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Prawn exoskeleton as an ingredient in the synthetic medium to culture an entomogenous fungus, *Zoophthora radicans* (Brefeld) Batko

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ABSTRACT: The growth parameters and infectivity of an entomogenous fungus, *Zoophthora radicans* (Brefeld) Batko were promoted significantly when cultured on Sabouraud Maltose Agar + 50 per cent prawn exoskeleton (biomass: 725 mg; radial growth : 84.75 mm ; sporulation 90.71% ; infectivity : 71.90%) followed by 75 and 25 per cent dosages, thus revealing it to be a suitable ingredient in the synthetic media.

KEY WORDS: *Cnaphalocrocis medinalis*, growth parameters, infectivity, prawn exoskeleton, *Zoophthora radicans*

Zoophthora radicans (Brefeld) Batko (Zygomycotina: Entomophthoraceae) has been recorded recently as a potential mycoparasite of rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) in India (Narayanasamy, 1994). Certain subgenera of *Zoophthora*, do not grow well on simple nutrient medium but when supplemented with carbon and nitrogen sources like egg yolk and yeast extract substantial fungal growth was observed.

Prawn exoskeleton which is thrown as waste in open spaces at the site of prawn processing industries and markets, is a rich source of carbon and nitrogen elements for nutritioning the fungal pathogen. It gets accumulated to the extent of 2.7 lakh tonnes annually, creating a highly contagious environment posing serious health hazards. Hence, an attempt was made to study whether the prawn waste would be useful in efficient growing insect fungi like *Z. radicans* in *in vitro* condition considering the nutritional content of its exoskeleton.

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MATERIALS AND METHODS

Fungus and insect culture

Indigenous strain of *Z. radicans* isolated from the field collected cadavers of rice leaf folder was cultured on Sabouraud Maltose Agar (SMA) plates and maintained at 25°C and 100 per cent RH. Leaf folder larvae collected from rice fields were reared on potted rice plants (var. IR 50) following the method developed at IRRI (Waldbauer and Marciano, 1979).

Preparation of prawn exoskeleton powder

The prawn waste collected from the shrimp farm and market was cleaned and washed thoroughly to remove the adhering tissues. Later it was thoroughly sun-dried and powdered finely. To get an uniform mixture with the synthetic medium, the ground powder was passed through sieve and fine powder of particle size, 37μ was obtained.

Three quantities, *viz.*, 1.62, 3.25 and 4.87g/100ml amounting to 25, 50 and 75 per cent of the powder in the synthetic medium were maintained in the SMA medium and the same quantities were added to Sabouraud Maltose Broth (SMB) separately and mixed thoroughly. The media were autoclaved at 1.15kg/cm^2 for 15 minutes. Four replications were maintained per concentration for each of the parameter. Besides, two control, one with synthetic medium alone and another with synthetic chitin alone were included.

Studies on biometric characteristics of *Z. radicans*

Biomass production

Discs cored (10mm diam) out of the fungal cultures of *Z. radicans* grown on SMA medium were inoculated in the SMB containing 25, 50 and 75 per cent prawn exoskeleton powder separately and incubated for 10 days at 25°C and 100 per cent relative humidity. Mycelial mats were collected separately by suction filtering on preweighed filter paper (Whatmann No.1), dried in hot air oven at 105°C for 24 h and weighed again. The difference in the weight was the biomass produced. Synthetic Medium Broth and synthetic Chitin Broth (SCB) were included as controls.

Radial growth

Mycelial discs (10mm) of *Z. radicans* collected from actively growing colony were seeded at the centre of agar medium containing 25, 50 and 75 per cent prawn exoskeleton powder separately in plates. The dishes were incubated at 25°C and 100 per cent relative humidity for 10 days. The diameter of the growth circle of the fungal colony in each treatment was measured. The control treatments included SMA and synthetic chitin + agar.

Spore germination

A drop of spore suspension (10^7spores/ml) of *Z. radicans* prepared from the respective treatment culture and control in broth was placed on 2mm agar discs seeded on glass slide. The slides were incubated

at 25°C in moist chambers having 100 per cent relative humidity, and per cent germination was determined after 24 h. The criterion of spore germination was development of germ tube equal to the diameter of the spore.

Infectivity

The spore suspension of *Z. radicans* with a spore load of 10^7/ ml prepared from respective treatment culture and control in broth was given as a fine mist spray on the leaf folder larvae confined in potted rice plants (var. IR 50) enclosed in mylar film cages. Third instar leaf folder larvae numbering twenty were tested in each replication and mortality due to mycosis was recorded four days after treament.

RESULTS AND DISCUSSION

Result on the effect of different dosages of the prawn exoskeleton on growth characterisitics of *Z. radicans* are presented in Table-1.

Biomass production

Highest production of biomass (725 mg) was witnessed in SMA mixed with 50 per cent of prawn exoskeleton powder followed by 75 and 25 per cent of prawn exoskeleteon powder (692.5 and 607.5mg, respectively). Synthetic chitin alone yielded the least biomass (235mg).

Radial growth

The radial growth of *Z. radicans* in

Table 1. Effect of different concentrations of prawn exoskeleton on the growth characteristics and infectivity of *Z. radicans*

Sl. No	Treatment/ Concentration	Biomass (mg)*	Radial growth (mm)* (%)**	Spore germination	Corrected mortality (%) of leaf folder **
1.	SMA+ Prawn exoskeleton (25%)	607.50 (24.65)^{c}	77.00 (8.77)^{c}	84.59 (66.89)^{bc}	65.65 (54.12)^{bc}
2.	SMA+ Prawn exoskeleton (50%)	725.00 (26.93)^{a}	84.75 (9.21)^{a}	90.71 (72.25)^{a}	71.90 (57.99)^{a}
3.	SMA+ Prawn exoskeleton (75%)	629.50 (26.32)^{b}	82.50 (9.09)^{b}	86.65 (68.57)^{b}	68.44 (55.82)^{ab}
4.	Synthetic chitin alone	235.00 (15.33)e	44.75 (6.69)^{e}	41.44 (40.07)^{e}	38.21 (38.18)^{e}
5.	Synthetic medium alone (SMA)	475.00 (21.79)^{d}	73.75 (8.59)^{d}	83.01 (65.66)^{d}	64.28 (53.30)^{d}
	SEM ±	0.07	0.02	0.78	1.23
	CD (P=0.05)	0.19	0.06	2.22	3.49

Figures in parentheses are square root*/ arcsine** transformed values

SMA containing 50 per cent prawn exoskeleton powder was on par with 75 per cent combination. SMA + 25 per cent prawn exoskeleton powder had 77mm growth which was higher than the control treatments namely synthetic chitin (44.75mm) and synthetic diet (73.75mm).

Spore germination

Maximum spore germination (90.71%) was recorded with 50 per cent prawn powder. Spore germination in 25 per cent combination (84.59%) and 75 per cent combination (86.65%) treatments was on par with each other. Synthetic chitin had very low germination (41.44%).

Infectivity

The per cent mycosis of leaf folder larvae was maximum (71.90%) with 50 per cent prawn exoskeleton powder followed by 75 per cent (68.44%). All the three doses of prawn exoskeleton exceeded the control medium in regard to the infectivity of *Z. radicans*.

It is evident that 50 per cent of prawn exoskeleton powder performed very well in enhancing growth and infectvity of *Z. radicans* and was superior over the control. However, increase in the concentration beyond 50 per cent did not enhance the biometric characteristics of *Z. radicans* to a considerable extent.

No work on the utility of prawn skin waste in this aspect has been carried out elsewhere though Shoemaker (1991) has proposed the utility of chitin and chitosan in agriculture in other ways.

Magalhaes *et al.* (1991) stated that various nitrogen and carbon sources influenced the conidium germination and fungal morphology. From the above findings, it can be inferred that chitin, a rich source of carbon and nitrogen can be utilized effectively by *Z. radicans* to putforth more growth and infectivity. But when synthetic chitin alone was used it remained underutilized. This is in agreement with that of Huber's (1958) observations.

Coudron *et al.* (1984) examined the production of chitinase and exochitinase activity in the germinating conidia of fungi like *Beauveria bassiana, Metarhizium anisopliae* and *Nomuraea rileyi*. Similarly to quicken the process of infection, *Z. radicans* might utilise the chitin by producing chitinase enzyme.

This gains more momentum as the prawn waste has been advocated already as seed coat to prevent soil fungal attack, as an anti-nematode agent and as poultry feed. This finding would expand the scope of using the prawn skin in agriculture. It is therefore concluded that prawn exoskeleton can be used as an ingredient in the synthetic media used in culturing insect fungus, *Z. radicans*. It is suggested that prawn exoskeleton extracts can be prepared similar to that of beef extract in making the media more useful.

In any biological control programme, mass production of the bio-agents is the ultimate goal for field use. Hence, prawn exoskeleton can find a suitable place in the synthetic media used for mass production of *Z. radicans*.

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