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Research Note

Mass production of *Trichoderma* spp. on spent meals of parasitoid hosts reared in laboratory

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ABSTRACT: **Suitability of the spent meals of laboratory reared insect parasitoid hosts, namely, *Corcyra cephalonica* and *Sitotroga cerealella* for the mass production of *Trichoderma harzianum*, *T. viride* and *T. virens* was studied. Assessment of optimum moisture level required in *C. cephalonica* spent meal for mass production of *Trichoderma* spp. was done. Approximately 45 per cent moisture yielded maximum sporulation of *Trichoderma* spp. While investigating the utilization of spent meals of *C. cephalonica* and *S. cerealella*, it was observed that *S. cerealella* spent meal supported maximum sporulation and viable propagules in all the three species of *Trichoderma* and was on par with sorghum as a substrate. However, *C. cephalonica* meal yielded lowest sporulation and viable propagules.**

KEY WORDS: *Corcyra*, mass production, *Sitotroga*, *Trichoderma harzianum*, *T. viride*, *T. virens*

Corcyra cephalonica (Stainton) and *Sitotroga cerealella* (Olivier) are the two commonly used parasitoid hosts for the multiplication of *Trichogramma*. These hosts are fed on sorghum-groundnut substrate and Barley grains, respectively. Disposal of the spent meal of these parasitoid hosts is a problem faced by most of the biocontrol laboratories. Exploring the feasibility of the spent meals for mass production of antagonistic fungi will help in disposal and recycling of the wastes. *Trichoderma* species have been extensively used as the biocontrol agents against a varied range of soil borne plant pathogens (Mukhopadhyay and Mukerjee, 1997). To make the antagonistic fungi readily available to the farmers it is essential to have mass production of these bioagents on relatively cheaper substrates. Several attempts have been made in this direction and various substrates such as barley (Moity and Shatala, 1981), sorghum (Upadhyay and Mukopadhyay, 1986), tapioca rind (Kousalya and Jeyarajan, 1988) and cow dung gas slurry (Jacob and Sivapraskasam, 1993) were tried successfully for mass production of *Trichoderma* spp.

The present study was aimed to assess the optimum moisture required in the *C. cephalonica* spent meal for mass multiplication of *Trichoderma* spp., and to evaluate the suitability of the spent meals of laboratory reared insect parasitoid hosts,

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namely, *C. cephalonica* and *S. cerealella* for mass production of *Trichoderma harzianum*, *T. viride* and *T. virens*.

C. cephalonica and *S. cerealella* spent meals were obtained from the mass production units of Project Directorate of Biological Control and Biotech International Ltd., Bangalore, respectively. *C. cephalonica* meal contained groundnut (100g), yeast (3g) and sorghum (2.5 kg). Whereas, *S. cerealella* meal contained the whole grain of barley with husk. Sorghum and barley grains were also used for comparative study to ascertain the effectiveness of the spent meals of the parasitoid hosts on sporulation of three promising PDBC isolates *viz.*, *T. harzianum* (PDBCTH-10), *T. viride* (PDBCTV-23) and *T. virens* (PDBCTVS-12).

a. Assessment of optimum moisture required in Corcyra spent meal for mass multiplication of *Trichoderma* spp.

The optimum moisture required for the maximum growth and sporulation of *Trichoderma* spp. is based on the nature of the substrate. It is therefore essential to assess the optimum moisture level needed in a particular substrate before its use for mass multiplication of antagonistic fungi. As the optimum moisture level required in *C. cephalonica* spent meal has not been reported hitherto, therefore, different moisture regimes from 40 to 60 per cent were tried to record the optimum moisture level for maximum growth of *Trichoderma* spp. The moisture assessment was done with the help of moisture analyzer. Hundred-fifty grams each of the substrate having different moisture levels was filled in autoclavable polypropylene bags (25x30cm), sealed and autoclaved at 1.2kg/cm^2 pressure for 30 minutes, twice at an interval of 24 hours. Each bag was inoculated with five ml spore suspension (1x10^6) of each *Trichoderma* spp. and incubated at room temperature (15-25°C). After ten days, viable propagules (colony forming units – cfu/g) from each moisture level were estimated on PDA using serial dilution technique.

b. Mass multiplication of *Trichoderma* spp. on different substrates

The moisture level supporting maximum growth of the fungi in the above set of experiment was chosen for the *C. cephalonica* spent meal in the next set of experiment wherein all the four substrates were compared for their relative efficacy in mass multiplication of *Trichoderma* spp. The remaining three substrates, namely, *S. cerealella* spent meal, barely, and sorghum were soaked overnight in water and the excessive free flowing water was drained off. Hundred-fifty grams each of all the four substrates were filled in separate autoclavable polyethylene bags, sealed and autoclaved at 1.2 kg/cm^2 pressure for 30 minutes, twice at 24 hours interval. Each bag was inoculated with five ml spore suspension of each *Trichoderma* species, obtained from seven-day old culture of respective *Trichoderma* spp. and incubated at room temperature. Another set of experiment was carried out in bottles where 50g of the substrate was inoculated with three ml of spore suspension, rest all procedures followed were same as in case of bags, described earlier.

The effect of different substrates on sporulation of *Trichoderma* spp. was recorded at weekly interval; by recording the spore count by Haemocytometer with improved Neubaue scale and viable propagule count (cfu/g) by serial dilution technique on PDA. All the experiments were conducted in completely randomized design with three replications.

Assessment of optimum moisture required in *C. cephalonica* spent meal for mass multiplication of *Trichoderma* spp.

All the three *Trichoderma* spp. produced significantly higher propagules (cfug^{-1}) on *C. cephalonica* spent meal at 45 per cent moisture (1.53, 1.67 and 1.63 X 10^6 cfu g^{-1}) than the count recorded at other moisture levels of 40, 50, 55, and 60 per cent (Table 1). Jacob and Sivaprakasm (1993)

observed that 40-60 per cent moisture in FYM supported faster multiplication of *T. harzianum* and *T. viride*. Elad *et al.* (1980) observed that *T. harzianum* grew and sporulated better when the moisture content of wheat bran + saw dust was 35 per cent. In soil, the growth and multiplication of *T. viride* and *T. harzianum* are favoured at 40 to 60 per cent moisture levels (Krishnamoorthy, 1987).

b. Mass multiplication of *Trichoderma* spp. on different substrates

Sporulation (spores/g) and viable propagule count (cfu/g) of *T. harzianum*, *T. viride* and *T. virens* produced on four different substrates are presented in Table 2, 3 and 4, respectively. All the three species of *Trichoderma* were observed to produce maximum sporulation and viable propagule count after 21 days on all the four substrates.

Maximum sporulation of *T. harzianum* was observed on *S. cerealella* spent meal ($16.63X10^6$spores/g^{-1}on 21 days) followed by the spore count on sorghum ($15.17X10^6$spores/g^{-1}on 21 days) (Table 2). Maximum viable propagules of *T. harzianum* were observed on sorghum ($37.33X10^6$ cfu/g^{-1}on 21 days), followed by the count on *S. cerealella* spent meal ($37.00X10^6$ cfu/g^{-1}on 21 days). These spore counts and viable propagule counts were significantly higher than the counts recorded on barley ($12.73X10^6$ spores/g^{-1} and $26.67X10^6$ cfu/g^{-1} on 21 days) and *C.*

Table 1. Effect of moisture levels in *C. cephalonica* spent meal substrate on the production of viable propagules of *Trichoderma* spp.

Moisture (%)	X 10^6 cfu g^{-1} substrate after 10 days		
	T. harzianum	*T. viride*	*T. virens*
40	0.87^{bc}	0.93^{b}	0.93^{b}
45	1.53^{a}	1.67^{a}	1.63^{a}
50	1.00^{b}	1.07^{b}	0.93^{b}
55	0.60^{c}	0.63^{c}	0.53^{c}
60	0.20^{d}	0.27^{d}	0.17^{d}
CD (P=0.05)	0.30	0.27	0.30

Table 2. Effect of substrates on sporulation and viable propagule count of *T. harzianum* at different time intervals

Substrate	Sporulation (X 10^6 spores g^{-1}) days after			Viable propagule count (X 10^6 cfu g^{-1}) days after		
	7	14	21	7	14	21
Corcyra spent meal	0.90^{c}	4.37^{c}	8.60^{d}	1.30^{b}	15.33^{b}	20.00^{c}
Sitotroga spent meal	3.73^{a}	8.60^{a}	16.63^{a}	7.00^{a}	19.67^{a}	37.00^{a}
Barley	2.57^{b}	6.90^{b}	12.73^{c}	5.00^{a}	15.33^{b}	26.67^{b}
Sorghum	3.40^{a}	8.83^{a}	15.17^{b}	6.67^{a}	20.00^{a}	37.33^{a}
CD (P=0.05)	0.67	1.22	1.43	2.44	3.11	5.38

Table 3. Effect of substrates on sporulation and viable propagule count of *T. viride* at different time intervals

Substrate	Sporulation (X 10^6 spores g^{-1}) days after			Viable propagule count (X 10^6 cfu g^{-1}) days after		
	7	14	21	7	14	21
Corcyra spent meal	0.47^c	3.23^c	8.70^d	0.90^c	7.67^c	22.00^c
Sitotroga spent meal	3.97^a	8.47^a	16.80^a	9.00^a	18.67^a	39.00^a
Barley	2.53^b	6.50^b	12.77^c	6.00^b	12.33^b	29.00^b
Sorghum	3.50^a	8.27^a	15.03^b	8.00^a	18.67^a	38.33^a
CD (P=0.05)	0.56	1.04	0.81	1.64	3.17	6.27

Table 4. Effect of substrates on sporulation and viable propagule count of *T. virens* at different time intervals

Substrate	Sporulation (X 10^6 spores g^{-1}) days after			Viable propagule count (X 10^6 cfu g^{-1}) days after		
	7	14	21	7	14	21
Corcyra spent meal	0.37^c	4.03^d	8.80^c	1.27^c	8.67^c	20.00^b
Sitotroga spent meal	3.77^a	9.10^a	16.90^a	9.33^a	19.67^a	39.33^a
Barley	2.87^b	6.77^c	13.57^b	6.33^b	16.33^b	26.67^b
Sorghum	3.57^a	8.97^b	15.60^a	8.67^{ab}	21.33^a	37.00^a
CD (P=0.05)	0.69	0.69	1.05	2.19	3.56	7.74

cephalonica spent meal (8.60X10^6 spores/g^{-1} and 20.00X10^6 cfu/g^{-1} on 21 days). Similar results were observed in case of *T. viride* and *T. virens* (Table 3 and 4). However, maximum viable propagules of these two antagonists were found on *S. cerealella* spent meal (39.00 and 39.33X 10^6cfu/g^{-1}, respectively on 21 days) followed by the viable propagule counts on sorghum (38.33 and 37.00X10^6 cfu/g^{-1}, respectively on 21 days). The viable propagule counts of all three species of *Trichoderma* spp. were significantly low on *C. cephalonica* spent meal (20.00, 22.00 and 20.00 cfu/g^{-1}, respectively on 21 days). This was mainly due to the problem of caking which resulted in poor and irregular sporulation. Therefore, further studies are required to minimize the caking so that maximum surface area is available to the antagonistic fungi for its mycelial spread. The results clearly indicate the superiority of *S. cerealella* spent meal over *C. cephalonica* spent meal as a substrate for mass production of the three species of *Trichoderma*.

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