

Optimization of *Ganoderma lucidum* Growth Contributing Concentration Gradient of Substrate Derived from Pine Forest Waste

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ABSTRACT:

Environment deterioration can be control by the suitable various techniques by use of agro forest wastes as supplements. These substance derived from agriculture & forest trashes contains macro or micro nutrients for new plant growth in the field of biotechnology. some macro fungus like mushrooms use these trashes in gradient conc. mix with the change in growth by substrate mix ratio. Most of the forest Himalayan region it contains maximum trees of deodar , pine trees and the forest ground is covered with their shreds parts like their leaves, stems, branches, wood chips, pollen grains, cones etc .At the time of maturity maximum fall takes place cause thick covering sheets attracts fires in it this leads to cause forest fires .It caused destruction to flora and fauna lives inside the forests. The fungi commonly known as *Ganoderma lucidum* form as bridge provide by nature to balance the plant waste by their different lignocellulosic and ligninolytic enzymes activity with respect to conc. gradient and substrate mix . These leaves wastes are consumed by mushrooms miracle to decrease the calamities, act as natural scavengers. In this observations includes evaluation of *Ganoderma lucidum* on pine waste leaves, wood bark, cone bundles etc. The minimum concentration test is carried out with the zone of exhibition (ZOE). The outer covering of pine cones, leaves are very rigid take a time to degrade in environmental condition ,so for cultivation purpose substrate mix preparation these forest pine waste is pretreated by cutting, chopping ,grinding ,water extraction .To evaluate the observation cone and pine leaves mixture shows max growth in plates than 40,30,20,10µl conc. by showing zones in media. The observation shows the mushroom enzymatic activity of the mushroom with minimum concentration of waste mixture substrate for yield. The pine forest waste shows the higher growth rating in pine leaves & cone mix substrate(PLCD) than pine leaves mix substrate30%(PLSC) than pine leaves mix substrate 20%(PLSB) than pine leaves mix substrate10% (PLSA) than sawdust without any

mixture(SAWD).as considering the BE of Ganoderma lucidum show different in different conc. BE is higher in PLCD then PLSC and lower in (SAWD) .The Biological efficiency of PLCD is 42.5%and PLSC 38.0% is near by then others after treatments. But the pine leaves are easily available in forest which are shredded by trees without cutting pine trees with less damage to forest . The wood chips and cones are not in large quantities they are less amount in forest than leaves .the reishi is cultivated by using local forest waste by products as substrates. The best mycelia growth and colonization were recorded mix with supplements and used as substrate for the growth of reishi .the PLSC mix show nearby in growth and yield of fruiting body. So PLSC is used in place of PLSD for economical efficient growth yield.

Keywords: Environment deterioration, lignocellulosic properties, ligninolytic properties

INTRODUCTION

Reishi is known as linzhi is one of the most popular medicinal mushroom in china ,Japan, Korea and other asian countries, is used by ancient ancestors[1] for the well being humans kind. The presence of divers vegetation in forest serves as heaven for mycoflora[26]. The reishi mushroom has various medicinal values ,ligninolytic and lignocellulosic properties breaking complex protein contents in very simple form, The hilly regions during rainy season, mushrooms are seasonally abundant and customarily collected from forest by the villagers and used as food[11].the reishi is having various nutritional ,medicinal value used in curing various diseases.[12] In new era the people come to know the beneficial properties of reishi ,this mushroom is in high demand the essential to constantly search for suitable cultivation techniques[2][3],several types of natural substrates and liquid media, solid state fermentation [21]are used for evaluation and enhancement[9].In nature the reishi grown on wood logs present in forests, it takes many months to grow and mature taking much time. various techniques are used to grow Reishi on sawdust ,agricultural wastes[15] [7],different types of straws[22], industrial wastes, on various brans ,tea waste[10], soybean residues[11], fishing industry wastes[16] may improve the yield of mushroom.[24]various local substrates are used for Ganoderma lucidum in mid hills of uttarakhand .For the growth of Ganoderma Lucidum effects by various conditions like pH, tempararture, compositoofsubstrate[4][5], suppliments, additives, agitation humidity, moisture conditions, composition of substrates on mycelium growth[8], biological yield, content of bioactive compounds ,carbon and nitrogen sources[17]. The substrate overgrowth and slow growth by mycelium are easily effected prone for fungal and bacterial attacks and decrease in yield[6], The aim of the presented study is to determine the different concentration gradient of substrate waste [13][14]mix ratio of pines wastes on mycelium growth ,fruiting body,quantity,quality , yield[24] and biological efficiency and economical efficiency.

METHODS AND MATERIALS

SOURCE OF STRAIN:

The (Reishi)Ganoderma lucidum culture strain is taken from DMR centre solan (H.P) an form subculture tubes for further testing procedures. The strain of basidiomycete fungi were cultured on Media Malt Extract powder and Agar Agar type I in Petri plates and grown on test tube slants at 27 °C for 24 to 48 hours. The slants were stored at 4 °C and sub-cultured every 4 weeks for further studies. the stain is inoculated on media plates were incubated .The plates are covered with Para film to prevent contamination, after 6-7 days the mycelia had covering most on the agar surface.

PROCEDURE:

SPAWN FORMATION

The dry ,healthy wheat grains are taken for spawn formation they are half boiled sterilized autoclaved, nurition are added autoclaved for 15 mins. Then the grains are cooled and inoculated with mycelia plugs in spawn formation bags and bottles ,in laminar air flow and incubated in specific controlled condition for two weeks.

SUBSTRATE PREPARTION

In the first step sawdust and pinleaf waste, wood chips, cones wastes used as raw materials. the pine wastes ,cones are crushed, grind, chopped to 2-3 cm size cleaned properly for second treatment. In second treatment pine leaf waste, cones content is extracted with water solvent extraction .the extracted content is collected in conical flasks and sterilized by autoclaving to further procedure in Petri plate formation. The conical flasks with media, scissors gauge, borer ,peri plates is sterilized. After sterilization the sample is moved to laminar air flow for sample loading in plates containing media with the help of micropipettes .the samples are cone pine leaf waste mix,pineleaf waste 30% pineleaf waste 20% ,pineleaf waste 10% ,without any waste as a control for Ganoderma lucidum. these samples are taken in 10µl,20µl 30µl,40µlare loaded in media wells formed by borer in media covered and move to incubator for mycelium growth .for substrate treatments consisted of mixtures of sawdust and wheat bran at varying ratios having a total weight 400gm/kg of sawdust of dry material All treatments were supplemented with 75 g wheat bran and 10 g of calcium carbonate and moistened 15with 2.5 liter water. The leaf waste are added 10%,20%,30% to the substrates forming ten bags after mixing of the materials for each treatments.

SUBSTRATE FORMULATION

The grain spawn from above process is aseptically inoculated in sterilized bags ten bags containing sawdust only(SAWD),pine leaves waste, cone wastes sawdust based(PLCD), pine cones waste 30%sawdust based(PLSC),

pine woodchips waste 20% sawdust based (PLSB) pine woodchips waste 10% sawdust based (PLSA)

The Polypropylene bags (inoculated fruiting bags) of reishi were incubated at room temperature in dark room to allow mycelia growth. the mycelia growth rate on substrate in number of days, thickness is recorded

STERILIZATION

substrate mixtures in bags were sterilized in an autoclave for one and half an hour at 126 °C under 1.5 A pressure to destroy the fungal spores and bacteria for mushroom diseases. When the sterilization of the substrates was finished, they were allowed to cool prior to inoculation.

INOCULATION PHASE

On day after sterilization the substrate mixtures in the bags were inoculated with *Ganoderma lucidum* spawn the inoculated substrate mixtures were reloaded into their bags, sealed and incubated for 15 days . Inoculation of the substrates with mushroom spawns mycelium growth and formation of basidiocarp. *Ganoderma lucidum* bags are shifted to their growing rooms, requiring darkness, and specific concentrations of oxygen and carbon dioxide, are entirely different from plant growth chambers. The bags containing the substrate mixtures, sterilized and inoculated by spawn were transported from the inoculation room to a cropping room having the over mentioned conditions and were allowed to develop mycelia. The growing room temperature was set to 24-25 o C and room is dark during mycelia development .The relative humidity of the room atmosphere was set around 85%.

FRUITING PHASE

PROCESS OF BASIDIOCARP FORMATION

When the mycelia is fully grown in bags the bags mouth is opened for primordial formation the bags are 8 -10 hours exposure and water is sprinkle twice in a day to maintain proper humidity and temperature aeration. After opening of cotton plugs 2 to 3 days cap formation is initiated and 60 -72 days the three flushes are harvested from these bags .*Ganoderma lucidum* have brilliant capacity to decompose lignin cellulose and lignin[29][18].the tea waste act as good supplement for cultivation of reishi mushroom.[19] wheat straw waste is used as a substrate for the cultivation of reishi is act as scavenger ,bioremediation for its protein breaking ability[26][27]. [20]The *Ganoderma lucidum* shows the high activity of ligninolytic enzymes[25] [29]at the time of vegetative growth the mushroom laccase & Mnp activities were high during colonization &decrease during primordial and fruit body formation[28]. The artificial cultivation bags are formed with poplar sawdust which is available near by wood machines(arramachines),wheat bran is added as supplement to increaease growth with 65% moisture is added ,calcium carbonate chalk powder is added to maintain the pH for growth.

Fig I SYSTEMATIC REPRESENTATION OF GANODERMA LUCIDUM GROWTH STAGES.

[A] Plate with wells [B]Zone of exhibition 10,20,30,40 µl conc.[C]Bag preparation [D] Fruit formation

DATA COLLECTION AND ANALYSIS:

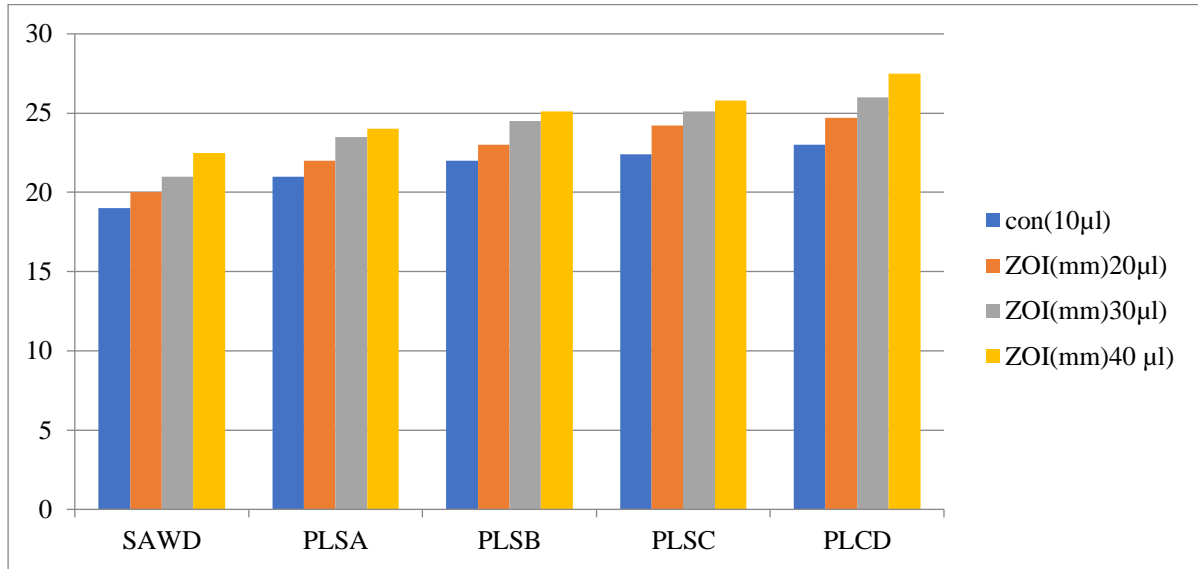
(I) Sample preparation for zone of exhibition

In Zone of exhibition the concentration of give sample is prepared by the addition of some culture from the given reference primary strain derived from mother culture strain of mushroom *Ganoderma lucidum* with gauge wire loop diameter of 2mm full with culture strain & mix with sterile and sterile water 10ml shake till mycelia threads get fragmented. Then used under sample examined with minimum concentration at 10,20,30,40µl to check there active growth effect on different substrate conc. mix of pine leaf waste and cone mix.

Table I.shows the growth of *Ganoderma lucidum* as zone of exhibition (mean and standard deviation) of Reishi on wells with the different concentration versus pine leaf wastes and cone pine waste mixture .the plates are used in triplicates.

S.no.		con(µl)10 µl	ZOI(mm)20 µl	ZOI(mm)30 µl	ZOI(mm)40 µl
1	Control(SAWD)	19	20	21	22.5
2	PINE LEAVES WASTE 10%(PLSA)	21	22	23.5	24
3	PINE LEAVES WASTE 20%(PLSB)	22	23	24.5	25.1
4	PINE LEAVES WASTE 30%(PLSC)	22.4	24.2	25.1	25.8
5	CONE &PINE LEAVESWASTE(PLCD)	23	24.7	26	27.5

The Graph I. shows Zone of exhibition VS on different substrates concentration mix pine leaf and conewaste with ganoderma lucidum.



ANALYSIS:

Collection of different sample are collected from nearby forest, for check the enzymatic activity action , enhancing mycelium & substrates of G.lucidum for bioremediation, All the experimental performed in triplicates and the data were expressed as the mean & standard deviations, ANOVA value of significance(P <0.05).The growth and development of mushroom were monitored daily. The time required from inoculation to completion of mycelium running, time elapsed between opening the plastic bags to pinhead formation and time required from opening the plastic bags to harvesting were recorded. growth parameters including number of pine formation, stipe length (cm), stipe diameter (cm), pileus diameter (cm), and pileus thickness (cm) . Yield parameters, such as number of fruiting bodies , and total fresh weight (g) of mushroom were also recorded at harvest time. Matured fruiting bodies . the growth performance of mushroom on different substrates, yield and biological efficiency were calculated

Biological yield (g) was determined by weighing the weight of fruiting bodies without removing the base of stalk

biological efficiency (%) was calculated as :where, BE is Biological efficiency (%); FWm is total fresh

weight (g) of mushroom yield a, and DW is substrate dry weight (g).

$$\text{Biological efficiency(\%)} = \frac{\text{weight of mushroom(FWm)}}{\text{Total dry substrate used(DW)}} \times 100$$

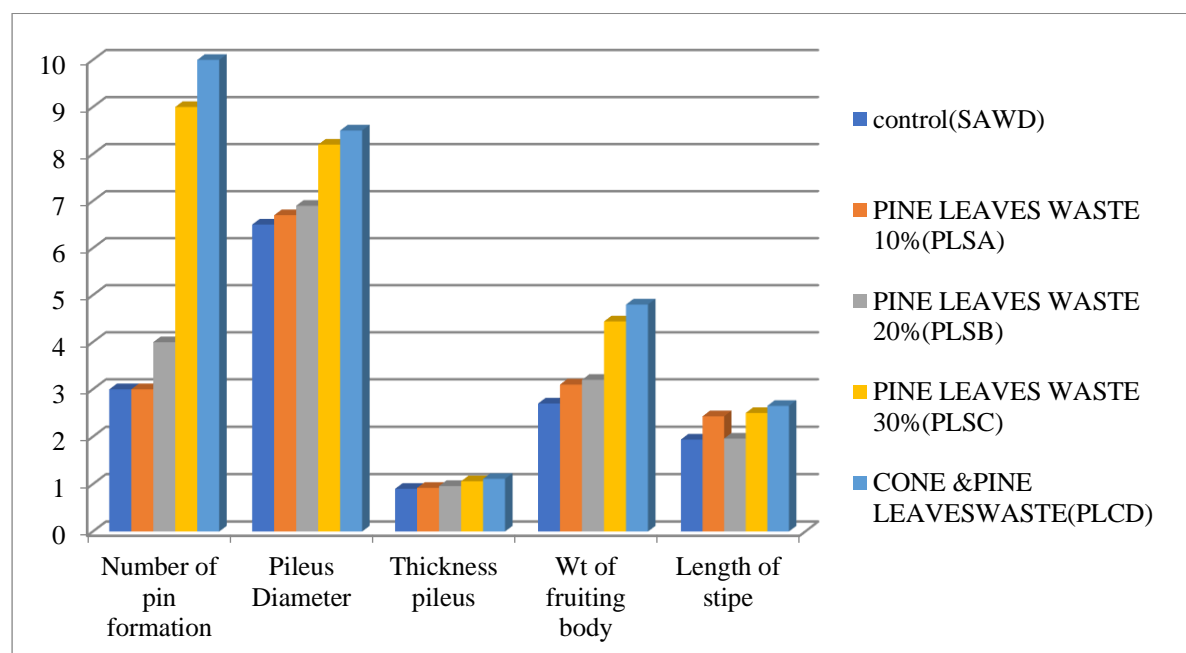
Total dry substrate used(DW)

Table II. Shows the different growth parameters of ganoderma lucidum on cone waste Pineleaf, pine leafwastes.

	Number pin formation	Pileus Diameter(cm)	Thickness pileus(cm)	Wt of fruiting body(g)	Length of stipe(cm)
Control(SAWD)	3	6.5	0.90	2.7	1.94
PINE LEAVES WASTE 10%(PLSA)	3	6.7	0.92	3.1	2.43
PINE LEAVES WASTE 20%(PLSB)	4	6.9	0.96	3.2	1.96
PINE LEAVES WASTE 30%(PLSC)	9	8.2	1.06	4.44	2.5
CONE &PINE LEAVESWASTE(PLCD)	10	8.5	1.11	4.80	2.65

Mean values in each column are significantly different ($p>0.05$)

The Graph II. shows number of pine formation ,pileus diameter, thickness of pileus,number of fruiting body, length of stipes ,of Ganoderma lucidum with different conc.pineleaf waste,conepine leaf waste substrate mix.

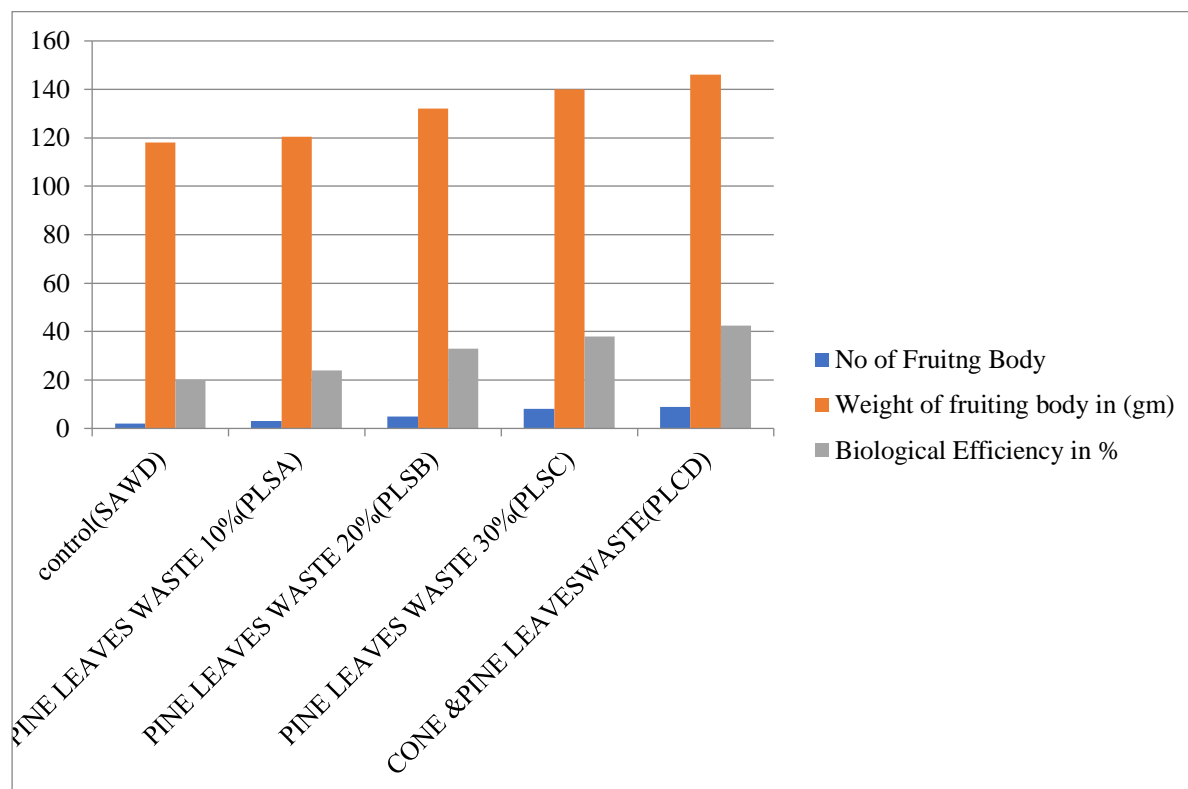


In the table III.shows the Yield and biological efficiency of Ganoderma lucidum grown on different pine waste substrates

	No of Fruitng Body	Weight of fruiting body in (gm)	Biological Efficiency in %
Control(SAWD)	2	118	20
PINE LEAVES WASTE 10%(PLSA)	3	120.5	26
PINE LEAVES WASTE 20%(PLSB)	5	132	33
PINE LEAVES WASTE 30%(PLSC)	8	140	38
CONE &PINE LEAVESWASTE(PLCD)	9	146	42.5

Mean values in each column are different superscript are significantly different (p>0.05)

Graph III.shows the Yield and biological efficiency of Ganoderma lucidum(Reishi) grown on different substrates conc mix of cone pine leaf wastes.



DICUSSION AND RESULTS

The observation shows that *Ganoderma lucidum* is used to convert pine waste to valuable nutritional medicinal forms. The forest waste is treated by crushing and extraction process used to producing ligninolytic & lignocellulosic enzymes. The *Ganoderma lucidum* is grown on higher in pine leaf & cone substrates than pine leaf substrate 30% than pine leaf substrate 20% than pine leaf substrate conc. 10% and lesser growth without forest pine waste as a substrate. The combination of pine needles and pine cones pretreated with various processes increase in the number of primordial, mycelium growth, yield are higher than pine needles mix substrates due enzymatic activity is higher than pine leaf mix at different concentration. The cone pine mix shows higher than others but the cones are not shredded abundantly and regular basis. They are grown on seasonal and generally collected used by near villagers as a fuel, so they are less available, less in number quantity, not cost efficient in collecting from trees. This is pine needles waste mix substrate with 30% is more economically sound and yield is nearby to cone leaves substrate mix. The drawback of pine cones is takes more labor and energy. The pine leaf waste substrate concentration 30% is somewhat near to cone pine needle waste mix in growth parameters and higher than pine needles waste mix substrate with 20% & pine needles waste mix substrate with 10% conc. the pine needles are regularly shredded, less consumed by human being, rarely eaten by animals. It is better option for growing Reishi (*Ganoderma lucidum*) mushroom with certain pine needle concentration mix used to increase yield, economical efficiency which is in demand for human well beings and get benefited. These findings further is useful in mycoremediation applications. This strategy of cleaning and changing the forest waste is environmentally, economically friendly for man kind and growers.

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