JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2015. Vol. **9**(2), p. 1579-1582

Studies on Development of Cost Effective Entomopathogenic Fungal Biopesticides for Sustainable Agriculture

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(Received: 15 January 2015; accepted: 02 March 2015)

A systematic study was conducted to develop the cost effective entomopathogenic fungal biopesticide. The different grains like Sorghum, Ragi, Paddy and Rice were selected based on their local availability and their lower in cost. However the carbon and nitrogen requirement was met by adding 1 per cent yeast extract. Among all the treatments, the half crushed rice kernels used treatment showed their best by producing highest spore load of 2.15 spores g^{-1}. This was followed by half crushed Sorghum and Maize. Rice kernels also retained the highest B:C ratio (7.91:1). All together it is concluded that Rice and Sorghum could be considered as the best source for mass production of entomopathogens.

Key words: Entomopathogens, Biopesticides, Rice.

Most of the entomopathogenic fungi are facultative fungal parasites, which grow on non-living media under unnatural conditions. Spores are required in large scale, for that they need to be used in natural conditions for biological control of the pest. Under natural conditions they are exposed to many of the variations in natural factors like temperature, RH, availability of the contact with host and optimal conditions for their growth, wind velocity and many other unexpected conditions. So we need to deposit a heavy amount of spore load in the ecosystem so that there will be increased efficiency of the biocontrol agent. Fungi readily sporulate on synthetic media like SMAY and PDA. But the synthetic diet will be expensive and can be used for experimental scale only and not for large-scale production. Carbon and nitrogen are the most vital nutrients required for growth and sporulation. Natural media, which are invariably rich in carbon so it support the growth and sporulation of the fungus at lower cost compared to synthetic media. In this sense a experiment was conducted to develop the economic feasible, cost effective entomopathogenic fungal biopesticides which can also lower the chemical insecticide environment pollution.

MATERIALS AND METHODS

Organisms that used for the study were *Fusarium* sp. *Verticillium lecanii* and *Metarrhizium anisopliae* all the cultures were used for inoculation to the substrates at with 6 mm disc of two weeks old cultures form the pure stock cultures. Selection of different grains for the study was done with the criteria of local availability and low cost. The grains of Sorghum, Ragi, Paddy, Rice and Maize. Dextrose and peptone, carbon and nitrogen sources could be effectively replaced with 1% yeast extract to the treated grains. Boiling treatment was done for the grains for easy availability of nutrients for the growth and development of fungi.

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The experiment was also conducted by using the different grains by varying the boiling temperature by 5min, 7min, 9min and 10min. Following treatments were done

A - Whole grains Boiled for 5min Sorghum, Ragi, Paddy, Rice, Maize 1%YE

B - Whole grains over night soaked Sorghum, Ragi, Paddy, Rice, Maize 1%YE

C - Grains extract used Sorghum, Ragi, Paddy, Rice, Maize 1%YE

Next set of treatments were done keeping the boiling factor for 5min common for all the treatments, and varying the intactness of the grains.

T_1 - Half crushed & boiled for 5min: Sorghum, Ragi, Paddy, Rice and Maize with 1%YE

T_2 - Full crushed & boiled for 5min: Sorghum, Ragi, Paddy, Rice and Maize with 1%YE

T_3 - Whole grain & Boiled for 5min: Sorghum, Ragi, Paddy, Rice and Maize with 1%YE

One kg of each cereal was half boiled and shade dried. In another set, equal weight of grains were half crushed in a local mill and the grains were sprayed 20 ml of distilled water after spreading them on a paper and mixed evenly by repeated heaping to retain moisture on the grains. The grains were divided into four equal parts by weight and kept in the polyethylene bags. The mouth of the bag was passed through a 50 mm thick PVC pipe ring (2cm diameter and 0.5 cm thick) and folded back over the ring and fastened with the help of an elastic band. Then the mouth of the bag was closed with a cotton plug and covered with paper and fastened with an elastic band. This resembled a bottle shape and was helpful easy inoculation of the different entomopathogenic fungi without any contamination. The bags with grains were sterilized in an autoclave at 121^0C at 15 lbs for 30 sec. The bags of the each cereal were inoculated with a 6 mm disc of two weeks old different entomopathogenic fungi. Culture grown in SMAY. One bag was not inoculated and kept as a control check. The bags were incubated at room temperature of 24 ± 1^0C at 71±2% RH for 20 days. After the prescribed period the bags were cut open under the laminar hood and the contents were transferred to a uniform size paper folds and kept for drying in an oven at 60^0C until the weight unchanged. Then the dry biomass weight of the different entomopathogenic fungi was computed by subtracting the dry weight of the grain from the fourth bag, which had received no fungus.

One gram of digested material of each substrate with spores after thorough mixing with a sterile rod was suspended in 10ml sterile distilled water and concentration of spore was estimated following standard counting procedure using Neubauer's haemocytometer.

RESULTS AND DISCUSSION

Various experiments were conducted by using the different grains by varying the boiling temperature by 5 min, 7 min, 9 min and 10 min, and 5 min boiling of the grains was found to be the best over other treatments. So that 5 min was treated as best boiling temperature, the same was used for further studies. Studies were done on the best release of nutrients for best growth of fungi and production of good amounts of viable and virulent spores by altering moisture levels in the grains. Three different treatments were done to provide different levels of water. A with minimum

Table 1. Spore production by entomopathogens and B:C ratio of the different cereals

Cereals	Maize		Paddy		Rice		Ragi		Sorghum	
	A	B	A	B	A	B	A	B	A	B
Spores/250g grains	1.40	1.67	1.62	1.25	1.99	2.15	1.0	0.90	1.59	1.68
Cost of grains (Rs. /Kg)	4.00	6.00	6.00	6.50	10.00	6.50	5.00	5.50	6.00	7.00
Cost of production (Rs. / Kg)	26.50	28.50	28.50	29.00	32.50	22.50	27.50	28.00	28.50	29.50
**Net Income (Rs/Kg)	174	172	172	171	167	178	173	172	172	171
B:C ratio	6.55:1	6.02:1	6.12:1	5.88:1	5.13:1	7.91:1	6.27:1	6.14:1	6.02:1	5.78:1

A - Uncrushed grains B - Crushed grains

water just sufficient for the growth and development of fungi. B maximum water in intact condition of the grains. C with maximum water. Out of the above-mentioned treatments A was found to be the best and cheaper as compared to that other two. Followed by B and C. The best A treatment was used for future studies. Next set of treatments were done keeping the boiling factor for 5 min common for all the treatments, and varying the intactness of the grains. T_1 - Half crushed, T_2 - Full crushed T_3 - Whole grain. T_2 treatment was incapable of producing either good amount of mycelium or spores so results are not shown in Table. The spore yields in low cost substrates like cheaply available cereal grains with different boiling and crushing treatments were comparable or significantly higher to the standard medium. A high spore yield of 2.15 g/250g of substrate was realized on crushed rice kernel. Similar studies were done and it was observed that aerial conidia produced on such substrates are indistinguishable with respect to morphology and infectivity to that of insect cadavers (Ball *et al*., 1994).

Many workers have reported mass production of commonly known entomopathogenic fungi. However, two recent pioneering reports from Italy on the mass production of potential entomofungi, *Fusarium larvarum* (Cozzi *et al*., 2002) and *F. semitectum* (Ganassi *et al*., 2000) claimed that these fungi were mass produced on rice kernels. Vimaladevi *et al*. (2002) pointed out that *Nomuraea rileyi* was successfully multiplied on crushed sorghum with the addition of 1% yeast extract. Hence locally available substrates were used to mass-produce the isolated different entomopathogenic fungi.

Different cereal grains were evaluated for their suitability to support the biomass and spore production of the mycopathogen. The isolates required 4 to 6 days to initiate mycelial growth on the cereal substrates. The surface of media almost covered by 20 days after inoculation. The biomass produced by the different entomopathogenic fungi isolates on different cereal grains is given in the Table 1.

The spore production of isolates was significant among the cereals tested. Half crushed rice kernel yielded the highest spore load of 2.15 spore/g, which was superior over other cereals. Half crushed sorghum and maize were next best followed by paddy. Ragi was not able to support a good biomass and spore load of the fungus. The crushed grains were superior in production of spore over whole grains. Rice kernel retained the highest B:C ratio due to its increased grain cost (7.91:1). Rice adjusted a low benefit cost ratio of 4.5-4.63:1 probably due to its grain cost compare to sorghum (5.13-6.02:1) however, rice would be the best choice due to the high spore production than sorghum. Altogether rice and sorghum could be considered as the best carbon source for mass production. In addition spore production of the mycopathogen varies greatly due to the strain variation and environmental conditions. The variety of cereals used also has an influence on the mass production of fungi. Spore production in all substrates was estimated four weeks after inoculation. When the virulence of the spores was analyzed, it was noticed that best virulence was observed in the spores if crushed rice followed by maize, paddy, sorghum and rice. The spores that were found in the ragi were very week and percent germination was below 40% under suitable conditions in the lab studies. Where as in case of the other substrates it was notified that there was 90% and above. And the spores that were produced in case of crushed rice and paddy had almost 98% spore germination under controlled conditions. This variation in the spore vitality and virulence may be due to variation in the nutrients and different essential vitamins in different cereal grains. Ragi was not suitable for mass production.

REFERENCES

1. Ball, B. V, Pye, B. J and Carreck, N. L., Laboratory testing of a mycoinsecticide on non target organisms: the effects of an oil formulation of *metarhizium flavoviridae* applied to *Aphis mellifera*. *Biocontrol Sci. Technol.* 1994; **4**: 297-307
2. Cozzi, G., Stornelli, C., Moretti, A., Logrieco, A and Porcelli, F., Field evaluation of *Fusarium larvarum* formulations in the biocontrol of *Sessetia oleae* on Olive in Apulia, *Acta hort.* 2002; **586**: 811-814
3. Ganassi, S., Moretti, A., Stornelli, C., Fratello, B., Bonvicini, P. A., Logrieco, A and Sabatini, M.

A., Effect of *Fusarium*, *Paecilomyces* and *Trichoderma* formulations against aphid *Schizaphis graminum*, *Mycopathologia.* 2000; **151**: 131-138

4. Vimala devi, P. S., Prasad, Y. G and Chowdary, A., Effect of drying and formulation of conidia on virulence of the entomofungal pathogen *Nomuraea rileyi* (f) samson, *J. Biol. Control*. 2002; **16**(1): 43 – 48