein rich mushroom fruit bodies. Average protein content of straw-grown *Pleurotus* mushrooms range between 25-30% of dry weight. Protein yield from high C/N ratio cereal straw composted with *Pleurotus* does not equivalence with nitrogen content of unsupplemented straw on per unit weight basis. We present here evidence of diazotrophic nitrogen fixation during 50-day growth of *Pleurotus citrinopileatus* in rice straw compost timed with spawn run to fruit body production.

P. citrinopileatus grown straw compost, simultaneous to loss in dry weight during 50-day incubation period showed incremental values of nitrogenase activity measured as acetylene reduction activity. There was a parallel increase in the population of nitrogen fixing bacteria as indicated by bacterial colony counts of composted straw in nitrogen-less specific nutrient medium. *Pleuorotus*, during its growth on straw breaks down hemicellulose, cellulose and lignin to liberate sugars that allow substantial growth of straw-associated nitrogen fixing bacteria in the moist, micro-aerophilic environment of the compost. Nitrogen fixed by the bacteria is utilized by the fungus to yield protein rich mycelium and fruit body in one of the most outstanding prokaryote-

eukaryote bioconversion systems of nature.

KEY WORDS: *Pleurotus mushroom, Nitrogen fixation, Bioconversion.*

INTRODUCTION:

High C/N ratio cereal straw, such as that of rice and wheat is an excellent substrate for cultivation of the wood rotting edible fungus *Pleurotus*, which ranks second in order of production and use in world mushroom market (Neupane et al., 2018). The white-rot fungus *Pleurotus* secretes a battery of lignin, cellulose and hemicellulose decomposing enzymes on moistened straw to biologically convert the straw's polymeric carbon to edible protein matter in the form of 'mushroom', which besides containing 25-30% protein is also rich in dietary fibers, vitamins and other nutrients (Sadler 2003; Khan and Tania, 2012). *Pleurotus* is commonly grown in moisture- soaked chopped straw in sealed polypropylene bags for 50-60 days in humid atmosphere. Upon removal of the polypropylene cover after 20-25 days, fruit bodies emerge from the surface of the compost in intermittent flushes during the next 30-35 days, subject to the compost remaining sufficiently moist for proliferation of the fungus on and over the straw mass. Mushroom yield vary with many factors, but on an average 75-80 g dry mushroom per kg dry straw is considered adequate (Bhatti et al., 2007). Nitrogen content of rice straw averaging between 0.6-0.7 % dry weight does not equivalence total protein yield from the compost on per unit weight basis of dry straw taking yield of fruit bodies and mycelium of the fungus into consideration. We studied the scope of nitrogen fixation by straw associated bacteria in *Pleurotus* grown rice straw compost to balance protein turn over as fruit bodies of the fungus from the composted straw substrate.

MATERIALS AND METHODS:

Spawn preparation

Disease free, unbroken wheat grain was washed and soaked in tap water for 30 minutes. Soaked grains were spread in a thin layer on a clean polythene sheet to remove excess water. Calcium sulphate and calcium carbonate, respectively @0.2% and 0.5% w/w, were mixed with the soaked grains. 200g portions of such prepared grains were taken in 500 ml milk bottles, plugged with non-absorbent cotton and sealed with aluminium foil. The bottles were sterilized at 20-22 lbs psi for 1.5 hours. Mycelial suspension of 15-day old *Pleurotus citrinopileatus* culture in potato-dextrose broth was aseptically transferred to these bottles with the help of a sterile hypodermic syringe. The inoculated bottles were incubated for 20 days at 28 +/- 1˚ C when the fungal mycelium overran the grains.

Preparations of substrates:

Good quality fresh rice straw after removal of dirt and debris was chopped into ca. 3 cm pieces. The straw pieces were washed in running water for 10-15 minutes and were allowed to remain

immersed in water for 14 hours. After draining excess water, the soaked straw pieces were left on a clean polythene sheet under a ceiling fan for an hour or so to evaporate the surface water from straw pieces.

Spawning and spawn run:

Such prepared straw pieces were inoculated by thorough mixing of wheat-grain mushroom spawn @ 200 g spawn / kg dry straw weight. Inoculated straw was taken in 1-liter black polythene bags and forcibly packed in full without leaving any vacant space. Straw filled bags tied with strings were hanged in a dark room made humid by leaving water-soaked jute carpet on the floor. Polythene bag cover was cut open after 20 days when the mycelium of the fungus had ran over the straw pieces forming a mound-like solid mass of straw compost. The spawn ran compost was allowed to incubate for another 30 days with occasional spraying of water to keep it fully moist for fruit body formation in flushes.

Enumeration of nitrogen fixing bacteria:

Enumeration of nitrogen fixing bacteria in compost samples drawn from 2-3 cm below the surface of the compost mound were done by serial dilution of suspended straw samples at 0 , 10 , 20 , 30 and 50 days of incubation using Burks nitrogen-less medium comprising of : 10g glucose, 0.41g KH_2PO_4, 0.05g K_2HPO_4, 0.05g $Na_2SO_4.7H_2O$, 0.005g $FeSO_4.7H_2O$, 0.0025g $Na_2MoO_4.2H_20$, 1.8g agar-agar per litre of double distilled demineralized water. All chemicals used were Analar grade. p^H of the medium was adjusted to 7.0 before autoclaving at 121˚ C for 15 minutes.

Acetylene reduction assay (ARA):

Nitrogenase activity in the compost at different stages of spawn run was determined by measuring acetylene reduction activity of sample quantity of compost mass by using a HP gas chromatograph fitted with flame ionization detector and a Porapak N column according to the method described by (Hardy et al., 1968; Fulwieler et al., 2015) .

Statistical Analysis:

SPSS 13 was used for statistical analysis. Error bar was applied at $\pm$1 level.

RESULTS AND DISCUSSION:

Pleurotus mycelium grew over moist straw mass held in polypropylene bags converting the same into solid mound in 20-25 days. Buttons of *Pleurotus* fruit bodies started appearing over the surface of the mound from 15 days. Oyster-like fruit bodies of *Pleurotus* started appearing from around 20-21 days and continued till 60 days or more in intermittent flushes. Time lapse determination of dry weight of 1 g replicate sample quantity of compost showed reduction in dry mass of straw due to decomposition effected by the fungus and other microorganisms residing on the straw surface (Figure 1). Nitrogenase enzyme activity of the

compost, 2-3 cm below surface, measured in terms of acetylene reduction activity registered significant increases with time indicating that there was heightened nitrogen fixation inside the compost mound, the rate of its increase being almost proportional to time of incubation up till 50 days (Figure 2).The same samples of composted straw showed significant increases in the counts of nitrogen fixing bacteria where the rate of increase was also almost directly proportional with time (Figure 3).

Cereal straw being a natural substance harbours a large population of microorganisms including aerobic and micro-aerophilic nitrogen fixing bacteria on its surface (Roper and Gupta, 2016). The lingo-cellulolytic mushroom fungus *Pleurotus* decomposes the hemicellulose, cellulose and lignin fractions of rice and other straws (Tsang et al., 1987; Adebayo and Carrera, 2015) to liberate sugars to support its own carbon requirement. Data revealed that there was progressive increase in the population of nitrogen fixing bacteria with time inside the compost approximating to more than 2.5 times at 50 days. It was obvious that sugars made available by the fungus by decomposition of the polymeric carbon compounds of straw besides supporting growth of the fungus supported the growth of a population of nitrogen fixing bacteria inside the compost mass. This actively growing population of nitrogen fixing bacteria was capable of fixing atmospheric nitrogen in the compost as revealed by progressively increasing nitrogenase enzyme activity whereby the compost was enriched with easily available inorganic nitrogen residues. We mention that the compost upon growth of the mushroom fungus is converted into a moisture-saturated, semi-aerobic, exothermically heated substrate which becomes micro-ecologically conducive for growth of the nitrogen fixing bacteria residing on straw surface. This actively growing bacterial population was responsible for the heightened activity of nitrogenase enzyme in the compost as revealed in the experiment. We propose that such microbially fixed inorganic nitrogen as gets available with the bacterial development would sustain nitrogen requirement of the fungus for high yield of cellular protein in the form of mushroom fruit bodies, mycelium included. Such prokaryotic-eukaryotic association in composting of straw with *Pleurotus* and such other fungi makes mushroom production from straw an exemplary case of bioconversion for protein yield from natural wastes.

CONCLUSION:

High protein yield of mushroom fungi such as *Pleurotus* cannot be balanced by the basal nitrogen content of high C/N ratio cereal straw commonly used as substrate for mushroom production. Nitrogen fixation by the fungus, as such a eukaryote, has been occasionally suggested (Ginterova and Maxianova, 1975; Rangaswami et al., 1975). Results of a time course investigation with rice straw composted with *Pleurotus citrinipileatus* revealed simultaneous to significant increase in the population of surface associated nitrogen fixing bacteria, a progressive, parallel and significant

increase in nitrogenous enzyme activity in the inside layer of the compost. A high rate of bacterial nitrogen fixation in the microaerophilic, moisture saturated, exothermically heated straw compost due to proliferation of surface residing nitrogen fixing bacteria upon decomposition of lingo-cellulose by the fungus in the straw compost is suggested. Nitrogen fixed by the bacteria shall add to the residual nitrogen in the straw to explain the high protein turn over by the fungus from the high C/N ratio cereal straw.

REFERENCES:

Adebayo EA, Martinez- Carrera (2015) Oyster mushroom (*Pleurotus*) are useful for utilizing lignocellulosic biomass. *African J of Biotechnol.* 14(1)52-67.

Bhatti MI, Jiskani MM, Wagan KH, Pathan M (2007) Growth, development and yield of oyster mushroom, *Pleurotus ostreatus* (Jacq. Ex. Fr.) Kummer as affected by different spawn rates. *Pakistan J Bot*. 39(7) 2685-2692.

Fulweiler RW, Heiss EM, Rogener MK, Newell SE, Leclair GR, Kortebein SM, Wilhelm SW (2015) Examining the impact of acetylene on N-fixation and the active sediment microbial community. *Frontiers in Microbiology.* 6:418.10,3389/fmicb. 2015.00418.

Ginterova A, Maxianova A (1975) The balance of nitrogen and composition of proteins in *Pleurotus ostreatus* grown on natural substrates. *Folia Microbial (Prague)* 20: 246-250.

Hardy RW, Holster R, Jackson E, Burns R (1968) The acetylene-ethylene assay for N_2 fixation: laboratory and field evaluation. *Plant Physiol.* 43: 1185-1207.

Khan MA, Tania M (2012) Nutritional and medicinal importance of *Pleurotus* mushrooms: An overview. *Food Reviews International.* 28 (3) 313-329.

Neupane S, Thakur V, Bhalla B, Pathak P, Gautam BB, Aryal L (2018) Performance of different substrates on the production of oyster mushroom (*Pleurotus florida*) at Gokuleshwar, Darchule. *International Journal of Scientific and Research Publications*. 8(6) 231-240.

Rangaswami G, Kandaswami TK, Ramasamy K (1975) *Pleurotus sajor-caju* (Fr.) Singer, a protein rich nitrogen fixing mushroom fungus. *Curr Sci* 44: 403-404.

Roper MM, Gupta V.V.S.R. (2016) Enhancing non-symbiotic N_2 fixation in agriculture. *The open Agri Journal* 10: 7-27.

Sadler M (2003) Nutritional properties of edible fungi. *Nutrition Bulletin* 28: 305-308.

Tsang LJ, Reid ID, Cox Worth EC(1987) Delignification of wheat straw by *Pleurotus* spp. under mushroom-growing conditions. *Appl Environ Microbial* 53 (6) 1304-1306.

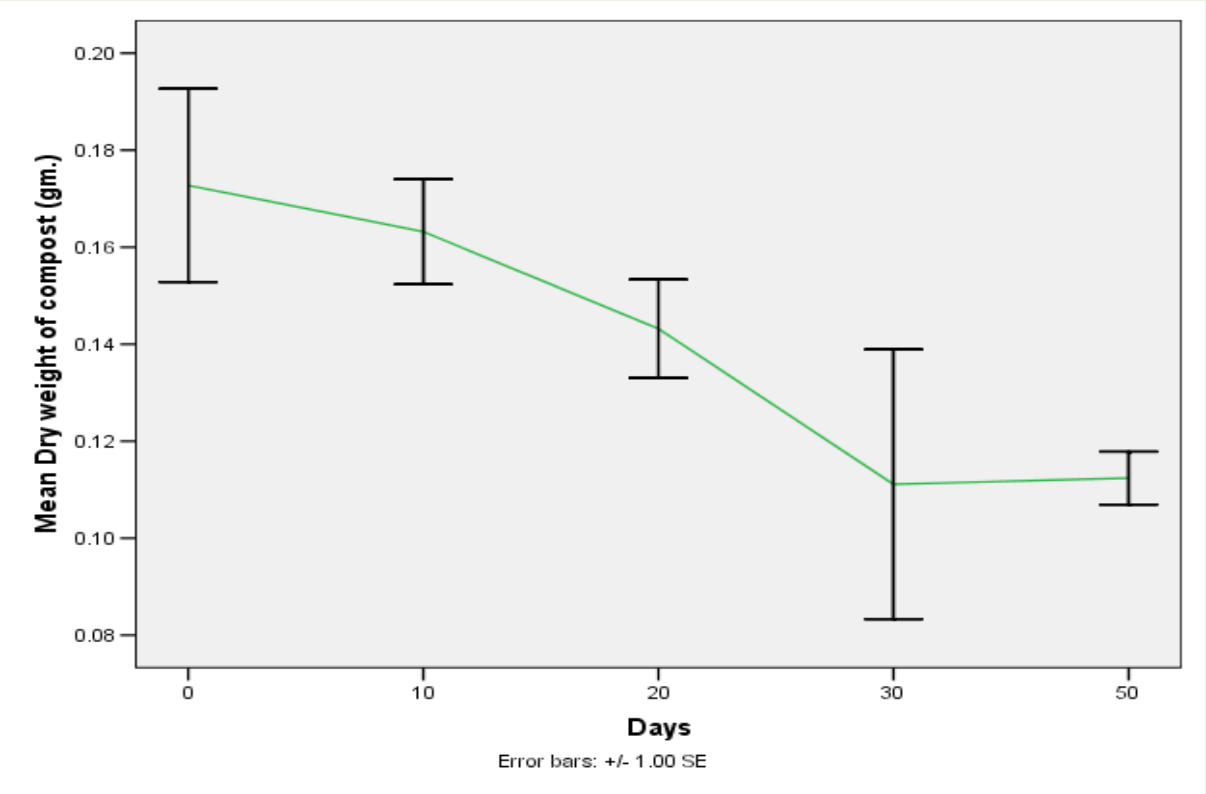

Figure 1: Time course change in dry matter content of rice straw composted with *Pleurotus citrinopileatus*

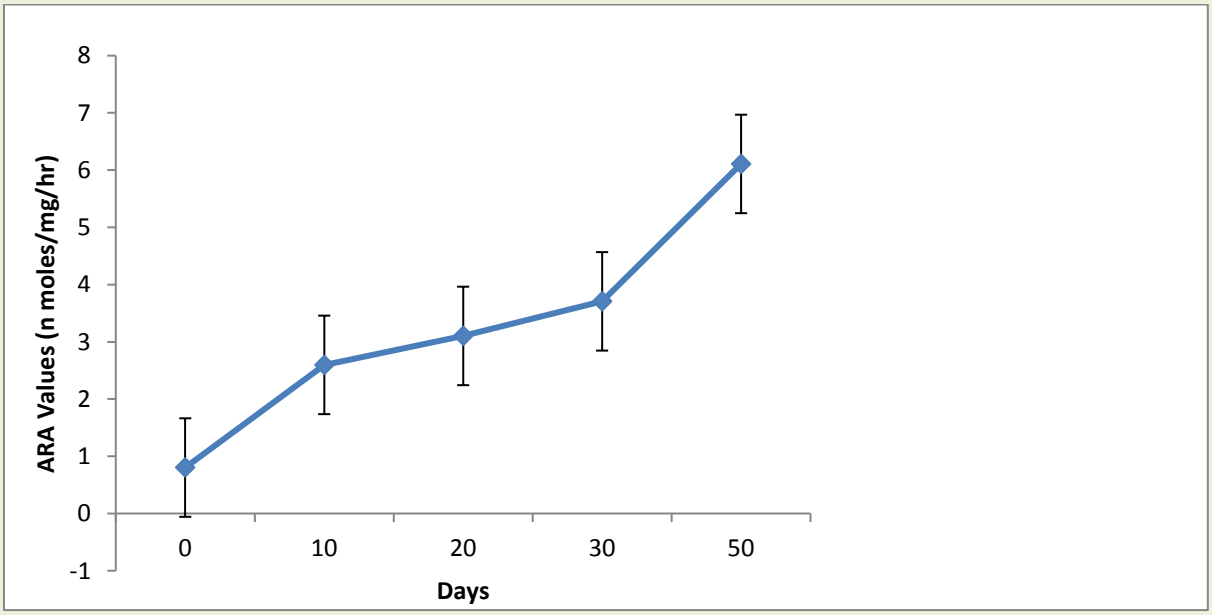

Figure 2: Time course changes in nitrogensae activity of rice straw composted with *Pleurotus citrinoplieatus*

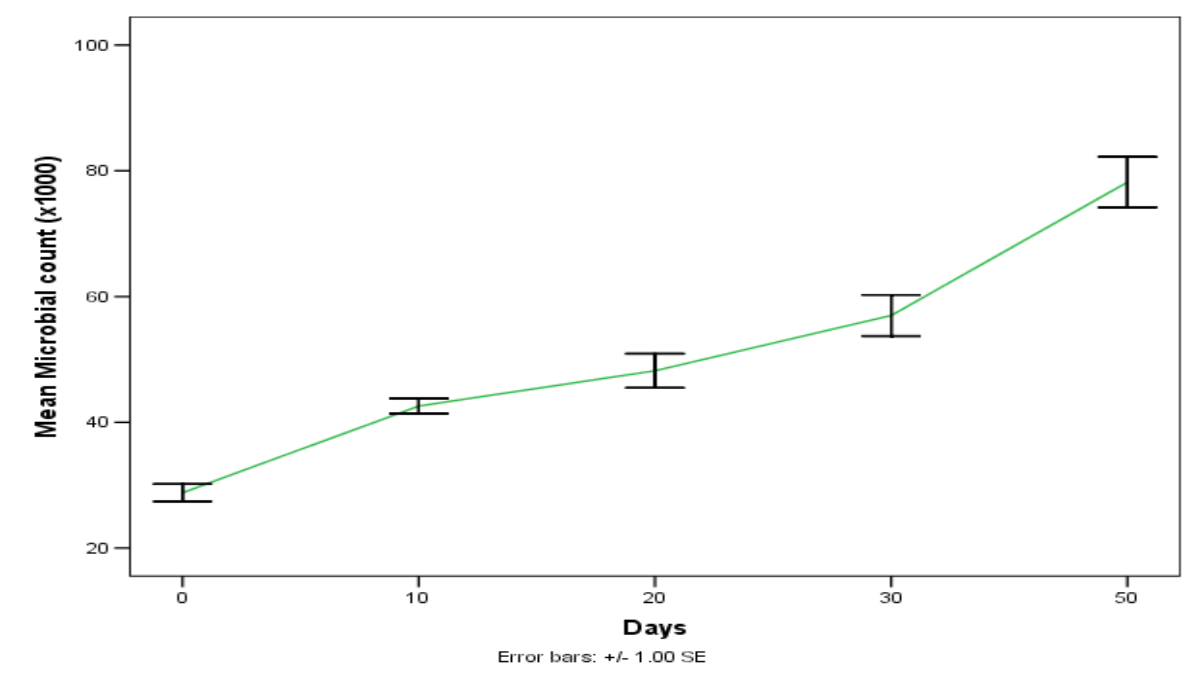

Figure 3: Time course changes in the count of nitrogen fixing bacteria in straw composted with *Pleurotus citrinopileatus*