

agricultural fields and floral waste was collected from different temples of Allahabad, the floral waste mainly contained of marigold flowers. After collection and removal of foreign particles the floral waste was spread in an open area to sundry for 20 to 30 days. After complete drying, the floral waste was autoclaved at 121°C, 15 psi for 20 minutes.

Hot water treatment of paddy straw

The paddy straw was completely dipped in water. The substrate was allowed to stay in water for overnight. After that excessive water was drained out. After draining, the paddy straw was again completely dipped in hot water (temperature 70°C to 80°C) for an hour. Then excess water was drained out.

Spawn preparation

Spawn is referred as the vegetative mycelium of the fungus, which is grown on cereal grains. Wheat grains were washed and then half boiled. After that water from wheat grains was drained out. This was followed by mixing of CaCO₃ and CaSO₄ in 3:1 ratio.

The wheat grains were now half filled in bottles and plugged by cotton. The half-filled bottles were autoclaved at the temperature 121°C and pressure 15 psi for 30 minutes then left for overnight followed by inoculation of bottles with *P. sajor-caju* from cultured plate. Then bottles were incubated in BOD incubator at temperature 25 ± 2°C. After 3-4 days of inoculation fungal mycelium started spreading on the grains. The mycelium is white net web like in appearance. The bottles were nearly half filled in 10-12 days and in 18-21 days these were completely filled with white mycelial growth.

Spawning

Process of mixing spawn in the sterilized substrates is known as spawning. Treated paddy straw and sterilized flower waste were mixed well and then 5% wet weight basis spawn grain was mixed to these substrates and filled to polythene bags. The mouth of the bags was tied with rubber band and holes were made to drain out extra water and for proper aeration. Different percentage of paddy straw and flower waste was used for the preparation of mushroom bags.

Nutritional analysis

The nutritional analysis of mushroom fruit bodies were done after drying the mushroom samples taken separately from each bag in hot oven at a constant temperature of 60°C to constant weight, ground in a blender and kept under refrigeration at 4°C for further analysis. Nutritional analysis of all the different flushes was carried out separately.

Biological efficiency

Three bags for each combination of *P. sajor-caju* were kept for evaluation of yield performance and biological efficiency in mushroom house under *in vivo* condition. The yield was expressed as of fresh fruit bodies produced per bag. Biological efficiency (B.E.) was calculated as the percentage conversion of dry substrates to fresh fruit bodies [9] i.e.,

$$\text{Biological Efficiency} = \frac{\text{Fresh weight of mushrooms per bag}}{\text{Dry weight of substrate per bag}} \times 100$$

Moisture content

It was done by picking fresh fruit body of the *P. sajor-caju* and dried in hot air oven at 105°C to a constant weight.

$$\text{Moisture content} = \frac{\text{Fresh weight of mushroom} - \text{Dry weight of mushroom}}{\text{Fresh weight of mushroom}} \times 100$$

Ash determination

It was done by keeping the samples at 500°C for 3-4 hours.

$$\text{Ash content} = \frac{\text{Weight of ash}}{\text{Weight of sample taken}} \times 100$$

Total sugar, reducing sugar, total protein, total lipid and crude fiber estimation

Total sugar estimation was done by using phenol sulphuric acid method [10] and the reducing sugar estimation was done by Dinitrosalicylic acid method. Protein estimation was done by Lowry et al. method [11] and total lipid was determined by the method of Folch et al. [12]. Crude fiber was determined by following the method of Alam et al. [5].

Total cholesterol and total triglycerides

Total cholesterol and total triglyceride was estimated by using kit (Autospan Liquid Gold. For cholesterol- CHOD-PAP Enzymatic end point assay and for triglyceride- GPO-PAP End point assay).

Statistical Analysis

All the measurements in this study were conducted on three replicates samples. The results are expressed as mean values and standard error (SEM). The results were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test (P<0.05). This treatment was carried out using the SPSS v.16.0 program.

Results and Discussion

Effect of flower waste on mushroom production

The edible mushroom *Pleurotus sajor-caju* was grown on paddy straw alone and in different percentage of paddy straw (PS) and flower waste (FW). Mycelia growth rate, time for complete substrate colonization and time from inoculation to the formation of the fruiting bodies were considerably different among *P. sajor-caju* groups cultivated on different substrate mixtures. 100% PS produced mushroom upto 4th flush but 80% PS + 20% FW and 60% PS + 40% FW produced only upto 3rd and 2nd flush, respectively. The probable reason for this may be, as the percentage of floral waste was increased the percentages of amorphous lignocellulosic materials were more than crystalline one. However, in 100% paddy straw, crystalline forms are more than the amorphous form. Mushroom mycelia first utilize amorphous form of lignocellulosic substrate.

Table 1 shows the yield, biological efficiency and moisture content and Figure 4 shows the biological efficiency and moisture content of *P. sajor-caju* cultivated on different percentage of floral and agro-wastes. The highest yield was obtained for 100% PS mushroom with 233.42 g per bag, followed by 80% PS + 20% FW and 60% PS + 40% FW, with 162.10 g per bag and 141.19 g per bag, respectively. Accordingly, the highest biological efficiency was for 100% PS mushroom followed by 80% PS + 20% FW and 60% PS + 40% FW, with 93.37%, 64.84% and 56.47%, respectively. 100% PS mushroom had the highest moisture content, followed by 80% PS + 20% FW and 60% PS + 40% FW, with 84.17%, 83.50% and 78.47% respectively. This moisture variability is dependent on the parameters such as environmental temperature, relative humidity during growth and relative amount of metabolic water that may be produced or utilized during storage [13].

Nutritional attributes

Table 2 shows the protein content, total sugar, reducing sugar, non-reducing sugar, lipid and energy of mushroom of different percentages

S. No	Substrates	Total Yield (g/bag)	Biological efficiency (%)	Moisture Content (%)
1	100% PS	233.42 ± 3.37 ^a	93.37 ± 1.35 ^a	84.16 ± 2.11 ^a
2	80% PS+20% FW	162.10 ± 4.04 ^b	64.84 ± 1.62 ^b	83.50 ± 1.05 ^a
3	60% PS+40% FW	141.19 ± 3.20 ^c	56.47 ± 1.28 ^c	78.46 ± 0.88 ^b

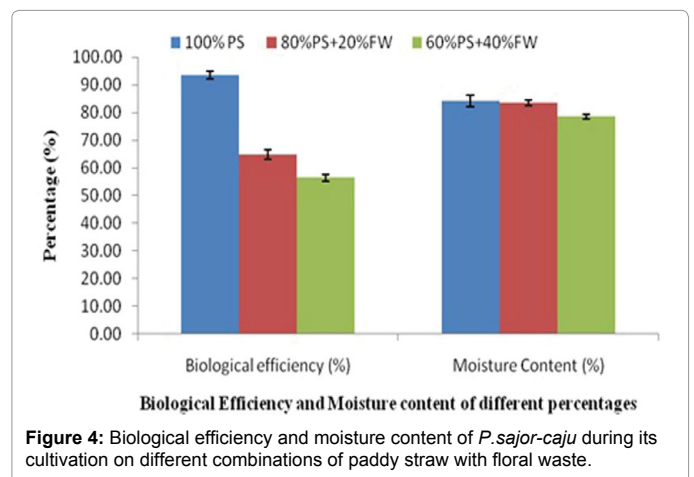
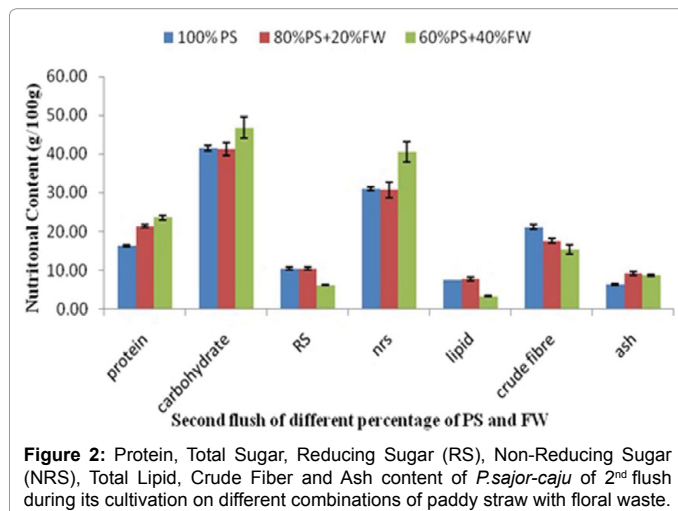
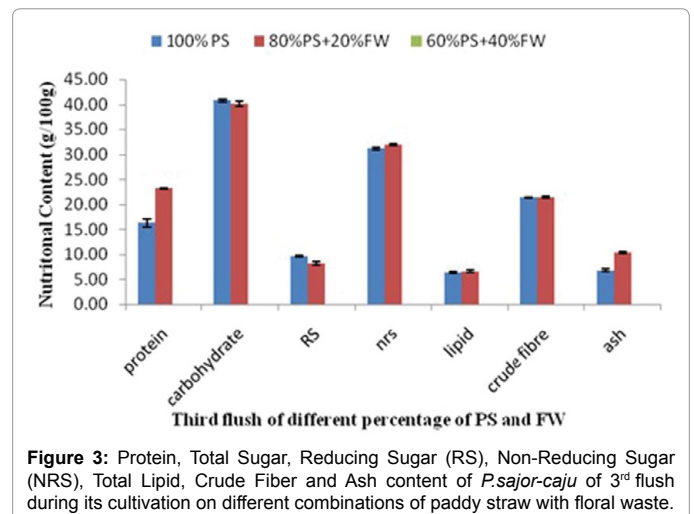
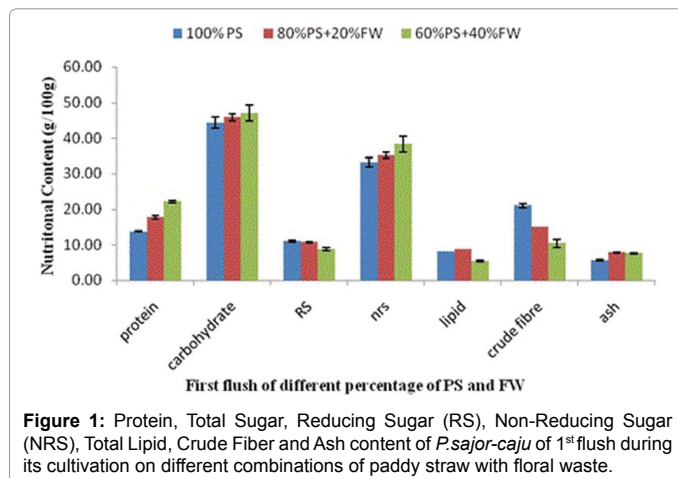
Results show mean ± SEM of 3 trials.
Values in the same column that do not share a common superscript are significantly different at P<0.05 (One-way ANOVA, then Duncan post hoc Comparison).

Table 1: Yield, biological efficiency and moisture content of *Pleurotus sajor-caju* cultivated on different combinations of floral and agro-waste.

Substrates	Flushes	Protein	TS	RS	NRS	Lipid	Energy
		(g/100g)	(g/100g)	(g/100g)	(g/100g)	(g/100g)	(kcal/100g)
100% PS	I st	13.76 ± 0.12 ^c	44.27 ± 1.56 ^b	11.07 ± 0.21 ^a	33.19 ± 1.42 ^c	8.02 ± 0.01 ^b	304.35 ± 6.68 ^b
80% PS+20% FW		17.76 ± 0.43 ^b	45.82 ± 0.95 ^a	10.68 ± 0.07 ^a	35.14 ± 0.88 ^b	8.75 ± 0.01 ^a	333.13 ± 5.46 ^a
60% PS+40% FW		22.13 ± 0.41 ^a	47.05 ± 2.21 ^b	8.77 ± 0.45 ^b	38.28 ± 2.28 ^a	5.46 ± 0.17 ^c	325.88 ± 6.38 ^a
100% PS	II nd	16.26 ± 0.19 ^c	41.53 ± 0.70 ^a	10.47 ± 0.38 ^a	31.03 ± 0.38 ^a	7.54 ± 0.09 ^a	298.94 ± 3.59 ^a
80% PS+20% FW		21.4 ± 0.35 ^b	41.27 ± 1.63 ^a	10.52 ± 0.43 ^a	30.74 ± 2.04 ^a	7.77 ± 0.45 ^a	320.64 ± 4.26 ^a
60% PS+40% FW		23.63 ± 0.58 ^a	46.81 ± 2.64 ^a	6.23 ± 0.11 ^b	40.58 ± 2.62 ^a	3.31 ± 0.07 ^b	311.64 ± 9.56 ^a
100% PS	III rd	16.3 ± 0.85	40.77 ± 0.30	9.62 ± 0.21	31.14 ± 0.21	6.40 ± 0.20	285.91 ± 6.01
80% PS+20% FW		23.16 ± 0.13	40.12 ± 0.49	8.15 ± 0.40	31.96 ± 0.27	6.57 ± 0.29	312.28 ± 4.19
100% PS	IV th	16.53 ± 0.27	40.76 ± 0.21	7.4 ± 0.18	33.36 ± 0.16	6.27 ± 0.27	285.61 ± 3.85

PS: Paddy Straw, FW: Flower Waste, TS: Total Sugar, RS: Reducing Sugar, NRS: Non-reducing Sugar
Results show mean ± SEM of 3 trials.
Values in the same column (in flushes) that do not share a common superscript are significantly different at P<0.05 (One way ANOVA then Duncan post hoc comparison) (In IIIrd and IVth flush post hoc cannot be applied because only two or one groups was to be compared).

Table 2: Nutritional content of *Pleurotus sajor-caju* cultivated on different combinations of floral and agro-waste (in different flushes).



in different flushes. Figures 1-3 show the protein, total sugar, reducing sugar, non-reducing sugar, total lipid, crude fiber and ash content of *P. sajor-caju* of 1st, 2nd and 3rd flush during its cultivation on different combinations of paddy straw with floral waste.

Protein content: The protein content in 100 g of dry *P. sajor-caju* on 100% PS increased with flushes, with protein content in 1st flush (13.76 g), 2nd flush (16.26 g), 3rd flush (16.3 g) and 4th flush (16.53 g). In 80% PS + 20% FW and 60% PS + 40% FW also, the protein content increased with flushes. In 80% PS + 20% FW, 1st flush (17.76 g), 2nd flush (21.4 g) and 3rd flush (23.16 g) and in 60% PS + 40% FW, 1st flush (22.13 g) and 2nd flush (23.63 g). Contrary to the earlier report, the protein content was increasing, the probable reason may be that some micro-organism was responsible for atmospheric nitrogen fixation and as the nitrogen was being fixed the protein content increased. However, the protein content when compared among the different groups of percentage, in different flushes it was observed that it increased with increasing percentage of flower waste. This can lead to conclusion that flower waste helped in enhancing the protein content in our mushroom. Similar variation in the crude protein content of *P. sajor-caju* cultivated on paddy straw, cotton waste, their mixture and cotton waste plus tea leaves has been observed [9].

Total sugar content: The total sugar content in 100 g of dry *P. sajor-caju* on all the three percentages i.e., 100% PS, 80% PS + 20% FW and 60% PS + 40% FW decreased with flushes, with total sugar content in 100% PS, 1st flush (44.27 g), 2nd flush (41.53 g), 3rd flush (40.77 g) and 4th flush (40.76 g). In 80% PS + 20% FW, 1st flush (45.82 g), 2nd flush (41.27 g) and 3rd flush (40.12 g) and in 60% PS + 40% FW, 1st flush (47.05 g) and 2nd flush (46.81 g). Earlier it was reported that amount of total carbohydrate in *P. sajor-caju* ranges from 39.0 to 50.7 g/100g of dry matter [9]. However, the total sugar concentration when compared among the different groups of percentage, in different flushes it was observed that it increased.

Reducing and non-reducing sugar content: The reducing sugar and non-reducing sugar content in 100 g of dry *P. sajor-caju* on 100% PS decreased with flushes, with reducing sugar content in 1st flush (11.07 g), 2nd flush (10.47 g), 3rd flush (9.62 g) and 4th flush (7.4 g) and non-reducing 1st flush (33.19 g), 2nd flush (31.03 g), 3rd flush (31.14 g) and 4th flush (33.36 g). In 80% PS + 20% FW and 60% PS + 40% FW also, the same result was observed, with reducing sugar content in 80% PS + 20% FW 1st flush (10.68 g), 2nd flush (10.52 g) and 3rd flush (8.15 g) and non-reducing 1st flush (35.14 g), 2nd flush (30.74 g) and 3rd flush (31.96 g). While in 60% PS + 40% FW, reducing sugar content was, 1st flush (8.77 g) and 2nd flush (6.23 g) and non-reducing, 1st flush (38.28 g) and 2nd flush (40.58 g). However, the reducing sugar content when compared among the different groups of percentage, in different flushes

it was observed that it decreased while opposite results were observed for non-reducing.

Total lipid content: The total lipid content in 100 g of dry *P. sajor-caju* on 100% PS decreased with flushes, with total lipid content in 1st flush (8.02 g), 2nd flush (7.54 g), 3rd flush (6.40 g) and 4th flush (6.27 g). In 80% PS + 20% FW and 60% PS + 40% FW, also the total lipid content decreased with flushes. In 80% PS + 20% FW, 1st flush (8.75 g), 2nd flush (7.77 g) and 3rd flush (6.57 g) and in 60% PS + 40% FW, 1st flush (5.46 g) and 2nd flush (3.31 g). The total lipid content was found to be higher than earlier reported which range from 4.3 to 4.9 g/100g of dry matter [14]. However, the total lipid concentration when compared between 100% PS and 80% PS + 20% FW, in different flushes it was observed that it increased. And when 60% PS + 40% FW were compared with 100% PS and 80% PS + 20% FW, in different flushes it was observed that it decreased.

Energy value: The energy value in 100 g of dry *P. sajor-caju* on all the three percentages i.e., 100% PS, 80% PS + 20% FW and 60% PS + 40% FW decreased with flushes, with energy value in 100% PS, 1st flush (304.35 kcal), 2nd flush (298.94 kcal), 3rd flush (285.91 kcal) and 4th flush (285.61 kcal). In 80% PS + 20% FW, 1st flush (333.13 kcal), 2nd flush (320.64 kcal) and 3rd flush (312.28 kcal) and in 60% PS + 40% FW, 1st flush (325.88 kcal) and 2nd flush (311.64 kcal). It has observed earlier that energy value of *P. sajor-caju* ranges from 272 to 316 kcal/100g dry matter [15]. However, the energy value when compared between 100% PS and 80% PS + 20% FW, in different flushes it was observed that it increased. And when 60% PS + 40% FW was compared with 100% PS, in different flushes it was observed that it increased, while decreased when compared with 80% PS + 20% FW.

Cholesterol content: Table 3 shows the cholesterol, triglyceride, ash and crude fiber content. The cholesterol content of dry *P. sajor-caju* on 100% PS increased with flushes, with cholesterol content in 1st flush (1259.27 mg/dl), 2nd flush (1427.11 mg/dl), 3rd flush (1449.19 mg/dl) and 4th flush (1501.14 mg/dl). While in 80% PS + 20% FW and 60% PS + 40% FW, the cholesterol content decreased with flushes, in 80% PS + 20% FW 1st flush (1559.18 mg/dl), 2nd flush (1476.11 mg/dl) and 3rd flush (1370.31 mg/dl) and in 60% PS + 40% FW, 1st flush (1410.08 mg/dl) and 2nd flush (1201.04 mg/dl). However, the cholesterol content when compared between 100% PS and 80% PS + 20% FW, in different flushes it was observed that it increased except in 3rd flush where it decreased. And when 60% PS + 40% FW was compared with 100% PS and 80% PS + 20% FW, in different flushes it was observed that in 1st flush it was more than 100% PS but less than 80% PS + 20% FW, while in 2nd flush it was less than both the percentages.

Triglyceride content: The triglyceride content of dry *P. sajor-*

Substrates	Flushes	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Ash (g/100g)	Crude Fiber (g/100g)
100% PS	I st	1259.27 ± 0.64 ^c	60.13 ± 0.59 ^c	5.70 ± 0.17 ^c	21.04 ± 0.59 ^a
80% PS+20% FW		1559.18 ± 0.60 ^a	211.29 ± 0.65 ^a	7.80 ± 0.12 ^a	15.02 ± 0.02 ^b
60% PS+40% FW		1410.08 ± 0.58 ^b	150.16 ± 0.60 ^b	7.51 ± 0.05 ^b	10.42 ± 1.15 ^c
100% PS	II nd	1427.11 ± 0.59 ^b	92.02 ± 0.58 ^c	6.33 ± 0.20 ^c	21.15 ± 0.58 ^a
80% PS+20% FW		1476.11 ± 0.59 ^a	170.09 ± 0.66 ^b	9.13 ± 0.41 ^a	17.66 ± 0.58 ^b
60% PS+40% FW		1201.04 ± 0.58 ^c	314.28 ± 0.64 ^a	8.70 ± 0.17 ^b	15.38 ± 1.16 ^b
100% PS	III rd	1449.19 ± 0.61	140.19 ± 0.61	6.80 ± 0.23	21.37 ± 0.14
80% PS+20% FW		1370.31 ± 0.66	130.23 ± 0.62	10.33 ± 0.22	21.40 ± 0.23
100% PS	IV th	1501.14 ± 0.59	776.08 ± 0.58	8.00 ± 0.29	21.41 ± 1.15

Results show mean ± SEM of 3 trials.

Values in the same column that do not share a common superscript are significantly different at P<0.05 (One way ANOVA then Duncan post hoc. comparison) (In IIIrd and IVth flush post hoc cannot be applied because only two or one groups was to be compared).

Table 3: Nutritional content of *Pleurotus sajor-caju* cultivated on different combinations of floral and agro-waste (in different flushes).

