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RESEARCH ARTICLE

Effect of *Mimosa pudica* L. (Fabaceae) as Organic Nitrogen supplementing material on the growth and yield of Oyster Mushrooms (*Pleurotus* spp.)

Tenya Rina* and Rajiv Kumar Singh

Department of Botany, Rajiv Gandhi University, Rono Hills, Doimukh, 791112, Arunachal Pradesh, India.

*Corresponding author email: tenyarina2017@gmail.com
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Abstract

The sundried biomass of *Mimosa pudica* L. (Fabaceae) was applied as organic nitrogen supplementing material (MP) @3:1 along with basal substrate, paddy straw (PS+MP) on dry weight basis for cultivation of four popular commercial species of oyster mushrooms viz: *Pleurotus sajor-caju*, *P.sapidus*, *P. flabellatus* and *P. citrinopileatus* to determine its effect on the biological efficiency of mushroom mycelia, its ability to efficiently utilize the basal substrate and mushroom yields. The study was conducted for three different growing months, viz: November, January and March in an outdoor, semi-technical mushroom house at Rajiv Gandhi University, Arunachal Pradesh. Paddy straw (PS) was used as the control substrate. Each treatment was kept with 5 replicates (n=5). Inoculation of ready substrates was done using 2-3 weeks old whole wheat grain spawn @3% by layering method. From the study, the mean biological efficiency (BE) value for all species of *Pleurotus* on supplemented substrates (PS+MP) was recorded at 103.1% which was significantly higher than the BE of 94.2% on untreated substrate paddy straw (PS). Also, a comparatively higher mushroom yield (fresh weight) was recorded from supplemented substrates over unsupplemented substrate paddy straw. The results indicate that supplementing the basal substrate paddy straw with *M. pudica*@3:1 is a better substrate giving enhanced biological efficiency and best mushroom yields ($p<0.001$) on these species of oyster mushrooms.

Keyword: Oyster mushrooms; Substrate; Supplementation; Biological Efficiency; Yield

1. Introduction

Oyster mushrooms (*Pleurotus* spp.) are the second largest commercially cultivated edible mushrooms in the world (Royse, 2014). They are the group of edibles, fleshy basidiomycete fungi cultivated worldwide for their high nutritional and therapeutic properties (Cohen et al., 2002). Owing to a simple and low-cost production technology, their cultivation is very popular in Asia and Europe particularly among small and medium scale growers who grow them both in indoor and outdoor conditions (Mane et al., 2007). *Pleurotus* species are efficient colonizers and degraders of lignocellulosic materials (Poppe, 2000)). They are the easiest, fastest growing and the cheapest cultivable mushrooms which require less preparation time, minimum space and very low economic investment and most importantly, this mushroom requires moderate climatic condition for growth and fruit body production (Royse, 2014) and hence can be cultivated throughout the year. Under a moderate range of temperature, they grow and produce sporophores on various agro-forest and other lignocellulosic wastes (Guzman, 2000; Sanchez, 2004; Royse, 2014) due to their excellent ability to synthesize relevant extracellular enzymes such as cellulases, hemicellulases and lignolytic enzymes (Kuforiji and Fasidi, 2008; Kurt and Buyukalaca, 2010). Hence, cultivation of *Pleurotus* spp. offers an opportunity to utilize renewable lignocellulosic agro-forest wastes in the production of consumable highly nutritive, protein-rich food.

Supplementation of substrate with inorganic nitrogen source such as ammonia and urea has been found to increase the fruit body yield and mushroom productivity of *Pleurotus* spp. (Chang, 1999; Royse, 2002) and shortens the cropping period (Curvetto et al., 2002).Organic nitrogen sources like alfalfa, soybean meal, spent agro-residues, wheat bran and millet grains (Royse, 1992), rice bran, cotton seed cake, gram flour, mustard cake and groundnuts have also been recommended as better supplementing materials (Ralph and Kurtzman, 1994; Upadhyay et al., 2002; Narain et al., 2009). Some aquatic weeds, viz: *Typha anguistica* (Typha), *Oryza nigra* (Tina) and *Scirpus locustris* (Gonn) in different proportions as supplementing materials has also been tried (Mishra and Singh, 2007). In some developing countries, water hyacinth (*Eichhornia crassipes*) has also been utilized (Murugesan et al., 1995; Nageswaran et al., 2003; Anakalo et al., 2008).

Arunachal Pradesh, the largest state of the North-East India, offers a highly conducive environment for mushroom cultivation due to a moderate temperature range (15-30° C) and high humidity (70-80%) prevailing throughout the year. However, due to less availability and scarcity of paddy straw in the open market, and it is reported to be very high-cost involvement in the adjacent locations of Assam, oyster mushroom cultivation could not emerge as a popular, established agri-business in the state. In the recent past, the state's annual commercial production of oyster mushrooms is recorded to be merely about 5 tons per annum (Wakchaure, 2011). Therefore, to develop a popular, low-cost oyster mushroom cultivation technology for the region, several agro-forest lignocellulosic substrates that are abundantly available in the region which do not have any other economic utilization, needs to be explored and incorporated as alternate high yielding substrates for wide scale cultivation of oyster mushrooms.

Mimosa pudica L., commonly known as *touch me not* plant belongs to the family Fabaceae. It is a small, perennial woody herb measuring about 1 meter tall with a short, prickly stem and semi-creeping shoot with its branches growing near to the ground. This plant is usually considered to be a weed, invasive species that grows abundantly in sub-tropical and tropical open forest, roadsides, moist grounds, lawns, wastelands and abandoned fields.

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Table 1. Duration of Spawn Running on PS+MP (Days taken for Substrate Colonization)

P. sajor-caju	*P. sapidus*	*P. citrinopileatus*	*P. flabellatus*
Spawning: October; Cropping: November			
Days taken for Spawn running			
[RT: 21 - 26ºC; RH: 76-96%; Average RH: 83%]			
17	19	18	18
Spawning: December; Cropping: January			
Days taken for Spawn running			
[RT: 11 - 22ºC; RH: 60-86%; Average RH: 69%]			
21	21	21	21
Spawning: February; Cropping: March			
Days taken for Spawn running			
[RT: 11 - 22ºC; RH: 60-86%; Average RH: 69%]			
19	19	19	19

PS: Paddy Straw; MP: *Mimosa pudica*; PS+MP: Paddy Straw + *Mimosa pudica*

Apart from some reports on its medicinal uses, this plant finds no other economic importance rather than being considered and removed as an unwanted, perennial weed from cultivation practices and fields.

In the present study, attempt was made to assess the effect of an abundantly growing invasive, perennial weed herb, *M. pudica* L. (Fabaceae) as organic nitrogen supplementing material for enhanced biological efficiency and high mushroom yields in Arunachal Pradesh. The dried biomass of *M. pudica* L. was applied as organic supplementing material @ 3:1 (on dry weight basis) along with the basal substrate paddy straw for enhanced biological efficiency and higher mushroom yields.

2. Materials and methods

2.1. Experimental design

The experiment was carried out in an outdoor, semi-technical mushroom house (Figure 1 and 2) constructed out of cheap, locally available forest materials such as bamboo, thatch grass and wood in the Department of Botany at Rajiv Gandhi University, Itanagar, Arunachal Pradesh. The experimental set up was based on the semi-natural cultivation of four popular commercial strains of oyster mushrooms on three different growing seasons, viz: January, March and November using paddy straw as control substrate and *Mimosa pudica* L. as wild organic nitrogen supplementing material. The substrate treatment combinations were designed as follows:

2.1.1. Substrate combination

PS=Paddy straw (control)
PS+MP= Paddy straw + *Mimosa pudica* (@3:1 on dry weight basis)

2.1.2. Supplementation

As organic nitrogen supplementing material, properly sundried biomass of *M.pudica*, except its root parts was directly mixed with ready basal substrate paddy straw @ 3:1 on dry weight basis (@25% per treatment of paddy straw

2.1.3. The experimental plot

Each treatment was kept with 5 replicates (n=5) for all four commercial species of oyster mushrooms, namely: *Pleurotus sajor-caju*, *P.sapidus*, *P. flabellatus* and *P. citrinopileatus* (Figure 2).

2.1.4. Inoculation

All treatments were inoculated with 2-3 weeks old, freshly prepared whole wheat grain spawn @3 (on wet weight basis) on ready substrates and filling was done in clear, pre-punched food grade polythene bags by layering method (Figure 1e).

2.1.5. Observation

The trial cultivation was carried out for three growing seasons, namely: November (October-November), January (December-January) and March (February-March). All cropping parameters data were observed and data recorded at different growth period for spawn running, pinning and mushroom yields.

(b) Starter culture

The starter cultures of all the four selected commercial strains of *Pleurotus* namely: *P. sajor-caju* (PL-1140); *P. sapidus* (PL-40); *P.flabellatus* (PL-50) and *P. citrinopileatus* (PL-100) were procured from the culture bank of Directorate of Mushroom Research (formerly National Research Centre for Mushroom, NRCM), Solan, Himachal Pradesh, India. Pure culture of all species of *Pleurotus* was maintained on Potato Dextrose Agar (PDA) at 25±2°C for 2-3 weeks.

(c) Spawn preparation

The spawn was prepared on superior quality whole wheat grains in heat resistant Tarsons polypropylene bags following standard techniques. Properly boiled whole wheat grains were mixed with 2% Gypsum powder (Calcium Dihydrate Phosphate Precipitate, Laboratory Grade, Merck Pvt. Ltd) and 0.5% Chalk powder (Calcium Carbonate Precipitate, Laboratory Grade, Merck Pvt. Ltd) on dry weight basis (final pH verified at ~ 7.0- 7.5). After mixing, prepared grain was filled in autoclavable polypropylene bags of 375 x 175 mm x 160 guage @350 g mixed prepared grain per bag and plugged with PP ring and non-absorbent cotton for sterilization by autoclaving. Finally, sterilized whole wheat grains in bags were inoculated with 3-4 weeks old pure mycelial culture under aseptic condition in Laminar Air Flow cabinet.

(d) Preparation of basal substrate and supplementing material

Being considered the most ideal substrate for oyster mushroom cultivation in terms of biological efficiency and mushroom yields, paddy straw (PS) was used as the base substrate for the study. Shining bright straw heaps was procured from nearby locality just after the harvesting season for their easy accessibility, abundance and mushroom yield potential. Freshly harvested, disease-free paddy

Table 2. Days taken for Pinhead formation and Period of cropping on PS+MP

P. sajor-caju	*P. sapidus*	*P. citrinopileatus*	*P. flabellatus*	*P. sajor-caju*	*P. sapidus*	*P. citrinopileatus*	*P. flabellatus*
Spawning: October; Cropping: November							
Pinhead formation (days)				*Flush duration (days)*			
[RT 20 - 25ºC; RH 70-84%; Average RH: 79%]							
2	2	2	2	20	18	21	21
Spawning: December; Cropping: January							
Pinhead formation (days)				*Flush duration (days)*			
[RT 13 - 20ºC; RH 66-90%; Average RH: 76%]							
2	2	3	3	24	28	29	27
Spawning: February; Cropping: March							
Pinhead formation (days)				*Flush duration (days)*			
[RT 13 - 20ºC; RH 66-90%; Average RH: 76%]							
3	3	3	3	13	15	18	14

PS: Paddy Straw; MP: *Mimosa pudica*; PS+MP: Paddy Straw + *Mimosa pudica*

Table 3. Yield of four summer *Pleurotus* spp. on PS+MP in outdoor condition during different seasons (Results are mean ± SEM (n=5). Values in a row with different letters indicate a significant difference ($p<0.05$) between *Pleurotus* species).

	Month	*P. sajor-caju*	*P. sapidus*	*P. citrinopileatus*	*P. flabellatus*
BE*	Nov.	108.2^{bc}	116.4^{ab}	102.6^{c}	118.7^{a}
	Jan.	114.0^{a}	106.4^{a}	90.7^{b}	108.6^{a}
	Mar.	96.1^{a}	86.2^{a}	96.4^{a}	87.9^{a}
First flush yield (g)	Nov.	174.1±7.9^{b}	177.6±4.6^{b}	153.6±5.4^{c}	200.6±8.0^{a}
	Jan.	174.4±9.3^{a}	171.2±8.8^{ab}	154.5±8.1^{b}	195.4±4.0^{a}
	Mar.	155.7±7.2^{ab}	126.8±5.8^{c}	172.7±4.6^{a}	142.9±15^{bc}
Av. Weight (g) of FB† in first flush	Nov.	3.20±0.21^{a}	2.85±0.07^{a}	2.86±0.22^{a}	3.34±0.20^{a}
	Jan.	3.22±0.16^{b}	3.61±0.29^{b}	4.29±0.41^{ab}	5.12±0.47^{a}
	Mar.	3.76±0.19^{a}	2.47±0.15^{b}	3.77±0.17^{a}	2.95±0.26^{b}
Weight of QFB‡ (g) in first flush	Nov.	10.6±0.19^{c}	12.4±0.25^{b}	10.6±0.29^{c}	14.0±0.45^{a}
	Jan.	10.0±0.32^{b}	9.6±0.10^{b}	8.8±0.26^{c}	12.4±0.29^{a}
	Mar.	10.2±0.12^{b}	11.0±0.32^{a}	9.6±0.10^{b}	11.2±0.20^{a}

*BE = Biological efficiency †FB = Fruit bodies, ‡QFB = Quality fruit bodies
PS: Paddy Straw; MP: *Mimosa pudica*; PS+MP: Paddy Straw + *Mimosa pudica*

straw (PS) was first properly sundried and chopped into small piecesof about 0.5 – 1.0 cm with a grass chaffer on a clean, raised cemented floor under shade. To enhance the biological efficiency and mushroom yields of four different popular commercial species of oyster mushrooms, *M. pudica* L. (Mimosaceae) was used as organic nitrogen supplementing material (Figure 1b,1c and 1d).

For this, the whole plant of *Mimosa pudica* L. was collected in heap by cutting off above ground biomass and properly sundried for few weeks until all green matters disappeared. The completely sun-dried biomass was then chopped into small pieces of about 0.5 – 1 cm and directly mixed with the basal substrate paddy straw @3:1 on dry weight basis.

(i) Substrate treatment and pH

The chopped basal substrate, paddy straw (as control) and paddy straw combined with supplementing material were separately pretreated and sterilized (Figure1c and 1d) by Chemical Sterilization method using 500 ppm formalin and 75 ppm carbendazim (Vijay and Sohi, 1987). The sterilized substrates were separately spread on clean tarpaulin sheets for about 30 minutes under shed for leaching out excess water so that the substrates retain only about 65% moisture at the time of inoculation.

(ii) Substrate pH

The pH of each untreated and treated substrates in all the experiments was measured using a digital pH meter (Systronics) as the initial and final pH (Table 3) following standard technique. For the initial pH, 10 g of pre-wetted substrate (priorly soaked 100 g dried sample in plain water overnight) was dissolved in 100 ml distilled water. For the final pH, similar method was followed by taking 10g each after substrate supplementation, before inoculation with mushroom spawn. For accuracy, 3 separate readings were taken from fresh samples for each substrate type.

(f) Substrate Inoculation and Incubation

Spawning of freshly prepared substrates was done @3% on wet weight basis by layering method (Royse, 2003). Filling of spawned substrates was done in fresh, pre-punched clear polypropylene food grade bags @1 kg substrate on wet weight basis. Filling was done in four layers, the first layer being the thinnest and the final layer being on the top above the substrate for maximum colonization. For each

bag, 30 g freshly prepared, matured whole wheat grain spawn was inoculated with the ready substrate. Inoculated bags were then incubated in total darkness in closed heaps covered with dark tarpaulin sheets at room temperature for substrate colonization of by the actively proliferating mushroom mycelia.

Complete mycelial run (spawn running) was estimated by observing the degree of the thick whitening mat of colonizing vegetative mycelia all over the substrate. The total time taken for a complete colonization of substrate for each treatment was recorded by observing the degree of mycelial colonization in 5 days interval from date of spawning till complete colonization occurred (Table 1).

3. Results

2.1. Days taken for Spawn Running, Pinhead formation and Crop duration

Comparison of data for spawn run (the days taken for complete substrate colonization), pinhead formation (appearance of fruit primordial) and crop duration for three cropping cycles, viz: November, March & January have been presented in Table 1 and 2. From Table 1, it was observed that complete colonization occurred with a difference of 1-2 days among all the species of *Pleurotus* taking 17-19 days in November and while it took 19 days in March for all the species of *Pleurotus*. During January, the complete substrate colonization was much delayed in comparison to the two other cultivation seasons taking 21 days for all the species of *Pleurotus*. Pinhead formation by all species occurred on 2nd day in November but it took 3 days in March and 2-3 days in January. The cropping duration for various species was shortest in March and longest in January taking 13-18 days and 18-29 days. In November, it was between 18-23 days. There was no definite pattern of variation among the species except that the cropping period for *P. citrinopileatus* was slightly longer.

2.2. Yield of four summer species on PS+MP in outdoor condition during different seasons

Four summer species of oyster mushrooms namely, *P. sajor-caju*, *P. sapidus*, *P. citrinopileatus* and *P. flabellatus* were grown on PS+MP in three different months, viz: November, January and March (Figure 2). The BE value for each month was analyzed separately by 1-way ANOVA and the effect of *Pleurotus* species on BE was found significant only in the month of November and January ($p<0.005$) but not in March.

(i) Biological Efficiency (BE): Comparison of biological efficiency of all species of *Pleurotus* on PS and PS+MP by t-test showed that the latter is a better substrate than the former ($p<0.001$). The mean BE value for all species on PS+MP was 103.1% which was significantly higher than that on untreated PS (94.2%). *P. flabellatus* and *P. sapidus* gave significantly higher BE in November on PS+MP than other two species which were on par with each other (Table 3). *P. flabellatus* gave highest BE (118.7%) followed by *P. sapidus* (116.4%), *P. sajor-caju* (108.2%) and *P. citrinopileatus* (102.6%). BE in January month was significantly not different in comparison to November ($p>0.05$). In January, BE of *P. sajor-caju*, *P. flabellatus* and *P. sapidus* on PS+MP was 114.0%, 108.6% and 106.4% respectively. *However, P. citrinopileatus* could give BE of 90.7% only which was significantly less than the former three species of *Pleurotus* tested. Among the cultivation period, the BE in the month of March was significantly less in comparison to both November and January. And all species of *Pleurotus* gave statistically equal BE of 86.0-96.0% ($p>0.05$) in the month of March.

(ii) Mushroom Yield in First Flush: The first flush mushroom yield on substrate supplemented with *Mimosa* (PS+MP) during November, January and March showed a significant difference among the four summer species of *Pleurotus* tested. The difference in the mushroom yield in first flush was much more pronounced in the month of November ($P<0.001$) than in January and March ($p<0.05$).

The first flush yield of a species did not show any variation between November and January (Table 3). In November, *P. flabellatus* gave

Figure 1. (A) Outdoor Mushroom House; (B) *Mimosa pudica* L.; (C) Substrate preparation; (D) Ready substrate; (E) Whole grain spawn; (F) Mushroom Harvests.

significantly more yield than *P. sajor-caju* and *P. sapidus* that were on par with each other. The yield of *P. citrinopileatus* was significantly less than these species. In January also, the yield of *P. flabellatus* was relatively more than *P. sajor-caju* and *P. sapidus* but not significantly different. The yield of *P. citrinopileatus* was also only slightly less than *P. sapidus* but not significantly different. The yield decreased in March for three species except *P. citrinopileatus* which gave first flush yield statistically equal to *P. sajor-caju* but significantly more than *P. sapidus* and *P. flabellatus*.

(iii) Average Weight of Fruit Body: Variation in average fruit body weight of four summer species was significant only in January and March but not in November. In November, *P. flabellatus* and *P. sajor-caju* had better size of fruit body than both *P. sapidus* and *P. citrinopileatus* (Table 3). In January, the average fruit body size of *P. flabellatus* was bigger than *P. sajor-caju* and *P. sapidus*. Next in the order was *P. citrinopileatus* whose fruit body size was not significantly different from *P. flabellatus* and *P. sapidus* and *P. sajor-caju*. In March, the average fruit body size of *P. citrinopileatus* and *P. sajor-caju* was similar and was significantly bigger than *P. flabellatus* and *P. sapidus*. The fruit body size of *P. citrinopileatus* in March was bigger than in November, but for *P. sapidus* and *P. flabellatus* it was smaller.

(iv) Weight of Quality Fruit Bodies: Size of the quality fruit bodies were significantly different in three different growing seasons, November, January and March. *P. flabellatus* produced large sized quality fruit bodies in November (14.0 g) which was significantly bigger than that of *P. sapidus* (12.4 g) (Table 3). The fruit body size of *P. sajor-caju* and *P. citrinopileatus* was 10.6 g each.

The size of the fruit bodies in January was smaller in comparison to November. The quality fruit body size of different species was also significantly different ($p<0.001$). *P. flabellatus* formed comparatively big sized quality fruit bodies (12.4 g) than *P. sajor-caju* (10.0 g) and

P. sapidus (9.6 g) that were not significantly different in terms of their fruit body size. The smallest quality fruit body among all was shown by *P. citrinopileatus* (8.8 g).

A significant difference among the species was absent in terms of number of fruit bodies formed in the month of ($p>0.05$). The size of the quality fruit bodies of four species in March showed significant difference ($p<0.001$). *P. flabellatus* and *P. sapidus* both formed bigger quality fruit bodies (11.2 and 11.0 g respectively) in comparison to *P. sajor-caju* and *P. citrinopileatus* (10.2 and 9.6 g respectively). The size difference in quality fruit bodies of *P. sajor-caju* and *P. citrinopileatus* was not significant.

4. Discussion

Since the basal substrate for growing mushroom has lower C:N ratio, supplementation with nitrogenous materials has been reported to enhance proliferation of mushroom mycelia on the substrate (Bisaria, 1997). The nitrogen content of *M. pudica* is almost equal to that of alfalfa as reported by Sreenath et al (2001). However, Nasrullah et al (2003), has estimated the crude protein content in *M. pudica* as 15.63% (~2.5% N). Application of the whole plant directly as sundried biomass of *M.pudica* as a nitrogen supplementing material in this study gave results showing increased biological efficiency of the proliferating mushroom mycelia on the substrate and enhanced mushroom yields over the ideal, untreated basal substrate (control), thus proving this perennial weed plant to be a good supplementing material for oyster mushroom cultivation. The studies on the effect of enhanced nitrogen by mixing the basal growing medium with biomass of *M. pudica* and *M.invisa* which resulted in increased crop yield and organic biomass has been documented by Barman et al (2005) and Barman et al (2007) respectively. Another study by Barua et al (2013) to evaluate the effect of compost prepared from biomass of *Mimosa invisa* showed significant results on the crop characters of Lentil (*Lens culinaris* L.) Similarly, the effect of *M.invisa* on the overall performance of plants resulted in increased growth rate, enhanced biomass production and improved soil organic matter (Mishra et al., 2011). These significant attributes of *Mimosa* plant and its parts resulting in enhanced effect on plant growth, biomass production and compost quality is an indicative to direct effect enhanced on the growth performance, enhanced biological efficiency and higher mushroom yields in this study. The fruiting body formation and enhanced mushroom yields are considered direct resultant of supplementing materials rich in C:N ratio, releasing better nitrogen and carbohydrate influx for the proliferating mushroom mycelia (Rai and Mohatarum, 2002). Also, the overall enhanced performance of oyster mushrooms in this study giving shorter crop duration and higher mushroom yield from substrates supplemented with *Mimosa pudica* due to its rich organic matter composition and C:N ratio (Curvetto et al., 2002; Isikhuemhen et al., 2009).

Therefore, the possibilities of increased nitrogen and other organic nutrients resulting in an enhanced biological efficiency of the actively proliferating mushroom mycelia on the basal substrate, giving subsequent higher mushroom yields in comparison to the unsupplemented basal substrate (control) can't be overlooked in this study. A proximate analysis of the plant biomass used in this study can be performed as supplemented substrate, unsupplemented substrate as well as the fruit bodies of mushrooms harvested to determine the effect of nitrogen composition alongwith several other lignocellulosic compounds, xyloses, glucoronic acids, amino acids and a host of organic compounds such a flavonoids, flavones, jasmonic acids, saponins etc. found on *M.pudica* that resulted in high biological efficiency and higher mushroom yields.

5. Conclusion

The present study indicates that supplementing the basal growing substrate paddy straw with dried biomass of weed herb, *Mimosa pudica* L. (Fabaceae) @3:1 (dry weight) has greatly enhanced the biological efficiency and the performance of four popular summer species of oyster mushroom yields, namely: *Pleurotus sajor-caju*, *P.sapidus*, *P. flabellatus* and *P. citrinopileatus* between cropping temperature range of 11 to 26°C and average relative humidity of 69 to 83% on three different growing seasons, viz: January (December-January), March (February-March) and November (October-November). This abundantly growing perennial weed herb having no significant economic importance can be utilized as organic nitrogen supplementing material for ensuring high yields in oyster mushroom cultivation.

Figure 2. Organic Nitrogen Supplementation of Paddy Straw (PS) with *Mimosa pudica* (MP). (A and B) *P. sajor-caju;* (C and D) *P. sapidus;* (E and F) *P. citrinopileatus* (G and H) *P. flabellatus*

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Conflict of interests

Authors have no conflict of interests for publication of this research work.

Author's contribution

Rajiv Kumar Singh conceptualized, designed and supervised the experiments of this research work. Tenya Rina carried out the experiments, recorded the data and observations, performed data analysis and prepared the manuscript.

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