Conversion of spent mushroom compost to a solid substrate for mass production of an entomopathogenic fungus, *Isaria fumosorosea* (Wize)

Rahim Eslamizadeh[1], Ahmad Said Sajap[2], Zolkifli Omar[3], Nur Azura Adam[4], Marziyeh Naiimifar[5]

1. Assistant professor, Agricultural Research, Education and extension Organization. Jolgeh Dez Agricultural company (Dezful mushroom)., Dezful, Khuzestan, Iran.
2. Professor, Forestry faculty, University Putra Malaysia, Malaysia.
3., 4. Professor, Associate professor, Agriculture faculty, University Putra Malaysia, Malaysia.
5. MSc plant pathology, Khuzestan, Jolgeh Dez Agricultural Company (Dezful mushroom), Dezful, Iran.
Corresponding author: Rahim Eslamizadeh, email: Reslamizadeh@gmail.com

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Abstract

Spent mushroom compost (SMC), an organic waste produced from mushroom production farms it is a complex waste management issue in many developing countries and developed countries. In this study, an effort to convert the waste into a beneficial use was carried out. Composts from varying stages of fermentations in a mushroom production line were chemically analysed and tested for their suitability for mass production of an entomopathogenic fungus, *Isaria fumosorosea* (Wize). The result shows that the compost taken from early fermentation stages did not support the growth of *I. fumosorosea*, the fungus, however, grew well thereafter on substrates from the mushroom harvesting stage and SMC. The conidia from the SMC not only remained as pathogenic as conidia from rice to its target pest, *Bemisia tabaci* but they were also more resistant to UV–B radiation and have a longer shelf life than conidia from rice. These properties indicated that SMC can be used as a solid substrate for mass production of the entomopathogenic fungus, *I. fumosorosea* for an environmentally friendly fungal–based biopesticide.

Keywords: biological pesticide, whitefly, asexual Spore, Agaricus bisporus, cereal

Introduction

Spent mushroom compost (SMC), composted organic waste remaining after mushroom crop has been harvested, poses disposal problems to many mushroom producing industries. Apart from its odour problem (Pecchia *et al*., 2001) the compost could leach into the underlying soil and contaminat the groundwater (Riahi *et al*., 1998; Lyons *et al*., 2008). Iran is currently one of the world producers of mushrooms producing 70,000 tons of mushrooms and discarding 390,000 tons of SMC annually; Like many other parts of the world this large amount of SMC could pose a pollution problem if it would not properly managed. Even though SMC has been tested and recycled as a soil amendment and fertilizer for plant production systems (Chong & Rinker, 1994; Medina *et al*., 2009; Gonani *et al*., 2011; Jonathan *et al*., 2011), animal feed (Zhang *et al*., 1995), soil bioremediation agents (Eggen, 1999; Marín–Benito *et al*., 2012) and biofuel (Balan *et al*., 2008) it remains to pose a serious waste management issue. One option that has yet to be tested is to utilize SMC for the production of entomopathogens. SMC consists of hemicelluloses, lignocellulosic materials, lignin, mushroom mycelia and mycelial metabolites (Jordan *et al*., 2008). Chemically, the compost contains varying amounts of compounds that changes over time during the fermentation process (Sharma *et al*., 2000), Biologically, SMC can sustain the growth of many microorganisms including fungi, *Paecilomyces* spp. (Kleyn & Wetzler, 1981). Currently, entomopathogens for controlling insect pests are mass–produced using either solid or liquid culture systems. A liquid or submerged culture has some limitations. The blastopores are less virulent and environmentally less resistant than conidia from solid substrates (Jenkin & Prior,1993).

Solid–state production features simpler production processes with cheaper equipment and culture media (Castilho *et al*., 2000; Krishna, 2005). Hence a solid substrate culture is a preferred technology for adoption in less developed countries. In many of these countries, the systems use cereal grains, such as rice, maize and barley for the solid substrates (Mar *et al*., 2012). Even though rice has been one of the best and most commonly used substrates for the production of *Metarhizium anisopliae* (Metschn) (Hunter *et al*., 2001), *Beauveria bassiana* (Bals–Criv) (Ye *et al*., 2006) and *Paecilomyces fumosoroseus* (Wize) (Cheong *et al*., 2013) the ever–increasing cost of this staple food grain could lead to uneconomical production of the fungi and conflict of resource utilization. Thus, a cheaper input such as spent mushroom compost may be preferable. Even though SMC may support the growth of the entomopathogenic fungi,

their virulence to the target insects may be impaired. Studies have shown that substrates on which entomopathogenic fungi are grown affects the growth and virulence of the fungi (Ali *et al.*, 2009). For example, growth and subsequent sporulation of *M. anisopliae* and *B. bassiana* isolates were affected by nutrient sources (Shah *et al.*, 2005), particularly the C: N ratio of the substrates (Gao, 2011). Apart from the nutrients present in the substrate, lignin added to the substrate could also affect the persistence of the fungi in the environment. The incorporation of lignin in fungal formulations has been shown to limit the adverse effect of UVB radiation on the conidia (Leland & Behle, 2005). The lignin from the wheat straw in the compost could have a similar function as a UV–B protectant. Thus, objectives of the study were (i) to chemically characterized *Agaricus bisporus* (J.E. Lange) SMC, (ii) to evaluate SMC as a raw material for mass production of an entomopathogenic fungus, *Isaria fumosorosea* (Wize) and (iii) to examine and compare the properties of the harvested conidia in terms of their resistance to UV–B radiation, shelf life and efficacy on the target pest, whitefly *Bemisia tabaci* (Gennadius, 1889) (Gennadius) (Homoptera: Aleyrodidae)

2. Materials and methods

Culture isolate

Conidia of *I. fumosorosea* originally isolated from *B. tabaci* in Malaysia were cultured singly on Potato –Dextrose Agar (PDA) kept at dark, 25 ±1 °C for 14 days (Behle and Robert 2009).

Mushroom and spent mushroom composts

Mushroom compost and SMC were taken from a mushroom farm in Iran. The composts were prepared following a method routinely practised by Iranian mushroom growers. The process utilized 100 units of wheat straw, 65 units of chicken manure 10 units of gypsum (calcium) and 5% lime (calcium carbonate) (wt/wt). The materials were thoroughly mixed, watered daily and allowed to ferment aerobically in the compost yard for three days. The compost was then transferred manually into a concrete tunnel for pasteurization for 12 hours at 58±1 and 4 days at 48±1. After pasteurization, the compost was mixed with the spawn, spread on a platform 1.4 m × 22 m, kept the dark at 25 ±1°C for 23 days and reduced the temperature to 19±1°C for pinning and subsequent cropping.

Sampling of compost for chemical analysis

The composts from the following stages of mushroom growth; in the bunker (6th day), in the tunnel (9th, 12th and 15th day), spawning and casing (18th, 21th and 30th day), cooling and picking (58th and 65th day) were sampled for the chemical analysis. Three kilograms of each composting stage were taken from four spots, 3 mm apart, across the composting platform. A portion of the sample was used to culture *I. fumosorosea*.

Chemical determinations

Immediately after sampling, the compost samples were taken to the laboratory and manually homogenized. The moisture content (MC) was measured by a standard gravimetric method and pH was determined using a pH meter. The total organic carbon (OC) and total Nitrogen (N) of the samples were determined by the Walkley–Black method (Walkley and Black 1934) and the Kjeldahl method (Bremner & Mulvaney, 1982), respectively. Lignin was determined based on TAPPI standard number T222–88.

Preparation of solid substrates for *I. fumosorosea* production

Samples taken from different composting stages, as indicated earlier, were air–dried for 3 days at 26 °C. In addition, solid substrates consisting of SMC+ wheat bran and rice medium were also prepared. SMC that had been air–dried for 3 days was mixed with wheat bran at a ratio of 7:3. The mixture was thoroughly mixed and kept in a 500 ml flask. The rice medium was prepared by placing 250 g of long–grain rice in a 1000 ml beaker containing 250 ml of distilled water and cooking in a microwave oven for 15 min. The rice was cooled at room temperature. All substrates were kept in closed containers at 5 °C refrigerators till usage.

Inoculation of substrates

From each treatment, 7.5 g of the solid substrate was placed in a test tube, 20 mm x 150 mm, and 5 ml of distilled water were added into the tubes. The samples were autoclaved for 60 min at 123°C and a pressure of 1.8kg/cm3. The tubes were then cooled for 24 h and 3 ml of 5 $\times 10^6$ conidia/ml suspension in 0.05% Tween 80 was subsequently inoculated into each tube. All inoculation procedures were carried out in a laminar airflow chamber. The tubes were agitated and incubated in the dark at 25 ± 1°C for 2 weeks.

Quantification of conidia

The yield of conidia from different solid substrates and SMC+wheat bran were quantified. One gram of each conidial substrate was placed in a 100 ml conical flask containing 10 ml sterilized distilled water with a few drops of Tween 80. The flask was shaken in an automatic shaker VELP Scientifica® twice for 10 min and the suspension was serially diluted and the conidia were counted using a Neubauer haemocytometer under a phase–contrast microscope at 400× magnifications.

Preparation of specimen for Scanning Electron Microscopy (SEM)

To determine the pattern of fungal growth and colonization on the SMC+wheat bran media, samples of SMCcontaining conidia and wheat bran were prepared for scanning electron microscopy (SEM). The conidial substrates were fixed in 4% Glutaraldehyde for 12 h at 4 °C. Then they were washed with 0.1M Sodium Cacodylate Buffer for three changes of 10 min each, fixed in Osmium Tetroxide for 2 h at 4 °C and washed again with 0.1M Sodium Cacodylate Buffer for three changes of 10 min each. The specimens were dehydrated in a series of acetone (35, 50, 75, and 95%) for 10 min in each and in100% for 15 min in 3 changes. The specimen was fixed in the basket and placed into the critical dryer for about 30 min, mounted on specimen stubs, sputter–coated and viewed under a Philips 400 Scanning Electron Microscope at the Institute of Bioscience, University Putra Malaysia (UPM).

Bioassay on second instar *Bemisia tabaci*

Whiteflies, *B. tabaci* (biotype Asia 1) were reared on potted eggplants, *Solanum melongena* L., maintained in cages at the insect rearing room of Faculty of Forestry, UPM, at 65%±5 RH, 27 °C±1, photoperiod 12L:12D. Whitefly adults were introduced onto two–week–old plants and were allowed to lay eggs for three days. Plants with eggs were transferred to new cages for subsequent development. The development was monitored and second instar nymphs were used for the bioassay. Conidia from the three substrates, rice, SMC and SMC+wheat bran, were bioassayed on second instars of *B. tabaci*. Before conducting the bioassay, the conidia were tested for their viability (Braga *et al*., 2001) and all conidia had more than 85% viability. Conidia suspension at 1×10^6 conidia per ml was prepared for each treatment. Eggplant plant leaves infested with at least 30–second instar nymphs of *B. tabaci* were dipped in the suspension for 2–3 sec. The leaves were placed separately in a 9 cm Petri dish lined a piece of moistened filter paper. Controls were treated with 0.1% Tween 80 water solution. The Petri dishes were sealed with parafilm and kept in a 25 ± 1 °C incubator, with a photoperiod of 12L:12D and 90% relative humidity. Mortality of *B. tabaci* was recorded daily and only dead nymphs with signs of fungal infection, as observed under a microscope, were counted. Each treatment was replicated four times.

Effect of UV B on conidia germination Conidia produced on white rice, PDA and SMC+wheat bran were suspended separately in 10 ml sterilized distilled water containing Tween 80 (0.05%) in a glass tube. The tubes were shaken in a vortex mixture (Stuart®) for 10 min and the conidial concentrations were adjusted to 10×10^5 spores/ml. A 0.1 ml aliquot of the suspension was pipetted and spread evenly onto PDA.

Petri dishes containing The Petri dishes with conidia were immediately exposed to UV–B irradiance (30 centimetres) (UV–B lamb, 302nm, 8 Watt, UVM–18 Ultra–Lum Inc™., California) for 30, 120, 240, 360 and 480 min. The UV–B lamb light source was calibrated using a Lutron UV–340® UV light meter. PDA plates were incubated at 25 ±1 °C for 24 and 48 h. Conidia viabilities were assessed for all treatments following a method described by Braga *et al*., (2001). Each treatment was replicated four times.

Shelf life of conidia

Isaria fumosorosea was cultured on white rice, SMC + wheat bran, SMC, and PDA at room temperature (26 ±1 ºC). After one and every 8^{th} week thereafter until the 54^{th}, the conidia from each substrate were harvested for viability assessment. The conidia were suspended in 10 ml in glass tubes containing sterilized distilled water with 0.05% of Tween 80. The tubes were shaken in a vortex mixture (Stuart®) for 10 min. The suspension was serially diluted to 10×10^5 spores/ml. A few drops of the suspension were plated onto Petri dishes containing PDA. The PDA plates were incubated at 25 ±1°C for 24 h and conidia viabilities were assessed for all treatments following a method previously described. Four replicates were used for each treatment.

Data analysis

The data were subjected to an ANOVA and the means were compared using Tukey's multiple range tests (0.05 level of significance), (SAS Institute 1999).

Results

Chemical changes in the substrates during the composting process

The quantity of main elements in the compost changed over time during composting (Table 1). The percentage of nitrogen (N) increased from 1.2% in the bunker stage to 1.8% in the SMC while the percentage of carbon decreased from 40% in the bunker to 26%. The changes in these two elements significantly affected the C: N ratio of compost. The C: N ratio significantly decreased over time (R2=0.66) to about 20, from 33 in the bunker to 13 in the SMC (Fig. 1). The C: N ratios in SMC+ wheat bran and rice were 8.07 and 13.08, respectively. The moisture content dropped by 20%, from 75% in the bunker to 54% in the SMC. During the fermentation process, the compost changed slightly from alkaline, pH=8.26, to slightly acidic, pH =5.42. The changes in the amount of lignin during composting were not significantly different. The proportion of lignin slightly fluctuated from 25% in the wheat straw at the initial stage of composting, decreased to 21% at the bunker and subsequently increased respectively to 24%, 26% and finally to 29% in the tunnel, spawning stage and SMC.

Table 1. Chemical properties, moisture content of solid media in various stages of composting

Stages	Nitrogen (%)	Carbon (%)	C: N ratio	Ammonia (%)	pH	M C. %
Bunker	1.25d	44.06b	35.40a	0.133a	8.56 a	76.00a
Tunnel	1.38c	40.10b	28.03c	0.083b	8.24ab	74.00ab
Spawning	1.86b	35.60c	19.43d	0.023c	7.70b	71.00bc
Harvesting	2.10a	34.26c	16.13e	0.002d	7.56b	69.50c
Rice	1.64b	54.20a	33.00 b	0.001e	4.81e	52.00d
SMC	1.83b	26.30d	13.80f	0.002d	6.39c	69.00c
SMC+ Bran	1.90ab	15.50e	8.07g	0.002d	5.42 d	54.00f

Mean values in the same column followed by different letters are significantly different (Tukey's HSD, p<0001)

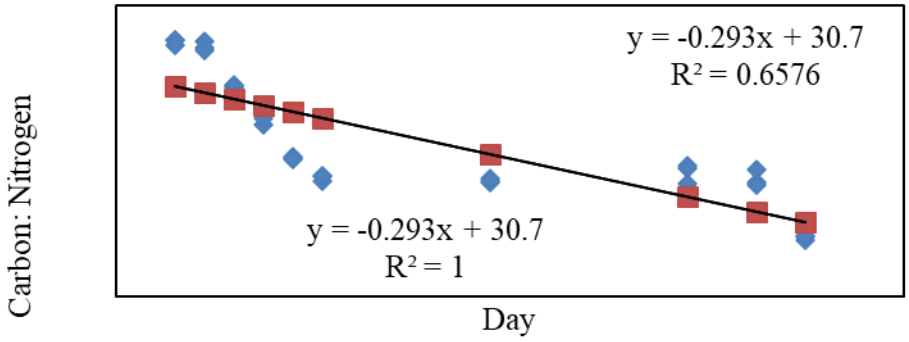

Fig. 1. Changes in C: N ratio during the compost fermentation process

Conidia production on different stages of the composting process

The solid substrates, taken from different stages of the mushroom composting process, significantly yielded a variable amount of conidia (Table 2). The fungus, *I. fumosorosea*, was unable to grow in the composts that were taken from the bunker and the tunnel and neither mycelia nor conidia were detected in samples from both substrates. The compost from the spawning stage was able to support the growth of the fungus but only 150 conidia per gram were obtained. A significantly greater conidia production occurred in the later composting stages. The composts from the mushroom harvesting stage and SMC yielded 4.8×10^8 and 8.5×10^9 conidia per gram of composts, respectively. The yield was greater than 2.17×10^{10} per gram of composts when 30% of wheat bran was added to the SMC. The C: N ratio of SMC+ wheat bran was 8.07. The yield of conidia was higher with a decreasing C: N ratio (R2 =0.65) of the substrate (Fig. 2). The rice media with a C: N ratio of 13.8 and served as the baseline control produced 9.3×10^8 per gram of rice.

Table 2. The yield of conidia of *Isaria fumosorosea* cultured on substrates from different stages of composting

Fermentation Stage	Number of conidia/g substrates	Weight of conidia (mg/100g substrate)
Bunker	0.01 ± 0e	0.0002 ± 0e
Tunnel	0.02 ± 0e	0.001 ± 0e
Spawning	150 ± 45d	0.003 ± 0.001e
Harvesting	$4.8 \times 10^8 \pm 3.1\times10^5$c	2600 ± 294d
Rice (Control)	$9.3 \times 10^8 \pm 4.7\times10^5$c	4925 ± 442c
Emptying stage (SMC)	$8.5\times10^9 \pm 6.7\times10^5$b	21450 ± 964b
SMC + Wheat bran	$2.17 \times 10^{10} \pm 5.9\times10^5$a	43400 ± 1290a

Mean values followed by different letters are significantly different (Tukey's HSD, p<.0001)

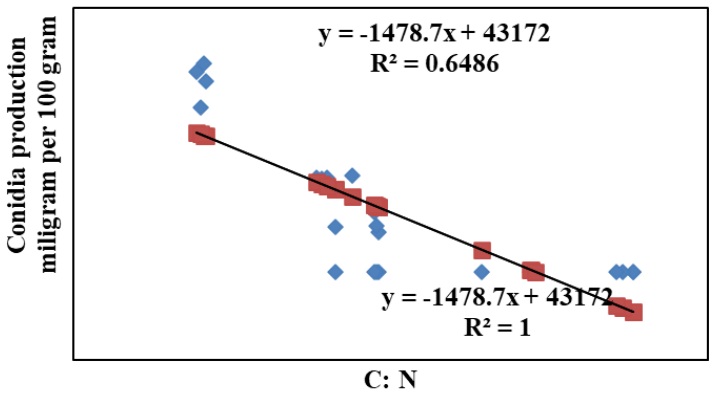

Fig. 2. Relationship between conidia production of *I. fumosorosea* and C: N ratio of the substrate

Effect of UV–B radiation on conidial germination

The effect of UV–B radiation on the viability of the conidia varied significantly with the substrate on which they were grown. Even though the percentage of germination from both substrates decreased with increasing exposure time to UV–B radiation, the rate of deactivation of conidia from SMC+Wheat bran was significantly slower compared with those conidia from rice (Fig. 3). After 120 min of UV–B exposure, the per centage of germination from SMC+Wheat bran and rice was 90.75% and 77.50%, a decline of 7% and 20%, respectively. The viability of conidia from rice decreased to 24% when the duration of exposure was extended to 480 min while conidia from SMC+wheat bran still retained 73.50% germination (Table 3). The data indicate that the germination of conidia from SMC+wheat bran was about three times more than those of conidia from rice.

Scanning electron microscope (SEM) examination shows that materials in the SMC+Wheat bran, wheat straw, gypsumand chicken manure, were well mixed (Fig. 4). Chicken manure and gypsum were uniformly blended with the wheat straws. Figure 5 represents a photomicrograph of substrate 14 days after incubation showing all parts of the substrate covered with the fungal mycelia. All stages of the fungus including mycelia, conidiophores, conidia, were found on the substrate.

Mortality of *B. tabaci*

I. fumosorosea regardless of the substrates cultured on were pathogenic to *B. tabaci* nymphs. More than 80% mortality was recorded from all treatments. Conidia taken from SMC + wheat bran caused the highest mortality 86.00 % but it was not significantly different from mortalities caused by conidia from rice, 84.00%, and SMC 82.00%. The nutrient content and growth conditions within substrates did not affect the virulence of the fungus (Table 4).

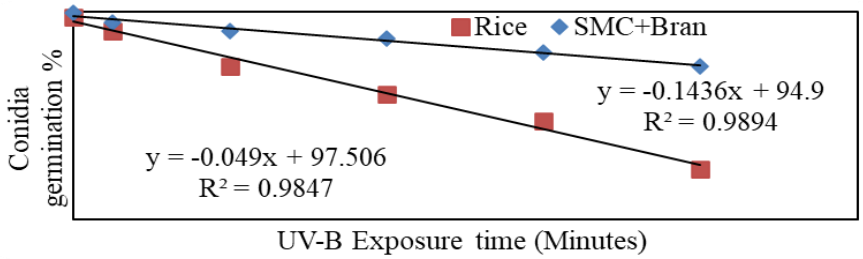

Fig. 3. Percentage of conidial germination for SMC+wheat bran and rice that had been exposed to different duration of UV–B

Table 3. Mean comparison of conidial germination based on the interaction between substrates and UV exposure time

Substrates	UV exposure time Minutes	Conidia germination% UVA	Conidia germination% UVB	Conidia germination% UVC
SMC70%+Wheat bran30%	0	97.2 a	97.2 a	97.2 a
	30	96a	94.5a	94.5a
	120	90.5ab	90.75ab	90.25ab
	240	89.5b	86.75bc	89ab
	360	86.5b	80.25cd	85.25bc
	480	72.5cd	73.5d	72d
White Rice	0	97.2 a	97.2 a	97.2 a
	30	90.25b	90.5ab	92.5a
	120	77.5c	73.75d	81.25c
	240	67.5d	60.25e	73d
	360	52.5e	47.25f	53.75e
	480	19.25f	24g	18.5f

Means in the column followed by different letters are significantly different (Tukey's test, $p<.0001$)

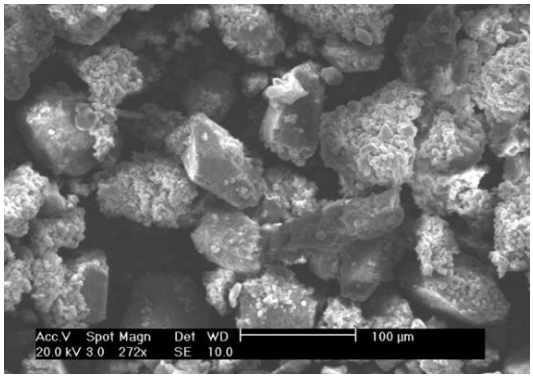

Fig. 4. Spent mushroom compost showing interparticle spaces created by gypsums

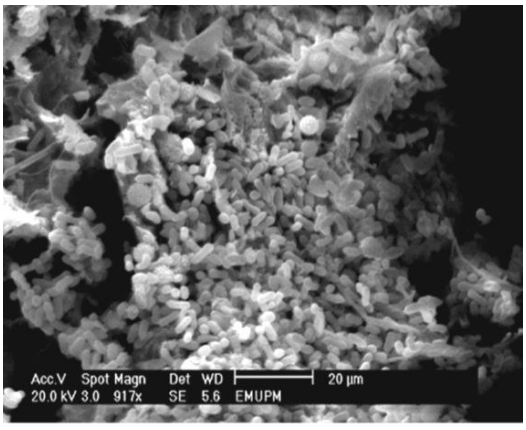

Fig. 5. *Isaria fumosorosea* colonizing SMC + wheat bran 14 days after incubation

Table 4. Effect of substrates for conidia production of *P. fumosoroseus* on mortality response of second nymph of *B. tabaci*

Substrate	Mean Mortality	Lower Bound	Upper Bound
SMC+Bran	86±3 a	82	88
Rice	84±4 a	78	85
SMC	82±3 a	82	88

Means followed by the same letter within each column are not significantly different (Tukey´s multiple range test at 0.05 level of significance)

Shelf life of conidia

The result indicates that percentage of germination was significantly affected by the type of substrates and the duration of storage under the study condition (26 ±1 ºC). The conidia from rice and PDA loss their viability faster than conidia from SMC and SMC + wheat bran (Fig. 6). During the first week, conidial viability from all treatments was more than 90%. After the 8th week, the germination of conidia from rice and PDA dropped to 59.33% and 56.33%, respectively, while those conidia from SMC and SMC + wheat bran still retained 94% and 93% viability, respectively. From the 16th week onwards conidial viability from rice, 13.33%, and PDA, 10.00% decreased sharply to zero when stored for 24 weeks. On the other hand, conidia from SMC and SMC + wheat bran still maintained more than 90% germination. They remained viable with 70% germination even after 64 weeks of storage.

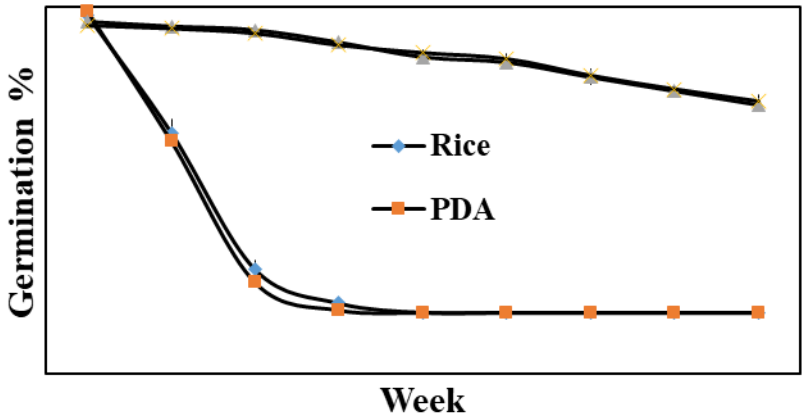

Fig.6. Germination of *Isaria fumosorosea* conidia cultured on different substrates after several weeks of storage

Discussion

The present study demonstrates that SMC from the Iranian mushroom farm is suitable to be converted into a solid substrate for mass production of entomopathogenic fungus *I. fumosorosea*. SMC + wheat bran has several advantages over the commonly used substrate, rice. The SMC+wheat bran not only retained its virulence against the target insect, *B. tabaci*, and it also enhanced fungal growth and conidia yield, providing greater conidial stability in terms of their shelf life and UV–B protection. Apart from the SMC, the substrates from the initial composting stages were not suitable for fungal production. This finding indicated that the yield of conidia depended on the maturity of the compost. Among the factors could be the high C: N ratio in the early stages of composting. A study by Gao & Liu (2009) showed that sporulation patterns of some fungi used as microbial control agents were affected by the C: N ratios of the substrates and the highest sporulation invariably occurred in substrates with low C: N ratios the same SMC. However, as the composting processes proceeded, changes in compost elements occurred.

The carbon compounds decreased while cation exchange capacity (CEC) and nitrogen increased as the SMC further decomposed. The addition of carbohydrate and fibre–riched wheat bran reduced the C: N ratio to 8±0.09, a level within a recorded range of matured organic composts, 7.8 –11.8 (Bernal *et al.*, 1998). This level of C: N ratios favoured the development and sporulation of many entomopathogenic fungi (Gao *et al.*, 2007). Calcium sulphate or gypsum added at the beginning of composting process still retained its physical functions. As applied in agriculture for soil

amelioration (Jayawardane & Chan, 1994) gypsum prevented clumping in the SMC+wheat bran substrate. It made the SMC porous hence creating a larger surface area that facilitated *I. fumosorosea* mycelia to penetrate and ramify uniformly within the entire substrate, and yielded a higher mass of conidia per unit weight of the substrate. With sufficient aeration and moisture, the fungus grew aerobically and produced conidia profusely on and within the substrate.

Conditions favourable for higher conidial production with extended shelf life may be lacking in rice or other cereal–based solid substrates. These cereal–based substrates tend to clump more than moisture thereby reducing the interparticle spaces. The extended viability of the conidia from the SMC+wheat bran was comparable with conidia from other entomopathogenic fungi that had been formulated and stored in different types of oils (Alves *et al*., 2002). Under a constant storage temperature, factors such as availability of nutrients, moisture and aeration could have contributed to the longer shelf life of the conidia. Compared with SMC + wheat bran, rice substrate which produced less conidial mass with a shorter shelf life could be less nutritive, more compacted and less aerated than SMC + wheat bran substrate. With its shelf life exceeding 12 m (64 weeks), *I. fumosorosea* mass–produced on SMC+ wheat bran fits the practical requirements of the fungus for field applications (Alves *et al*., 2002). The high lignin content in SMC + wheat bran could have provided better protection to the conidia from UV–B radiation than those grown from rice. Earlier studies have proven that lignin did enhance UV–B protection when added to preparations and formulations of entomopathogenic fungi (Alves *et al*., 2002). The composition of elements in SMC used in this study, however, may be different compared with other SMC from other regions. This variability may be due to the types of materials used in the preparation of the mushroom composts (Jordan *et al*., 2008), it is therefore essential that chemical analysis be conducted on its composition before it can be utilised for mass production of entomopathogenic fungi. In conclusion, apart from alleviating environmental problems, SMC can be an economically attractive resource for mass–producing fungal based–biopesticide.

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تبدیل بستر رویشی قارچ خوراکی پس از اتمام برداشت (SMC)، به بستر جامد برای تولید انبوه قارچ بیمارگر حشرات *Isaria fumosorosea* (Wize)

رحیم اسلامی زاده[۱]، احمد سعید ساجاپ[۲]، زولکفلی عمر[۳]، نور آزورا آدام[۴]، مرضیه نعیمی‌فر[۵]

۱– استادیار، سازمان تحقیقات، آموزش و ترویج کشاورزی. شرکت کشاورزی جلگه دز (قارچ دزفول)، دزفول، خوزستان، ایران.
۲–استاد، دانشگاه پوترا مالزی، دانشکده جنگل ، مالزی.
۳، ۴– استاد، دانشیار، دانشگاه پوترا مالزی، دانشکده کشاورزی، مالزی.
۵– کارشناس ارشد بیماری شناسی گیاهی، شرکت کشاورزی جلگه دز (قارچ دزفول)، دزفول، خوزستان، ایران.
مسئول مکاتبات: رحیم اسلامی‌زاده، ایمیل: Reslamizadeh@gmail.com

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چکیده

مدیریت بستر قارچ خوراکی پس از اتمام مراحل برداشت (SMC)، که یکی از پسماندهای ارگانیک تولید شده از مزارع قارچ خوراکی محسوب می‌شود، یکی از مهم‌ترین مسائل حل نشده در کشورهای در حال توسعه و پیشرفته است. هدف این مطالعه، تبدیل SMC به‌عنوان یک پسماند، یک ماده کاربردی‌تر است. آنالیز شیمیایی بستر قارچ دکمه‌ای در مراحل مختلف تخمیر در خط تولید آن انجام شد و مناسب بودن آن‌ها برای تولید انبوه قارچ بیمارگر حشره، *Isaria fumosorosea* مورد بررسی قرار گرفت. نتایج نشان داد که بستر حاصل از مراحل اولیه تخمیر تولید، برای رشد *I. fumosorosea* مناسب نمی‌باشد، با این حال قارچ بیمارگر پس از آن روی بسترهای مرحله برداشت قارچ و SMC به خوبی رشد کرد. کنیدی‌های تولید شده در SMC نه تنها به اندازه کنیدی‌های برنج به آفت هدفش، مگس سفید *Bemisia tabaci* بیماری‌زا باقی ماندند، بلکه در برابر اشعه UV–B نیز مقاوم‌تر بودند و ماندگاری طولانی‌تری نسبت به کنیدی‌های تولید شده در برنج دارند. این ویژگی‌ها نشان داد که SMC می‌تواند به عنوان یک بستر جامد برای تولید انبوه قارچ بیمارگر *I. fumosorosea* برای یک آفت‌کش زیستی مبتنی بر قارچ سازگار با محیط زیست تجاری‌سازی و استفاده شود.

واژه‌های کلیدی: آفت‌کش‌های زیستی، سفیدبالک، اسپور غیر جنسی، قارچ دکمه‌ای، دانه غلات