Saudi Journal of Biological Sciences 28 (2021) 2709–2717

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Growth performance of *Ganoderma lucidum* using billet method in Garhwal Himalaya, India

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ARTICLE INFO

Article history:
Received 16 February 2021
Revised 6 March 2021
Accepted 8 March 2021
Available online 23 March 2021

Keywords:
Mushroom
Ganoderma lucidum
Billet method
Growth
Fruiting bodies

ABSTRACT

Medicinal mushrooms have been used in various treatments from a very long time, among which, *Ganoderma lucidum* is one of the most important medicinal mushroom. It is cultivated worldwide to meet its ever-increasing demand in the market. It is generally cultivated by bed log (Sawdust) and wood log (billet) method. This study was an attempt to observe the growth performance of *G. lucidum* on poplar billets (*Populus deltoides*) in the Sherpur Village (Dehradun) and Manjgaun village (Tehri Garhwal) of Garhwal Himalaya, India. The farmers' field with empty house/ rooms having proper growing conditions especially humidity and light were used for the cultivation of *G. lucidum*. The *G. lucidum* spawn was inoculated in poplar wood billets and these billets were installed in well prepared soil. The results demonstrated that cropping cycle of *G. lucidum* was shorter (132–136 days) in Sherpur Village (Dehradun) as compared to Manjgaun village (141–145 days) in Tehri Garhwal. Further the results also revealed that yield was decreased in the subsequent flushes. In Village Sherpur, the fruiting bodies of *G. lucidum* were harvested between 64-66 days, 100-101 days and 135-136 days during first, second and third flush after the installation of billets, respectively. However; in village Manjgaun, the fruiting bodies of *G. lucidum* were harvested between 69 and 71 days, 107-108 days and 144-145 days in first, second and third after the installation of billets respectively. Warmer temperature in Village Sherpur resulted in the early emergence and development of the fruiting bodies as compared to village Manjgaun where pinhead and fruiting body development was delayed due to the lower temperature during cropping cycle.

1. Introduction

Ganoderma lucidum or Lingzhi is a fungus belonging to the Polyporaceae family of class Basidiomycetes. It is a wood decaying pathogenic fungus associated with the root and butt rot of the trees. It has been acknowledged and being used significantly in Chinese and Japanese culture since 2000 years (Sliva, 2006). The name *Ganoderma* came from Greek word 'ganos' which means shining and 'derma' meaning skin as the fruiting body of this mushroom is bright and shiny (Liddell and Scott, 1980). It's fruiting body is called "Lingzhi", Chi–zhi" or "Rui–zhi" in China, "Reishi", "Munnertake" or "Sachitake" in Japan and "Youngzhi" in Korea

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.03.030
1319-562X/

(Wagner et al., 2003; Paterson, 2006). This medicinal mushroom has gained popularity worldwide due to its usage as health tonic to strengthen the body since thousands of years. Due to the presence of various medicinal properties, this mushroom is often called as "Mushroom of Immortality", "God of herbs", "Herb of spiritual potency" and "Celestial Herb" (Wasser, 2005; Lin, 2009; De Silva et al., 2012, Al-Obaidi et al., 2016). The fruiting body, spores and mycelium of this mushroom are sold and used globally for manufacturing of various edible, pharmaceutical and cosmetic products. Commonly, the fruiting body of *G. lucidum* is kidney shaped with thick, irregular margins and dark brown in colour (Singh et al., 2014). This mushroom is distributed worldwide and just like other mushrooms, it prefers hot and humid climate.

The presence of >400 bioactive compounds like- polysaccharides, nucleosides, alkaloids, polypeptides steroids, fatty acids, inorganic elements and triterpenoids makes this mushroom useful in the treatment of various ailments. These bioactive compounds possess pharmacological properties like anti-viral, anti-bacterial, immunomodulation and anti-ageing etc. (Sanodiya et al., 2009). The collection of this mushroom from wild was difficult and unable to meet the increasing demand of its raw material in global market so the attempts to artificially cultivate this mushroom started. The first successful cultivation of *G. lucidum* was done in Institute of Microbiology, Chinese Academy of Sciences, Beijing in 1969 (Yu & Shen, 2003). Since then, several attempts have been made with significant results in different parts of the world. This study is an attempt toassess the growth performance of *G. lucidum* fruiting bodies on farmer's field in the villages of Garhwal Himalaya with the short wood log cultivation method commonly known as 'billet method'.

2. Material and methods

2.1. Study site

The present investigation was carried out at two study sites in Garhwal Himalaya: Village Sherpur in Dehradun district and Village Manjgaun in Tehri district of Uttarakhand (India). The village Sherpur (Vikasnagar block, Dehradun, India) is spread between 30°32′N and 77°81′E with an altitude of 618 m above the mean sea level, while the village Manjgaon (Jaunpur block, Dehradun) is located at 30°36′N and 78°25′ with an altitude of 1625 m above the mean sea level. During the study, the average day temperature of village Sherpur was 32 ± 3 °C, whereas it was 28 ± 3 °C in village Manjgaun.

2.2. Preparation of pure culture

For the experiment, the pure culture of *G. lucidum* was prepared from its fruiting body. The fruiting body was incised into small pieces and was inoculated in Potato Dextrose Agar (PDA) medium in a Petri-dish at room temperature (25 ± 3 °C) (Ayodele et al., 2007; Suberu et al., 2013). The mycelia of *G. lucidum* took 7–8 days to properly colonize on the PDA medium and were ready for the preparation of spawn.

2.3. Preparation of spawn

The wheat grains were used as substrate for the preparation of *G. lucidum* spawn. For this, one kg wheat grain was boiled and filled in the polypropylene bags. In order to maintain the pH, 1 g lime along with 0.5 g gypsum was added to the boiled wheat grain. These polypropylene bags were sealed with cotton plug by using polyvinyl chloride (PVC) tube and neck of the bags were covered with sterile brown paper and tied with rubber. The bags were kept in autoclave for one hour at 121 °C and 1.5 kg/cm^2 pressure for sterilization and subsequently transferred to aseptic chamber for cooling. The pure culture of *G. lucidum* was inoculated in sterilized bags by removing the cotton plug inside the laminar air flow to avoid any chance of contamination. The inoculated bags were cotton plugged and placed in B.O.D. incubator for 15 days at 25 ± 1 °C temperature (Azizi et al., 2012; Singh et al., 2014).

2.4. Ganoderma lucidum cultivation

The billet method was opted in this study for the cultivation of *G. lucidum* fruiting bodies using methodology mainly of (Singh et al., 2014). The wood logs of *Populus deltoids* were debarked and cut down to 10 to 15 cm. length and 4 to 5 cm thickness. diameter which are also called 'billets'. These billets were soaked overnight in 1% malt solution. The billets were further tied and kept in polypropylene bags for sterilization and autoclaved (Fig. 1). The

Fig. 1. Sterilization of debarked poplar billets inside polypropylene bags.

Fig. 2. Ready to install *Ganoderma lucidum* mycelium run billets.

Fig. 3. *Ganoderma lucidum* mycelium run billets installed in soil.

(Zhou, 2017)was also taken into consideration during this experiment.The pressure of 1.5 kg/cm^2 was maintained in steam sterilizer for 1.5–2 h. After sterilization, the bags were kept for cooling in a chamber for a day. Later, *G. lucidum* spawn was inoculated in these bags under laminar air flow. The spawn at the rate of 20 gm/bag was used for the inoculation after which the bags were tied. The incubation of these billets for spawn run was done at 26 ± 1 °C and 60–70% RH for 15 days. A good mycelial growth was supplemented by providing proper ventilated and sanitized conditions (Fig. 2).

Table 1
Different stages of *Ganoderma lucidum* cultivation (First Flush) in district Dehradun (Village Sherpur) and district Tehri Garhwal (Village Manjgaun).

Pinhead Formation			Cap Formation			Fruiting Body Development			Harvest		
No. of Days from billets installed	**No. of billets with pinheads (Sherpur)**	**No. of billets with pinheads (Manjgaun)**	**No. of Days from billets installed**	**No. of billets with cap formation (Sherpur)**	**No. of billets with cap formation (Manjgaun)**	**No. of Days from billets installed**	**No. of billets with fruiting bodies (Sherpur)**	**No. of billets with fruiting bodies (Manjgaun)**	**No. of Days from billets installed**	**No. of billets harvested (Sherpur)**	**No. of billets harvested (Manjgaun)**
15	5		**43**	3		**60**	2		**64**	20	
16	9		**44**	5		**61**	5		**65**	23	
17	11		**45**	9		**62**	6		**66**	16	
18	11		**46**	15		**63**	11		**67**		
19	14	8	**47**	18	5	**64**	14		**68**		
20		13	**48**		6	**65**	12	3	**69**		19
21		12	**49**		11	**66**		7	**70**		21
22		17	**50**		13	**67**		9	**71**		10
			51		15	**68**		10			
						69		12			
						70		9			

Table 2
Different stages of *Ganoderma lucidum* cultivation (Second Flush) in district Dehradun (Village Sherpur) and district Tehri Garhwal (Village Manjgaun).

Pinhead Formation			Cap Formation			Fruiting Body Development			Harvest		
No. of Days from first harvest	**No. of billets with pinheads (Sherpur)**	**No. of billets with pinheads (Manjgaun)**	**No. of Days from first harvest**	**No. of billets with cap formation (Sherpur)**	**No. of billets with cap formation (Manjgaun)**	**No. of Days from first harvest**	**No. of billets with fruiting bodies (Sherpur)**	**No. of billets with fruiting bodies (Manjgaun)**	**No. of Days from first harvest**	**No. of billets harvested (Sherpur)**	**No. of billets harvested (Manjgaun)**
73	9		89	5		96	6		100	27	
74	13		90	9		97	8		101	14	
75	19		91	13		98	15		102		
76			92	8		99	9		103		
77			93	6		100	3		104		
78			94			101			105		
79			95			102			106		
80		12	96		6	103		4	107		25
81		18	97		11	104		3	108		12
82		7	98		10	105		12			
			99		7	106		11			
			100		3	107		7			

Table 3
Different stages of *Ganoderma lucidum* cultivation (Third Flush) in district Dehradun (Village Sherpur) and district Tehri Garhwal (Village Manjgaun).

Pinhead Formation			Cap Formation			Fruiting Body Development			Harvest		
No. of Days from second harvest	**No. of billets with pinheads (Sherpur)**	**No. of billets with pinheads (Manjgaun)**	**No. of Days from second harvest**	**No. of billets with cap formation (Sherpur)**	**No. of billets with cap formation (Manjgaun)**	**No. of Days from second harvest**	**No. of billets with fruiting bodies (Sherpur)**	**No. of billets with fruiting bodies (Manjgaun)**	**No. of Days from second harvest**	**No. of billets harvested (Sherpur)**	**No. of billets harvested (Manjgaun)**
108	8		125	4		131	4		135	20	
109	11		126	12		132	8		136	14	
110	15		127	7		133	13		137		
111			128	6		134	5		138		
112			129	5		135	4		139		
113			130			136			140		
114			131			137			141		
115			132			138			142		
116		7	133			139			143		
117		11	134		4	140		5	144		18
118		10	135		6	141		5	145		10
			136		11	142		11			
			137		4	143		4			
			138		3	144		3			

2.5. Billets installation and fruiting body management

Well drained sandy soil was mixed with 1% lime and left for two days for soil solarization. The mycelium run were distributed to the farmers under this project out of which, a sample of 50 billets in each study site was critically observed for the growth performance of *G. lucidum*. These billets were then installed in this soil vertically with one end at level surface but covered with thin layer of soil (Fig. 3). Proper soil moisture and adequate ventilation was maintained throughout the season and the temperature was regularly recorded. These installed billets were kept in dark until the emergence of pinheads after which proper light conditions were provided. After the maturation of fruiting bodies and spore shedding, the fruiting bodies were harvested with the help of sharp knife. These fruiting bodies were harvested, dried, measured, weighed, packed and stored in air tight bags in cool and dry place. *2.6.*

2.6. Growth Performance of G. lucidum

The growth performance of *G.lucidum* was recorded in terms of number of days required to attain different growth stage *viz.* pinhead formation, cap formation, fruiting body development and maturation in various flushes at both the study sites. Further, the growth parameters such as stipe length, diameter and weight of the individual fruiting body were recorded after harvest in each flush. The performance of different growth stages (pinhead formation, cap formation, fruiting body development and maturation) in the installed billets were observed in relation to temperature at both the study sites.

3. Results

3.1. Ganoderma lucidum cultivation at Village Sherpur, Dehradun

After installing the billets having *G. lucidum* mycelium in the cropping room, the pinheads started to emerge on 15th day, gradually increased and continued to emerge till the 19th day (Table 1, Figs. 4 and 9a). The cap formation took place between 43 and 47 days of billets installation (Figs. 5 and 9b). At this stage, the top of the antlers started flattening and white coloured caps appeared on top of the antlers. After this stage, the cream coloured fruiting bodies started to develop with flat and tough top. Later, the fruiting body became oval or kidney bean shaped with brown colour spreading towards its margins (Fig. 6). The fruiting bodies were completely developed after 60–65 days when they turned into dark brown in colour (Figs. 7 and 9c). These fruiting bodies were left for 1–2 days for spore shedding after which they were harvested (Fig. 8). The matured fruiting bodies were harvested after 64–66 days of billets installation in first flush at Sherpur village

Fig. 4. *Ganoderma lucidum* pinhead formation stage.

Fig. 5. *Ganoderma lucidum* Cap Formation stage.

Fig. 6. *Ganoderma lucidum* fruiting body development.

Fig. 7. *Ganoderma lucidum* mature fruiting body ready to harvest.

Fig. 8. Weighing of harvested *Ganoderma lucidum* fruiting bodies.

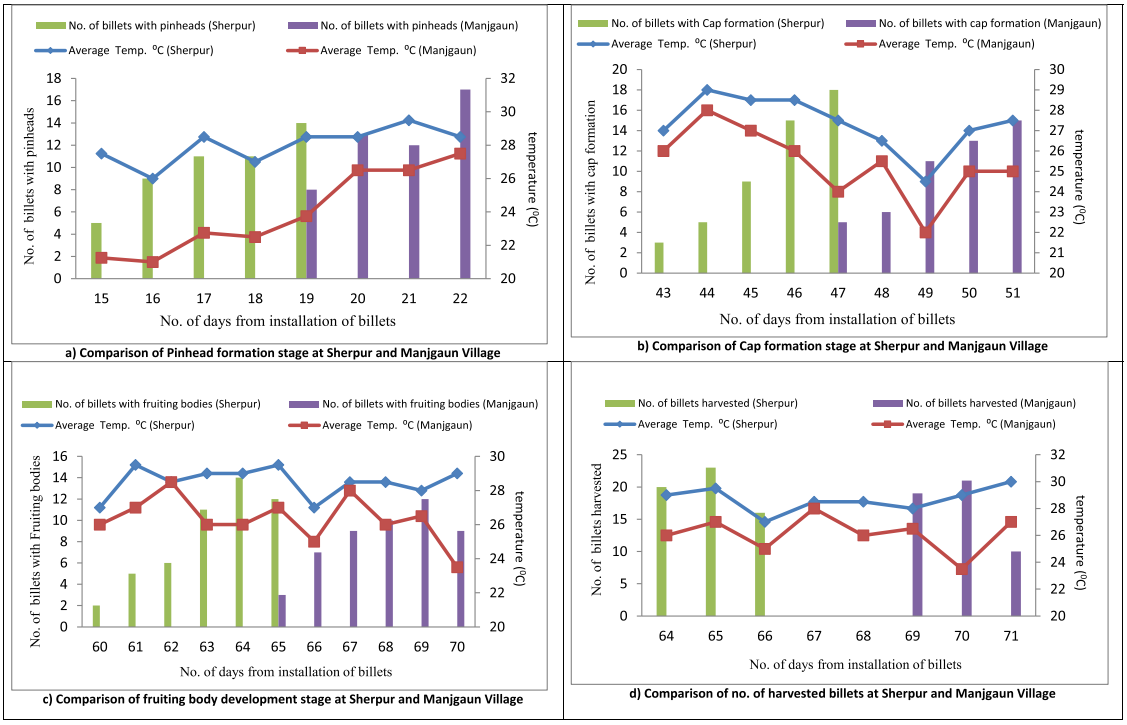

Fig. 9. Different stages of *Ganoderma lucidum* cultivation (First Flush) in district Dehradun (Village Sherpur) and district Tehri Garhwal (Village Manjgaun).

(Table 1 and Fig. 9d). A total of 58 numbers of *G. lucidum* fruiting bodies weighing 160.1 gm (dry weight) were produced from 50 billets in the first harvest (Table 4). The average weight of one fruiting body was 3.2 gm with average stipe length of 2.29 cm and 3.7 cm fruiting body diameter (Table 4). The second pinning started 73 days after installation of billets and continued till 75th day (Table 2 and Fig. 10a). Out of 50 billets, only 41 billets were able to produce pinheads in the second flush. The cap formation and fruiting body development in these billets took place from 89 to 93 days and 96–100 days after billets installation, respectively (Table 2, Fig. 10b & c). A total of 43 number fruiting bodies weighing 116.7 gm (dry weight) were produced from 41 billets in the second harvest (Table 5). The average weight of one fruiting body was 2.85 gm with average stipe length of 2.15 cm and 3.65 cm fruiting body diameter (Table 5).

During the third flush, pinheads emerged on 108 days and continued till 110th day after installation of billets (Table 3 and Fig. 11a). Out of 50 billets, only 34 billets produced pinheads. The cap formation and fruiting body development took place between 125 and 129 days and 131–135 days after installation of billets, respectively (Table 3, Fig. 11b & c). A total of 34 numbers of *G. lucidum* fruiting bodies weighing 86 gm (dry weight) were harvested in the third flush (Table 6). The average weight of fruiting body was 2.53 gm with stipe length of 2.28 cm and 3.89 cm fruiting body diameter (Table 6). In village Sherpur, the crop cycle of *G. lucidum* through billet method was 132–136 days (excluding the time taken for the spawn preparation or spawn run). A total of 362.8 g dried fruiting bodies of *G. lucidum* was produced from 50 billets.

3.2. Ganoderma lucidum cultivation at Village Manjgaun, Tehri Garhwal

In contrast to Sherpur village, the pinheads emerged later in Manjgaun village on 19th day and continued till 22nd day of billets installation (Table 1 and Fig. 9a). The cream coloured small pinheads turned dark brown towards the cap formation stage which took place between 47 and 51 days after installation of billets (Table 1 and Fig. 9b). The fruiting body developed between 65 and 70 days after installation of billets with the tip of cap becoming flat and kidney bean shaped (Table 1 and Fig. 9c). The matured fruiting bodies were harvested between 69 and 71 days of billet installation (Table 1 and Fig. 9d). A total of 56 numbers of *G. lucidum* fruiting bodies weighing 151.2 gm (dry weight) were harvested from 50 billets in the first harvest (Table 4). Average weight of fruiting body was 3.02 gm with an average stipe length of 2.42 cm and 4.28 cm average fruiting body diameter (Table 4). In the second flush, pinhead emergence and cap formation took place between 80 and 82 days and 97–100 days after installation of billets, respectively (Table 2, Fig. 10a & b). Further, the development of fruiting bodies took place between 103 and 107 days after installation of billets (Table 2 and Fig. 10c). A total of 39 fruiting bodies of *G. lucidum* weighing 101 gm (dry weight) were harvested from 37 billets in the second harvest (Table 5). The average weight of fruiting body was 2.73 gm with stipe length of 2.11 cm and 3.92 cm fruiting body diameter (Table 5).

During third flush, the pinheads emerged between 116 and 118 days after installation of billets (Table 3 and Fig. 11a). Only 28 out of 50 billets were able to produce pinheads in the third flush. Following pinhead formation, the cap formation took place between 134 and 138 days after installation of billets (Table 3 and Fig. 11b). The fruiting body development started on 140th day and continued till 144th day after installation of billets (Table 3 and Fig. 11c). These fruiting bodies were matured and harvest on 144 and 145 days after installation of billets (Table 3 and Fig. 11d). A total of 28 numbers of *G. lucidum* fruiting bodies weighing 64.3 gm (dry weight) were produced from 37 billets in the third harvest (Table 6). Average weight of fruiting body was 2.3 gm with stipe length of 2.27 cm and 3.91 cm fruiting body diameter (Table 6). In village Manjgaun (Tehri Garhwal), the crop cycle of *G. lucidum* was 141–145 days excluding the time taken for the spawn preparation or spawn run. A total of G. lucidum dried fruiting body production from 50 billets was 316.5 gm in three flush.

Table 4
Ganoderma lucidum cultivatiom data for the first flush.

	Village Sherpur (Dehradun)			Village Manjgaun (Tehri Garhwal)		
S. No.	**Weight of fruiting body (g)**	**Diameter Of fruiting body (cm)**	**Stipe length (cm)**	**Weight of fruiting body (g)**	**Diameter Of fruiting body (cm)**	**Stipe length (cm)**
1	3.5	2.5	1.5	2.5	5	2.5
2	4.5	2.5	1	3.5	6.5	3.8
3	2.8	4	3.5	3	5	3
4	4	2.5	4	2.5	3	3
5	3.2	5	2.8	6	6	2
6	4	2.5	1.5	4	2.5	2
7	5	1	2	4.5	2.5	0.5
8	3.2	2.5	1.2	3	5	2
9	3.4	4	2.5	2.5	3	2
10	3	4.6	1	2	3	2
11	2.6	2.5	3	4.5	5	2.7
12	3	4	1	3	6	3
13	3.5	4.5	4	2	3.8	1
14	2.6	3.5	1.2	3	4	2
15	3	4	4	4	3	3
16	3	1	2	4	7	3
17	3.5	3	1	1.8	2	2
18	3.7	3.5	3	1.8	4	3
19	3.2	2.2	3	2.5	2	3
20	2.7	4	2.5	1.7	3	2
21	3.6	4.5	1	1.6	3	2
22	2.7	5	3	4	5	3
23	3.2	4.8	3	3.5	3.5	1/2
24	3	5	1	4	5.5	3
25	2.8	4.1	0.5	3	5	4
26	3.5	4.5	1	3	5	4
27	4	2	2	2.5	5	3.6
28	2.7	3.8	3	3	4	2
29	2.8	4	0.5	7	5	1
30	2.3	4	3	2	4	3
31	2.8	4	2	1.6	2	1
32	2.4	4.5	3	1.6	4	0.5
33	3.5	4	2.5	3.5	4	1
34	2.8	4.5	3	3	4	2.2
35	2.5	6	1.5	2.5	5	3
36	4.5	3	1.5	3	5	0.5
37	3.4	4	3	3.6	6	3
38	3	4	3.3	3.5	6	2
39	4.5	2.3	1	3	5	4
40	3.2	3.5	2	2	4	2
41	2.4	4	3.4	4	4	3
42	2.5	5	3.2	2	6	0
43	2.3	4.5	3.5	4	5	3
44	2.7	4	2.4	3.5	5.5	4
45	2.8	4	1.1	3.5	4	2
46	3.5	4	3.5	2	5	4
47	3	2.5	1.1	4	5	2
48	2.5	4.5	3.5	1.5	4	2
49	4	2.8	3	1.5	2	3
50	3.8	5	3.3	2.5	2	3.5
Avg.	**3.20**	3.70	2.29	3.02	4.28	2.42

4. Discussion

Temperature is an important factor that determines the mycelial growth of fungi. The results of the present study indicated that growth of *G. lucidum* was temperature dependent. For the optimum growth of most of the mushrooms, the higher temperature favours mycelial invasion whereas fruiting body forms at relatively lower temperature (Dawit, 1998; Oei, 2003). The pinhead and cap formation was early in Sherpur village as compared to Manjgaun village due to warmer temperature in the Sherpur village leading to faster mycelial growth on the billets. These findings were similar to the results reported by Tesfaw et al. (2015) who also reported early pinhead and cap formation at higher temperature. Alam et al. (2010) demonstrated the maximum mycelia growth and pinhead formation at 25^{0}C which gradually decreased with the lower temperature. In the village Sherpur, early pinhead and cap formation subsequently led to more fruiting body development. Although, lower temperature triggers mycelial growth but temperature downshift induces fruiting body formation in most of the mushrooms (Stamets, 2000; Sakamoto, 2018). However, too low temperature delays and reduces fruiting body formation. The optimum downward shift of temperature at Sherpur village resulted in early and more fruiting body formation as compared to delayed and less fruiting body development at Manjgaun village due to lower temperature during the developmental period. (Sakamoto, 2018) demonstrated that temperature downshift induces fruiting body formation by expression of specific proteins. There was also a difference in cropping cycle and fruiting body production in both the study sites possibly due to the variation in the diurnal temperature. The cropping cycle in village Sherpur (Dehradun) was shorter with increased yield as compared to village Manjgaun (Tehri Garhwal) possibly due to prevailing warmer climate. Maxi-

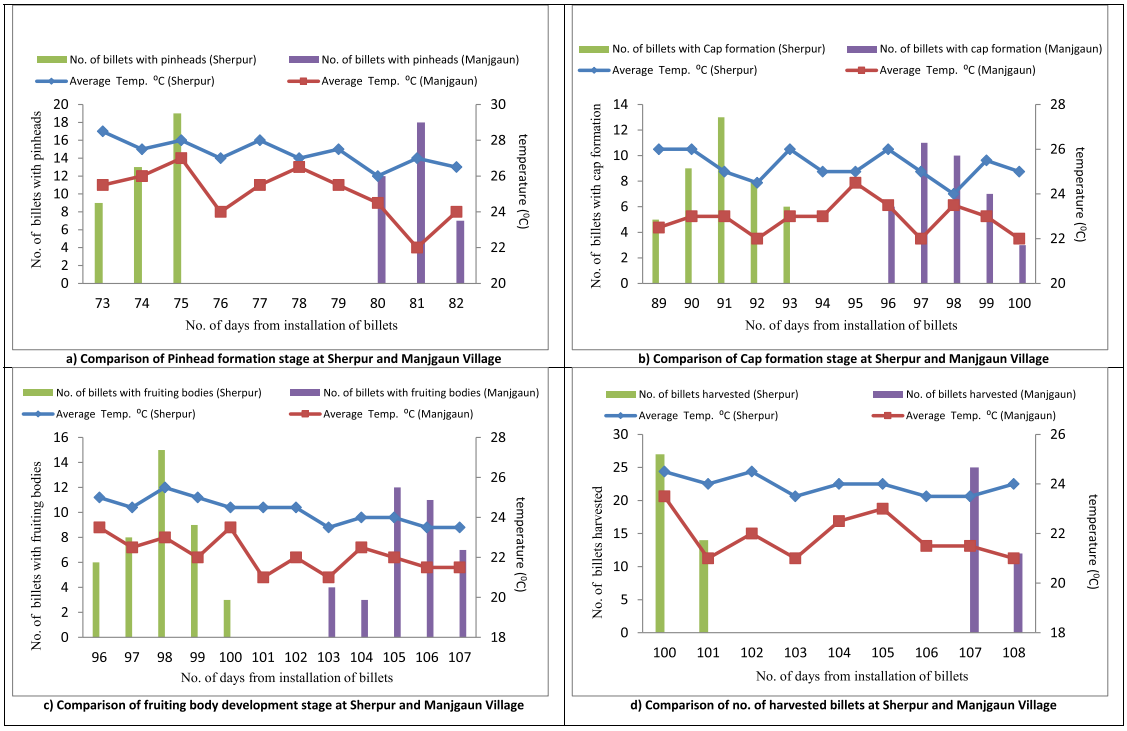

Fig. 10. Different stages of *Ganoderma lucidum* cultivation (Second Flush) in district Dehradun (Village Sherpur) and district Tehri Garhwal (Village Manjgaun).

Table 5
Ganoderma lucidum cultivatiom data for second flush.

	Village Sherpur (Dehradun)			Village Manjgaun (Tehri Garhwal)		
S. No.	**Weight of fruiting body (g)**	**Diameter Of fruiting body (cm)**	**Stipe length (cm)**	**Weight of fruiting body (g)**	**Diameter Of fruiting body (cm)**	**Stipe length (cm)**
1	2.5	4.5	2	3.4	5	2
2	2	4	2	3.2	5	2.2
3	2.8	4	0.5	2.5	4.6	3
4	2.8	4	1.8	3	3	0.5
5	3.5	5.5	2.2	2	4	1
6	4	4	3	3	4	1.5
7	2.2	3	3	2.6	3.5	1.5
8	3.5	2.5	2.2	3	4.5	1
9	2.5	3	2	2.5	4	2
10	3	4.5	1.5	3.2	2.5	3
11	4	3.8	1	3	3	1
12	2.8	4	2.5	2.2	4.5	0
13	3.5	2	2.7	1.9	3.5	5
14	2.5	4.5	3.5	2.6	4	2
15	2.2	3	3.2	3.5	4	1
16	2.5	2.5	1.5	3	5.7	2
17	3	4	3.9	3.5	4	3
18	3	4	0.5	1.8	3	2
19	2.5	4	1	3.4	4	3.5
20	3.2	4	1.8	2	2.5	3.5
21	2	2.5	1	2.5	3	1.5
22	2	3.4	3	2.5	3.8	1.5
23	3	4.6	2	2.2	3.5	2
24	2	3	2	3.5	5	1.5
25	3.5	5.2	3	2.2	4.5	1
26	3	5	2	2	4	3
27	2.7	4.5	2	2.8	2.8	3
28	3.5	3	3	3.4	3	2
29	3	3.5	3	1.8	2.5	2.5
30	3	2.2	3	3.5	5	2
31	2	2.8	4	2.8	6	2.5
32	3.2	4	1	3.3	4.5	3
33	3.4	2.5	2	2.8	5.5	2
34	3.4	4.2	3	2.5	4	3.5
35	3.6	3.2	1.2	3.5	2.9	1.5
36	2.5	4	1	1.9	2.8	3
37	2	5	3	2.5	4	2
38	3.2	5	2			
39	2.5	2.5	2.8			
40	2.2	2.8	1			
41	3	2	1.5			
Avg.	**2.85**	**3.65**	**2.15**	**2.73**	**3.92**	**2.11**

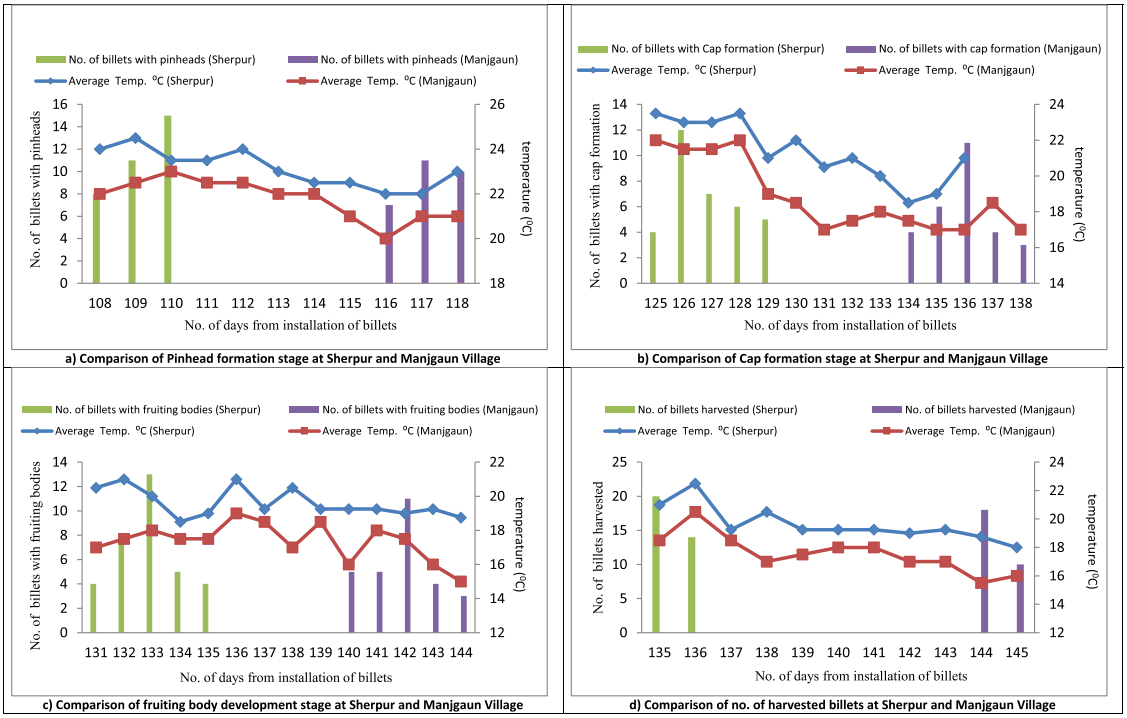

Fig. 11. Different stages of *Ganoderma lucidum* cultivation (Third Flush) in district Dehradun (Village Sherpur) and district Tehri Garhwal (Village Manjgaun).

Table 6
Ganoderma lucidum cultivatiom data for third flush.

	Village Sherpur (Dehradun)			Village Manjgaun (Tehri Garhwal)		
S. No.	**Weight of fruiting body (g)**	**Diameter Of fruiting body (cm)**	**Stipe length (cm)**	**Weight of fruiting body (g)**	**Diameter Of fruiting body (cm)**	**Stipe length (cm)**
1	2.2	2.5	1.5	1.9	3	1
2	3.5	5	1	2	2.5	0.5
3	2.9	4	0.5	2.2	3	3.5
4	2.8	2.5	3	1.9	2.8	3
5	3.1	3.8	0.5	2.6	4.6	0.5
6	1.2	5.5	3	2.2	3	1
7	1.9	4	4	1.9	4	1
8	2.4	5.7	3	2.6	5.7	5
9	2	4	0.5	3.5	4	3
10	2.8	3	1	3	3	2.5
11	3	4	2	2.2	4	3
12	2.5	2.5	2	3.5	2.5	4.5
13	2.1	2.5	2.5	2.2	5.5	3.5
14	3.5	3	2	1.5	2.5	3
15	2.2	3	2.5	1.5	5	0.5
16	1.9	2.5	3	2	6	3
17	2.6	4	1	2.2	3	0.5
18	3	2.5	5	2	2.5	1
19	2.2	5	2	3.5	5	2
20	3.5	6	3.5	1.2	4	2.5
21	2.8	5.7	1.5	1.9	3	2
22	2	4	4	2.6	5	2
23	4	3	4	2	6	3.5
24	3.5	4	3	3	2.5	1.5
25	2.5	2.5	0.5	1.9	3.8	2.5
26	3	5.5	3	2.3	5.5	2
27	2.8	4.2	3.5	2	4.2	2.5
28	1.1	4	3	3	4	3
29	2	5	0.5			
30	2.1	4.6	1			
31	1.9	3.5	2.5			
32	2.2	3	2			
33	3	3.3	2.5			
34	1.8	5	3			
Avg.	**2.53**	**3.89**	**2.28**	**2.30**	**3.91**	**2.27**

mum yield of *G. lucidum* (3.7–20.37% BE) was obtained within 57 days by Kaur et al., 2015 and Erkel, 2009. Like other mushrooms, the yield of *G. lucidum* continuously decreased with the consequent flushes. Average weight of a single fruiting body also decreased with each flush in both of the study sites. The highest yield was obtained during the first flush followed by second and third flush. The decreased yield in the consequent flushes can be attributed to less number of fruiting body development along with reduced availability of nutrients from the substrate in the later flushes.

5. Conclusion

Owing to its medicinal value, *G. lucidum* is gaining popularity across the world. The present study revealed that the growth performance of *G. lucidum* in both the study sites mainly dependent on temperature resulted higher fruiting body development in the warmer site having shorter cropping cycle under billet method. Considering, temperature as major factor for the growth of this medicinally and economically important mushroom, the billet method can be an effective method for its cultivation.

CRediT authorship contribution statement

Arvind Bijalwan: Conceptualization, Supervision, Methodology, Validation, Formal analysis, Investigation, Data curation, Resources, Writing - original draft, Writing - review & editing. **Kalpana Bahuguna:** Conceptualization, Supervision, Methodology, Validation, Formal analysis, Investigation, Data curation, Resources, Writing - original draft, Writing - review & editing. **Amol Vasishth:** Conceptualization, Investigation, Writing - review & editing. **Alankar Singh:** Conceptualization, Investigation, Writing - review & editing. **Sumit Chaudhary:** Conceptualization, Investigation, Writing - review & editing. **Ankit Dongariyal:** Writing - review & editing. **Tarun Kumar Thakur:** Writing - review & editing. **Sandeep Kaushik:** review & editing. **Mohammad Javed Ansari:** review & editing. **Saleh Alfarraj:** Writing - review & editing. **Sulaiman Ali Alharbi:** Writing - review & editing. **Milan Skalicky:** review & editing. **Marian Brestic:** review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are extremely thankful to National Mission on Himalayan Studies (NMHS), GB Pant National Institute of Himalayan Environment, Almora, Ministry of Environment, Forest and Climate change, Government of India for awarding the project ‘‘Adoption of Medicinal and Edible Mushroom integrated with climate resilient interventions for up-scaling livelihoods in Garhwal Himalaya” (Project ID- NMHS/2018-19/SG61/61) vide sanction letter No. NMHS/2018-19/SG 7/212. The authors are grateful to the Vice Chancellor and University authorities of VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Uttarakhand, India for all support and encouragement. The authors are thankful to Prof. S.P. Singh, President, Center for Business Development and Entrepreneurial Development (CBED) and Dr Anil Tyagi, Director, CBED, Dehradun, Uttarakhand, India for guidance and all support. The authors are highly thankful to Dr. N.S.K. Harsh, former Scientist, Forest Research Institute, Dehradun, India and advisor and consultant CBED, Dehradun, Uttarakhand, India for technical support and his time-to-time guidance for *G. lucidum* cultivation. The researchers and beneficiaries/farmers and all supporters involved in this study are deeply acknowledged for their support and co- operation. This research was also supported by Researchers Supporting Number (RSP-2021/7) King Saud University, Riyadh, Saudi Arabia. The authors also express their gratitude to the anonymous reviewer for improving the quality of this manuscript.

References

Alam, N., Lee, J., Lee, T., 2010. Mycelial growth conditions and phylogenetic relationships of Pleurotus ostreatus. World App. Sci. J. 9 (8), 928–937.
Al-Obaidi, J.R., Saidi, N.B., Usuldin, S.R., Hussin, S.N., Yusoff, N.M., Idris, A.S., 2016. Comparison of different protein extraction methods for gel-based proteomic analysis of ganoderma spp. Protein J. 35, 100–106.
Ayodele, S.M., Akpaja, E.O., Anyiador, F., 2007. Evaluation of the yield of *Lentinus squarrosulus*. Pak. J. Bio. Sc. 10, 4283–4286.
Azizi, M., Tavana, M., Farsi, M., 2012. Yield performance of lingzhi or reishi medicinal mushroom, Ganoderma lucidum (W.Curt.:Fr.) P. Karst. (higher basidiomycetes), using different waste materials as substrates. Int. J. Med. Mushroom. 14, 521–527.
Dawit, A., 1998. Mushroom Cultivation: A Practical Approach. Birhannaselam Printing Enterprise, Addis Ababa.
De Silva, D.D., Rapior, S., Fons, F., 2012. Medicinal mushrooms in supportive cancer therapies: an approach to anti–cancer effects and putative mechanisms of action. Fungal Div. 55, 1–35.
Erkel, E.I., 2009. Yield performance of *Ganoderma lucidum* (Fr.) Karst cultivation on substrates containing different protein and carbohydrate sources. African J. Agri. Res. 4 (11), 1331–1333.
Kaur, H., Sharma, S., Khanna, P.K., Kapoor, S., 2015. Evaluation of *Ganoderma lucidum* strains for the production of bioactive components and their potential use as antimicrobial agents. J. App. Nat. Sc. 7 (1), 298–303.
Liddell, G.H., Scott, R.A., 1980. Greek-English Lexicon. Oxford University Press, United Kingdom.
Lin, Z.B., 2009. Lingzhi: From Mystery to Science. Peking University Medical Press, Beijing, pp. 1–162.
Oei, P., 2003. Mushroom Cultivation: Appropriate Technology for Mushroom Growers. Backhuys Publishers.
Paterson, R.R.M., 2006. Ganoderma a therapeutic fungal bio factory. Phytochem. 67, 1985–2001.
Sakamoto, Y., 2018. Influences of environmental factors on fruiting body induction, development and maturation in mushroom-forming fungi. Fungal Bio. Rev..
Sanodiya, B.S., Thakur, G.S., Baghel, R.K., Prasad, G.B., 2009. *Ganoderma lucidum*: a potent pharmacological macrofungus. Curr. Pharma Biotechnol. 10, 717–742.
Singh, S., Harsh, N.S.K., Gupta, P.K., 2014. A novel method of economical cultivation of medicinally important mushroom, *Ganoderma lucidum*. Int. J. Pharma Sci. Res. 5, 2033–2037.
Sliva, D., 2006. *Ganoderma lucidum* in cancer research. Leukemia Res. 30, 767–768.
Stamets, P., 2000. Growing gourmet and medicinal mushrooms. Int. J. Med. Mushrooms. 2.
Suberu, H., Bello, A., Yusuf, D., 2013. Mycelia biomass yield of *Ganoderma lucidum* Mushroom by submerged culture. Nigerian J. Tech. Res. 8.
Tesfaw, A., Tadesse, A., Kiros, G., 2015. Optimization of oyster (*Pleurotus ostreatus*) mushroom cultivation using locally available substrates and materials in Debre Berhan, Ethiopia. J. App. Biol. Biotech. 3 (01), 015–020.
Wagner, R., Mitchell, D.A., Sassaki, G.L., 2003. Current techniques for the cultivation of *Ganoderma lucidum* for the production of biomass, ganoderic acid and polysaccharides. Food Technol. Biotechnol. 41, 371–382.
Wasser, S.P., 2005. Reishi, or Ling Zhi *(Ganoderma lucidu*m). In: Coates, P.M., Blackman, M.R., Cragg, G.M., Levine, M., Moss, J., White, J.D. (Eds.), Encyclopedia of dietary supplements. Marcel Dekker, New York, pp. 603–622.
Yu, Y.N., Shen, M.Z., 2003. The history of Lingzhi *(Ganoderma* spp.) cultivation. Mycosyst. 22, 3–9.
Zhou, X., 2017. Cultivation of *Ganoderma lucidum*: technology and applications. Edible Med. Mushrooms., 385–413