In vitro Comparative Evaluation of Antibacterial Activity of Fruiting Body and Mycelial Extracts of *Ganoderma lucidum* against Pathogenic Bacteria

Sheetal Mehta* and Savita Jandaik

Faculty of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Solan, Himachal Pradesh - 173 212, India.

(Received: 08 April 2012; accepted: 14 May 2012)

A wide variety of organisms are emerging as resistant to antibiotics, and multiple drug resistant organisms pose a serious threat to the treatment of infectious diseases. Hence, mushroom derived antimicrobial substances have received considerable attention in recent years. In this study antagonistic effects of the methanol, acetone and water extracts of mycelia and fruiting body of *Ganoderma lucidum* were tested against seven bacterial species: *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Salmonella typhimurium*. All the extracts exhibited various degree of inhibition against all test bacteria. Widest inhibitory zone (33mm) was obtained with mycelial acetone extract of *Ganoderma lucidum* against *Pseudomonas aeruginosa*. Lowest zone of inhibition (7mm) was observed with fruiting body aqueous extract against *Staphylococcus aureus* and *Klebsiella pneumoniae*. Minimum inhibitory concentration (MIC) of acetone extract of fruiting body and mycelial extract was determined, for mycelial extract it ranged between 4 to 12mg/ml and for fruiting body extract it ranged between 15 to 35 mg/ml for test bacteria.

Key words: *Ganoderma lucidum*, Antibacterial activity, Bioactive molecules, Extraction.

Mushrooms are defined as “macrofungi” with distinctive fruiting bodies that are large enough to be seen by the naked eye and to be picked by hand. In recent years, more varieties of mushrooms have been isolated and identified, and the number of mushrooms being cultivated for food or medicinal purpose has been increasing rapidly. Development of bacterial resistance to currently available antibiotics has made it necessary to search for new antibacterial agents; natural plant products are being investigated because medicinal plants have been widely used for treatment of many types of acute and chronic diseases and many plants with antimicrobial activity have been reported. Several mushroom species belonging to the Polyporaceae family are now being regarded as the next candidate producers of valuable medicines. *Glucidum* (Curtis: Fr) Karst is a basidiomycetous fungus used as a traditional medicine for more than 2000 years. It’s a popular remedy to treat many diseases like chronic hepatitis, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, leucopenia, diabetes, anorexia. The fruiting body, mycelia and spores of *Glucidum* contain approximately 400 different bioactive compounds, which mainly include polysaccharides, triterpenoids, fatty acids, nucleotides, protein/peptides, sterols, vitamins, minerals etc. These metabolites are reported to be responsible for the medicinal properties of...
this mushroom. Many natural products are active as anti-HIV agents\textsuperscript{11}, these compounds belong to a wide range of different structural classes viz, terpenes, polysaccharides, coumarins and flavonoids etc.\textsuperscript{12}. Because of presence of some of these compounds (triterpenoids) in this mushroom, it has potent inhibitory activity against HIV. Some other constituents such as ganomycin, triterpenoids and aqueous extracts from \textit{Ganoderma} species have a broad spectrum of \textit{in-vitro} antibacterial activity against gram positive and gram negative bacteria\textsuperscript{13}. \textit{Ganoderma lucidum} and other \textit{Ganoderma} species are used to treat various bacterial diseases in combination with other therapeutic agents also\textsuperscript{15}. The present study, for the first time reports the comparison of antimicrobial activity of fruiting body and mycelial extracts of \textit{Ganoderma lucidum}.

**MATERIALS AND METHODS**

**Fungal sample**

The fruit bodies of \textit{Ganoderma lucidum} used in this study were collected from different areas of Himachal Pradesh, and were identified on the basis of microscopic and macroscopic morphological traits with the standard description of Stamet 1993\textsuperscript{6}. Mycelial biomass was obtained by inoculating 200ml of potato dextrose broth (Hi Media) with 6mm disc of inoculum from 7 days old plate of \textit{G. lucidum} culture. Flasks were incubated at 28°C for 15 days and biomass was separated by filtration and was dried.

**Test Organisms**

\textit{In vitro} antimicrobial susceptible studies were performed using seven human pathogenic bacteria (procured from MTCC Chandigarh): \textit{Escherichia coli} (MTCC 739), \textit{Staphylococcus aureus} (MTCC 737), \textit{Bacillus subtilis} (MTCC 441), \textit{Pseudomonas aeruginosa} (MTCC741), \textit{Enterobacter aerogenes} (MTCC 111), \textit{Klebsiella pneumoniae} (MTCC 109), and \textit{S. typhimurium} (MTCC 98). Antibacterial activity of extracts was screened by filter paper disc diffusion method and activity was measured in terms of zone of inhibition size (mm) obtained after 24 h incubation at 37°C.

**Preparation of bacterial inoculum**

Test bacterial colonies were transferred in nutrient broth and were incubated at 37°C. In order to standardize the inoculums density for test, a BaSO\textsubscript{4} turbidity standard (equivalent to 0.5 Mc Farland std. = turbidity equals to 0.5 =1 to 2 x10\textsuperscript{8} cfu /ml) was used.

**Preparation of fungal extract**

The fruit bodies were cut into bits and dried at 40 °C. These dried fruit bodies and dried mycelial biomass were pulverized in a blender. The extraction of the mushroom fruit bodies and mycelial biomass was carried out using three solvents (water, methanol and acetone). For water extraction, 1 litre of sterile distilled water was dispensed into conical flasks containing 100g of powdered mushroom fruit bodies and mycelial sample. These were allowed to stand for 72 h with intermittent agitation. For methanol and acetone extraction, 100g of the pulverized fruit bodies and mycelial biomass was separately soaked in 1 litre of absolute methanol and acetone in conical flasks. These were covered with aluminum foil and allowed to stand for 7 days for extraction. The mixtures were filtered using Whatman filter paper No. 1 and the filtrate was concentrated under a reduced pressure in a rotator evaporator until a semi solid substance was obtained. These were dried inside the crucible under a controlled temperature (45°C) to obtain solid extract. The left residues were kept in refrigerator until used\textsuperscript{20}.

**Antimicrobial activity of extracts**

The concentration of the extracts used was 60 mg/ml and sterile distilled water was used to reconstitute the extract residues. For determination of antimicrobial activity sterile filter paper discs (6mm diameter) were soaked with the test extracts (60mg/ml) and dried at 40 °C for 1 h. The discs were placed on bacteria seeded Muller Hinton Agar (Hi Media) plates and placed in the refrigerator for 12 h to allow the diffusion of the extracts into the growing medium. The plates were incubated for 24 h at 37°C after which the zone of inhibition was observed and measured\textsuperscript{20}.

**Minimum Inhibitory Concentration (MIC)**

This study aimed in finding out the lowest concentration of acetone extract that will inhibit the growth of the test microorganisms. It was carried out by following the method described by Hirasawa \textit{et al} (1999)\textsuperscript{21}. Different concentrations (1- 40mg/ml) were prepared using sterile distilled water as the diluent. Filter paper disc diffusion method was used\textsuperscript{22}.
In this study antibacterial activity of methanol, acetone and aqueous extracts of *G. lucidum* was determined by disc diffusion method. The antimicrobial activity of samples varied according to the solvent. Results presented in Table I and Table II shows the antibacterial activity of different extracts of fruiting body and mycelial biomass respectively. Table III shows MIC value of acetone extract of fruit body and mycelial biomass. It is clear from the data presented in Table 1 and Table 2 that the acetone extract of *Ganoderma lucidum* showed maximum antibacterial activity followed by methanol and aqueous extract. Mycelial extract of the *Ganoderma* showed high antibacterial activity as compared to fruit body extract. Maximum inhibitor activity of acetone extract of mycelial biomass (Table 2) was shown against *P. aeruginosa* (33mm), followed by *E. coli* with zone size of 30mm. This extract showed equal inhibitory effect against *K. pneumoniae* and *E. aerogenes* (24mm). A 14mm zone was shown for *B. subtilis* and least zone was shown against *S. aureus* (12mm) at the same concentration. In case of methanol extract, maximum activity was found against *K. pneumoniae* and *E. coli* (18mm). Aqueous extracts were found to be comparatively less effective against all bacterial strains. MIC of only acetone extract was determined because it exhibited maximum antagonistic activity. MIC value of mycelial biomass was found to be 4mg/ml against *P. aeruginosa* followed by *E. coli* with a value of 6 mg/ml. In case of fruit body MIC was 15 mg/ml for *P. aeruginosa* and *E. coli*.

### DISCUSSION

Resistant bacteria are emerging world wide as a serious threat to the outcome of common infections in community and hospitals. Therefore, novel antimicrobial agents from different biological sources are continuously sought. Rosa *et al.* (2003) detected 14 mushroom isolates with high antimicrobial activity against target microorganisms\(^2\). Zjawiony (2004) observed that 75% of polypore fungi that have been tested show strong antibacterial activity\(^2\). *Ganoderma lucidum* was reported to be best among other *Ganoderma* species that generally exhibited high antagonistic activity against test bacteria\(^2\). Recently, more studies demonstrated that *Ganoderma* contain antibacterial constituents that are able to inhibit gram-positive and /or gram-negative bacteria\(\text{18, 17, 14, 15, 13}\). It is evident from the results of
present investigation that *Ganoderma* extracts had inhibitory activity against both Gram positive and Gram negative bacteria. These results also affirm the claims of traditional herbalists in the south western Nigeria that *Ganoderma* species could be used as feed supplement to resist microbial infections in human being.

All the extracts were different in their antimicrobial effectiveness depending on their extractive solvent used. Our results agree favorably with the suggestions of Oloke and Kolawole (1988) that bioactive components may differ in their solubility depending on the extractive solvents used\(^2^6\). Kawagishi *et al.* (1988), observed that some of the active phytochemicals are soluble in alcohol but insoluble in water as in case of *Agaricus blezei*\(^2^7\). Cowan (1999) reported that the most active components are generally water insoluble, hence it is expected that low polarity organic solvents would yield more active extracts\(^2\), our findings of present study are consistent with these observations, the organic extracts exhibited high antimicrobial activity than aqueous extract.

Yamac and Bilgili (2006) investigated antimicrobial activity of chloroform and dichloromethane extracts of fruit body/mycelial cultures of some (20 isolates) mushrooms against 7 bacterial strains and observed good antimicrobial activity with all the extracts for all tested organisms, in their findings mycelial extract of many fungi possess high inhibitory activity against Gram negative bacteria\(^2^8\), our results are in collaboration with this finding, mycelial extract had greater inhibitory effect against *E. coli* and *P. aeruginosa* in the present study. Mycelial biomass extracts exhibited high inhibitory activity as compared to fruit body extracts possibly because of varying content of bioactive molecule in fruit body and mycelia. It is essential to carry out further research regarding this difference in antagonistic activity pattern of fruit body and mycelia; unfortunately we were unable to perform this due to limited facilities.

CONCLUSION

In conclusion, this study has shown that different extracts (aqueous, methanol and acetone) have been used in vitro to inhibit the growth of some pathogenic bacteria. It can therefore be suggested that, *Ganoderma lucidum* is promising antimicrobial fungus and could be employed to combat several bacterial diseases.

ACKNOWLEDGMENTS

The authors wish to thank IMTECH, Chandigarh, for providing bacterial cultures and Shoolini University of Biotechnology and Management Sciences, Solan (HP), for supporting research.

REFERENCES


Effect of Cost-Effective Substrates on Growth Cycle and Yield of Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Higher Basidiomycetes) from Northwestern Himalaya (India)

Sheetal Mehta,¹* Savita Jandaik,¹ & Dharmesh Gupta²

¹Shoolini University, Faculty of Biotechnology, Solan, HP, India; ²Dr. Y.S. Parmar University of Horticulture and Forestry Nauni, HP, India

*Address all correspondence to: Sheetal Mehta, Faculty of Biotechnology, Shoolini University of Biotechnology and Management Sciences, Bajhol, Solan, HP 173212, India; Tel.: +91-941-835-6611; Email: mehta.sheetal1@gmail.com.

**ABSTRACT:** To find a cost-effective alternative substrate, the medicinal mushroom *Ganoderma lucidum* was grown on sawdusts of sheesham, mango, and poplar. Optimum spawn level was determined by spawning in substrates at various levels (1, 2, 3, and 4%). To determine the effect of supplementation, substrates were supplemented with wheat bran, rice bran and corn flour at different concentrations (10, 20, and 30%). Duration of growth cycle, mushroom yield, and biological efficiency data were recorded. Among substrates, mango sawdust was superior, with 1.5-fold higher yields than poplar sawdust, which was the least suitable. However with respect to fructification, mango sawdust produced the first primordia earlier (21±1 days) compared with the other investigated substrates. 3% spawn level was found to be optimal irrespective of the substrate. Yield and biological efficiency (BE) were maximally enhanced by supplementation with wheat bran, whereas rice bran was the least suitable supplement among those tested. Growth cycle shortened and mushroom yield increased to a maximum at the 20% level of supplements. Mango sawdust in combination with 20% wheat bran, if spawned at the 3% level, resulted in a high yield (BE = 58.57%).

**KEY WORDS:** medicinal mushrooms, *Ganoderma lucidum*, sawdust, supplement, yield, biological efficiency

**ABBREVIATIONS:** CF, corn flour; ITS, internal transcribed spacer; MS, mango sawdust; PS, poplar sawdust; PVC, polyvinyl chloride; RB, rice bran; SS, sheesham sawdust; WB, wheat bran.

**I. INTRODUCTION**

Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. (Ganodermataceae, higher Basidiomycetes) has become the object of intensive research because of its multiple health benefits with apparent absence of side effects.¹,² All pharmacological properties have been correlated to large pool of bioactive compounds produced by its fruiting bodies, mycelia and spores.³ *G. lucidum* is rare in nature; consequently, the supply of wild mushrooms is insufficient for commercial exploitation, as more than 100 brands of *Ganoderma* are sold on the market.⁴ Chang and Wasser reported the total global production of this mushrooms in 2012.⁵ In India, the market for *Ganoderma*-based nutri-cteicals is growing very rapidly and was estimated to be approximately US $20 million in 2012. Even though the cultivation technology has advanced in many Asian countries, very little is known about the cultivation of this mushroom in India, where *Ganoderma* products are imported from Asian countries, even though *Ganoderma* mushrooms are indigenous to India. Considering the increased demand for this mushroom in domestic and export markets, its cultivation has become an essential focus of research.

*G. lucidum* can be grown with large variety of substrates, including lignocellulosic components (cellulose, hemicellulose, and lignin) that provide soluble inorganic and organic materials. In recent years, successful artificial cultivation of mushrooms has been reported on solid substrates containing...
agricultural residues such as rice, banana, wheat, sorghum, varagu, sugarcane, poplar, oak, corn and beech, nepal alder, Indian horse chestnut, red cedar, black jack oak, sunflower seed hulls, and whey permeate. One strategy for improving yield may include supplementation of the substrate with nitrogen-based supplements such as millet, gram, rye, corn, etc. Some of these raw materials may not be available or are available at relatively higher prices in some parts of India. Thus, growers are perpetually searching for alternative substrates that may be more readily available and economical and that provide higher yield and better quality.

Cost-effective production of mushrooms depends on the reliability, availability, and cost of substrate ingredients. Mango, sheesham, and poplar sawdusts are easily available agro-wastes in India, and as hardwoods, they are likely to support mushroom growth. Wheat, corn, and rice are also among the top crops of India, offering easy availability and inexpensive and nutrient-rich supplements.

In the present mushroom cultivation study, we specifically aimed (1) to determine optimum spawn level, (2) to investigate the suitability of various sawdusts as growth media, and (3) to determine the effect of various supplements at different spawn levels.

II. MATERIAL AND METHODS

A. Substrate and Supplement Materials

For this study, sawdusts of sheesham (Dalbergia sissoo), mango (Mangifera) and poplar (Populus); brans of wheat (Triticum), rice (Oryza sativa); and corn flour (Zea mays) were collected from different regions of Punjab and Himachal Pradesh in India.

B. Mushroom Cultures

During the mycobiotic survey of different regions of Punjab and Himachal Pradesh, G. lucidum fruit bodies were collected. Polysaccharide content and antimicrobial activities of all isolates were evaluated, and one isolate (strain no. GL04) with maximum polysaccharide content and antimicrobial activity was chosen for cultivation studies at a mushroom farm. Identity of the culture was confirmed by amplifying and sequencing the Internal Transcribed Spacer (ITS) DNA, according to a previous report (GenBank accession number: KF648564). A pure culture was grown on malt extract agar slants. These slants were incubated at 28°C until substantial mycelial growth was observed. This mycelium (raised from single fruit body of the GL04 strain) was used as the inoculum for the mother culture and for spawn preparation on wheat grains (Triticum durum).

C. Experimental Design

In the first part of the experiment, the substrates were spawned at various levels. Each treatment had three replicates, resulting in 36 experimental units. The second part of the experiment investigated the most suitable substrate by spawning all test substrates at the optimal level. Each treatment had three replicates, resulting in nine experimental units. The third part of the experiment consisted of supplementing various substrates with three different supplements at variable concentrations. Each treatment had three replicates, resulting in 81 experimental units. The effects of these experimental conditions were evaluated by their effect on the complete spawn run period, the time needed for primordia formation, yield, and the biological efficiency (BE) of G. lucidum.

D. Substrate Preparation and Inoculation

Substrate preparation includes setting pH, establishing moisture content, and substrate sterilization. All sawdusts were stacked on a cemented floor and wetted thoroughly for 24 h to raise the moisture content to approximately 65%. Calcium sulphate (gypsum) and calcium carbonate (chalk powder) were added to achieve a pH of 5.5. The test substrates were loaded into polypropylene bags (20×35 cm, without filter) each weighing 1 kg (350 g dry weight). Then the bags were labeled and closed with PVC (polyvinyl chloride) rings (3 cm
diam.) and plugged with cotton to prevent contamination by airborne organisms while allowing air exchange. Bags were autoclaved at 121°C for 2 h, were allowed to cool, and were then spawned.

E. Analysis of Effect of Spawn Level

This experiment was performed on all substrates (MS, SS, and PS). To determine the optimal spawn level, different doses of spawn were inoculated at 1, 2, 3 and 4% dry weight of all substrates. Spawn was mixed thoroughly in the substrate material by shaking the bags properly (i.e., ‘thorough spawning’) under aseptic conditions.

F. Analysis of Best Substrate

To determine best growth substrate, all test substrates were spawned at the 3% level.

G. Analysis of Effect of Supplementation

To find a superior combination of media or to assess effect of supplementation, sawdusts and test supplements were mixed in different ratios (sawdust:supplements, 90:10, 80:20, and 70:30), spawned at the 3% level, and were then exposed to cultivation conditions.

H. Cropping and Harvesting

Inoculated bags were arranged on pest-resistant shelves in a cropping room to attain the five stages of mushroom growth: spawn run, primordial initiation, stalk formation, cap differentiation, and maturation. The temperature and relative humidity were controlled at 28 ± 2°C and 65%, respectively, during the first stage (spawn run period was completed without artificial lighting). The temperature and relative humidity were controlled at 28 ± 2°C and 90–95% during the second stage with light exposure (10 h, provided by white fluorescent bulbs).
The temperature and relative humidity were controlled at 28±2°C and 70–80% during the third stage with light exposure. The temperature and relative humidity were controlled at 25°C and 85–90% during the fourth stage with light exposure and at 28±2°C and 60% relative humidity during the last stage. All inoculated bags remained closed until the substrate in the bags was fully colonized. Completely colonized bag tops were cut at the level of the substrate to allow the development of fruiting bodies. Days needed for the complete spawn run and primordia initiation were recorded. Fruit bodies were harvested when they had fully matured, and total yield (g) was measured. The BE percentage \([(\text{fresh weight of harvested basidiomata/dry weight of the substrate}) \times 100]\) was calculated.

I. Statistical Analysis

One-way ANOVA followed by Tukey- Kramer multiple comparison test was performed using GraphPad Prism, version 5.02 (GraphPad Software, Inc.), to determine the significance of differences between different study groups.

III. RESULTS AND DISCUSSION

A. Effect of Different Spawn Levels

Spawn level is defined as the weight ratio of spawn to substrate on a dry weight basis. This factor relates to the production cost of mushroom cultivation, as spawn is a relatively expensive item. An attempt was made to determine the optimal spawn level so that minimal inoculum could be used without sacrificing mushroom yield to achieve the best economics. Among the tested spawn levels (1, 2, 3, and 4%), the 3% level was optimal, resulting in the shortest growth cycle and maximum yield (Table 1) with all substrates, and was statistically similar to the 4% spawn level. However, the 1% spawn level was least efficient in all aspects, with the longest spawn run period and a negligible yield with all substrates, which proves that a spawn level that is too low results in a longer growth cycle and lower yield. No similar study has been reported so far; thus, this study is the first of its kind to our knowledge. In our study, an increase in the spawn level from 2 to 4% demonstrated an increase in yield and shortened the growth cycle duration in \(G.\) \(lucidum.\) This result may be explained by the fact that increased spawn level accelerates colonization and narrows the gap of opportunity for competitor invasion. Deviation in optimum spawn levels in various studies may be attributed to the type of substrate, mushroom species, spawn quality, and cultivation conditions.

B. Effect of Different Sawdusts

To determine the best substrate, test sawdusts were inoculated with 3% spawn and exposed to spawning and cropping conditions. The prerequisite criteria for selecting these agro-wastes as substrates were their suitability as growth media, continuous support to growth, and the related activity of mycelium of some other mushrooms. Initiation of mycelial growth occurred on day 8 post inoculation in all substrates. This uniformity in the initiation of mycelial growth in all of the substrates may have been due to the low enzyme activity during substrate colonization. The implication of this result is that the level of breakdown of substrate materials for the release of nutrients is low or nonexistent within this short period of 8 days. Hence, nutrients in the substrate may have not been released and consequently were not available to the mycelia and thus had no influence on the initial growth of the mycelia. Spawn run refers to the propagation of mycelia in the colonized substrate bags during the vegetative growth stage before fruit body formation. In this study, this period ranged from 15 to 20 days as shown in Table 2. The minimum time for initiation of pin heads occurred with MS followed by SS and PS, respectively. The highest yield (weight of fresh mushrooms) was obtained with MS, which was 1.5-fold higher than the yield on PS; however, the yield on SS was between that of MS and PS (Table 2). The lowest yield with PS could be due to the carbon:nitrogen (C:N) imbalance in the sawdust, which is in agreement with the
previous observations of Stamets.\textsuperscript{14}

**C. Effect of Combinations of Sawdust and Supplements**

The experiment was conducted to assess the best combination of media or the role of different organic supplements in stimulating the growth of *G. lucidum* so that productivity can be enhanced. Sawdusts and brans were mixed in different ratios (90:10, 80:20, or 70:30); inoculated with the 3% spawn level, and exposed to proper spawning and fructification conditions. Our results (Table 3) reveal that supplementation enhanced the yield on all substrates at all concentrations, which can be attributed to the change in decomposition rate and the

### TABLE 1: Effect of Different Doses of Spawn on Growth Cycle and Yield of *Ganoderma lucidum*

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Spawn rate (%)</th>
<th>Complete spawn run (Days)</th>
<th>Primordia initiation (Days from spawning)</th>
<th>Yield (g/kg)</th>
<th>BE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>1 22 ± 1.0</td>
<td>29 ± 0</td>
<td>2.33 ± 1.45\textsuperscript{B,C,D}</td>
<td>0.66</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 20 ± 1.0</td>
<td>27 ± 0.58</td>
<td>25 ± 5.0053\textsuperscript{A,C,D}</td>
<td>7.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 17 ± 0.58</td>
<td>24 ± 0.58</td>
<td>120 ± 4.93\textsuperscript{A,B}</td>
<td>34.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 17 ± 1.0</td>
<td>25 ± 0</td>
<td>120 ± 5.51\textsuperscript{A,B}</td>
<td>34.28</td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td>1 17 ± 0</td>
<td>24 ± 0</td>
<td>7 ± 2.08\textsuperscript{B,C,D}</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 16 ± 0.58</td>
<td>24 ± 0</td>
<td>65 ± 4.04\textsuperscript{A,C,D}</td>
<td>18.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 15 ± 0.58</td>
<td>20 ± 1.0</td>
<td>150 ± 2.64\textsuperscript{A,B}</td>
<td>42.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 16 ± 1.0</td>
<td>24 ± 0</td>
<td>146 ± 4.58\textsuperscript{A,B}</td>
<td>41.71</td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td>1 22 ± 1.0</td>
<td>31 ± 1.0</td>
<td>2 ± 1\textsuperscript{C,D}</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 22 ± 0.0</td>
<td>30 ± 0.58</td>
<td>1 ± 1\textsuperscript{C,D}</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 20 ± 0.58</td>
<td>28 ± 1.0</td>
<td>100 ± 4.93\textsuperscript{A,B,D}</td>
<td>28.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 21 ± 0.58</td>
<td>29 ± 0.0</td>
<td>80 ± 4.36\textsuperscript{A,B,C}</td>
<td>22.86</td>
<td></td>
</tr>
</tbody>
</table>

All substrates were inoculated with various spawn levels i.e., 1, 2, 3, and 4%, in order to determine optimum spawn level on the basis of total yield at the end of harvest. Results represent the mean ± SEM of the three independent experiments followed by different letters indicating significant differences according to Tukey-Kramer HSD (P < 0.05), n=3. SS, sheesham sawdust; MS, mango sawdust; PS, poplar sawdust; BE, biological efficiency.

### TABLE 2: Effect of Different Sawdusts on Growth Cycle and Yields of *Ganoderma lucidum*

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Complete spawn run (Days)</th>
<th>Primordia initiation (Days from spawning)</th>
<th>Yield (g/kg)</th>
<th>BE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>17 ± 0.58</td>
<td>24 ± 0.58</td>
<td>120 ± 4.93\textsuperscript{B,C}</td>
<td>34.28</td>
</tr>
<tr>
<td>MS</td>
<td>15 ± 0.58</td>
<td>21 ± 1.0</td>
<td>150 ± 2.64\textsuperscript{A,C}</td>
<td>42.86</td>
</tr>
<tr>
<td>PS</td>
<td>20 ± 0.58</td>
<td>29 ± 1.0</td>
<td>100 ± 4.93\textsuperscript{A,B}</td>
<td>28.57</td>
</tr>
</tbody>
</table>

Spawn was added to different substrates at the 3% level, exposed to spawning and cropping conditions. After maturation, fruit bodies were harvested, total mushroom yield in grams per kg was recorded. Results represent the mean ± SEM of the three independent experiments followed by different letters indicating significant differences according to Tukey-Kramer HSD (P < 0.05), n=3. SS, sheesham sawdust; MS, mango sawdust; PS, poplar sawdust; BE, biological efficiency.
sequence of decomposition of substrate components by supplements.\textsuperscript{15,16} Of three different supplements (WB, CF, and RB) at variable concentrations (10, 20, and 30%), 20% of all supplements supported higher reduction in growth cycle duration and enhanced yield and BE on various substrates, though there was no statistical difference compared with 10% and 30% supplementation. Maximum yield was obtained with a mixture of MS and WB (205 g at 20%), followed by a mixture of SS and WB.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Substrate & Supplement & Supplement Ratio (%) & Spawn run (Days) & Primordia initiation (Days from spawning) & Yield (g/kg) & BE (%) \\
\hline
SS & WB & 10 & 17 ± 0.58 & 24 ± 0.58 & 152.33 ± 3.18 & 43.52 \\
 & & 20 & 15 ± 1.0 & 20 ± 0.58 & 171.66 ± 3.84 & 49.04 \\
 & & 30 & 17 ± 1.0 & 25 ± 0.0 & 159.33 ± 2.03 & 45.52 \\
 & & 20 & 17 ± 0.0 & 21 ± 0.58 & 156.66 ± 2.60 & 44.76 \\
 & & 30 & 17 ± 1.0 & 22 ± 0.58 & 145.33 ± 3.93 & 41.52 \\
CF & & 10 & 18 ± 1.0 & 21 ± 0.0 & 136.66 ± 3.48 & 39.04 \\
 & & 20 & 17 ± 1.0 & 21 ± 0.58 & 156.66 ± 2.60 & 44.76 \\
 & & 30 & 17 ± 1.0 & 22 ± 0.58 & 145.33 ± 3.93 & 41.52 \\
RB & & 10 & 17 ± 1.0 & 23 ± 0.0 & 134.66 ± 3.84 & 38.47 \\
 & & 20 & 17 ± 1.0 & 23 ± 0.0 & 148 ± 7.64 & 42.28 \\
 & & 30 & 17 ± 0.0 & 24 ± 0.0 & 140.66 ± 7.75 & 40.19 \\
MS & WB & 10 & 13 ± 0.0 & 18 ± 0.58 & 177 ± 4.04 & 50.57 \\
 & & 20 & 13 ± 0.58 & 17 ± 0.0 & 205 ± 4.04 & 58.57 \\
 & & 30 & 13 ± 0.58 & 18 ± 1.0 & 170.66 ± 4.98 & 48.76 \\
 & CF & 10 & 14 ± 1.0 & 20 ± 0.58 & 170 ± 6.08 & 48.57 \\
 & & 20 & 14 ± 0.0 & 19 ± 0.0 & 194 ± 1 & 55.43 \\
 & & 30 & 14 ± 1.0 & 20 ± 0.0 & 165 ± 5.86 & 47.14 \\
 & RB & 10 & 15 ± 0.58 & 21 ± 1.0 & 168 ± 4.62 & 48.0 \\
 & & 20 & 15 ± 0.0 & 20 ± 0.58 & 185 ± 3.78 & 52.86 \\
 & & 30 & 16 ± 0.0 & 20 ± 1.0 & 163 ± 8.89 & 46.57 \\
PS & WB & 10 & 20 ± 0.0 & 28 ± 0.58 & 139.33 ± 1.85 & 39.80 \\
 & & 20 & 18 ± 0.58 & 25 ± 1.0 & 156 ± 4.72 & 44.57 \\
 & & 30 & 19 ± 0.0 & 27 ± 0.58 & 142.33 ± 3.76 & 40.66 \\
 & CF & 10 & 20 ± 0.0 & 28 ± 0.58 & 131.66 ± 2.90 & 37.52 \\
 & & 20 & 21 ± 0.58 & 29 ± 1.0 & 147 ± 5.51 & 42 \\
 & & 30 & 21 ± 0.58 & 29 ± 0.0 & 135 ± 7.37 & 38.57 \\
 & RB & 10 & 20 ± 0.58 & 29 ± 0.58 & 122.66 ± 4.70 & 35.04 \\
 & & 20 & 20 ± 0.58 & 30 ± 1.0 & 144 ± 3.78 & 41.14 \\
 & & 30 & 21 ± 1.0 & 30 ± 0.0 & 136.66 ± 3.84 & 39.04 \\
\hline
\end{tabular}
\caption{Effect of Combinations of Various Sawdusts and Supplement Doses on Growth Cycle and Yield of \textit{Ganoderma lucidum}}
\end{table}

Sawdusts and supplements were mixed in different proportions (90:10, 80:20, and 70:30) for cultivation trials. SS, sheesham sawdust; MS, mango sawdust; PS, poplar sawdust; WB, wheat bran; CF, corn flour, RB, rice bran; BE, biological efficiency. Results represent the mean ± SEM of the three independent experiments followed by different letters indicate significant differences according to Tukey-Kramer HSD (P < 0.05), n=3.
Effect of Cost-Effective Substrates on Growth Cycle and Yield of Ganoderma lucidum from India

The major findings of the current study are that shorter growth cycle and higher yield and BE in G. lucidum can be obtained with mango sawdust supplemented with 20% wheat bran spawned at the 3% level. Cultivation of G. lucidum may become one of the most inexpensive and profitable agri-businesses, producing medicinal and food products with agro-waste materials, and helping dispose of agricultural residues in an environmentally friendly manner. Thus, these findings may be useful in many mycoremediation applications.

ACKNOWLEDGMENTS

The authors wish to thank the Department of Science and Technology, Govt. of India, for financial support (SR/FT/LS-888/2009 dated 04-09-2009) and Shoolini University of Biotechnology and Management Sciences, Solan (HP), for providing facilities.

REFERENCES
