Mycelial Growth and Fruit Body Formation of Lyophyllum decastes in Livestock Compost

Pokhrel, Chandra Prasad
Laboratory of Forest Resource Management,Division of Ecosphere Sciences and Management,Graduaate School of Bioresource and Bioenvironmental Sciences,Kyushu University

Yoshimoto, Hiroaki
Laboratory of Forest Resource Management,Division of Ecosphere Sciences and Management,Graduaate School of Bioresource and Bioenvironmental Sciences,Kyushu University

Iida, Shigeru
Laboratory of Forest Resource Management,Division of Ecosphere Sciences and Management,Department of Forest and Forest Products Sciences,Faculty of Agriculture,Kyushu University

Ohga, Shoji
Laboratory of Forest Resource Management,Division of Ecosphere Sciences and Management,Department of Forest and Forest Products Sciences,Faculty of Agriculture,Kyushu University

https://doi.org/10.5109/4587

出版情報：九州大学大学院農学研究院紀要. 49 (2), pp.273-282, 2004-10-01. Faculty of Agriculture, Kyushu University

J. Fac. Agr., Kyushu Univ., **49** (2), 273–282 (2004)

Mycelial Growth and Fruit Body Formation of *Lyophyllum decastes* in Livestock Compost

Chandra Prasad POKHREL[1], Hiroaki YOSHIMOTO[1], Shigeru IIDA and Shoji OHGA*

Laboratory of Forest Resource Management, Division of Ecosphere Sciences and Management, Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, Fukuoka 811–2415, Japan

(*Received June 30, 2004 and accepted July 13, 2004*)

Eight composted substrates were evaluated for mycelial growth, spawn run and the fruit body formation of *Lyophyllum decestes*. The experiments were carried out on livestock bedding materials such as sawdust, bark dust and rice husk after being composted. The composted livestock species were cow, horse, pig and chicken. Composted materials were supplemented. Cow livestock composts were the best materials examined in the study.

INTRODUCTION

Mushroom is a good source of protein, minerals and vitamins, which are essential for nutritional purposes (Khan and Kausar, 1981). Not only food it is valuable for medicines, besides them it has great important in forestry, agriculture and environmental aspect. The production of mushroom is constantly increasing throughout the world because of their high demand, the total world output 6,161,000 t of edible mushroom in 1997 (Royse *et al.*, 2004). High quality mushroom, more efficient cultivation methods and cost effective alternative substrates are the present needs in the commercial production of mushroom.

Lyophyllum decastes (Hatakeshimeji) a gray Basidiomycetes, is an edible and medicinal fungus which is of the same genus as *L. shimeji*, is a rot fungus and the mycelium from its fruit body is connected with the buried wood in the ground (Imazeki and Hongo, 1987). It is more delicious than *Pleurotus ostreatus* and *Hypsizygas marmoreus*. It was found that the antitumor activity of Hatakeshimeji was the highest (Ukawa, *et al.*, 2000), the decrease in the number of leukocytes due to X–ray irradiation was eased by the medication of Hatakeshimeji (Gu *et al.*, 2002). Regarding its nutritional and medicinal value it must be set at a higher value in the market. Technology for the artificial cultivation of Hatakeshimeji in pots has been developed (Hara *et al.*, 1990) and recently cultivated mushrooms have appeared on the market (Ukawa *et al.*, 2000).

The selection of improved strains, new and cost effective cultivation techniques and substrates are the basic for the successful commercial mushroom cultivation. Biological efficiency has been improved by optimizing various factors (Ohga *et al.*, 2001). Superior

[1] Laboratory of Forest Resource Management, Division of Ecosphere Sciences and Management, Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University
* Corresponding author (E–mail: ohgasfor@mbox.nc.kyushu–u.ac.jp)

spawn to be one of the best way to improves crop yield and better quality fruit body without increasing cost of production. Cheap and easily available substrates even waste products are also important factors in mushroom cultivation. Various studies have been conducted to find out the suitable substrate for the cultivation of shiitake mushroom (Ito, 1978; Han *et al.*, 1978; Royse, 1985), farmers are perpetually searching for alternative substrates that may be more rapidly available or cost effective (Royse *et al.*, 2004).

The purpose of present investigation was the observation of mycelial growth and fruit body formation in different substrates. Various livestock compost were evaluated for suitability of practical cultivation of *L. decastes*.

MATERIALS AND METHODS

Organism

A commercial strain of *L. decastes* was used for experiment. The isolated strain was maintained in Potato Dextrose Agar (PDA, Nissui) at Kyushu University Laboratory. The PDA medium stock colonies were bored with cork borer (4.2 mm in diameter) and each bored piece was inoculated on prepared media.

Material preparation

The experiments were conducted in three kinds of animal bedding materials made of agriculture and forestry by–products (waste) sample such as rice husk, sawdust and bark dust. The collected samples were used as a bedding material for livestock species such as

Table 1. Name, composted livestock, source, physical properties and chemical composition of compost.

Sample No.	1	2	3	4	5	6	7	8
Livestock	Cow	Cow	Pig	Chicken	Horse	Pig*	Cow	Cow
Substrate	Sawdust(F)	Bark	Sawdust	Husk	Sawdust	Sawdust	Husk	Sawdust(M)
Source	Fukuoka Ken	Fukuoka Ken	Miyagi Ken	Miyagi Ken	Miyagi Ken	Miyagi Ken	Miyagi Ken	Miyagi Ken
Water (%)	–	–	22.9	29.3	52.0	23.8	67.1	63.6
Ash (%)	–		21.9	32.9	12.4	17.8	34.0	16.5
PH	–	–	8.17	8.83	8.73	7.96	8.75	9.16
EC mS/cm	–	–	4.10	5.72	1.28	2.75	1.23	1.89
Nitrogen (%)	–	–	4.24	4.08	1.65	2.99	1.53	1.78
Carbon (%)	–	–	38.8	33.5	43.1	41.9	32.2	42.7
C/N ratio	–	–	9.2	8.2	26.2	14.0	21.0	23.9
Ammonia (ppm)	–	–	889.9	800.0	624.0	1399.8	469.8	359.9
P_2O_5 (%)	–	–	4.01	4.39	0.31	3.13	1.59	1.44
K_2O (%)	–	–	2.69	4.07	0.86	1.28	1.30	2.50
CaO (%)	–	–	5.45	8.01	0.92	5.01	1.49	3.15
MgO (%)	–	–	2.36	2.31	0.23	1.34	1.03	1.45
Copper (ppm)	–	–	209.4	86.7	14.8	178.3	13.7	26.3
Zink (ppm)	–	–	547.7	416.1	43.5	375.8	142.3	129.4

*Special fermented

cow, chicken, horse and pig. The comparative mycelial growth performance and fruit body formation were evaluated in bedding materials after being composted. (Bedding materials were used in pens (bed) or directly contact with livestock about 10 cm height for a week, took out and then left for a year for composting). The cow compost sawdust and bark dust were shived and other samples were grinded for experiment (only for mycelial growth). During the experiment composted bedding materials were supplemented by PDA medium and broad leaved sawdust based (with 15% wheat and barley bran). Composted samples were collected directly from two different companies: one from Fukuoka and another from Miyagi. Source, name of materials, composting livestock species, physical and chemical composition of used samples are shown in Table 1.

Experimental design and conditions

The experiment was designed as PDA medium and sawdust–based substrate (broad leaved sawdust with 15% wheat and barley bran) supplements. Mycelial growth and fruit body formation were observed.

Firstly, mycelial growth was examined in different percentages of sample 1 (sawdust) and 2 (bark dust). The percentage levels of samples were 0, 0.5, 0.7, 1 and 2% with PDA medium. The ingredients were mixed with distilled water and autoclaved at 120 °C for 40 min. The percentage levels of samples with sawdust based supplements were 0, 25, 50 and 75%, tap water added to give a final moisture contain of 60%. The substrate of 100 g (40 g dry wet substrate and 60 g water) were autoclaved at 120 °C for 40 min and then cooled. The ratio of content of various samples and supplements for mycelial growth and bag cultivation are shown in Table 2. Each Petri dish was inoculated from previously maintained spawn. The inoculated Petri dishes were incubated at 23 °C. After obtaining the results, other six samples (3–8) were experimented in 1% sample with PDA and 50% with sawdust based supplements.

Fruit body formation was experimented (only in sawdust based supplements) in bag cultivation. 2.5 kg wet substrate was packed in polypropylene bag, was sterilized using an autoclave and inoculated with sawdust spawn. The culture bags were kept in the incubation room at 23 °C. The completely colonized substrate bag was removed and transferred to the production room. Temperature was maintained at 17 °C, relative humidity was kept at 90% and light intensity was 500 lux in 12 hrs interval per day. Fruit body was picked when fully mature and weighed.

Table 2. Contain of sawdust substrate testing for various livestock compost.**

Sample ratio %	gram	Sawdust g (dw) (Broad leaved)	Wheat bran (dw) gram	Barley bran (dw) gram
0		34	3	3
25	10	24	3	3
50*	20	14	3	3
75	30	4	3	3

** Mycelial growth: Each petridish contain100 g. wet weight (40 g. dry substrate),
** Final moisture contain was 60%
* Various eight livestock compost used for fruit body formation, weight was 2.5 kg, content ratio of each bag was same as Table 2.

Measurement of colony

Data on mycelial growth was taken on PDA medium and sawdust based substrate after 16 and 14 days, respectively. The diameter of colonies were measured by verneairs calipers. Spawn run days and fruit body formation were observed and recorded. The experiments were conducted in triplicates to minimize the errors.

RESULTS AND DISCUSSION

Cow livestock

First, cow livestock composts were examined for vegetative mycelial growth of Hatakeshimeji. Two samples were used, one was based on sawdust and the rest one was bark dust. The influence of mycelial growth on different percentages of cow compost with PDA medium supplement is shown in Fig. 1.

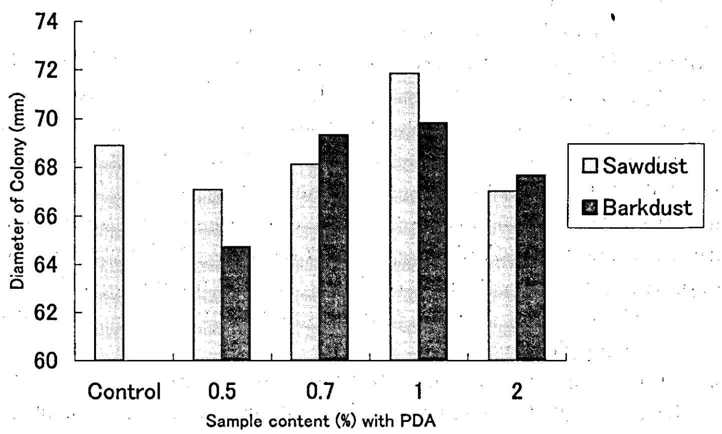

Fig. 1. Influence on vegetative mycelial growth of *L. decastes* in different percentages of cow livestock compost with PDA supplement (16 days).

The mycelial growth was greater than that of control and best in 1% substrate content with PDA medium in both samples followed by 0.7, 2, 0.5% in sawdust and 0.7, 0.5, 2% in bark dust, but 0.7% was also better than control in bark dust.

Influence of mycelial growth on different percentages of cow livestock compost with sawdust based supplement is shown in Fig. 2. Mycelial growth was the best in 50% followed by 75 and 25% with sawdust based supplements in both samples, but all percentages were better than control. Based on the results obtained, it is thought that 1% with PDA medium and 50% with sawdust based supplements were sufficient and comparatively better for promoting vegetative mycelial growth. However, further research was conducted in other six various livestock species samples with both supplements. The vegetative mycelial growth in different percentages with PDA medium and sawdust based supplements are shown in Figs. 3 and 4, respectively.

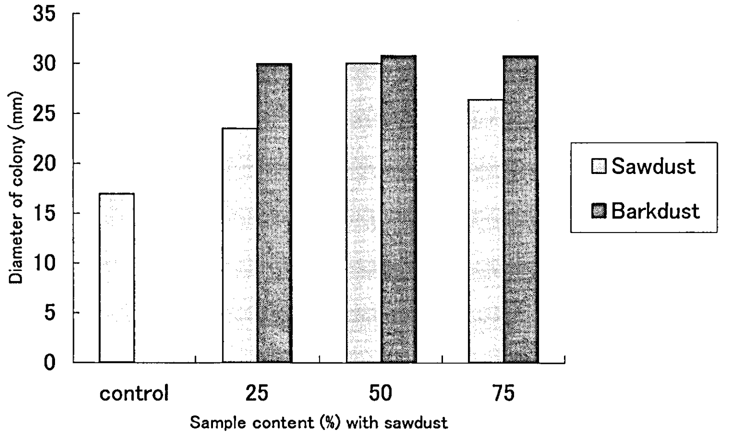

Fig. 2. Influence on vegetative mycelial growth of *L. decastes* in different percentages of cow livestock compost with sawdust based supplements (14 days).

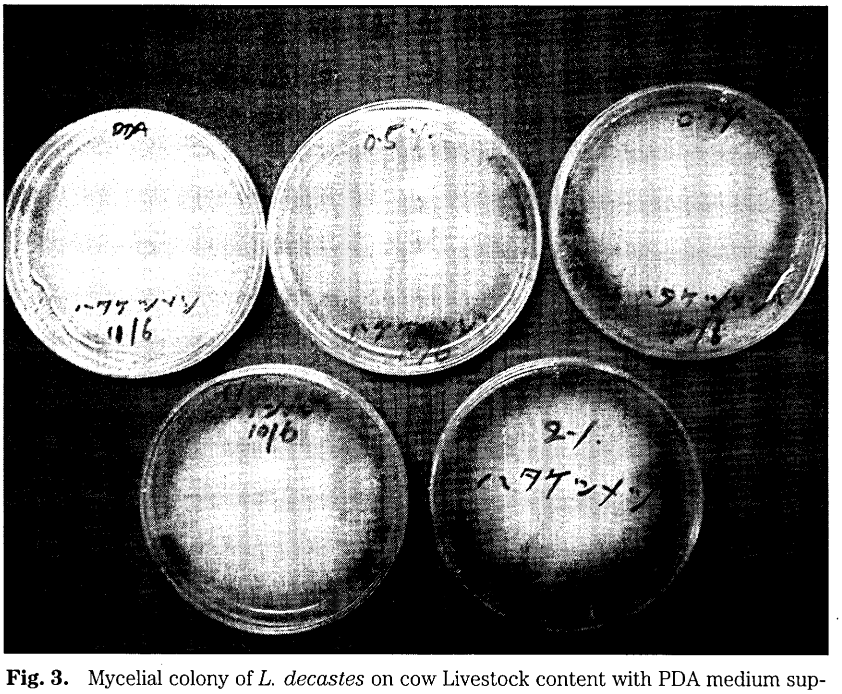

Fig. 3. Mycelial colony of *L. decastes* on cow Livestock content with PDA medium supplement after 16 days.

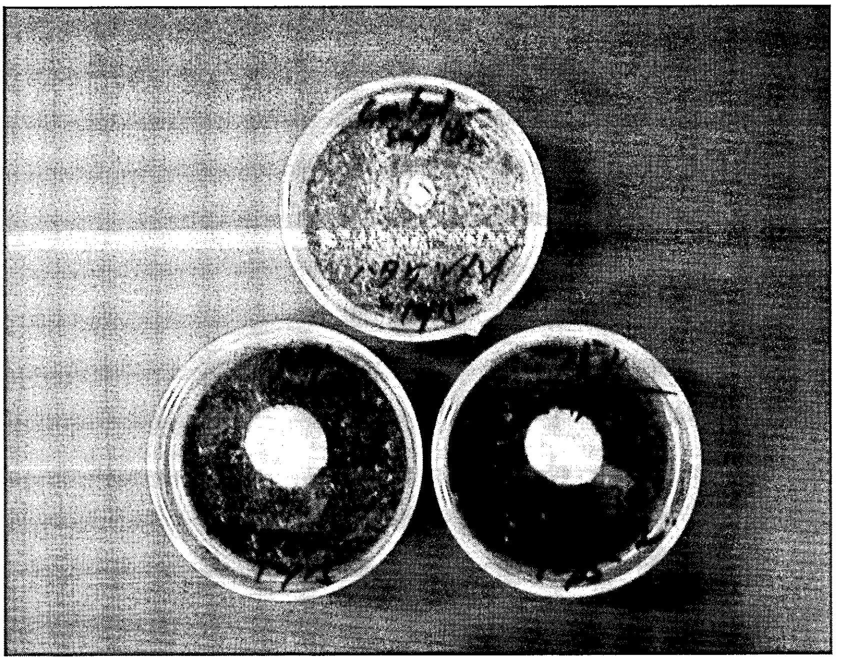

Fig. 4. Mycelial colony of *L. decastes* on cow Livestock content with sawdust based supplements after 14 days.

Various eight livestock

The influence on vegetative mycelial growth in 1% substrate of eight different kinds of samples with added PDA supplement is shown in Fig. 5. The maximum mycelial growth was found in 1% sample content with PDA supplement in rice husk bedding materials compost (cow) followed by bark dust, sawdust (Fukuoka (F) and Miyagi (M)) and sawdust bedding material compost (pig). They showed high inhibition effect for mycelial growth compared with those of control and other samples. There was no significant difference in mycelial growth between control and composted samples with supplement. Mycelial growth was almost identical in all samples and to the control.

Cow composted rice husk, barkdust, sawdust (F and M) and sawdust compost (pig) showed the stronger promotive effect on mycelial growth with PDA medium. Differences in nutritional physiology were suggested to reflect mycelial growth (Arai *et al.*, 2003). Kitamoto *et al.* (1994) reported that rapidly growing species rely on mycelium and medium nutrients, whereas slow growing species depend almost entirely on nutrients in mycelia for fruit body development. Mycelial growth was significantly inhibited in cow composted rice husk, sawdust, bark dust and sawdust compost (pig). Based on the results, mycelial growth was the best in all kinds of cow bedding material composts. Therefore this result showed that cow livestock composts were good performance for mycelial growth with PDA medium. Mycelial growth in rice husk compost (chicken), sawdust compost (horse), and sawdust compost (pig) was poorer than that of control.

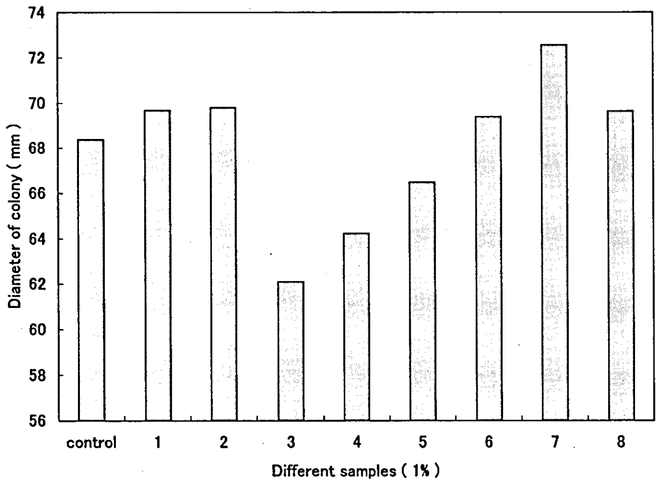

Fig. 5. Influence on vegetative mycelial growth of *L. decastes* in 1% sample content of different livestock species with PDA medium supplement (16 days).

They were not significant in mycelial growth.

The influence on vegetative mycelial growth in 50% substrate of eight different kinds of samples with added sawdust based supplements is shown in Fig. 6. The optimum mycelial colony was grown in rice husk bedding material compost (cow) followed by sawdust (F), bark dust, sawdust compost (pig), sawdust compost (cow M), and sawdust compost (horse). Mycelial growth was superior than that of control in all substrates. They showed the high inhibition effect on mycelial growth. The mycelial growth was poorer than that of control in rice husk compost (chicken) and sawdust compost (pig).

Cow composted rice husk, sawdust (F), barkdust, sawdust (M), sawdust compost (pig) and sawdust compost (horse) showed stronger promotive effect on mycelial growth with sawdust based supplements. Mushroom use glucose as their primary carbon source, but when the glucose and nitrogen concentration are high in the medium, mycelial growth is inhibited (Azuma and Kitamoto, 1994; Boyle, 1998). Livestock bedding materials after being composted seems to be having enough nutrients for mycelial growth of *L. decastes*. Mycelial growth was almost identical in sawdust compost (F) (cow), bark dust compost (cow) and sawdust compost (Pig). They were superior than sawdust compost (cow) (M) and sawdust compost (horse). Rice husk was comparatively best for mycelial growth among all. Based on the results, mycelial growth was better in all kinds of cow bedding material composts. Mycelial run was approximately double in all six samples compare to the control. This result showed that cow livestock composts were especially good inhibitor for mycelial growth, spawn preparation and fruit body formation of *L. decastes*. Mycelial growth in husk compost (chicken) and sawdust compost (pig) was poorer than that of control. Rice husk compost (chicken) and sawdust compost (pig) were not significant in mycelial growth, both were not promotor for mycelial growth.

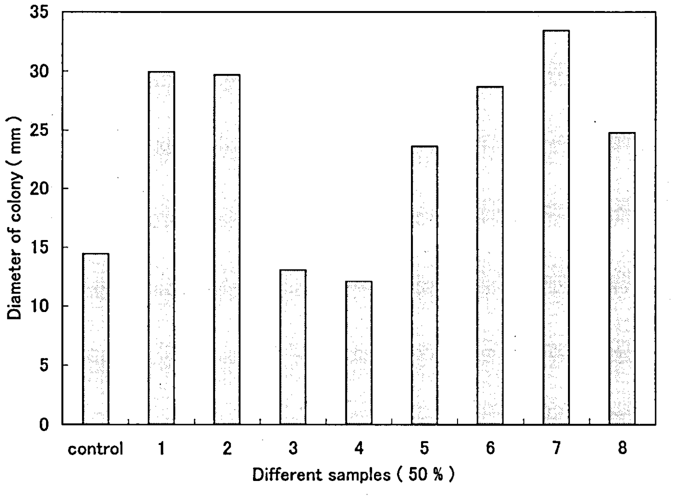

Fig. 6. Influence on vegetative mycelial growth of *L. decastes* in 50% sample content of different livestock species with sawdust based supplements (16 days).

Vegetative mycelial growth and spawn preparation are very important for successful mushroom cultivation and high yield. Fruiting ability of mushrooms reflect to substrate constitutions (Royse, 1985; Ohga, 1990) and length of vegetative stage (Royse and Bahler, 1986; Ohga *et al.*, 1992). Spawn of *L. decastes* was completely observed after 60 days incubation at 23°C in cow sawdust with sawdust based supplements. To optimize mushroom yield, the incubating culture must be at the proper stage of maturity when induce to shift from the vegetative phase to the fruiting phase (Ohga and Donoghue, 1998). Maximum yield (weight of fresh mushroom harvested at maturity) was obtained in sawdust compost followed by bark dust and rice husk of cow compost. All samples were able to produce fruit body more than that of control. The yield of mushroom per 2.5 kg wet substrate in various livestock compost is given in Fig. 7 and fruit body of *L. decastes* is shown in Fig. 8.

In the present study, the influence of mycelial growth was promotive in 5 different samples of bedding material composts with PDA medium and 6 with sawdust based supplements. Rice husk compost (cow) was the highest performance on mycelial growth with both supplements and cow composted substrates were comparatively best for mycelial growth. It is showed that cow livestock was not only inhibitor on mycelial growth but also improve the nutrients for fruit body formation of *L. decastes*. There was no significant relationship between chemical composition and mycelial growth, because the samples were supplemented. The colony diameter with PDA medium was larger than that of sawdust based supplements. Growing pattern of mycelium in both supplements was mostly identical. Poor mycelial growth is not able to grow sufficient fruit body. Poor mycelial growth was observed in 3 samples with PDA and 2 with sawdust based supplements.

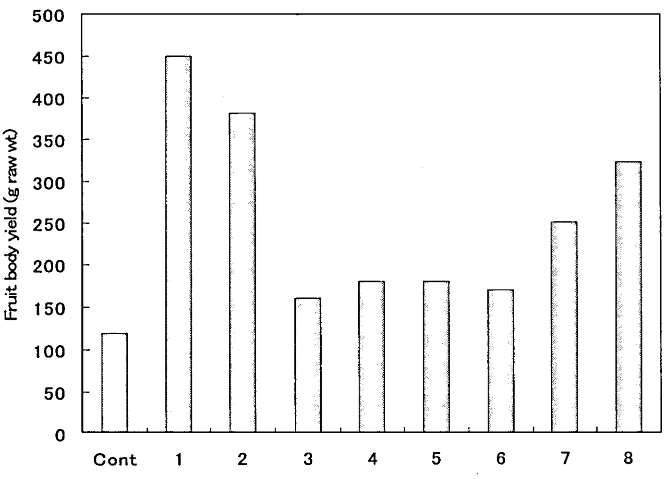

Fig. 7. Fruit body yield g raw wt. per 2.5 kg. in various livestock compost substrate.

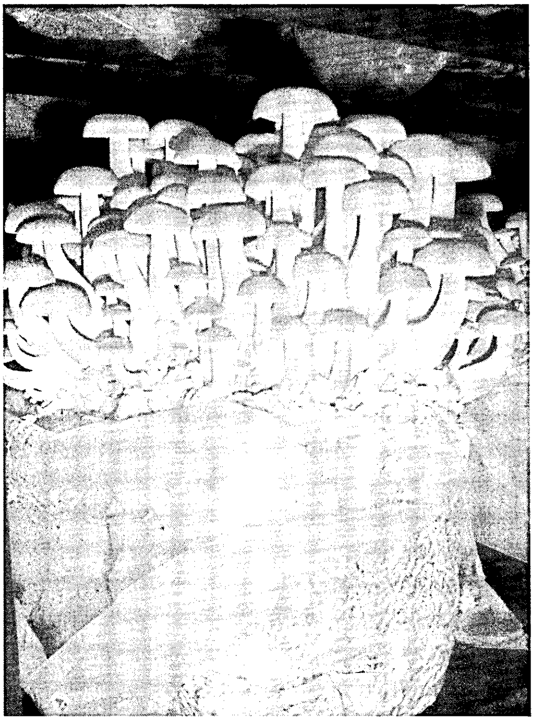

Fig. 8. Fruit–body formation of *L. decastes* in maintained experimental condition.

Fruit yield was better than control in all composted samples. Cow composted sawdust, bark dust and rice husk were comparatively better for yield. Fruit yield on rice husk compost (chicken) and sawdust compost (horse and pig) was comparatively identical but poorer than that of cow composted substrates. Cow compost sawdust and bark dust were comparatively superior than rice husk for yield, but unexpectedly mycelial growth was higher in rice husk. Yield increases may be due to several factors (Royse *et al.*, 2004). Excessive use of supplement is only support for mycelial growth but not fruit body yield. Rice husk was best only for mycelial growth.

On the basis of results mycelial growth and fruit body formation were successful in the study. The livestock species compost of bedding materials, especially cow livestock in our experiments seems to influence significantly to the waste substrate utilization for vegetative and fruit–body formation of *L. decastes*.

REFERENCES

Arai, Y., M. Takao, R. Sakamoto, K. Yoshikawa and T. Terashita 2003 Promotive effect of the hot water–soluble fraction from corn fiber on vegetative mycelial growth in edible mushroom. *J. Wood Sci.*, **49**: 437–443

Azuma, S. and Y. Kitamoto 1994 Nutritional environment for mycelial growth and fruit body formation in *Lentinus edodes* (in Japanese). *Mushroom Sci. Biotechnol.*, **1**: 7–13

Boyle, D. 1998 Nutritional factors limiting the growth of *Lentinus edodes* and other white–rot fungi in wood. *Soil. Biol. Biochem.*, **30**: 817–823

Gu, Y., Y. Ukawa, S. Park, I. Suzuki, K. Bamen and T. Iwasa 2002 Radiation protection effect on Hatakeshimeji (*Lyophyllum decastes* sing.). *Int. Congress Ser.*, **236**: 495–499

Han, Y. H., W. T. Veng, L. C. Chen and S. Cheng 1978 Physiology and ecology of *Lentinus edodes*. *Mush. Sci.*, **11**: 387–388

Hara, H., T. Sonoda and M. Itoh 1990 Japan Kokai Tokkyo Koho, H2–41295 (Feb. 23)

Imazeki, R. and T. Hongo 1987 *Colored illustration of mushroom of Japan.* Hoikusha, Osaka

Ito, T. 1978 Cultivation of *Lentinus edodes*. *In* "the biology and cultivation of edible mush". ed. by O. Chang and O. Hayes, Academic Press, New York, pp. 461–467

Khan, S. M. and A. G. Kausar 1981 Prospects of mushroom culture in Pakistan. *Mushroom Sci.*, **11**: 50–55

Kitamoto, Y., T. Horikoshi and A. Suzuki 1994 Physiological chemistry of fruit body development on basidiomycetes (in Japanese). *Protein Nucleic acids Enzyme*, **16**: 276–278

Ohga, S. 1990 Effect of nutritional components on mycelial grwoth and fruit body yield of the shiitake mushroom, *Lentinus edodes*, on sawdust substrate. *J. Fac. Agr. Kyushu Univ.*, **34**: 405–412

Ohga, S. and J. D. Donoghue 1998 Evaluation of methods for determining microbial activity and maturity of sawdust–based culture of shitake (*Lentinula edodes*). *Mushroom. Sci. and Biotech.*, **6**: 115–123

Ogha, S., S. Iida, C–D. Koo and N. S. Cho 2001 Effect of electric impulse on fruit body production of *Lentinula edodes* in the sawdust–based substrate. *Mush. Sci. and Biotech.*, **9**: 7–12

Ohga, S., F. V. Roozendael, M. Aspinwall and M. Miwa 1992 Yield and size response of the shitake mushroom *Lentinus edodes*, depending on incubation time on sawdust–based culture. *Trans Mycol. Soc. Japan*, **33**: 349–357

Royse, D. J. 1985 Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. *Mycologia*, **77**: 756–762

Royse, D. J. and C. C. Bahler 1986 Effect of genotype, spawn run time, and substrate formulation on biological efficiencies of shitake. *Appl. Environ. Microbiol.*, **52**: 1425–1427

Royse, D. J., T. W. Rhodes, S. Ohga and J. E. Sanchez 2004 Yield, mushroom size and time to production of *Pleurotus cornucopiae* (Oyster Mushroom) grow on switch grass substrate spawned and supplemented at various rates. *Biores. Tech.*, **91**: 85–91

Ukawa, Y., H. Ito and M. Hisamatsu 2000 Antitumor Effect (1→3)–β–D–Glucan and (1→6)–β–D–Glucan purified from newely cultivated mushroom Hatakeshimeji. *J. Biosci. Bioeng.*, **90**: 98–104