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Physico-chemical properties and characterization of thermophilic flora from button mushroom compost prepared using SMS of different mushroom as supplement

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ABSTRACT

The white button mushroom (*Agaricus bisporus*) is widely cultivated worldwide using a substrate composed of wheat straw, horse manure, chicken manure, gypsum, and urea. In this study, spent mushroom compost was also used in varying quantities for button mushroom compost preparation. Compost preparation occurs in two phases, both significantly involving thermophilic fungi. These fungi affect the growth of mushroom mycelia and mushroom yield in three distinct ways i.e. they reduce the concentration of ammonia in the compost, immobilize nutrients in a form that is apparently available to the mushroom mycelia and support the overall composting process. Four fungal species were isolated from the compost samples using the serial dilution method and were further identified by the National Centre of Fungal Taxonomy, New Delhi. The four species associated with *Agaricus bisporus* compost formation are *Aspergillus niger*, *Aspergillus fumigatus*, *Scytalidium thermophilum*, and *Torula compostis*. *Scytalidium thermophilum* dominated the fungal biota of the compost, while *Torula compostis*, a newly identified species, plays a significant role in the first phase of composting.

Keywords: *Agaricus bisporus*¸ compost, microscopic characters, fungal biota

Mushrooms are the product of transforming inedible waste into edible biomass. They are increasingly being accepted as a food of high quality, flavor and nutritional value. *Agaricus bisporus* alone accounts for a little more than one-third of the world's total mushroom production (Chang and Buswell, 1996). This species is grown on a specially prepared substrate, known as compost, which becomes a selective medium for its growth. In India, *Agaricus bisporus* and *Pleurotus* sp. are the two main edible fungi exploited for large-scale production. The profitability of the mushroom industry depends on the use of an economically prepared selective growth medium that maximizes mushroom yields. *Agaricus bisporus* is cultivated on a selectively prepared medium called "synthetic compost" or more commonly "straw-based compost," where wheat straw constitutes the bulk of the substrate. The waste left after *Agaricus bisporus* cultivation is referred to as "spent compost," while the waste left after harvesting *Pleurotus* sp. is called "spent straw" or "*Pleurotus* waste." Typical spent mushroom compost contains 1 to 2% nitrogen, in the form of mushroom mycelium, unused supplements, and non-decomposed fibrous raw material components. Till (1962) concluded that significant nutrients remained in spent mushroom compost (SMC) that could be utilized by the mushrooms after the crop was terminated, sterilized,

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re-spawned, and re-supplemented. So spent mushroom substrate were utilized for the preparation of button mushroom compost. The purpose of composting is to prepare a substrate that promotes the growth of mushroom mycelia while excluding other microorganisms. The compost is prepared through an aerobic process facilitated by the fermentation activities of various microorganisms. Among the microorganisms, thermophilic fungi play a key role and contribute significantly to the preparation of compost (Seal and Eggins, 1976; Eicker, 1977; Ross and Harris, 1983). The effects of these fungi on the growth of mushroom mycelia and mushroom yield have been described at three distinct levels (Wiegant, 1992). First, they decrease the concentration of ammonia in the compost, which would otherwise inhibit the growth of the mushroom mycelium. Second, they immobilize nutrients in a form that is apparently available to the mushroom mycelia. Third, they may have a growth-promoting effect on the mushroom mycelia. The aim of the present investigation was to isolate the thermophilic fungi present in the compost prepared by using spent mushroom compost through short method of composting.

MATERIALS AND METHODS

Compost Preparation

Different formulations of compost were prepared by using spent mushroom compost in different quantities. There were five treatments in the study. The details are given in Table 1. The compost was prepared by standard short method of composting (Sinden and Hauser, 1953).

All the treatments were evaluated for quality parameters defined for a good compost i.e. pH, Electrical conductivity, Organic carbon, Nitrogen (%), Moisture (%) and bulk density.

Physical properties (pH, EC, Moisture, bulk density) of the compost samples

pH of compost was measured with the help of a pH meter as described by the standard procedure given by Jackson (1973). EC of the compost samples were measured as per the standard procedure given by Jackson (1973). Moisture content of compost were determined by Gravimetric method as per the standard procedure given by Black (1965). Bulk density was measured following the method given by Singh (1980).

Chemical properties (Organic Carbon and nitrogen) of the compost samples

Organic Carbon of compost samples were measured as described by Walkley and Black (1934) chromic acid titration method while nitrogen per cent were measured following Macrokjeldahl digestion and distillation method following the procedure given by Jackson (1973).

Table 1. Formulation for button mushroom compost preparation using SMS of button and oyster mushroom

Ingredients	Treatment1 (Control)	Treatment 2	Treatment 3	Treatment 4	Treatment 5
Wheat straw	1000kg	800kg	1000kg	1000kg	1000kg
Chicken manure	500kg	390kg	390kg	390kg	390kg
Wheat bran	70kg	120kg	120kg	120kg	120kg
Gypsum	40kg	35kg	35kg	35kg	35kg
Urea	14.5kg	12kg	12kg	15kg	12kg
SMS (button)	—	445kg	200kg	300kg	—
SMS (oyster)	—	—	—	—	200kg

Isolation of thermophilic fungi

One kg of compost was randomly sampled in 100 g portions, thoroughly mixed, and used for isolation. Thermophilic fungi were isolated from different composting phases, namely Phase-1 and Phase-2, using the serial dilution method on Emerson's modified Yeast Starch Agar (YpSs) plates (Yeast extract, 4.0 g; K_2HPO_4, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; Soluble starch, 15 g; Agar, 20.0 g; Distilled water, 750 ml; Tap water, 250 ml) supplemented with Streptomycin and Rose bengal at 50 mg/L each. In this method, 10 grams of compost sample were placed in a 250 ml Erlenmeyer flask containing 90 ml of sterile water and shaken on a rotary shaker for 1 hour. Various dilutions (10^{-2}, 10^{-3} and 10^{-4}) were used for the isolation of thermophilic fungi. Plates were then incubated at 45°C in the dark and screened daily for up to 5 days. Representative isolates were purified and maintained on YpSs agar slants at 4°C.The pure cultures of the isolated fungi were identified at the National Centre of Fungal Taxonomy, Inderpuri, New Delhi.

RESULT AND DISCUSSION

The samples of composts prepared with five different formulations were drawn at the time of spawning. The samples were dried at 55-60 ºC in a hot air oven, ground and analysed. The composts prepared with different proportions of SMS substitutions were found to vary in their quality characteristics as shown in Table 2.

Moisture content was highest in control treatment (68.33%) followed by treatment 3 and treatment 2. According to one study, moisture was highest in compost prepared with standard formulations (68.33) (Anonymous, 2016). It was at same level (65.67%) in composts prepared with SMS of oyster and button mushroom.

Maximum pH was observed in control treatment (6.82) whereas minimum (6.32) was found in treatment 2. Hawker (1966) indicated that acidic pH is favourable for mushroom cultivation. The bulk density was found maximum in treatment 2 (0.31g/ cm3).

Bulk density of compost should be 0.34g/cm3. In the present investigations, it ranged between 0.24-0.31 indifferent treatments. Similar results have been recorded under the present investigation. Medina *et al.* (2012) studied the relationship between soil physico-chemical, chemical and biological properties in a soil amended with spent mushroom substrate.

The electrical conductivity was maximum in treatment 2 containing 445 Kg button mushroom SMS (2.89 Ds/m). Electrical conductivity was highest in compost prepared with button mushroom SMS. Shandilya and Hayes (1987) concluded that there is decrease in number of pin heads with increase in conductivity and vice versa. Eigenberg *et al.* (2002) reported that the slight increase in electrical conductivity with the addition of SMC is due to

Table 2. Quality characteristics of the compost in different compost treatments

Treatments	Moisture content (%)	pH	Bulk density (g/cm^3)	EC (deci S m^{-1})	Organic carbon (%)	Nitrogen content (%)	C:N ratio
T1	68.33	6.82	0.29	2.45	37.50	1.85	20:1
T2	67.07	6.32	0.31	2.89	36.15	2.19	17:1
T3	67.80	6.45	0.27	2.73	38.70	2.01	19:1
T4	65.64	6.51	0.26	2.81	37.20	1.91	20:1
T5	65.55	6.49	0.24	2.64	37.80	1.98	19:1

increased salinity which, could be further correlated with the organic matter mineralisation. Similar kinds of results were observed in the present investigations also.

The values of organic carbon did not vary in different compost treatments. However, the organic carbon tends to decrease with increasing quantity of SMS used in replacement of wheat straw. The Nitrogen content was observed to be the highest (2.19) in treatment 2 followed by treatment 3. High nitrogen content may be because of addition of additional SMS in compost. Overall, the nitrogen per cent was observed to be highest in compost prepared with button mushroom SMS. The nitrogen content was higher in 20 and 30% SMS substitution treatments without nitrogen balancing compared with 20% SMS substitution with nitrogen balancing and compost for standard formulation. This increase in nitrogen content may be related to the increase of bacterial communities by addition of spent mushroom substrate. Similarly, Jordan *et al.* (2008) reported that the supplementation of spent mushroom substrate increased organic matter content in soil.

Table 3. Isolation of Thermophilic fungi from newly prepared compost formulations

Treatments	Thermophilic flora isolated
T1	*Aspergillus fumigatus, Scytalidium thermophilum, Torula compostis*
T2	*Scytalidium thermophilum, Torula compostis*
T3	*Scytalidium thermophilum, Aspergillus niger, Torula compostis*
T4	*Scytalidium thermophilum,Torula compostis*
T5	*Aspergillus niger, Scytalidium thermophilum, Torula compostis*

The samples of the compost for isolation of thermophilic fungi were isolated from the five different formulations mentioned above. The thermophilic fungal flora isolated in different treatments are listed in Table 3. Out of total 13 thermophilic fungi isolated from 5 different treatments of button mushroom compost, 4 samples were accessioned by NCFT (National Center of Fungal Taxonomy) New Delhi. Under T1 *Aspergillus fumigatus, Scytalidium thermophilum, Torula compostis* were isolated and identified as dominating thermophilic fungi whereas in T2 *Scytalidium thermophilum* and *Torula compostis*, in T3 *Scytalidium thermophilum, Aspergillus niger* and *Torula compostis*, T4 *Scytalidium thermophilum* and *Torula compostis*, T5 *Aspergillus niger, Scytalidium thermophilum* and *Torula compostis*.

In total, four species were isolated from the compost samples by serial dilution method. These samples were further identified by National Centre of Fungal Taxonomy, New Delhi. Four fungal species were found associated with *Agaricus bisporus* compost formation. Those four species *Aspergillus niger*, *Aspergillus fumigatus*, *Scytalidium thermophilum* and *Torula compostis* are listed in Table 4 along with their accession numbers.

Detailed colony morphology and microcopical properties is given below.

Table 4. Identification Report by NCFT (National Center of Fungal Taxonomy) New Delhi

Sr. No.	Isolate No.	Source	Acc No.	Final Identification
1.	Isolate-1	Agaricus compost	9440.19	*Torula compostis* (New sp.)
2.	Isolate-2	Agaricus compost	9441.19	*Scytalidium thermophilum*
3.	Isolate-3	Agaricus compost	9442.09	*Aspergillus niger*
4.	Isolate-4	Agaricus compost	9443.19	*Aspergillus fumigatus*

Aspergillus niger

This fungus was isolated from the phase-1 of composting. This fungus is ubiquitous present in soil and present in indoor environment. *Aspergillus niger* culture initially exhibits white growth, but after a few days, it changes to black as it produces conidial spores (Fig 1a). Under microscopic view, *Aspergillus niger* exhibits smooth, dark brown colored conidiophores and conidia.

The conidiophores protrude from septate and hyaline hyphae. The conidial heads appear radial and are arranged in columns (biseriate). The conidiophore vesicle produces sterile cells known as metulae, which in turn support the phialides on the conidiophores (Fig.1b).

Aspergillus fumigatus

Aspergillus fumigates was isolated from the phase-1 of composting. This fungus is present in soil and decaying organic matter such as compost heaps and play important role in carbon and nitrogen recycling. It produces white, yellow, yellow-brown, brown to black or green colored colonies (Fig 2a). The morphology of *Aspergillus fumigatus* is characterized by its hyphal conidia and conidiophores. The conidia are green and spiked, with small spikes covering their surface. They measure 2.5-3 µm in diameter and can have either a smooth surface or a spiked surface. The conidia are produced in basipetal column chains from green phialides that measure 6-8 µm by 2-3 µm in size (Fig 2b).

Scytalidium thermophilum

This fungus was isolated from both phase-1 and phase-2 of composting. This is mainly a compost fungi and play important role on formation of compost. Fresh cultures typically turn dark green-grey to black quickly due to sporulation. It also exhibit white to light grey mycelial overgrowth, especially at the margins, which may appear completely white, while the center remains green-grey (Fig 3a). Chains of thick-walled, dark swollen cells were observed directly under microscope. These chains did not detach easily, and the cells were cylindrical or globose in shape, often becoming darkly pigmented. Conidia are thick-walled, globose to ellipsoidal smooth, mostly 8-12 µm in diameter. They were found singly or in short and long

Fig. 1a. Pure culture of *Aspergillus niger*

Fig. 1b. Conidia and Conidiophores

Fig. 2a. Pure culture of *Aspergillus fumigatus*

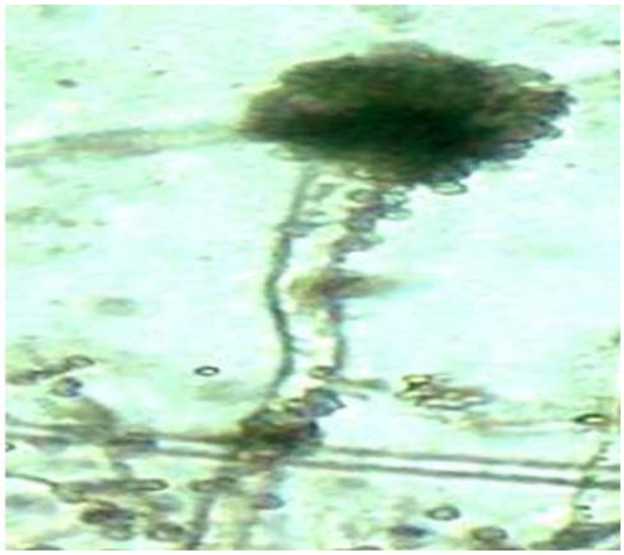

Fig. 2b. Conidia and Conidiophores

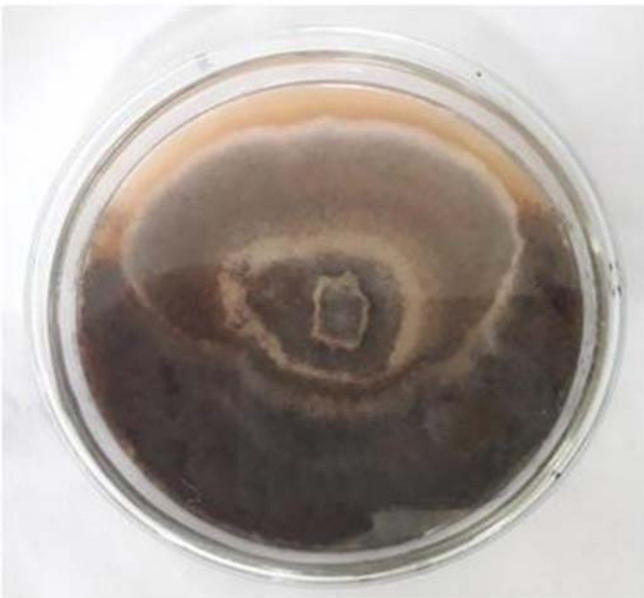

Fig. 3a. Pure culture of *Scytalidium thermophilum*

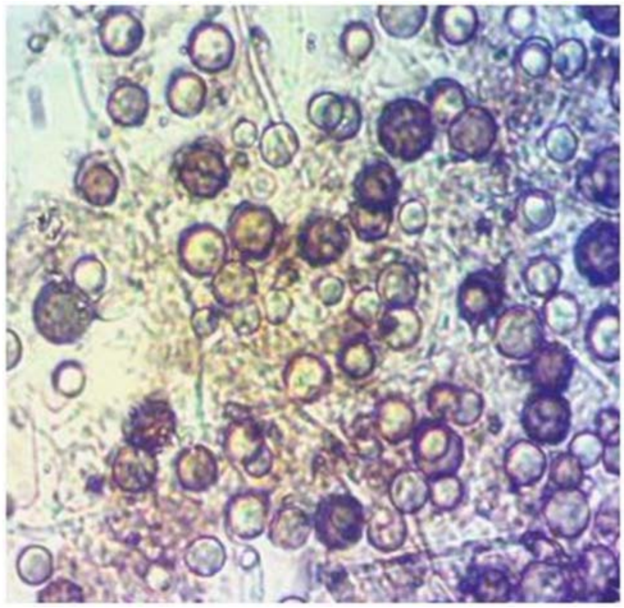

Fig. 3b. Type 1 and Type 2 Conidia

chains, and formed in terminal or intercalary positions. Two distinct types were identified: type 1, characterized by single very dark spores borne on short lateral hyphal branches and type 2, characterized by intercalary slightly pigmented spores (Fig 3b).

Torula compostis

This fungus was isolated from the phase-1 of composting. The Colonies were fast growing on Potato Dextrose Agar as floccose, cottony growth at 25^{o} C after five days than turning to brown, dark blackish releasing the pigment of the same color (Fig. 4a). The colony developed conidiophore directly from thick walled mycelium, initially semi macronematous, turning to macronematous, brownish in color, straight or flexuous, smooth walled unbranched up to 30 µm long x 5-8 µm wide bearing terminal conidiogenous cell of 28 µm x 10 µm at bottom and widening up to

Fig. 4a. Pure culture of *Torula compostis*

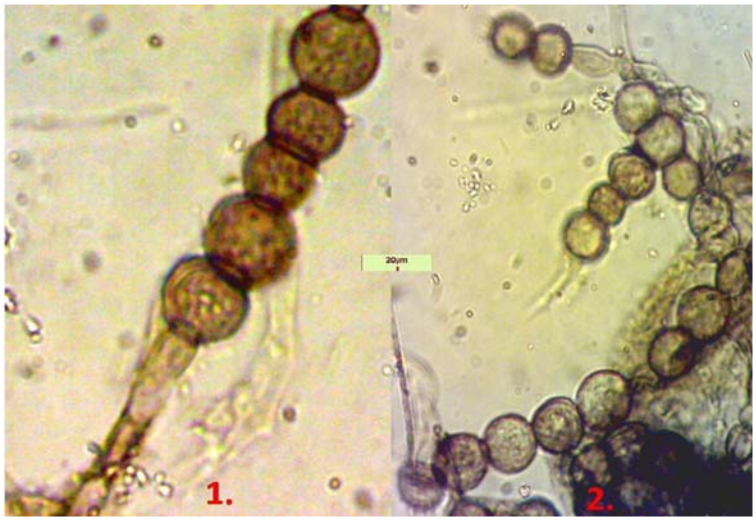

Fig. 4b1. Conidiophore and Conidia **; Fig. 4b2.** Conidia in branched chains

13 µm. Developing conidia in long and branched chain, olivaceous to light brown, smooth thick walled, globose to spherical in shape, having acropetal or basipetal development, 15-25 µm in diameter (Fig. 4b).

Salar and Aneja (2007) isolated eighteen species of thermophilic and thermotolerant fungi from mushroom compost. They studied the growth of *Agaricus bisporus* on sterile compost pre-colonized with four thermophilic fungi: *Chaetomium thermophile*, *Malbranchea sulfurea*, *Thermomyces lanuginosus*, and *Torula thermophila*. Each of these fungi was inoculated singly and in various combinations on sterilized compost to evaluate their potential to promote the growth and yield of *A. bisporus*. A mixed inoculum of *Malbranchea sulfurea* and *Torula thermophila* was found to be the best treatment, promoting the growth of *A. bisporus* to a rate of 7.7 mm/day and almost doubling the mushroom yield compared to the pasteurized control. The effect of *T. lanuginosus*, whether inoculated singly or in combination with other thermophilic fungi, was insignificant, resulting in lower growth rates. The study examined the mycelial extension rate, compost pH before spawning, mushroom yield, and biological efficiency for various treatments. The findings reveal that thermophilic fungi provide compost selectivity and protect against the negative effects of compost bacteria on the mycelial growth of *A. bisporus*. This is relevant for the commercial production of high-yielding mushroom compost for *A. bisporus*. Fergus (1964) isolated *Aspergillus fumigates, Humicola grisea, H. insolens* and a new species of *Stilbella thermophila* from phase-2 of composting. According to Vijay (1996), *Scytalidium thermophilum* and *Humicola* sp. dominated the entire composting process at various N levels. They were frequently isolated at 47°C and 52°C and however not so common at 42°C. Vijay (1996) found positive relation between density of *Scytalidium thermophilum* in compost and yield of button mushroom. Thermophilic fungi play a significant role in the preparation of compost for cultivated mushrooms. They have been shown to have important effects on composts. Eddy and Jacobs (1976) observed that thermophilic fungi selectively thrive in composts with very low levels of readily available nutrients, such as sugars and amino acids. It is highly likely that these fungi exert their effects by rapidly utilizing the readily available substrates. They could reduce NH_3 levels by competing with ammonifying organisms for substrates or possibly by assimilating the NH_4^+ ion. The growth of good mushroom mycelium often follows the growth of thermophilic fungi.

CONCLUSION

In the present study, composts were prepared by supplementing spent mushroom substrate of button and oyster mushroom. The physico-chemical properties of different treatments were analysed for the compost quality. The parameters of compost showed the quality of compost at par with the control. A total of four thermophilic fungal species were isolated from the compost prepared from different formulations. These thermophilic fungi were found to play important role in the formation of compost on which mushroom mycelium can grow. *Torula compostis* was identified as new species and *Scytalidium thermophilum* as the most dominating thermophilic fungi present in compost. The thermophilic fungi isolated from different compost are previously reported from compost formulations and plays an important role for quality compost production for button mushroom. The study concludes that the substitution of wheat straw with SMS of button mushroom @ 20-30% results in a good compost production, which will result in to reduced cost of production of button mushroom compost and recycling of SMS in mushroom Industry.

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REFERENCES

1. Chang S.T. and J.A. Buswell. 1996. Mushroom nutraceuticals. *World Journal of Microbiology and Biotechnology* **12:** 473-476.

2. Eddy, B.P. and L. Jacobs. 1976. Mushroom compost as a source of food for *Agaricus bisporus*. *Mushroom Journal* **38:** 56-59.

3. Eicker, A. 1977. Thermophilic fungi associated with the cultivation of *Agaricus bisporus*. *South African Journal of Botany* **43:** 193-207.

4. Fergus, C.L. 1964. Thermophilic and thermotolerant molds and actinomycetes of mushroom compost during peak heating. *Mycologia* **56:** 267-284.

5. Ross R.C. and P.J. Harris. 1983. The significance of thermophilic fungi in mushroom compost preparation. *Scientia Horticulturae* **20**: 61-70.

6. Salar, R.K. and K.R. Aneja. 2007. Significance of thermophilic fungi in mushroom compost preparation: effect on growth and yield of *Agaricus bisporus* (Lange) Sing. *Journal of agriculture Technology* **3:** 241-253.

7. Seal, K.J. and H.O.W. Eggins. 1976. The upgrading of agricultural wastes by thermophilic fungi, p. 58-78. In G. G. Birch, K. J. Parker, and J. T. Worgan (ed.), Food from waste. Applied Science Publishers, London.

8. Sinden, J.W. and E. Hauser. 1953. The nature of composting process and its relation to short composting. *Mushroom Science* **2:** 123-130.

9. Till, O. 1962. Champignonkultur auf sterilisiertem Nahrsubstrat und die Wiederverwendung von abgetragenem Kompost. *Mushroom Science* **5:** 127-133.

10. Vijay, B. 1996. Investigations on compost mycoflora and crop improvement in *Agaricus bisporus* (Lange). Ph.D. thesis, H.P. University, Shimla, India.

11. Wiegant, W.M. 1992. A simple method to estimate the biomass of thermophilic fungi in composts. *Biotechnology* **5:** 421-426.

12. Jackson, M.L. 1973. Soil Chemical Analysis. Pp 111-26. Printice Hall of India Private Limited NewDelhi..

13. Black, C.A. 1965. Methods of Soil Analysis, Agronomy No. 9, Am. Soc. Of Agron., Madison, Wisconsis.

14. Singh, R.A. 1980. Soil physical analysis. pp 321-325. Kalyani Publishers, New Delhi.

15. Walkely, A.J. and C.A. Black. 1934. Estimation of soil organic carbon by thechromic acidtitration method. *Soil Science* **37:** 259-60.

16. Anonymous. 2016. Annual report. ICAR-Directorate of Mushroom Research Chambaghat, Solan (HP), India. pp 38-60

17. Hawker, L.E.1966. Environmental influences on reproduction in the fungi II. G Ainsworth and AS Sussman (eds.). pp. 435-69. Academic Press, New York.

18. Medina, E., C. Paredes, M.A. Bustamante, R. Moral and J. Moreno-Caselles. 2012. Relationship between soil physic-chemical, chemical and biological properties in a soil amended with spent mushroom substrate. *Geoderma* **173:** 152-61.

19. Shandilya, T.R. and W.A. Hayes. 1987. Available elements in some casing soil. *Indian Mushroom Science* **2:** 10-15.

20. Eigenberg, R.A., J.W. Doran, J.A. Nienaber, R.B. Ferguson and B.L. Woodbury. 2002. Electrical conductivity monitoring of soil condition and available nitrogen with animal manure and cover crop. *Agriculture, Ecosystems and Environment* **88:** 183-93.

21. Jordan, S.N., G.J. Mullen and R.G. Courtney. 2008. Utilization of spent mushroom compost for the revegetation of lead-zinc tailings : effects on physic-chemical properties of tailings and growth of *Lolium perenne*. *Bioresource Technology* **99:** 8125-29.