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EFFECTS OF NITROGEN SUPPLEMENTATION WITH WHEAT STRAW ON PRODUCTIVITY OF *PLEUROTUS DJAMOR* (RUMPH. EX FR.) BOEDIJN

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Abstract

The present experiment was conducted in order to prepare a suitable substrate composition using wheat straw and also to ascertain the effect of various levels of nitrogen supplementation on the yield and biological efficiency of *Pleurotus* mushroom. The strain DMRP-205 of *Pleurotus djamor* was used in the study. Among different treatment combinations, maximum biological efficiency of 75.11% was recorded in T_2 with 0.5% of urea supplementation. It was also observed that, though the higher nitrogen supplementation does not increase the yield proportionately, it has significant effect on the spawn run period, cropping duration and colour intensity of the basidiocarp. The study necessitates the nitrogen supplementation at optimum levels (0.5%) to realize the maximum yield in *P. djamor* cultivation.

Introduction

Pleurotus species popularly known as oyster mushrooms are the important cultivated edible mushrooms in the South Asian countries and contributing around 19% of the total mushroom (Royse *et al*. 2017). *Pleurotus djamor* is highly valued for its role in anti-hypercholesterolemia activity and lowering the cardiovascular diseases (Jegadeesh *et al*. 2014). It is also an important source of minerals (Das *et al*. 2014). They are gaining wide acceptance among growers, due to their diversity and simple method of cultivation (Shirur *et al*. 2017).

In comparison to its inherent yield potential, the oyster mushroom production in India is far lesser (Wakchaure 2011). To realize the full yield potential, five underlying components need be harmonized *viz.*, high yielding strains, enzyme system, congenial crop environment, substrate nutrient composition and efficient pest and disease management. Early fruiting and high yield in pink oyster mushroom is enabled by its prolific enzyme system. The enzyme system is mainly driven by the availability of nutrition in the substrate and more particularly of nitrogen (Singh *et al*. 2008). Nitrogen also plays critical role in utilization of carbon sources. However, the lignocellulosic materials used for *Pleurotus* cultivation has relatively low nitrogen content ranging from 0.03 to 1.0% (Machado *et al*. 2016). Hence, nitrogen supplementation in the substrate is a major determinant of the yield. In view of this, an experiment was conducted to study the effects of nitrogen supplementation on yield and cropping pattern of *Pleurotus djamor*.

Materials and Methods

The present investigation was conducted at ICAR-Directorate of Mushroom Research, Solan. Dry wheat straw (150 kg) was used as substrate. The straw was wetted with water to hold 65 -70% moisture and stacked into four different piles of 5 feet width and 3.5 feet height. Each of these piles was supplemented with 1% lime (dry substrate basis) and 0 (T1), 0.5(T2), 1(T3) and 2% (T4)

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nitrogen (amide form present in urea), respectively. The four stacked piles were partially composted for 6 days in open compost yard with turnings on alternate day followed by steam pasteurization in pasteurization tunnel for 4 hrs at 58 - 62°C. The pasteurized substrate was then cooled to 28°C and spawning was done with 10% spawn on dry weight basis.

The strain DMRP–205 of *Pleurotus djamor* from ICAR-DMR germplasm collection was selected for the trial. The spawned bag of 5 kg wet straw was kept in cropping room maintaining 24 - 26°C temperature and 70 - 80% humidity.

The trial was conducted following RCBD with three treatments and control. Six replications were maintained. Eighteen parameters were recorded in experiment for all the treatments. The observations on days to spawn run, days to pinning, biological yield of each flush (%), duration for each flush, average fruit body weight in each flush (g), moisture content of fruit bodies in each flush (%), days to each flush up to three flushing, total biological yield (kg) and biological efficiency (BE%) were taken. Morphological observations of fungus were studied (Atri *et al.* 2005). The color terminology used is that of Kornerup and Wanscher (1978).

The samples were examined at 10x, 40x and 100x for different microscopic characters *viz.* type of hyphal system, shape and reaction of basidiospores, types of cystidia and basidia. All the microscopic characters were observed by cutting hand cut sections and crush mount in different reagents like congo red, cotton blue etc. ANOVA was done for significance testing as followed by Panse and Sukhmate (1964) and DMRT was used to identify the superior treatment for enhancing yield. The data were analyzed using R (R Core team 2013).

Results and Discussion

Fructification was up to 10 cm in height, stout, fleshy pink shell like, pleurotoid, caespitose. Pileus was up to 10 cm in diameter; surface pinkish white (10 A2) to pastel pink (11A3) at maturity, smooth, scales on the pileus surface, moist; veil absent; margin inrolled, depressed, flesh up to 0.5 cm thick, pinkish white, unchanging on exposure; taste and odour mild. Lamellae pastel red (10 A5), decurrent, extending down on to the stipe, subdistant (up to 0.3 cm apart from each other), unequal, divided into four tiers, ventricose, gill edges smooth and spore print whitish. Stipe small, pinkish white (10A2), excentric, up to 3.5 cm long, 2.5 cm broad, slightly narrow at the base, solid, smooth, hairy tomentose towards the base and flesh pinkish white underneath.

Basidiospores 6.5 - 9.0 × 2.5 - 4.0 µm, oblong, elliptical bean shaped, smooth, thin walled, inamyloid. Basidia 16.0 - 22.5 × 4.02 - 4.8 µm, club shaped, tetrasporic, tetra-sterigmatic; sterigmata 2.4 - 4.0 µm long; gill edges heteromorphous. Pileus cuticle 60 - 112 µm thick, with thin hyphae forming a scurfy layer over the pileus surface. Pileus context composed of elongated, branched, thick walled, sepatate hyphae measuring 2.2 - 4.8 µm in breadth; pileocystidia small 2.4 - 4.9 × 2.4 - 4.0 µm. Gill trama consisting of interwoven generative hyphal elements measuring 2.2 - 4.8 µm in width. Cheilocystidia 17.7-26.9 × 4.6-7.4 µm, clavate, very few. Hyphal pegs present. Stipe cuticle 12 - 20 µm thick: context composed of thick walled, clamped, septate, branched hyphae measuring 4.8 - 6.4 µm in breadth, hyphal construction monomitic, and generative hyphae with clamps (Fig. 1). Collection examined**:** ICAR-DMR culture bank collection, DMRP 205. The species can be easily identified by its attractive pinkish shell like, pleurotoid, caespitose, basidiocarps with pileus having pinkish to pastel red surface with inrolled, pink rose margins. Stipe small to with hairy tomentum at the base. This species is widely distributed around the world and has been described several times showing great phenotypic plasticity, mainly in pileus colour, varying from deep pink salmon to slightly pinkish and even white from distinct geographical localities by many authors (Singer *et al.* 1961, Saccardo *et al.* 1888, Guzman *et al.* 1993, Vilgalys *et al.* 1994, Guzman *et al.* 1995, Pegler *et al.* 1997).

The analysis of variance showed that, except the duration of third flush, all other parameters showed significant results for the applied treatments. The parameters with significant difference were considered for further analysis. The DMRT results showed that biological efficiency was affected by urea concentration in substrate (Fig. 2). The DMRT graph for water content in each flush suggested that, although a non-linear relationship of water content with concentration of

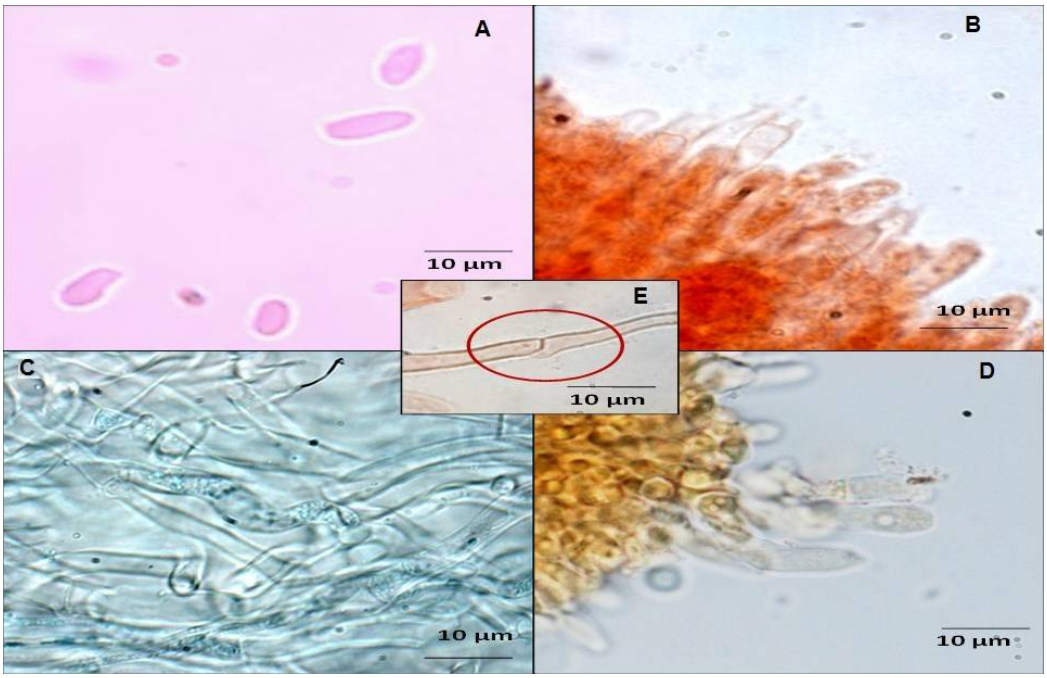

Fig. 1. Light microscope photographs A. Basidiospores, B. Basidia, C. Pileus context hyphae, D. Cheilocystidia, E. Clamp connection.

urea, but water level was comparatively higher in the control treatment with no urea supplementation in the substrate. The 0.5% nitrogen formed a distinct group highest biological efficiency. In duration of flushes, the treatments with 1 and 2% concentration were increasing the flushing days which means that the synchronization of flushes may depend upon nitrogen utilization. The average fruit body weight of different flushes though showed no distinct effect of treatment, was observed that fruit body weight from first flush was higher compared to other flushes for all treatments. Days taken for complete spawn run was found inversely related to nitrogen concentration in all the treatments. The minimum was found for the 2% nitrogen level. Similar trends were observed for the days to pinning parameter. The overall production - represented as biological efficiency was found maximum in treatment grown on substrate supplemented with 0.5% nitrogen level. Treatments with 1 and 2% nitrogen supplementation showed no superior yield signifying the importance of using an optimum level of nitrogen supplementation to enhance the biological yield.

The major pest incidence observed during the experiment was *Megaselia halterata* (Fig. 4). These insects were small hump backed black or light to dark brown flies measuring 1.9 - 2.0 mm in size. These insects lay an egg which varies with the species. Adult female lays eggs singly or in

batches. Adult insects have normal life span ranging from a few days to few weeks. The length of life was correlated with fecundity, death usually occurring a short time after the completion of mating or oviposition activities. In the current experiment the infestation was recorded for all treatments at each flush.

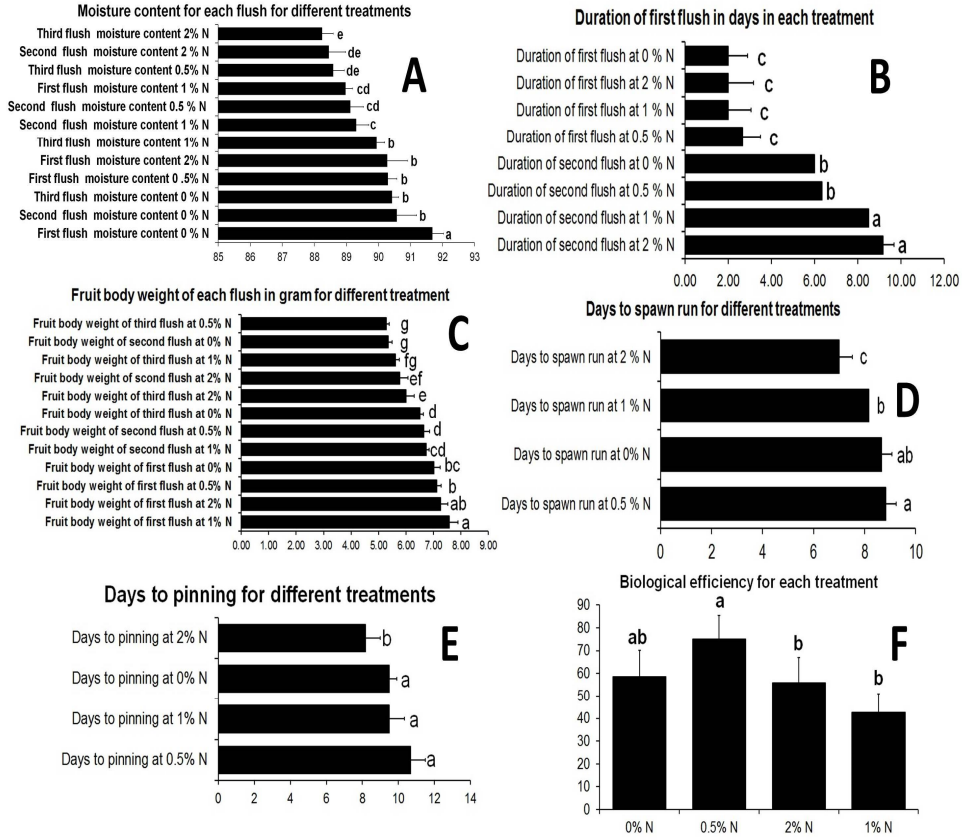

Fig. 2. DMRT (Duncan multiple range test) graph of different parameters (A - Moisture content of each flushes, B - Duration of first flush in days, C - Fruit body weight of each flush in gram for different treatments, D - Days to spawn run for different treatment, E - Days to pinning for different treatments and F - Biological efficiency for each treatment).

Fig. 3. Gill side photograph of different treatment in *Pleurotus djamor*.
(a) T1, 0% nitrogen, (b) T2,0.5% nitrogen, (c) T3, 1.0% nitrogen, (d) T4, 2.0% nitrogen

Fig. 4. Infestation of *Megaselia halterata* during experiment

The infestation was found higher in 1.0% urea concentration and minimum in control treatment (Table1). However, the reasons of pest incidence and the replicability of these observations need further investigations.

Table 1. Per cent infestation of the flushes with *Megaselia halterata* insect larvae.

Treatments (% nitrogen)	% infestation during thee different flushes		
	First flush	Second flush	Third flush
T2 0.5	0	12	15
T3 1	0	28	22
T4 2	0	17	20
T1 Control	0	10	15

Pleurotus enzyme system played a critical role in its biological yield with economic implications on its commercial cultivation. The enzyme system was in turn influenced by nitrogen which was known to impact in the development of protein and nucleic acids. Nitrate is a nitrogen source for mushrooms (Martínez-Espinosa *et al*. 2011). The supplementation with nitrate form of nitrogen will enhanced the mushroom productivity, but knowledge about its optimum level is essential for all practical purposes. In the present experiment 0.5% nitrogen supplementation found to have improved the effect on productivity of pink oyster mushroom. Similar results were recorded in supplementation in *Pleurotus* sp. "florida" which showed that the nitrogen up to certain level can increase crop productivity, as higher nitrogen can adversely affect the fruiting of mushrooms (Maganhotto *et al*. 2005). The same results were evident in the present experiment, as nitrogen level up to 0.5% was found useful and higher doses of nitrogen showed incidence of flies and larvae during the cropping. This could be due to the softness of the fruit bodies caused by

increased moisture content in 1 and 2% of nitrogen. The interaction of pH with supplementation was another grey area which needs further understanding. In the current experiment, the pH recorded at the time of spawning for control, 0.5, 1 and 2% nitrogen was 7.0, 7.2, 7.4 and 7.6, respectively, which after first flush was reduced to 5.4, 4.9, 5.6 and 5.5, respectively. The result clearly indicated that amide form of nitrogen increased the substrate pH initially which needs more examination especially on its mode of action.

The analysis of variance showed that effect of nitrogen played a significant role during second flush and onwards. This also proved that additional nitrogen increases the yield of the crop especially after the first flush thus helping to improve the sustainability of substrate in the bag by replenishing the exhausted nutrients. The results of the present work paves the way for similar experiments with other raw materials like paddy straw, sugarcane bagasse, dry stalks of soybean, cotton wastes, groundnut hulls, etc. as substrate to grow pink oyster mushrooms.

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