Internat. J. Agric. Sci. Vol.3 No.2 June, 2007 : 153-157

Effect of combination of grains in media on the sporulation of *Beauveria bassiana* (Balsamo) Vuill.

K. ELANCHEZHYAN
Department of Agricultural Entomology, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

ABSTRACT
Laboratory experiments were conducted to study the effect of combination of grains in media on the sporulation of *Beauveria bassiana* (Balsamo) Vuill. Among the different combination of grains tested, rice in combination with pearl millet, sorghum, finger millet, maize (3:1), pearl millet + sorghum (1:3), maize + pearl millet (1:3) were superior to finger millet + pearl millet (1:3).

Key words : Agricultural products, Mass production, Entomopathogenic fungi.

INTRODUCTION

Entomopathogenic fungi particularly, white muscardine, *Beauveria bassiana* (Balsamo) Vuill. have been reported to be useful to control insect pest (Sandhu *et al*., 2001). The use of entomopathogenic fungi, due to their amenability to mass production, has potential in future strategies of pest management. *Beauveria bassiana* has one of the largest host list among the imperfect fungi. A number of successful commercial products based on conidia/spores of fungal pathogens have already been developed and sold internationally (Jenkins *et al*., 1998). The present studies were undertaken to evaluate the different culture media for the growth and sporulation of an isolate of *B. bassiana* obtained from Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore.

MATERIALS AND METHODS

The influence of different cereal nutritive substrates in combination was studied against *B. bassiana* in six different experiments including five preliminary experiments separately. The treatments were rice alone, rice + cereals at ratios 3:1, 1:1 and 1:3 (experiment 1); pearl millet alone, pearl millet + cereals at ratios 3:1, 1:1 and 1:3 (experiment 2); sorghum alone, sorghum + cereals at ratios 3:1, 1:1 and 1:3 (experiment 3); finger millet alone, finger millet + cereals at ratios 3:1, 1:1 and 1:3 (experiment 4) and maize alone, maize + cereals at ratios 3:1, 1:1 and 1:3 (experiment 5).

Fifty gram of each media of different combinations were prepared in three replicates as in previous experiment. Aliquots of 10 µl containing 10^5 spores were dispensed using micropipette and the cultures were incubated at 25 ± 0.5°C for 15 days. The observations on conidia yield, productivity ratio and the computation of cost was done as described earlier.

In the sixth experiment, media that yielded maximum conidia of *B. bassiana* or that gave the highest cost benefit ratio based on media cost were compared. Three replicates of each media was prepared and compared for the different parameters.

RESULTS AND DISCUSSION

Rice in combination with other grains :

Multiplication of *B. bassiana* in medium containing rice alone or rice + pearl millet (3:1) was significantly higher (2.53-2.62 x 10^9 conidia/g) than other combinations (Table 1). Combination of rice with finger millet and maize significantly reduced the spore yield. The productivity ratio of various media was not better than rice alone in this experiment. Combination of rice + pearl millet (3:1), rice+ sorghum (3:1) and rice + pearl millet (1:3) were marginally lower than rice alone (0.93-0.97) considering the productivity factor (Table 1). The required quantity of media to produce $1.5x10^{12}$ spore units was lower in rice alone (0.51 kg) and rice + pearl millet (3:1) (0.59 kg) than others. Among the different treatments, the highest cost/ benefit ratio was recorded in the combination of rice + pearl millet (1:3) (1:1.40) followed by rice + sorghum (1:3) (1:1.25).

Sorghum in combination with other grains :

Combination of sorghum and rice (1:3) resulted in $2.58x10^9$ conidia/g which was significantly superior to sorghum alone ($2.08x10^9$ conidia/g) or its combination with other grains in differing ratios (Table 2). Of the remaining treatments, sorghum + rice (1:1) was found to be the next effective treatment recording $2.35x10^9$ conidia/g.

Table 1 : Influence of rice in combination with other grains on the production of *B. bassiana*

Medium*$	Conidia/g ($x10^9$)	Conidia/kg ($x\ 10^{12}$)	Productivity ratio	Media (kg) for $1.5x10^{12}$ spores	Cost of media for $1.5x10^{12}$ spores (Rs.)	C:B ratio
R + S (3:1)	2.44 bcd	2.44	0.93	0.61	9.28	1:1.05
R + PM (3:1)	2.53 ab	2.53	0.97	0.59	9.02	1:1.08
R + FM (3:1)	2.32 def	2.32	0.89	0.65	9.85	1:0.99
R + M (3:1)	2.30 ef	2.30	0.88	0.65	9.98	1:0.87
R + S (1:1)	2.35 cde	2.35	0.90	0.64	8.45	1:1.15
R + PM (1:1)	2.46 bc	2.46	0.94	0.61	8.08	1:1.20
R + FM (1:1)	2.05 g	2.05	0.78	0.73	9.86	1:0.99
R + M (1:1)	1.89 h	1.89	0.72	0.79	8.77	1:1.11
R + S (1:3)	2.20 g	2.20	0.84	0.68	7.75	1:1.25
R + PM (1:3)	2.43 bcd	2.43	0.93	0.62	7.01	1:1.40
R + FM (1:3)	1.79 h	1.79	0.68	0.84	9.83	1:0.99
R + M (1:3)	1.55 I	1.55	0.59	0.97	11.54	1:0.84
Rice alone	2.62 a	2.62	--	0.51	9.72	--

* In a column, means followed by similar letters are not significantly different (P=0.05) by DMRT
$ R = Rice; S = Sorghum; PM = Pearl millet; FM = Finger millet; M = Maize

Combination of finger millet or maize with sorghum was found to reduce the spore yield significantly. The productivity ratio was higher in combinations of sorghum + rice or sorghum + pearl millet compared to sorghum alone, in general. Among them, sorghum + rice (1:3) gave the highest productivity of 1.24 followed by sorghum +

Table 2 : Influence of sorghum in combination with other grains on the production of *B. bassiana*

Medium*$	Conidia/g ($x10^9$)	Conidia/kg ($x10^{12}$)	Productivity ratio over sorghum alone	Media (kg) required to produce $1.5x10^{12}$ spores	Cost of media for $1.5x10^{12}$ spores (Rs.)	C:B ratio
S + R (3:1)	2.27 c	2.27	1.09	0.70	7.51	1:0.91
S + PM (3:1)	2.16 d	2.16	1.04	0.69	6.60	1:1.04
S + FM (3:1)	1.95 f	1.95	0.93	0.77	7.40	1:0.93
S + M (3:1)	1.88 g	1.88	0.90	0.80	7.13	1:0.89
S + R (1:1)	2.35 b	2.35	1.13	0.64	8.46	1:0.91
S + PM (1:1)	2.24 c	2.24	1.08	0.67	6.36	1:1.08
S + FM (1:1)	1.79 h	1.79	0.86	0.84	8.19	1:0.84
S + M (1:1)	1.67 i	1.67	0.80	0.90	8.86	1:0.78
S + R (1:3)	2.58 a	2.58	1.24	0.58	8.79	1:0.78
S + PM (1:3)	2.27 c	2.27	1.09	0.66	6.28	1:1.09
S + FM (1:3)	1.66 i	1.66	0.80	0.90	8.93	1:0.77
S + M (1:3)	1.47 j	1.47	0.71	1.02	10.25	1:0.67
Sorghum alone	2.08 e	2.08	--	0.72	6.85	--

* In a column, means followed by similar letters are not significantly different (P=0.05) by DMRT
$ R = Rice; S = Sorghum; PM = Pearl millet; FM = Finger millet; M = Maize

rice (1:1). The quantity of media needed to produce $1.5x10^{12}$ spore units was lower in sorghum + rice (1:3) (0.58 kg) than others. Considering the cost for the above target, combination of sorghum + pearl millet (1:3) was found to be the cheapest which was followed by sorghum + pearl millet (1:1). The cost/benefit ratio, however, was marginally higher in these treatments and than sorghum alone (Table 2).

Pearl millet in combination with other grains :

The sporulation of *B. bassiana* was significantly the highest in pearl millet + rice (1:3) followed by their combination at 1:1 and 3:1. The yield in these treatments was 2.55, 2.46 and $2.39x10^9$ conidia/g respectively which was superior to pearl millet alone or other combinations (Table 3). The productivity ratios were marginally better only in these treatments compared to pearl millet alone. It was found that the quantity of media required to arrive at $1.5x10^{12}$ spore units in pearl millet + rice (1:3) was minimum (0.59 kg). However, none of the treatments were showing increased cost benefit ratios than pearl millet alone (Table 3).

Finger millet in combination with other grains :

Combination of finger millet + rice (1:3) resulted in the maximum sporulation of $2.24x10^9$ *B. bassiana* /g compared to finger millet alone ($1.49x10^9$/g). Finger millet + pearl millet (1:3) and finger millet + rice (1:1) were the second best treatments yielding 2.07-2.09 conidia/g. All other treatments were found to be statistically inferior to the former combinations. The productivity ratio was the highest in finger millet + rice (1:3) followed finger millet + pearl millet (1:3) and finger millet (1:1). The quantity of media required was the lowest in finger millet + rice (1:3) (0.67 g) followed by finger millet + pearl millet (1:3) (0.72 kg). The highest cost benefit ratio of 1:1.46 was observed in finger millet + pearl millet (1:3) followed by finger millet + sorghum (1:3) (1:1.33) (Table 4).

Maize in combination with other grains :

Among the media, maize + rice (1:3) yielded the maximum quantity of $2.24x10^9$ conidia/g (Table 5). This was followed by maize + pearl millet (1:3) in the order of significance ($2.07x10^9$ conidia/g). It was found that maize + finger millet (1:3) or maize alone resulted in the lowest yield of $1.26x10^9$ conidia/g. The productivity ratio was the highest in maize + rice (1:3) (1.78) followed by maize + pearl millet (1:3) (1.64). The quantity of media needed for production was lower (0.67 kg) in maize + rice (1:3) followed by maize + pearl millet (1:3) that required 0.73 kg. The cost/benefit ratio was found to be the maximum in maize + pearl millet (1:3) (1:1.75) followed by maize +

Table 3 : Influence of pearl millet in combination with other grains on the production of *B. bassiana*

Medium*$	Conidia/g ($x10^9$)	Conidia/kg ($x\ 10^{12}$)	Productivity ratio over pearl millet alone	Media (kg) required to produce $1.5x10^{12}$ spores	Cost of media for $1.5\ x10^{12}$ spores (Rs.)	C:B ratio
PM + R (3:1)	2.39 bc	2.39	1.03	0.63	7.17	1:0.86
PM + S (3:1)	2.27 de	2.27	0.97	0.66	6.27	1:0.97
PM +FM (3:1)	2.13 gh	2.13	0.91	0.70	6.65	1: 0.91
PM + M (3:1)	2.05 h	2.05	0.88	0.73	7.03	1:0.86
PM + R (1:1)	2.46 b	2.46	1.06	0.61	8.08	1:0.75
PM + S (1:1)	2.23 ef	2.23	0.95	0.67	6.37	1:0.95
PM + FM (1:1)	1.93 i	1.93	0.83	0.78	7.61	1:0.80
PM + M (1:1)	1.81 j	1.81	0.18	0.83	8.20	1:0.74
PM + R (1:3)	2.55 a	2.55	1.09	0.59	8.92	1:0.68
PM + S (1:3)	2.16 fg	2.16	0.93	0.69	6.56	1:0.93
PM + FM (1:3)	1.71 k	1.71	0.73	0.88	8.69	1:0.70
PM + M (1:3)	2.12 gh	2.12	0.91	0.71	7.14	1:0.85
Pearl millet alone	2.33 cd	2.33	--	0.64	6.08	--

* In a column, means followed by similar letters are not significantly different (P=0.05) by DMRT

$ R = Rice; S = Sorghum; PM = Pearl millet; FM = Finger millet; M = Maize

Table 4 : Influence of finger millet in combination with other grains on the production of *B. bassiana*

Medium*$	Conidia/g ($x10^9$)	Conidia/kg ($x\ 10^{12}$)	Productivity ratio over finger millet alone	Media (kg) required to produce $1.5x10^{12}$ spores	Cost of media for $1.5x10^{12}$ spores (Rs.)	C:B ratio
FM + R (3:1)	1.78 d	1.18	1.19	0.84	9.87	1:1.02
FM + S (3:1)	1.93 c	1.93	1.30	0.78	7.70	1:1.31
FM + PM (3:1)	1.69 d	1.69	1.13	0.89	8.79	1:1.16
FM + M (3:1)	1.32 f	1.32	0.89	1.13	11.37	1:0.89
FM + R (1:1)	2.07 b	2.07	1.39	0.72	9.72	1:1.04
FM + S (1:1)	1.81 d	1.81	1.21	0.83	8.09	1:1.25
FM + PM (1:1)	1.89 cd	1.89	1.27	0.79	7.70	1:1.31
FM + M (1:1)	1.35 f	1.35	0.91	1.11	11.24	1:0.80
FM + R (1:3)	2.24 a	2.24	1.50	0.67	10.22	1:0.99
FM + S (1:3)	1.88 cd	1.88	1.26	0.79	7.60	1:1.33
FM + PM (1:3)	2.09 b	2.09	1.40	0.72	6.93	1:1.46
FM + M (1:3)	1.31 f	1.37	0.92	1.09	11.00	1:1.091
FM alone	1.49 e	1.49	--	1.01	10.00	--

* In a column, means followed by similar letters are not significantly different (P=0.05) by DMRT
$ R = Rice; S = Sorghum; PM = Pearl millet; FM = Finger millet; M = Maize

sorghum (1:3) (1:1.57) (Table 5).

Combinations of media in varying proportions were studied to identify the prospective media that could yield the maximum conidia was highly productive with minimal media requirement and realization of increased cost/ benefit ratio (Table 1-5). The results of the investigations showed that rice in combination with other cereals, yielded higher quantities of conidia and permitted quicker growth

Table 5 : Influence of maize in combination with other grains on the production of *B. bassiana*

Medium*$	Conidia/g ($x10^9$)	Conidia/kg ($x\ 10^{12}$)	Productivity ratio over maize alone	Media (kg) required to produce $1.5x10^{12}$ spores	Cost of media for $1.5x10^{12}$ spores (Rs.)	C:B ratio
M + R (3:1)	1.58 f	1.58	1.25	0.95	11.34	1:1.08
M + S (3:1)	1.48 g	1.48	1.17	1.01	10.16	1:1.20
M + PM (3:1)	1.54 fg	1.54	1.22	0.97	9.76	1:1.25
M + FM (3:1)	1.34 h	1.34	1.06	1.12	11.31	1:1.08
M + R (1:1)	1.87 c	1.87	1.48	0.80	10.90	1:1.12
M + S (1:1)	1.67 c	1.67	1.32	0.90	8.89	1:1.37
M + PM (1:1)	1.79 d	1.79	1.42	0.84	8.30	1:1.47
M + FM (1:1)	1.35 h	1.35	1.07	1.11	11.24	1:1.09
M + R (1:3)	2.24 a	2.24	1.78	0.67	10.26	1:1.19
M + S (1:3)	1.87 c	1.87	1.48	0.80	7.75	1:1.57
M + PM (1:3)	2.07 b	2.07	1.64	0.73	6.97	1:1.75
M + FM (1:3)	1.26 I	1.26	1.00	1.19	11.97	1:1.02
Maize alone	1.26 I	1.26	--	1.19	12.20	--

* In a column, means followed by similar letters are not significantly different (P=0.05) by DMRT
$ R = Rice; S = Sorghum; PM = Pearl millet; FM = Finger millet; M = Maize

often, requiring minimal quantity of substrate to achieve a set target as an entrepreneur would plan to develop. Commercial consideration in production of mycoinsecticides revolves around production feasibility (McCoy *et al.* 1988). Hence, in the present investigations computations were done to achieve a target, arbitrarily $1.5x10^{12}$ spores. (Tables 1-5). In the confirmation experiment, rice based combinations together with few other cereal combination were compared. The conditions applied for the choice of media in this experiment was maximum conidial yield and cost-effectiveness. The study revealed that rice in combination with pearl millet, sorghum, finger millet, maize (3:1), pearl millet + sorghum (1:3), maize + pearl millet (1:3) were superior to finger millet + pearl millet (1:3). Earlier reports on the use of solid substrates for multiplication of *B. bassiana* indicate that it could be cultivated on rice husk, rice bran, and saw dust in combinations. The yield of conidia was superior to PDA in a combination of rice husk + saw dust + rice bran (75:25:100) (Puzari *et al.* 1997). They suggested that the compactness of the solid substrate media was very vital for the growth of fungus, besides the nutritional status and this was achieved by combining two more nutritional sources that could provide loose substrate. Deva Prasad (1989) reported that carrot, rice and jack seed were the most favourable media for culturing *B. bassiana*.

REFERENCES

Deva Prasad, V. (1989). Studies on certain entomopathogens of *Heliothis armigera* Hb. and *Spodoptera litura* F. and their interaction with some botanicals. Ph. D. Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Jenkins, N. E. and Prior, C. (1998). Growth and formation of true conidia by *Metarhizium flavoviridae* in a simple liquid medium. *Mycological Research*, **97:** 1489-1494.

McCoy, C. W., Samson, R. A. and Boucias, D. G. (1988). Entomogenous fungi. In: Ignoffo, C. and Mandava, N.B. (eds.) CRC Handbook of natural pesticides, Vol.5. Microbial insecticides, Part A: Entomogenous protozoa and fungi. CRC Press, Boca Raton, Florida, pp. 151-23.

Puzari, K. C., Sarmah, D. K. and Hazarika, L. K. (1997). Medium for production of *Beauveria bassiana* (Balsamo) Vuillemin. *Journal of Biological Control*, **11:** 97-100.

Sandhu, S. S., Unkles, S. E., Rajak, R. C. and Kinghorn, J. R. (2001). Generation of Benomyl resistant *Beauveria bassiana* strains and their infectivity against *Helicoverpa armigera*. *Biocontrol Science and Technology*, **11:** 245-250.

Received : September, 2006; Accepted : February, 2007