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Bio-efficacy of agro-industrial wastes treated with neem leaf extract on the growth and yield of *Pleurotus sajor-caju*

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ABSTRACT

This research work was carried out to use locally available agro-industrial wastes such as wheat straw and maize stover as substrates for cultivation of *Pleurotus sajor-caju* and to check the efficacy of neem leaf extract as bio-control agent for oyster Mushroom cultivation. In the present work for cultivation *P. sajorcaju,* spawn run was completed after 19-25 days of inoculation and pinheads were formed 2-3 days after the spawn running that is 22-27 days after inoculation. The fruiting bodies appeared 5-7 day after pinhead formation and took 27-34 days after spawning. The total cropping duration of 82 days was recorded for *P. sajor-caju* from treatment T_5 (wheat straw + maize stover + NE as substrate). Maximum average yield 4.4 kg/kg dry weight of substrate was obtained from the treatment T_5 (wheat straw + maize stover + NE as substrate) in which neem leaf extract treatments showed increased yield with highest biological efficiency of 89.6%. Neem leaf extract treatments showed decrease in incidence of mycopathogenic infection in mushroom beds. The present work focuses on the exploration of locally available agro-industrial wastes for mushroom bed preparation and introduction of neem leaf extract treatment during the course of oyster mushroom cultivation as it exerts antimicrobial activity that inhibited various fungal pathogens in mushroom compost.

Keywords: Maize stover, neem leaf extract, oyster mushrooms, substrate, wheat straw

Oyster mushrooms also called by name dhingri mushrooms are the trendiest among all edible mushrooms and belongs to the genus *Pleurotus* and the family *Pleurotaceae*. It is on the third rank on the basis of acceptance in the world and holds second position in India (Sharma *et al.,* 2017). *Pleurotus* species like *P. ostreatus, P. sajor-caju, P. pulmonarius, P. eryngii, P. cornucopiae, P. tuber-regium, P. citrinopileatus* and *P. flabellatus* are commercially very important, found all over the world (Barh *et al.,* 2019). High nutritional value and ability to grow on diverse agricultural wastes, made oyster mushroom very popular in the recent years. Consuming this delicious dhingri mushrooms can solve the problems of malnutrition and disease as they are rich in proteins, minerals and vitamins (Caglarirmak, 2007; Gupta *et al.,* 2018). The cultivation of edible mushroom like dhingri is easy and employs cost-effective method for the bioconversion of agro-lignocellulose wastes. Therefore, in India, the cultivation of this mushroom has increased startlingly (Patel and Trivedi, 2013). It requires simple and inexpensive cultivation techniques (Chang and Miles, 2004) to produce a highly nutritious food and of high commercial value (Hamde and Solunke, 2013).

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The production technology offers one of the feasible ways to combat air pollution associated with burning agriculture wastes as well as to decrease environmental pollution due to unutilized agricultural wastes that would otherwise increase the pollution load of the earth (Patil, 2012). These white-rot fungi are useful decomposers of various agricultural wastes (Barh *et al.,* 2018). *Pleurotus* spp. are the most versatile group among the cultivated mushrooms, which have ability to degrade many lignocellulosic substrates and are capable to colonize successfully on different lignocellulosic substrates. Various lignocellulosic agricultural residues are being used as substrates for cultivation of dhingri mushrooms. Soybean straw, groundnut haulms or straw, wheat straw, leaves and stalks of pigeon pea, cotton stalks (Mane *et al.,* 2007; Hamde and Solunke, 2013), paddy straw, sunflower stalk (Patil, 2012), apple leaf and chinar leaf substrates (Pala *et al*., 2012), maize stalk, pea residue (tendrils) and banana leaves (Pokhrel *et al.,* 2013). Sugarcane bagasse, sun flower stalks, domestic waste, used tea leaves, fruit waste, semal flowers, newspaper, bamboo leaves, saw dust (Dehariya *et al.,* 2013) various grasses, weeds, reed stems, sorghum stover, coffee pulp and coffee husk, cottonseed and sunflower seed hulls, peanut shells, rice husks and wood chips (Mosisa, 2014; Kathiravan *et al*., 2016) are the left out agricultural wastes reported many a times to be used as substrates for the cultivation investigation of the yield performance of *Pleurotus* spp. These agricultural residues are rich in protein and carbohydrate therefore it provides carbon and nitrogen sources to mushrooms, and can be used alone or in combinations (1:1 proportion) to determine their effect on yield, growth and BE. Different substrates with different compositions like 50+50% of saw dust and wheat straw, 75+25% of saw dust and leaves and 50+50% of wheat straw+ paddy straw, paddy straw+ maize stover, maize stover+ sugarcane baggasse, sugarcane baggasse + wheat straw, wheat straw + maize stover, paddy straw + sugarcane baggasse (Toppo and Chandravanshi, 2018) have been utilized to grow mushrooms.

Another important factor that has deleterious effect on the cultivation of oyster mushrooms are fungal pathogens infection, plant extract have shown considerable promises as an effective alternatives for reducing the infection with these myco-pathogens and diseases of oyster mushroom (Biswas, 2015; 2016). With the aim to use regional lignocellulosic residues for bioconversion of agro-industrial wastes into protein rich food, wheat straw and maize stover were collected from different regions of Chhattisgarh, sterilized with conventional method and used neem leaves extract as bio-control agent to control competitive moulds.

MATERIALS AND METHODS

The present study deals with the diversification of some common and abundantly available agricultural wastes such as wheat straw and maize stover into protein rich food; *Pleurotus sajorcaju* and to check its yield potential in treated and untreated substrate with neem leaves extract.

Neem leaf extract preparation

The preparation of aqueous neem leaf extract was carried out according to the method as described by Biswas (2015; 2016). 100 gram neem leaves were collected, washed in tap water, air dried and homogenized with equal amount of distilled water (100 ml) by crashing them with mixer grinder machine. The extract was filtered through double – layered muslin cloth and centrifuged at 4000 rpm, for 10 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper which was considered as standard solution.

Substrates collection and preparation

Agro-industrial wastes such as wheat straw and maize stover were used as substrate for the cultivation of *P. sajor-caju.* Wheat straw was collected from small holder farmers of Mainpat, Sarguja region of Chhattisgarh. Also maize stover was collected from small local vendors of Bilaspur city during rainy

season. The methodology for substrate preparation in this study includes the chopping of substrate to 2-3 cm. pieces. The substrate was soaked in water overnight amended with 10% neem leaves extract and washed with water thoroughly as suggested by Kathiravan *et al.* (2014). A total of 6 treatments were made (T1- wheat straw+ NE, T2-wheat straw, T3-maize stover+ NE, T4- maize stover, T5-wheat straw + maize stover + NE, T6- wheat straw + maize stover). Excess of water was drained and substrate was then steam sterilized at 121°C for 30 min. in an autoclave (Pala *et al.,* 2012; Patil, 2012). Treatment without neem leaves extract was taken as control (C) and treatments with wheat straw was taken as standard.

Mushroom culture collection and cultivation

The pure culture of *P. sajor-caju* was collected from Karuna Vikas Samiti (NGO) at Maharana Pratap Chowk Bilaspur, Chattisgarh. Spawn i.e. *Pleurotus sajor-caju* seeds were inoculated in the sterilized substrate by multi layered spawning technique. The polythene bag of the size 35x45cm was used for preparing substrate bed for mushroom cultivation. Each bag was filled with sterilized substrate on the basis of 500gm/bag. The mouth of the bag was then tied with rubber bands and holes were made to drain out extra water and for proper aeration and hanged in the mushroom cultivation room in dark for mycelium spreading. The temperature of mushroom cultivation room was maintained at 20-25°C and 80-90% relative humidity. When the substrate was completely covered by the white cottony mycelium of Mushroom fungus, the polythene bags were removed and white light was switched on (Patel and Tiwari, 2013). Observations on days for spawn run, appearance of pinhead stage and harvest were recorded up to three flushes. In two days of appearance of pin heads, the mushroom fruiting bodies were in its fullest growth and then first harvest was made. Successively second and third harvest was also made (Kathiravan *et al*., 2016). Fresh weights of mature fruit bodies were recorded up to third flush to calculate the total yield and corresponding biological efficiency. Total yield was calculated as the fresh weight of mushrooms harvested up to third flush per 500g of dry substrate used for its cultivation (Pala *et al.,* 2012). Total yield of mushroom was measured by taking total weight of all the fruiting bodies harvested from all the three replicates of each treatment. The biological efficiency (yield of mushroom per kg substrate on dry weight basis) was calculated by the following formula (Chang *et al.,* 1981; Patil, 2012)

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

RESULTS AND DISCUSSION

The results obtained from this research work are presented in table 1, 2 and 3. The spawn run, pinhead's formation and fruiting bodies formation are three important phases in the cultivation of mushroom require proper humidity and temperature. Temperature of 25°C for spawn running and 17-20°C for fructification and 80-85% humidity was maintained throughout the project. The time interval for the completion of spawn running, pinhead formation and fruiting body formation on different substrates was found to be more or less significant and evident from the table 1. Spawn run in all the substrates was

Table 1. Days for completion of spawn running and pinhead formation of *P. sajor-caju* on different substrates

S. No.	Substrate	Mean no. of days taken	
		Spawn run	Pinheads formation
1.	Wheat straw + NE	19.5	22.5
2.	Wheat straw	20.0	23.5
3.	Maize stover + NE	23.5	26.5
4.	Maize stover	23.5	26.0
5.	Wheat straw + maize stover + NE	23.0	26.5
6.	Wheat straw + maize stover	23.5	26.5

Table 2. Days for mushroom harvesting

S.N.	Treatments	Substrate	Mean No. of days taken			
			I Flush	II Flush	III Flush	IV Flush
1.	T1	Wheat straw + NE	27	38	67	74
2.	T2	Wheat straw	29	37	67	72
3.	T3	Maize stover + NE	36	45	67	
4.	T4	Maize stover	35	47	66	
5.	T5	Wheat straw+maize stover + NE	34	43	72	82
6.	T6	Wheat straw+maize stover	35	44	74	

completed in 19-25 days of inoculation. All substrates were inoculated at the same day. After spawn run, pinhead's formation is the second stage of mycelial growth during cultivation of mushroom. The pinheads were formed 2-3 days after the spawn run that is 22-27 days after inoculation. These results are agreement with the findings of Pala *et al.* (2012) who stated that *P. sajor-caju* pinhead's formation occurred in (paddy straw substrate) 17-19 days, (wheat straw) 22-24 days and apple leaf substrate (25-28days).

The third and final stage during the cultivation of mushroom is the formation of fruiting bodies. The fruiting bodies appeared 5-7 days after pinhead's formation and took 27-34 days later after inoculation of spawn. The time duration for the formation of fruiting bodies varies in different substrate, was found longer in case of chinar leaf substrate (47-49 days), followed by apple leaf substrate (42-44), wheat straw (32-34 days) and paddy straw substrate (25-27 days) as reported by Pala *et al.* (2012).

The present study reveals that total cropping duration of *P. sajor caju* was higher for T5 (82 days) followed by T1 (74days) and T2 (72days) as compared to the other treatments. First fructification occurred after 22-27 days of inoculation. The mushrooms were harvested at different intervals ranging from 8-12 days between 1st- 2nd flushes and cropping cycle lasts up to 4th flush in case of T1, T2 and T5. The mushroom was harvested at 11 days interval between 4-7 flushes was reported by Shah *et al.* (2004) whereas Bughio (2001) recorded 8.53 to 14.33 days between flushes.

Table 3. Yield performance of *P. sajor-caju* on different substrates with and without neem extract treatments

S.No.	Treatments	Substrate	Yield fresh weight of mushrooms (g)/ 500g dry substrate (Total of 3 flushes in each Replicates)					Total yield FW (g)	Avg. yield FW in (g)	B.E. (%)
			R1	R2	R3	R4	R5			
1.	T1	Wheat straw + NE	448	498	416	450	411	2223	444.6	88.92%
2.	T2	Wheat straw	406	414	425	401	412	2058	411.6	82.32%
3.	T3	Maize stover + NE	235	190	207	242	211	1085	217	43.4%
4.	T4	Maize stover	204	181	195	160	187	0927	185.4	37.08%
5.	T5	Wheat straw+Maize stover + NE	427	495	478	429	411	2240	448	89.6%
6.	T6	Wheat straw+Maize stover	346	404	390	302	230	1672	334.4	66.88%

Fig. 1. A1. Substrate preparation A2. Spawning by layering method A3. Spawn run A4. Pin head formation A5. Fruit body formation, A6-A7 Harvesting, B1 and B2 - Untreated bags with fungal pathogens (*Aspergillus, Trichoderma*) and B3 Fungal weeds (*Coprinus* spp.)

The crop of *P. sajor-caju* was harvested between 3-4 flushes. Significantly maximum yield of *P. sajor-caju* was obtained from T5 (wheat straw + maize stover) that is 2.24 kg/2.5 kg of substrate, this was followed by yield on T1 (wheat straw) 2.223 kg/ 2.5 kg of substrate as compare to other treatments. Comparing the six treatments of lignocellulosic residues as substrates, T5 (wheat straw + maize stover) supported best growth of *P. sajor-caju,* as it showed complete and heavy colonization forming a compact white mass of mycelium within 3 weeks of inoculation.

So, by this study maize stover in combination with wheat straw is recommended as a best substrate for the cultivation of oyster mushroom *P. sajorcaju.* In the findings of Shah *et al.*(2004) maximum average yield was recorded 646.9 gm from the sawdust and they recommended it as a best substrate for the cultivation of oyster mushroom. The highest yield on paddy straw substrate (747.1g/500g dry weight), followed by wheat straw (623.7/500g) dry weight was reported for *P. sajor-caju* by Pala *et al.* (2012).

The biological efficiency was worked out against the dry weight of each substrate. It is clear from the table 3 that, as a substrate T5 (wheat straw + maize stover) showed best biological efficiency 89.6% followed by T1 (wheat straw) 88.92%, T2 wheat straw 82.32%, T6 (wheat straw+ maize Stover) 66.88%, T3 maize stover 43.08% and T4 maize stover 37.08% and least biological efficiency was recorded from T4 sugarcane bagasse 0.044%. Shah *et al.,* (2004) recorded 44.72% biological efficiency of *P.*

ostreatus on wheat straw. The biological efficiency of *P. sajorcaju* mushroom was 149.4 and 124.7 on paddy straw and wheat straw respectively, was reported by Pala *et al*. (2012). Results of Patil (2012), revealed significantly highest yield of *P. sajorcaju* (836.66 gm/Kg) on soybean straw with B.E 84.56%, followed by paddy straw as substrate with 83.66%. The findings of Manimuthu and Rajendran (2015) reveals that maximum biological efficiency value of 84.39% was obtained in paddy straw, which was followed by the grass (BE=70.65%) as substrate for cultivation of *P. florida*.

Also, treatments with neem leaf extract amendment in T1, T3 and T5 showed increased yield in comparison to control ranging from 6.32% (BE) in T1 to 22.72% (BE) in T5. The reason for the increase in yield of mushroom would be due to increase in protein content of the substrate in T5 (wheat straw and maize stover) and antimicrobial activity of neem leaf extract that inhibited various fungal pathogens (*Aspergillus, Trichoderma*) and fungal weed (*Coprinus* spp.) weed in mushroom compost. Our result was supported by the findings of Sharma and Gothalwal, (2011) and Mahmoud *et al.* (2011). *A. indica* (neem) extract exhibited maximum inhibitory effect (54.1 to 71.6 %) against *Aspergillus* spp., *Trichoderma* spp., *Coprinus* spp., and *Penicillium* spp. was also reported by Biswas (2015; 2016).

CONCLUSION

Conventionally, wheat straw is used for cultivation of oyster mushrooms. Besides this, maize stover can also be used as a substrate for cultivation of mushroom solely or in combination with wheat straw for obtaining better yield of mushroom. An increase in production of the *P. sajor caju* was obtained which were cultivated using combinations of wheat straw and maize stover as the substrate treated with neem extract, the increase in yield was due to protein and carbohydrate rich substrate and antifungal activity of neem extract. Mushroom being saprophytes, absorbs chemicals from environment in which they are grown, therefore the use of natural and organic biochemical such as neem extract for disinfection of substrates along with conventional sterilization techniques for better growth and yield of mushroom is suggested.

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