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SUPPLEMENTATION OF RICE STRAW WITH VARIOUS NITROGEN SOURCES TO IMPROVE THE YIELD OF *PLEUROTUS SAJOR*-CAJU

TASNIM KAUSAR AND S. H. IQBAL*

PCSIR Laboratories Complex, Lahore-54600, Pakistan

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Pleurotus sajor-caju was cultivated on rice straw. Supplementation of rice straw with different pulse (2% w/w), inorganic and organic sources (0.92% Nitrogen level) increased the yield from 72.3 to 83.5% maximum being on addition of 'Lobia' *(Vigna cajang)* followed by corn gluten meal (79.7%). Supplementation of rice straw with gram, black gram, red gram and french beans showed almost non-significant stimulatory effect whereas ammonium sulphate, ammonium nitrate, urea and mustard seed meal retard the growth of *P. sajor*-caju.

Key words: Pleurotus, Yield, Nitrogen sources, Fructification.

Introduction

Cultivation of mushrooms is widely practiced in Europe, North America, Taiwan, China and Japan. The oyster mushrooms (*Pleurotus* spp.), the best converters of straws into useful products, have been cultivated in large quantities in Japan for several years. Commercial production of mushrooms has increased dramatically during the past few years in Europe, Asia and the United States. Today *Pleurotus ostreatus* is the second most important commercially grown mushroom in Europe exceeded only by button mushroom, *Agaricus bisporous* [1,2].

Thirty six million meteric tonnes of crop residues are currently available in Pakistan [3], only a small portion of these residues is being used by industry or as ruminant feed whereas, a sizeable chunk is creating pollution and disposable problems. These wastes can be utilized for growing mushrooms which have great prospects as cottage industry in Pakistan. Mushrooms are nature's recyclers which have the capability to convert agricultural and industrial wastes into good quality protein rich food. If even half the quantity of wheat and paddy straws available in Pakistan is used as bedding material for cultivation of oyster mushrooms, it would yield 2.13 million metric tonnes of mushrooms. Thus, it will also bring a substantial amount of valuable foreign exchange annually [4]. Kausar *et al.,* [5, 6] have reported the effect of temperature, quality and quantity of substrate i.e. rice straw and rice husk on the growth and production of oyster mushrooms.

This present investigation have been carried out to see the effect of nitrogen sources on the yield of *Pleurotus sajor*-caju using rice straw as a substrate.

* Botany Department, Punjab University, Lahore, Pakistan.

Materials and Methods

Culture maintenance. The stock cultures of *Pleurotus sajor*-caju were maintained on standard malt extract agar medium in test tube slants at 3-5°C. The inoculum was grown on standard malt extract agar medium in 100 mm petri-plates. The petri-plates were dried in an oven at 105°C and sterillized in petri plate container by keeping at 160°C for 6 hr. The malt extract medium previously sterilized at 15 lbs for 15 min, was poured aseptically into petri plates. After cooling, each petri plate was aseptically inoculated with mycelial plugs (1-2 mm) of *Pleurotus sajor*-caju from the stock culture. The petri plates were incubated at 25°C and culture was allowed to develop for 7-10 days.

Spawn preparation. Spawn was prepared on grains of gram, rye, millet, sorghum or wheat. The grains were softened by soaking in boilling water for 15 min. The soft grains were sterilized in wide mouthed, cotton plugged bottles and subcultured by aseptically transferring small mycelial plugs (6 mm diameter). The bottles were placed in an incubator at 25 ± 0.5°C for 7-10 days.

Pasteurization of raw material. Rice straw was chopped into small pieces of 3-5 cm length and used as substrate. The substrate was soaked in boiling water. The excess water was allowed to drain off, so that moisture content of the substrate was nearly 80%.

Preparation of bed. A known quantity (1.5 kg) of pasteurized substrate was transferred while hot into wooden trays (52 x 38 x 10 cm) having two vertical growing surfaces. These trays were wrapped with polythene sheet.

Spawning and ramification. The bed was unwrapped when the temperature of the bed became nearly 25°C, 5% spawn of *Pleurotus* (w/w basis) was spread in two layers. The bed was wrapped with polythene sheet again and mycellium was allowed to ramify the substrate in spawn running room under controlled temperature of 30°C. The relative humidity (RH) of the room was maintained between 70-85% with the help of a humidifier (Defensor AG Zurich Model 505, switzerland.

Fructification and harvest. The bed was uncovered after 21 days and temperature was brought down to 10-15°C for fructification. In a week time pinheads appeared which later turned into full oyster shaped mushrooms. The full sized mushrooms were cut with the help of a sharp knife after interval of 8-10 days. The weight of different crops was added to get the total yield of the crop. The yield (%) of the crop was calculated as follows:

$$\text{Yield (\%)} = \frac{\text{Total weight of different flushes} \times 100}{\text{Weight of rice straw}}$$

Addition of nitrogen sources. Various pulses i.e. gram *(Cicer arietinum)* Green gram *(Phaseolus mungo).* Red gram *(Cajanus indicus),* french beans *(Phaseolus vulgaris),* Lobia *(Vigna cajang)* were supplemented to the rice straw at the rate of 2% w/w basis. Corn gluten meal, leaf protein concentrate, mustard seed meal, amongst the natural and ammonium sulphate, ammonium nitrate and urea as synthetic sources in an equivalent amount (0.92 N, w/w basis) as described in Table 1, were mixed with chopped rice straw before spawning.

Results and Discussion

The preliminary investigation by Kausar *et al.* [6] indicated that *P. florida, P. sajor*-caju showed maximum mycelial growth at 30°C whereas a temperature of 25°C was found optimum for *P. sapidus* and *P. ostreatus.* All *Pleurotus* species showed higher yield (51.5 to 72.3% on fresh mushroom/dry substrate basis) on rice straw than rice husk i.e. 14.7 to 23.9%. Maximum yield of *Pleurotus* spp. was observed when 1.5 kg of chopped rice straw was taken per bed for 52 cm x 38 cm x 100 cm.

Nitrogen has an indispensible role as a functional and structural element. The effect of supplementing rice straw with nitrogen source i.e. different pulses, on the yield of *P. sajor*-caju after 52 days is shown in Table 2. The yield of *P. sajor*-caju varied from 72.3 to 83.5% minimum being in control and maximum when rice straw was supplemented with 'Lobia'. Addition of green gram showed 78.3% yield whereas gram, black gram, red gram and french beans showed almost non-significant stimulatory effect on the yield (73.9 to 74.8%) of *P. sajor*-caju. The results are comparatively better than those reported by other researchers [7-10] that supplementation of different substrates with rice bean, oat meal, alfa alfa flour, rape and soya straws and cotton seed hulls improved the yield of *Pleurotus* species.

The effect of supplementing rice straw with three synthetic and three natural nitrogen sources in equivalent amount is shown in Table 3. The growth of mycelium was highly increased with all the additives except leaf protein concentrates, urea and mustard seed meal. But the yield product i.e. mushroom sporophore, was increased only with green gram, Lobia and corn gluten meal. The yield of *P. sajor*-caju varied from 18.6 to 79.7%, minimum being in case of ammonium sulphate and maximum with corn gluten meal. Addition of ammonium sulphate and ammonium nitrate decreased the yield from 72.3% (Control) to 18.6 and 25.5% respectively whereas leaf protein concentrate, gram, black gram, red gram and french bean showed non-significant effect. Supplementation with urea completely inhibited the mycelial growth whereas rudimentary, dark coloured sporophores of *P. sajor*-caju did appear on addition of mustard seed but were unfit for harvesting. The decrease in the yield on addition of ammonium sulphate, ammonium nitrogen and urea appeared to be due to the change of pH by release of ammonia which inhibited the growth of *P. sajor*-caju. The under developed sporophore formation of *P. sajor*-caju after supplementation with mustard seed meal could be attributed to the presence of toxic factor producing glucosinolates [11] in the meal which inhibited its growth.

The highest yield (83.5%) of *P. sajor*-caju by the addition of Lobia *(Vigna cajang)* followed by corn gluten meal (79.7%) seemed to be due to an increase in the availability of digestible nitrogen present in these sources. The proximate analysis of

TABLE 1. SUPPLEMENTATION OF RICE STRAW WITH NITROGEN SOURCES.

Nitrogen source	Amount added % (w/w)
Natural	
Gram ver-channa *(Cicer arietinum)*	2.0
Black gram ver. mash *(Phaseolus radiatus)*	2.0
Green gram ver. mungo *(Phaseolus mungo)*	2.0
Red gram ver. arhar *(Cajanus indicus)*	2.0
French bean ver. moth *(Phaseolus vulgaris)*	2.0
Lobia ver. rawan *(Vigna cajang)*	2.0
Corn gluten meal*	7.6
Leaf protein concentrate*	14.4
mustard seed meal*	15.0
Synthetic	
Ammonium sulphate*	4.4
Ammonium nitrate*	5.5
Urea*	2.0

*Equivalent to 0.92% nitrogen, determined by AOAC method [12].

TABLE 2. EFFECT OF DIFFERENT PULSES ON THE YIELD OF *P. SAJOR*-CAJU.

Name of the pulses added (2% w/w)	Completion of mycelial growth (days)	Pin head formation (days)	Yield* (g)/tray				Yield** (%)
			F_1	F_2	F_3	Cumulative weight (g)	
Control	23	33	750	230	105 (52)	1085	72.3
Gram (*Cicer arietinum*)	26	32	598	358	156 (51)	1112	74.1
Black gram (*Phaseolus radiatus*)	28	34	636	335	145 (53)	1116	74.4
Green gram (*Phaseolus mungo*)	27	34	875	222	78 (52)	1175	78.3
Red gram (*Cajanus indicus*)	27	34	571	376	162 (53)	1109	73.9
French bean (*Phaseolus vulgaris*)	25	31	632	348	142 (52)	1122	74.8
Lobia (Red) (*Vigna cajang*)	25	31	956	206	90 (51)	1252	83.5

*Average of four replicates **$\frac{\text{Total weight of different flushes X 100}}{\text{Weight of rice straw}}$. F= Flushes - F_1, F_2 & F_3 mean different cropping stage.

- Number in the paranthesis indicates days taken for harvesting three flushes.

TABLE 3. EFFECT OF DIFFERENT NITROGEN SOURCES ON THE YIELD OF *P. SAJOR*-CAJU.

Nitrogen source	Completion of mycelial growth (days)	Pin formation (days)	Yield* (g)/tray				Yield** (%)
			F_1	F_2	F_3	Cumulative weight (g)	
Control	21	31	750	230	105 (52)	1085	72.3
Ammonium sulphate	25	51	234	45 (89)	-	279	18.6
Ammonium nitrate	24	48	287	102 (75)	-	389	25.9
Urea	47[a]	-	-			-	-
Corn gluten meal	19	33	784	271	140 (55)	1195	79.7
Leaf protein concentrate	19	34	735	275	98 (58)	1108	73.8
Mustard seed meal	21[b]	35	C			-	

*Average of four replicates. ** Percent of dry substrate. F= Flushes - F_1, F_2, F_3. a = 6 sq. inch mycelial growth. b = 4 sq. inch mycelial growth. c = Rudimentary, dark coloured mushrooms, unfit for harvesting.

- Number in the paranthesis indicates days taken for harvesting three flushes.

oyster mushroom showed that it contained 30.5% crude protein, 1.8% fat, 11.3% crude fibre and 12.2% ash on dry matter basis.

The present investigations showed that *pleurotus sajor-caju*, a delicate and protein rich oyster mushroom can be successfully grown on agro-industrial wastes. This would help to produce a large quantity of unconventional protein source at low rate so that those who need protein can get it at reasonable price.

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