Evaluation of *Volvariella volvacea* Strains for Yield and Diseases/Insect-Pests Resistance Using Composted Substrate of Paddy Straw and Cotton Mill Wastes

O. P. Ahlawat · Rajender Singh · Satish Kumar

Received: 10 April 2009 / Accepted: 24 June 2009 / Published online: 25 January 2011
© Association of Microbiologists of India 2011

Abstract Out of the 3 parent strains and 4 single spore isolates of *Volvariella volvacea* evaluated, strain, OE-274 gave earliest yield in 11.25–11.50 days post-spawning in all 4 trials. The yield varied in different strains in different trials and it was highest in strain, OE-272 in trial 1, SSI, OE-55-08 in trial 2, and strain, OE-274 in trial 3 and 4. In overall average, highest yield was in strain, OE-272, closely followed by strain, OE-274. The number of fruiting bodies per q substrate also varied in different strains in different trials. Highest numbers were in strain, OE-272, SSIs, OE-55-08 and OE-12-22, and strain, OE-210 in trial 1, 2, 3 and 4, respectively. Highest fruiting body wt was in strain, OE-274 in all 4 trials. The yield during different weeks of cropping varied in different strains but invariably it was highest in first week, which accounted for 60–70% of the total yield. The fruiting bodies of strain, OE-274 were of bigger size, brownish, toughest and with least tendency of veil opening, while that of strain, OE-272 and SSI, OE-55-08 were whitish to grayish-white, oblong, medium size, delicate and lesser tendency of veil opening. The strain, OE-274 and SSI, OE-55-08 exhibited higher resistance against the growth of competitor moulds and infestations of insect-pests, while strain, OE-272 exhibited highest susceptibility to insect-pests infestation.

Keywords *Volvariella volvacea* · Strain · Single spore isolate · Yield · Quality · Disease/insect-pest

Introduction

The paddy straw mushroom, *Volvariella volvacea*, is known for its unique aroma and texture, and grows well between 28 and 35°C [1]. It is a fast growing mushroom compared with most other cultivated mushrooms [1] and has significant pharmacological properties, including anti-tumor polysaccharides, immunosuppressive proteins and immunomodulatory lectins [2, 3]. This mushroom ranks sixth in the global production of cultivated mushrooms and accounts for 5–6% of world production [4]. Compared to many cultivated species of mushrooms, the productivity of *V. volvacea* is very low (about 10–15% on rice straw) [5]. The productivity in this mushroom is attributed to the hydrolytic enzyme production potential of the mushroom [6, 7], quality of substrate used, method of substrate preparation and the growing conditions. The hydrolytic enzyme production potential and quality of substrate used for cultivation has direct bearing on its mushroom production potential. However, other characteristics of superior yielding strains viz., mycelial growth rate, mycelial density, and formation of aerial hyphae and chlamydospores on growing medium vary under varied growth conditions [8]. The present study thus aimed at evaluation of pre-screened parent strains and SSIs for their yield potential, and diseases and insect-pests resistance on composted substrate of paddy straw and cotton mill wastes.

Materials and Methods

Strains and Single Spore Isolates

Three parent strains (OE-272, OE-274 and OE-210) of *V. volvacea* (Bull. ex Fr.) Sing. pre-screened for mycelial

O. P. Ahlawat (✉) · R. Singh · S. Kumar
Directorate of Mushroom Research, Solan, Himachal Pradesh
173 213, India
e-mail: ahlawat22op@gmail.com

growth characteristics, extracellular lignocellulolytic enzymes activity profiles, genetic distinctness and superior yield potential on paddy straw substrate [7, 9] were used in the present study. Similarly, 4 single spore isolates (OE-12-06, OE-12-22, OE-55-08 and OE-55-30), 2 each from parent strains, OE-12 and OE-55, respectively, isolated by following the standard method [8] and pre-screened for mycelial growth characteristics, extracellular lignocellulolytic enzymes activity profiles and genetic distinctness [10] were used in the present study. The cultures were multiplied and maintained on Malt Extract Agar (MEA) medium slants at 32 ± 2 and $18 \pm 2^\circ\text{C}$, respectively in BOD incubator.

Mushroom Spawn

The ready to mix spawn (seed) of different strains and single spore isolates was prepared by using chopped paddy straw following improved method of Chang and Miles [11]. The fresh dried paddy straw was first cut into pieces of 2.5–5.0 cm long and soaked in clean water for 2–4 h. The water soaked straw was put on a sieve for 30 min, followed by mixing of calcium carbonate and calcium sulphate @ 2.0 and 1.0%, respectively on dry wt basis. The spawn substrate was filled up to one half portion in polypropylene (PP) bags of (25 cm $\times$ 12 cm) of 100 gauge thickness. The PP bags were closed first by creating necks with the help of plastic rings and plugging with non-absorbent cotton. The plugged bags were sterilized for 1.5 h at 22 psi. After sterilization, the bags were put under UV light for 30 min on laminar flow bench. Rests of the steps were of standard protocol followed for mushroom spawn making. The inoculated bags were incubated at $32 \pm 2^\circ\text{C}$ for 8 days in BOD incubator. After 8 days, the fully colonized bags were placed at ambient temperature of 16–25°C for chlamydospores development and storage till further use.

Crop Raising

First 3 trials were carried out by using paddy straw + blow room waste from cotton spinning mill in 1:1 ratio (w/w) as the substrate (w/w), while the fourth trial was carried out with paddy straw + cotton ginning mill waste in 1:1 ratio (w/w). Composting was carried out by following the method of Quimio [12] with some modifications in ingredients, supplements and the schedule of substrate turnings, pasteurization and conditioning. Chicken manure and CaCO_3 were added @ 5.0 and 1.5% (dry wt basis), respectively on day 0 and day 3 of stacking, while turnings were given on days 1, 2, 3 and 4 of out door composting. Beds of 180 cm $\times$ 70 cm $\times$ 12 cm (l $\times$ w $\times$ h) size were prepared with 35 kg wet substrate on shelves of iron racks in cropping room. Four replications were kept for each

strain/SSI and the experiment was conducted in a randomized block design. The data recorded was subjected to statistical analysis using AGRES software. The fresh mushroom yield obtained was converted into percent biological efficiency (kg q^{-1} dry substrate) by using equation $\text{BE (\%)} = \text{Fresh mushroom yield in kg/quantity of dry substrate used} \times 100$. Data was also recorded for the time taken for first harvest in days post-spawning, numbers of fruiting bodies harvested per q dry substrate, average fruiting body wt (g), visible growth of competitor moulds like green mould and ink cap on substrate, and presence of mites, nematodes, sciarid and phorid flies both on substrate as well as in fruiting bodies.

Resistance against Diseases and Insect-Pests

The in vitro studies were carried out first by growing different strains and SSIs up to full growth on MEA medium in petri dishes and then 5 larvae either of sciarid and phorid flies were released in separate plates, followed by incubation of the plates at $32 \pm 2^\circ\text{C}$ for 4 days in BOD incubator. Three plates were kept for each strain/SSI and recorded daily for mycelial-clearing zones created if any due to larval feeding of mushroom mycelia. The level of resistance in different strains was recorded based upon the dia of mycelial clearing zone created by the feeding larvae. The in vivo studies were carried out by observing the mushroom beds daily starting from spawn run to crop end for the growth of competitor moulds, and appearance of mites, nematodes, sciarid and phorid flies per unit area of the compost.

Results and Discussion

Mushroom Yield

The data depicted in Table 1 reveal that in all 4 trials, mushrooms were harvested earliest in strain, OE-274. In overall average of 4 trials, earliest harvest was in 11.38 days in strain, OE-274, followed by 12.19 days in SSI, OE-12-22. Amongst different trials, mushrooms were harvested earliest in trial 4. The mushroom yield obtained in different strains/SSIs varied in different trials (Table 2) and it was significantly higher in strain, OE-272 in trial 1, SSI, OE-55-08 in trial 2 and strain, OE-274 in trial 3 and 4 (Fig. 1). In average of 4 trials, parent strain, OE-272 exhibited highest yield, closely followed by strain, OE-274 and SSI, OE-55-08. Earlier study carried out with pasteurized and composted paddy straw has also reported early first harvest and higher yield in strains, OE-272 and OE-274 [9]. However, the higher yield was attributed to higher hydrolytic enzymes activity in these strains and the

Table 1 Time taken for first harvest in different strains of *V. volvacea*

Strain	Time taken for first harvest (days post-spawning)				
	Crop 1	Crop 2	Crop 3	Crop 4	Average
OE-272	14.25	11.75	11.33	12.25	12.40
OE-274	11.50	11.50	11.25	11.25	11.38
OE-210	13.75	12.25	12.50	11.50	12.50
OE-12-22	12.50	12.00	12.00	12.25	12.19
OE-12-06	14.25	13.00	12.00	NS	13.08
OE-55-08	NS	13.00	13.00	12.50	12.83
OE-55-30	NS	12.00	14.50	NS	13.25
Average	13.38	12.09	12.37	11.95	12.45
CD (0.05%)	1.0026	0.7862	0.9366	0.9770	–

NS Not studied

Table 2 Mushroom yield potential in different strains of *V. volvacea*

Strain	Mushroom yield (kg/q dry substrate)				
	Crop 1	Crop 2	Crop 3	Crop 4	Average
OE-272	39.85	28.09	23.79	25.73	29.37
OE-274	23.94	32.53	25.73	32.44	28.66
OE-210	24.72	23.58	16.21	23.57	22.02
OE-12-22	23.79	20.78	20.52	18.06	20.79
OE-12-06	14.38	11.61	9.75	NS	11.91
OE-55-08	NS	33.98	14.98	24.51	24.49
OE-55-30	NS	28.30	8.31	NS	18.31
Average	22.94	25.29	17.04	24.86	22.53
CD (0.05%)	6.1188	2.8096	6.4570	6.2176	

NS Not studied

same is applicable here. Yield evaluation trials for selecting a better performing strain or single spore isolate have also been performed earlier by several workers [13, 14]. However, trials by using composted substrate of 1:1, w/w combination of paddy straw and cotton mills waste has not been carried out.

The numbers of fruiting bodies harvested from different strains/SSIs varied in different trials and it was highest in strain, OE-272 in trial 1, SSI, OE-55-08 in trial 2, SSI, OE-12-22 in trial 3 and strain, OE-210 in trial 4. In overall average of 4 trials, highest numbers of fruiting bodies were recorded in strain, OE-272. This strain gave 57.74–61.20% of total yield during first week in first 2 trials (Fig. 2) and it increased to 74% in trial 3 as this trial was of much shorter duration. The trend for another high yielding strain, OE-274 was bit different as it gave almost 57–64% yield during first week in first 2 trials, but its yield level went on decreasing with increasing cropping time and it was almost negligible during fourth week. The high yielding SSI, OE-55-08 gave bit lesser yield than the strains,

Fig. 1 Crop photograph of high yielding strain, OE-274 of *V. volvacea* **Fig. 2** Weekly yielding pattern of different strains of *V. volvacea* in trial 2

OE-272 and OE-274 during first week but its yield level was more consistent during later weeks (Fig. 2), which contributed towards its superior yield in trial 2 and 4. In all strains and SSIs, the yield level in trial 3 was very high (71.56–96.41%) during first week, the reason being more favourable temperature during early phase of the crop. In trial 4, the cotton ginning mill waste and paddy straw based compost gave yield at par with paddy straw and cotton spinning mill waste but in a shorter duration of 2 weeks than 4 weeks in trial 1 and 2. The numbers of fruiting bodies harvested in different strains also varied during 2 flushes and majority were harvested during first flush. In second flush, the number was highest in strain, OE-210, followed by SSI, OE-55-08.

Table 3 Average fruiting body weight in different strains of *V. volvacea*

Strain	Average fruiting body weight (g)				
	Crop 1	Crop 2	Crop 3	Crop 4	Average
OE-272	13.87	10.75	13.23	13.15	12.75
OE-274	18.34	15.64	17.83	14.05	16.47
OE-210	13.45	12.36	10.21	9.62	11.41
OE-12-22	9.15	9.35	12.93	9.02	10.11
OE-12-06	10.32	9.00	11.75	NS	10.36
OE-55-08	NS	11.12	10.53	10.16	10.60
OE-55-30	NS	10.12	12.69	NS	11.41
Average	12.54	11.19	12.74	11.20	11.87
CD (0.05%)	4.0254	2.5862	3.6548	3.0050	

NS Not studied

Mushroom Quality

The average fruiting body wt in different strains varied in different trials and it was highest in strain, OE-274 in all 4 trials (Table 3). The second higher wt was in strain, OE-272 in trial 1, 3 and 4. In overall average of 4 trials, wt was highest of 16.47 g in strain, OE-274, followed by 12.75 g in strain, OE-272. The wt in rest of the strains and SSIs showed little variations. The fruiting bodies were whitish, oblong shaped and delicate in SSIs, OE-55-08 and OE-55-30 but with least tendency of veil opening (Table 4). The fruiting bodies in strain, OE-272 were of average size, medium level toughness and typically match the birds' eggs but with bit higher tendency of veil opening on delayed harvesting. The toughest fruiting bodies with least tendency of veil opening along with highest wt and size were harvested from strain, OE-274. The higher wt of fruiting bodies of strain, OE-274 contributed towards its superior yield even after lagging behind over other strains/SSIs in numbers of fruiting bodies harvested per q substrate. Similar observations with respect to size, shape, wt and colour of fruiting bodies, and tendency of veil opening were made in an earlier study [9]. However, average fruiting body wt and toughness recorded in the present

study is much higher than that was recorded in earlier studies and it is attributed to superior nutrient status of the growing medium used in the present study. Except some studies on its preservation [15, 16], not many references are available on comparative analysis of fruiting body quality in different strains of *V. volvacea*.

Resistance Against Diseases and Insect-Pests

Among competitor moulds, Ink cap (*Coprinus* sp.) was recorded as the major mould and it appeared during later stages of crop mainly on lower shelves (Table 5). Its appearance can be attributed to improper pasteurization of substrate and cropping conditions existed in and around lower shelves rather than strains. However, beds of SSIs were more prone to ink cap growth than the parent strains. Growth of green mould was recorded mainly on cotton fibers during trial 1 and it is again attributed to improper pasteurization of substrate. Mites also appeared during trial 1 and their intensity was higher on beds of strain, OE-210 and SSI, OE-12-06. Nematodes were also recorded in compost and in damaged fruiting bodies during trial 1 and their intensity was highest in beds on strains, OE-272 and OE-274. Highest yield loss of 20–40% mainly due to nematodes infestation was in strain OE-272, followed by 20–30% in SSI, OE-12-06. Nematodes infestation was also recorded in SSIs, OE-55-08 and OE-55-30. Under in vitro screening of different strains and SSIs, the SSI, OE-12-06 and strain, OE-272 were recorded to be more resistant against sciarid larvae, while strain, OE-210 and SSI, OE-55-08 were more resistant against phorid larvae (Table 6). Under in vivo conditions, infestation of only phorid flies was recorded and it was lowest in strains, OE-274 and OE-210, and SSI, OE-55-08.

In the present study, parent strain, OE-274 and SSI, OE-55-08 gave higher mushroom yield and superior quality fruiting bodies. These were also recorded to have higher resistance against competitor moulds growth and insect-pests infestation, hence proved as better strains. The other strain, OE-272 although gave higher mushroom yield

Table 4 Fruiting bodies quality in different strains of *V. volvacea*

Strain	Fruiting body colour	Fruiting body size (dia./length in cm)	Toughness	Veil opening
OE-272	Whitish with grayish spot	3–4/4–5	++++	+
OE-274	Brownish	4–5/5–6	Toughest	–
OE-210	Brownish	2–3/3–4	+++	–
OE-1206	Brownish	2–3/3–4	++	–
OE-1222	Brownish	3–4/4–5	+++	–
OE-55-08	Whitish	2.5–3/5–6	Delicate	–
OE-55-30	Whitish	3–4/5–6	Delicate	–

Table 5 Incidence of competitor mould and insect-pest in mushroom beds of different strains of *V. volvacea* and their effect on yield loss

Strain	Incidence of competitor moulds/insect-pests													
	<i>Coprinus</i> sp. ^a			Green mould ^b			Mites ^c			Nematodes			Yield loss (%)	
	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2	Crop 3	Crop 1	Crop 2 and 3
OE-272	+	+	+	++	–	–	+	–	–	+	–	–	1–1.13	20–40
OE-274	–	+	+	+	–	–	–	–	–	+	–	–	0.83–1.04	1–2
OE-210	+	+	+	+	–	–	++	–	–	–	–	–	0.61–0.81	2–3
OE-12-22	–	+	++	+	–	–	–	–	–	–	–	–	0.34–0.42	3–5
OE-12-06	++	+	+	+	–	–	++	–	–	–	–	–	0.69–0.83	20–30
OE-55-08	NS	+	++	NS	–	–	NS	–	–	NS	–	–	NS	2–20
OE-55-30	NS	+	+++	NS	–	–	NS	–	–	NS	–	–	NS	2–40

NS Not studied

^a *Coprinus* appeared during later stage of crop mainly in lowest shelves^b Green mould appeared after 1st flush only on cotton waste^c Mites appeared after 5–6 days of bed opening**Table 6** Susceptibility of different strains of *V. volvacea* against larvae of sciarid and phorid flies under in vitro and in vivo conditions

Strain	In vitro conditions		In vivo conditions	
	Sciarid larvae	Phorid larvae	Sciarid larvae	Phorid larvae
OE-272	+, –	+++	–	+
OE-274	+, ++	+++	–	–
OE-210	+++ , ++	–	–	–
OE-12-06	–, –	+++	–	+
OE-12-22	–, ++	++++	–	+
OE-55-8	++, +	+	–	–
OE-55-30	+, ++	++	–	+

and superior quality fruiting bodies but was most susceptible to insect-pests infestation and yield loss recorded was very high. Among substrates, the compost prepared with cotton ginning mill waste + paddy straw proved as better substrate as it gave same level of yield in 2 weeks time as was obtained in 4 weeks from spinning mill waste + paddy straw compost. The strains, OE-272 and OE-274 have also been reported as superior yielding strains in earlier studies carried out with pasteurized and composted paddy straw [9]. Similar study covering all facets of mushroom yield, fruiting body quality and resistance to diseases/insect-pests has not been carried out earlier in this mushroom. The present study has provided 2 high yielding parent strains and one SSI for their use at different levels of mushroom growing aiming either for the fresh mushroom sale or caning of mushrooms for export purpose.

Acknowledgment We thank the Director, Directorate of Mushroom Research (ICAR), Solan for constant encouragement and providing the requisite facilities for conducting the study.

References

- Chang ST (1978) *Volvariella volvacea*. In: Chang ST, Hayes WA (eds) The biology and cultivation of edible fungi. Academic Press, New York, pp 573–605
- Kishida E, Kinoshita C, Sone Y, Misaki A (1992) Structures and antitumor activities of polysaccharides isolated from mycelium of *Volvariella volvacea*. Biosci Biotechnol Biochem 56:1308–1309
- She QB, Ng TB, Liu WK (1998) A novel lectin with potent immunomodulatory activity isolated from fruiting bodies and cultivated mycelia of the edible mushroom *Volvariella volvacea*. Biochem Biophys Res Commun 247:106–111
- Buswell JA, Chen M (2005) Cultivation, biochemical, molecular biological and medical aspects of the culinary-medicinal straw mushroom *Volvariella volvacea* (Bull.: Fr.) Singer (Agaricomycetidae). Int J Med Mushrooms 7:157–166
- Chang ST (1974) Production of the straw mushroom (*Volvariella volvacea*) from cotton waste. Mushroom J 21:348–354
- Ahlawat OP, Ahlawat K, Dhar BL (2005) Influence of lignocellulolytic enzymes on substrate colonization and yield in monosporous isolates and parent strains of *Volvariella volvacea* (Bull. Fr.) Sing. Indian J Microbiol 45(3):205–210
- Ahlawat OP, Pardeep G, Shwet K, Dhar BL (2008) Development of molecular and biochemical markers for selecting a potential high yielding strain of paddy straw mushroom (*Volvariella volvacea*). J Plant Biochem Biotechnol 17(1):57–63
- Chang ST, Miles PG, Wai CC (1981) A study of monosporous isolates of *Volvariella volvacea*. Mushroom Sci XI:603–621
- Ahlawat OP, Pardeep G, Shwet K, Dhar BL (2008) Profile of the extracellular lignocellulolytic enzymes activities as a tool to select the promising strains of *Volvariella volvacea* (Bull ex Fr.) Sing. Indian J Microbiol 48:389–396
- Ahlawat OP, Pardeep G, Shwet K, Dhar BL (2010) Variability in intra-specific and monosporous isolates of *Volvariella volvacea* based on enzyme activity, ITS and RAPD. Indian J Microbiol 50(2): 192–198
- Chang ST, Miles PG (2004) Cultivation, nutritional value, medicinal effect, and environmental impact. CRC Press LLC, Boca Raton
- Quimio TH (1993) Indoor cultivation of the straw mushroom *Volvariella volvacea*. Mushroom Res 2(2):87–90

13. Graham KM (1975) Studies on the paddy mushroom (*Volvariella volvacea*). V. Performance of single-basidiospore isolates. Malays Agric Res 4(1):31–36
14. Iqbal SM, Muhammad B (1993) Performance of exotic and local strains of Chinese mushroom *Volvariella volvacea* (Bull ex. Fr.) on different substrates. Pak J Sci Ind Res 36:6–7
15. Belmonte CP, Aguilar DA (1993) Effect of methods of drying, sulfite and final moisture content on the quality of dehydrated mushroom (*Volvariella volvacea*). CLSU Sci J (Philipp) 14(1): 24–32
16. Raj D, Pardeep G, Ahlawat OP, Rai RD (2005) Effect of pre-treatments on the quality characteristics of the dehydrated paddy straw mushroom (*Volvariella volvacea*). Indian J Mushrooms 22(1 and 2):24–28