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Effect of different saw-dusts and supplements on the yield of *Ganoderma lucidum* in lab scale cultivation

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Abstract
The effects of various kinds of saw-dusts and supplements on the yield of *Ganoderma lucidum* were determined in lab scale cultivation. The saw-dust of *Mangifera indica*, *Syzygium cumini* and *Tectona grandis* & supplements of rice bran, wheat bran, corn flour and gram flour were used as substrates in *Ganoderma lucidum* cultivation. Saw-dusts (*M. indica*, *S. cumini* and *T. grandis*) alone were used as control substrate in the cultivation. Significant differences (P< 0.03) in yields were found among different varieties of saw-dusts and supplements used. Similarly, significant differences (P<0.03) were found in saw-dust with supplements and without supplements in yield as well as biological efficiency. *Tectona grandis* saw-dust could not give yield under ambient conditions while *Mangifera indica* was found to have minimum yield and biological efficiency. *Syzygium cumini* gave good yield and biological efficiency as compared to the other saw-dusts used. The highest yield and biological efficiency were obtained from wheat bran as compared to the other brans. *Syzygium cumini* saw-dust supplemented with wheat bran showed highest yield among all the treatments. Supplementation showed positive role in mycelia growth and yield of *G. lucidum*.

Keywords: *Ganoderma lucidum*, saw-dust, substrate, cultivation, biological efficiency, supplements, yield

Introduction
Scientifically mushrooms are the macrofungi with fleshy, sub-fleshy, or sometimes leathery, umbrella like sporophores that bear their fertile surface either on lamella or on lining the tubes, opening out by means of pores. Over 2000 species of edible mushrooms are known worldwide. Out of these, 300 species belonging to 70 genera reported from India are found to be edible. Around 80 mushrooms have been grown experimentally, 20 cultivated commercially and 4-5 species are being produced on industrial scales throughout the world [1]. Mushroom cultivation has become one of the most profitable agribusiness that could produce food products from different substrates and help to dispose them off in an environment-friendly manner [2].

Red mushroom i.e. *Ganoderma lucidum* is a medicinal mushroom. *Ganoderma lucidum* (Curt. Fr.) P. Karst. (Lingzhi in Chinese; Reishi, mannentake, or Sachitake in Japanese; and Youngzi in Korian, a species of Class Basidiomycetes, belongs to the family Polyporaceae or Ganodermataceae of the order Aphyllophorales[3]. The fruiting bodies and mycelia of *G. lucidum* contain immunomodulating polysaccharides, some of which inhibit the growth of several cancer cells [4, 5].

For cultivating most of the medicinal mushrooms, the basic substrate is hardwood saw-dust (a mixture of fine and coarse saw-dust to ensure good aeration) 75-80%, supplemented with wheat bran 20%, gypsum 1%, sucrose 1%, moisture content 60-65% and pH 5.5- 6.5 [6]. A successful artificial cultivation has been reported on solid substrates utilizing saw-dust and different agricultural wastes e.g. rice bran, wheat bran, sugarcane bagasse, rice husks, coconut fiber, peanut hulls, banana leaves etc. Erkel [7] investigated the effect of different kinds of saw-dusts (Poplar, Oak and Beech) and brans (Wheat, Rice and Corn) on the yield of *G. lucidum*. The highest yield and biological efficiency were obtained from oak saw-dust compared to the other saw-dusts and from wheat bran while comparing with other supplements. *Ganoderma lucidum* grows on a wide variety of dead or dying trees like Deciduous trees, especially *Quercus*, *Acer*, *Alnus*, *Betula*, *Pyrus*, *Magnolia* etc. These days, the methods most commonly adopted for commercial production are the wood log, short wood segment, tree stump, saw-dust bag and bottle procedures [8].

Materials and Methods
Fruiting bodies of *Ganoderma lucidum* were collected, worked out and identified from deciduous forests of District Bilaspur of Himachal Pradesh, India. The fungus was isolated in the form of pure culture on Potato Dextrose Agar (PDA) medium at 25ºC temperature. The pure culture of the fungus was used in subsequent studies.

Preparation of spawn
For preparing spawn, wheat grains were used as substrates. The grains were washed thoroughly and then soaked overnight. Dead grains or those float on the surface of water were removed. The following day, the grains were washed again and boiled for at least 30 minutes. Care was taken not to over boil the grains so that they may not rupture. Excess water was drained off and the grains were allowed to cool. Calcium carbonate and gypsum in the ratio 1:3 were added to the grains and then filled in bags up to 2/3rd of their capacity. These bags were properly plugged with non-

absorbent cotton and autoclaved at 22 psi pressure for two hours. After cooling down, these bags were immediately transferred to inoculation chamber and kept under UV light for one hour for surface disinfection. Mother culture was used for inoculation and bags were incubated for 15-20 days or till such time; the grains were fully impregnated with mycelium of the test fungus. The bags were stored at 2-4 ^{0}C in the refrigerator for further use.

Lab scale cultivation trials of *Ganoderma lucidum* on agricultural wastes

Saw-dusts of three plants (*Syzygium cumini, Mangifera indica and Tectona grandis*) with four kinds of supplements comprising rice bran (RB), wheat bran (WB), corn flour (CF) and gram flour (GF) were used. The saw-dust was used with different supplements as well as in controlled condition (without supplements) as artificial medium materials. The saw-dust and supplement were mixed in a ratio of 8:2 (W/W). The substrate was soaked overnight in tap water for further use. About 500ppm formaldehyde was also added for sterilization of the substrate. Next day, excess water was drained out from the agro-waste and it is filled in polypropylene bags. Bags were closed tightly. They were autoclaved at 22 psi pressure for two hours. After cooling, these bags were immediately transferred to inoculation chamber and kept under UV light for one hour for sterilization. Spawn prepared was used for inoculation. The temperature of the incubation room was maintained with the help of room heater. The temperature was recorded during the incubation period regularly and it was maintained at 28-30^{0}C. The substrates were kept in the incubation room till full colonization of the mycelium. When the mycelium colonized the substrate completely, the bags were shifted to cropping room [room having high temperature i.e. 30-32^{0}C, high humidity and light exposure] for the formation of fruiting bodies. The mouth of the bags were opened and kept on metal tray containing water to make high humidity. In growth stage frequent watering was done in order to raise relative humidity approximately 80-90%.

Primordial formation and harvesting

Primordial formation of *G. lucidum* was initiated at different times in different treatments. Fruiting bodies were harvested when the caps became completely red and the white margin disappeared. The total yield for each treatment was measured from two flushes in a harvest period of 75 days. The colonization period, primordial formation days and first harvest period were recorded and compared for all the treatments. The biological efficiency (BE) percentage of fresh weight of harvested mushrooms/dry matter content of the substrate was calculated thereby [9].

Results and Discussion

Mycelial Colonization

Mycelium of *G. lucidum* took equal time (35 days) to colonize bags of *Mangifera indica* saw-dust which was supplemented with gram flour, wheat bran, corn flour and rice bran. The colonization period of *M. indica* in control could not be obtained due to slow mycelial growth and got contaminated with other moulds and hence discarded. The average mycelia colonization period for the saw-dust of *M. indica* with supplements was longer (44 days) than that of *Syzygium cumini* (40 days).

Cold treatment for primordial Initiation

Bags inoculated with spawn which did not respond to primordial initiation by exposure to light and air were subjected to cold shock treatment. This cold shock was found effective for triggering primordial formation.

Primordial formation

The primordial formation period for *S. cumini* with supplements was 38 days. The primordial got matured in 20 days in *M. indica* while in case of *S. cumini*, the primordial maturation took place in 15 days.

Harvesting Period

The average time taken for fruiting body formation by *Ganoderma lucidum* on saw-dust of *Mangifera indica* was 60 days while it was 54 days in case of *Syzygium cumini* saw-dust with different supplements. *S. cumini* saw-dust showed shorter period for fruiting body formation than that of *M. indica* saw-dust. In case of *Tectona grandis*, there was no fruiting body formation due to very poor mycelial growth.

Effect of different types of saw-dusts on the yield and biological efficiency of *Ganoderma lucidum*

To investigate the feasibility of using three kinds of saw-dusts as basal substrates, *Mangifera indica, Tectona grandis* and *Syzygium cumini* saw-dusts were tested with wheat bran, gram flour, rice bran and corn bran as supplements and saw-dusts alone. The highest yield of 15.61g/500 g and BE of 9.67 % were recorded on saw-dust of *S. cumini* which was followed by saw-dust of *M. indica* i.e. 10.54g/500 g and BE of 4.85%. There were significant differences ($P<0.03$) among saw-dusts of *S. cumini* and *M. indica*. The Biological efficiency of *Syzygium cumini* recorded in the present investigation is in agreement with the work of Gururang *et al.* [10], who reported Biological efficiency of 15.69% from *Alnus nepalensis* saw-dust and low BE from *Shorea robusta* saw-dust (0.51%). The difference in BE of *M. indica* and *S. cumini* can be attributed to porous texture of the first one. The difference may also be due to variations in nutritional quality and water holding capacity of the different saw-dusts.

Effect of different types of supplements on the yield and biological efficiency of *Ganoderma lucidum*

Significant differences ($P<0.03$) in the yield of *G. lucidum* were found among various types of supplements used with different substrates as shown in Table 1 and 2. The highest yield (18.22 g/500 g) and BE (11.42%) were obtained in case of Wheat bran followed by Corn bran (16.37g/500 g and BE 9.67%). Corn bran was again followed by gram flour (13.86g/500 g and BE 7.40%). Rice bran gave lowest yield (12.12g/500 g and BE 5.47%) among different supplements.

Control (saw-dust without supplements) gave lowest yield of 4.75g/500 g and BE of 2.35%. There were found significant differences between substrate with supplements and control in yield and BE. Wheat bran showed significant difference with Corn bran and Gram flour in yield statistically.

This observation was contrast to results obtained by Gurung *et al.*, 2012. They found the highest yield (20.75 g/400 g) from gram flour which was followed by wheat bran (17.00 g/400 g). Corn flour gave the lowest yield among brans (14.00 g/400 g). This contrast seems to be arisen from the ratio of supplements used. These results suggest that the yield and biological efficiency are different for different substrates and different supplements.

Table 1: Effects of various kinds of saw-dusts on yield and biological efficiency of *G. lucidum*

Saw-dust	Supplement	Yield g/500g	Mean	Biological efficiency (%)	Mean
Syzygium cumini	Control	9.50	15.616	4.71	9.672
	Wheat bran	20.0		14.19	
	Corn bran	18.25		12.09	
	Gram flour	16.33		9.47	
	Rice bran	14.00		7.90	
Mangifera indica	Control	0.00	10.51	0.00	4.858
	Wheat bran	16.45		8.66	
	Corn bran	14.50		7.25	
	Gram flour	11.40		5.33	
	Rice bran	10.25		3.05	

Table 2: Effects of different types of supplements and control on yield and biological efficiency of *G. lucidum*

Supplement	Saw-dust	Yield g/500g	Mean	Biological efficiency (%)	Mean
Control	*S. cumini M. indica*	9.50 0.00	4.75	4.71 0.00	2.35
Wheat bran	*S. cumini M. indica*	20.0 16.45	18.22	14.19 8.66	11.42
Corn bran	*S. cumini M. indica*	18.25 14.50	16.37	12.09 7.25	9.67
Gram flour	*S. cumini M. indica*	16.33 11.40	13.86	9.47 5.33	7.40
Rice bran	*S. cumini M. indica*	14.00 10.25	12.12	7.90 3.05	5.47

a

b

c

d

e f

Fig 1: (a) *Ganoderma lucidum* in its natural habitat (b) Pure culture of *G. lucidum* on PDA medium (c) Spawn of *G. lucidum* on wheat grains (d) Cold treatment given for primordial initiation in *G. lucidum* (e) Fruiting bodies on *Mangifera indica* saw-dust (f) Fruiting bodies on *Syzygium cumini* saw-dust

Conclusion

The investigation was carried out on the effect of various kinds of saw-dusts and supplements on the yield of *Ganoderma lucidum* in our study. Yield and biological efficiency of *G. lucidum* varied widely depending on the kind of saw-dusts and supplements thus used. Therefore, it is important to use the proper substrate for the commercial production of *G. lucidum*. *Syzygium cumini* saw-dust supplemented with wheat bran showed highest yield among all the treatments. As wheat bran is industrial by-product and economically cheaper than gram flour, it is recommended for use as a supplement with *Syzygium cumini* saw-dust to cultivate *Ganoderma lucidum*. Further, Corn bran can be used as an alternative supplement. Supplementation showed positive role in mycelial growth and on the yield of mushroom, thus supplementation is required for commercial production of *G. lucidum*.

Conflict of interest

The authors hereby declare that there is no conflict of interest.

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