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Effect of combination of grains in media on the sporulation of *Nomuraea rileyi* (Farlow) Samson

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ABSTRACT
Laboratory experiments were conducted to study the Effect of combination of grains in media on the sporulation of *Nomuraea rileyi* (Farlow) Samson. Of the different combinations studied, rice alone favoured significantly the maximum sporulation of $4.49x10^8$ conidia/g. Rice + sorghum and rice + pearl millet (3:1) were the next effective treatments (4.16-4.25 conidia/g).

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

INTRODUCTION
Nomuraea rileyi is a slow growing fungus with a preference for maltose as the carbon source (Glare, 1987). In India, natural occurrence of this fungus has been reported on a variety of insects (Vimala Devi, 1999). Although the entomopathogenic fungus. *N. rileyi* was first described more than 100 years ago, no attempt was made to mass-produce and use it for biological control until 1955 (Samson, 1974). Conidiation of *N. rielyi* occurs readily on semi-synthetic media in general. *N. rileyi* could be multiplied on polished rice grains (Silva and Loch, 1987). However, only a few isolates sporulate on cereal grains although mycelial growth occurs readily. Taking into consideration, an attempt has been made to develop a mass production medium for the fungus using agricultural products.

MATERIALS AND METHODS
The influence of different cereal nutritive substrates in combination was studied against *N. rileyi* 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 *N. rileyi* 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
Of the different combinations studied, rice alone favoured significantly the maximum sporulation of $4.49x10^8$ conidia/g. Rice + sorghum and rice + pearl millet (3:1) were the next effective treatments (4.16-4.25 conidia/g). The productivity ratio of various media was not better than rice alone. Among the combinations studied, rice + sorghum, rice + pearl millet, (3:1) and rice + sorghum (1:1) were better than others but not equal to rice. The quantity of media required to produce $1.5x10^{12}$ spore units ranged from 3.34-3.60 kg in the above treatments. Of them, media composition with rice alone or rice + sorghum (3:1) required minimal quantities to achieve the projected yield. Cost of media for production of $1.5x10^{12}$ spore units was however, lower in rice + sorghum (1:3), rice + pearl millet (1:3) (1:1.16) and rice + sorghum (1:1) (1:161) (Table 1). Treatments involving in rice + pearl millet (1:3) and rice + sorghum (1:1) were also cost-effective (Table 1).

Sorghum in combination with other grains
The evaluation showed that combination of sorghum + rice either at 1:3 or 1:1 was significantly superior to sorghum alone and its combination with other cereals in varying proportions. A maximum of 4.07-4.10 $x10^8$

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

Medium*$	Conidia / g ($x10^8$)	Conidia / kg ($x10^{11}$)	Productivity ratio over rice alone	Media (kg) required to produce $1.5x10^{12}$ spores	Cost of media for $1.5x10^{12}$ spores (Rs.)	C: B ratio
R + S (3:1)	4.16 bc	4.16	0.93	3.60	54.45	1: 1.04
R + PM (3:1)	4.25 b	4.25	0.95	3.53	53.39	1: 1.06
R + FM (3:1)	3.81d	3.81	0.85	3.94	60.08	1: 0.95
R + M (3:1)	3.60 e	3.60	0.80	4.17	63.84	1: 0.89
R + S (1:1)	3.78 d	3.78	0.84	3.97	52.60	1: 1.08
R + PM (1:1)	4.08 c	4.08	0.91	3.68	48.76	1: 1.16
R + FM (1:1)	3.27 f	3.27	0.73	4.59	61.97	1: 0.92
R + M (1:1)	2.70 g	2.70	0.60	5.56	61.49	1: 0.92
R + S (1:3)	3.50 e	3.50	0.78	4.29	48.80	1: 1.16
R + PM (1:3)	3.81d	3.81	0.86	3.94	44.81	1: 1.27
R + FM (1:3)	2.80 g	2.80	0.62	5.36	62.98	1: 0.90
R + M (1:3)	1.86 h	1.86	0.41	8.06	96.24	1: 0.59
Rice alone	4.49 a	4.49	-	3.34	56.78	-

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

conidia/g could be produced in the former two media. The productivity ratio was also higher in these two treatments only. As a result, the lowest quantity of these media (3.66-3.68 kg) was required to achieve the projected yield of $1.5x10^{12}$ spore units. The cost of media however, was more in them compared to sorghum alone and hence, the data on cost/benefit ratio was favourably disposed towards sorghum alone (Table 2).

Pearl millet in combination with other grain

Multiplication of *N. rileyi* in media composed of pearl millet + rice (1:1), pearl millet + finger millet, pearl millet + sorghum, pearl millet + maize (3:1), pearl millet + rice, pearl millet +sorghum, pearl millet + maize (1:1), pearl millet + rice, pearl millet + sorghum (1:3) and pearl millet alone gave the maximum yield of conidia ($3.16-4.09x10^8$ /g) and were on par. Combination of pearl millet + maize (1:3) recorded the lowest level of sporulation ($1.6x10^8$conidia/g). The productivity ratio was marginally higher in pearl millet + rice at the ratio 3:1 or 1:1 (1.02-1.07). Correspondingly, the media requirement in these treatments also decreased (3.85-3.67 kg) for the production of $1.5x10^{12}$ spore units. The media requirement was the lowest in pearl millet + rice (1:1) (3.67 kg) and highest in pearl millet + maize (1:3) (9.37 kg). However, none of the treatments showed increased cost/benefit ratio over pearl millet alone (Table 3).

Finger millet in combination with other grains

The sporulation of *N. rileyi* in media containing finger millet + rice (1:3) was significantly higher ($3.79x10^8$ conidia/g) compared to finger millet alone ($2.23x10^8$ conidia/g) or the remaining substrates. Finger millet + rice (1:1) and finger millet + pearl millet (1:3) were ranked second to the superior treatment in sporulation recording $3.25-3.79x10^8$conidia/g. The productivity ratio was the maximum in finger millet + rice (1:3) followed by finger millet + pearl millet (1:3) (1.70 and 1.51) respectively. The media requirement was also lower in them. The cost of media for production of $1.5x\ 10^{12}$ spore units was however, lower in finger millet + pearl millet (1:3) which led to the highest cost/benefit of 1:1.57 compared to finger millet + rice (1:3) that was significantly the best media for sporulation (Table 4).

Maize in combination with other grains

Combination of maize + rice (1:3) resulted in the maximum sporulation of $3.52x10^8$conidia/g and the highest productivity ratio of 2.69. As a result, the media required to produce $1.5x10^{12}$ spore units was lowest in the treatment. However, the cost/benefit ratio was the maximum (1:2.51) in maize + pearl millet (1:3) only followed by maize + pearl millet (1:1) netting a cost/benefit ratio of 1:1.90 though they were not significantly superior in sporulation. Maize + rice (1:3) was the third cost

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

Medium*[$]	Conidia / g ($x10^8$)	Conidia / kg ($x10^{11}$)	Productivity ratio over rice 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)	2.65 e	2.65	0.74	5.70	64.83	1: 0.62
S + PM (3:1)	2.41 fg	2.41	0.67	6.22	59.09	1: 0.67
S + FM (3:1)	2.17 h	2.17	0.61	6.91	66.51	1: 0.59
S + M (3:1)	1.89 i	1.89	0.53	7.94	76.90	1: 0.51
S + R (1:1)	4.07 a	4.07	1.14	3.68	48.76	1: 0.81
S + PM (1:1)	3.41 c	3.41	0.95	4.40	41.80	1: 0.95
S + FM (1:1)	2.92 d	2.92	0.82	5.14	50.12	1: 0.79
S + M (1:1)	2.33 g	2.33	0.65	6.44	63.59	1: 0.62
S + R (1:3)	4.10 a	4.10	1.15	3.66	55.25	1: 0.72
S + PM (1:3)	3.32 c	3.32	0.93	4.52	42.94	1: 0.92
S + FM (1:3)	2.50 f	2.50	0.70	6.00	59.25	1: 0.67
S + M (1:3)	1.72 j	1.72	0.48	8.72	87.72	1: 0.45
Sorghum alone	3.58 b	3.58	-	4.19	39.80	-

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

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

Medium*[$]	Conidia / g ($x10^8$)	Conidia / kg ($x10^{11}$)	Productivity ratio over rice alone	Media (kg) required to produce $1.5x10^{12}$ spores	Cost of media for $1.5x10^{12}$ spores (Rs.)	C: B ratio
PM + R (3:1)	3.90 ab	3.90	1.02	3.85	43.79	1: 0.85
PM +FM (3:1)	3.59 ab	3.59	0.94	4.18	39.61	1: 0.94
PM + S (3:1)	3.40 ab	3.40	0.89	4.41	42.44	1: 0.88
PM + M (3:1)	3.16 ab	3.16	0.83	4.75	46.00	1: 0.81
PM + R (1:1)	4.09 a	4.09	1.07	3.67	48.62	1: 0.77
PM +FM (1:1)	3.50 ab	3.50	0.92	4.29	40.76	1: 0.92
PM + S (1:1)	2.58 bcd	2.58	0.68	5.81	56.65	1: 0.65
PM + M (1:1)	3.19 abc	3.19	0.84	4.70	46.41	1: 0.80
PM + R (1:3)	3.77 ab	3.77	0.99	3.98	60.20	1: 0.62
PM +FM (1:3)	2.88 abc	2.88	0.75	5.21	49.50	1: 0.75
PM + S (1:3)	1.98 cd	1.98	0.52	7.57	74.75	1: 0.50
PM + M (1:3)	1.60 d	1.60	0.42	9.37	94.26	1: 0.40
PM alone	3.82 ab	3.82	-	3.93	37.34	-

*In a column, means followed by similar letters are statistically not 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 *N. rileyi*

Medium*[$]	Conidia / g ($x10^8$)	Conidia / kg ($x10^{11}$)	Productivity ratio over rice 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)	2.74 e	2.74	1.23	5.47	64.27	1: 1.05
FM + S (3:1)	2.41 g	2.41	1.08	6.22	61.42	1: 1.10
FM + PM (3:1)	1.60 f	1.60	1.16	5.77	56.98	1: 1.18
FM + M (1:1)	1.90 i	1.90	0.85	7.89	79.37	1: 0.85
FM + R (1:1)	3.25 b	3.25	1.46	4.62	62.37	1: 1.08
FM + S (1:1)	2.76 e	2.76	1.24	5.43	52.94	1: 1.26
FM + PM (1:1)	3.05 c	3.05	1.37	4.92	47.97	1: 1.40
FM + M (1:1)	1.32 j	1.32	0.59	11.36	115.02	1:0.59
FM + R (1:3)	3.79 a	3.79	1.70	3.96	60.39	1: 1.11
FM + S (1:3)	2.90 d	2.90	1.30	5.17	49.76	1: 1.35
FM + PM (1:3)	3.36 b	3.36	1.51	4.46	42.93	1: 1.57
FM + M (1:3)	1.33 j	1.33	0.60	11.28	114.92	1: 0.59
FM alone	2.23 h	2.23	-	6.73	67.3	-

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

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

Medium*[$]	Conidia/g ($x 10^9$)	Conidia/kg ($x 10^{12}$)	Productivity ratio over rice alone	Media (kg) required to produce $1.5 x 10^{12}$ spores	Cost of media for $1.5 x 10^{12}$ spores (Rs.)	C:B ratio
M + R (3:1)	1.83 f	1.83	1.40	8.20	97.89	1:1.20
M + S (3:1)	1.51 i	1.51	1.15	9.93	99.90	1:1.17
M + PM (3:1)	1.70 g	1.70	1.30	8.82	88.73	1:1.32
M + FM (3:1)	1.33 j	1.33	1.02	11.28	114.92	1:1.02
M + R (1:1)	2.70 c	2.70	2.06	5.56	75.76	1:1.55
M + S (1:1)	2.11 e	2.11	1.61	7.11	70.21	1:1.67
M + PM (1:1)	2.40 d	2.40	1.83	6.25	61.72	1:1.90
M + FM (1:1)	1.60 h	1.60	1.22	9.38	94.97	1:1.24
M + R (1:3)	3.52 a	3.52	2.69	4.26	65.22	1:1.80
M + PM (1:3)	3.11 b	3.11	2.37	4.82	46.68	1:2.51
M + S (1:3)	1.87 f	1.87	1.43	8.02	77.67	1:1.51
M + FM (1:3)	1.02 k	1.02	0.18	14.70	147.88	1:0.79
Maize alone	1.31 j	1.31	--	1.45	117.36	--

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

effective media (1:1.80) (Table 5).

In the present studies attempts were made to evaluate cheaper nutritive media (Table 1-5) in different experiments. The results indicated that maximum sporulation achievable with grain media was $4.12x10^8$ conidia/g in rice and rice based combinations. Kulkarni and Lingappa (2002) reported that crushed sorghum and rice grains with yeast extract (1%) favoured the production of *N. rileyi*. Growth of *N. rileyi* and the cost of production have been studied on complex media (Bell *et al.,* 1982), on basal salts and on media containing a number of inorganic and organic compounds including addition of cuticular extract. Larval cuticle of *H. zea* and yeast extract added to a minimal medium induced germination of conidia of *N. rileyi*. Yeast extract increased mycelial yield, but when cuticle was added, mycelial yield significantly decreased (El-Sayed *et al*., 1993). The growth medium containing silkworm pupae and potato was shown to be most economical for the production of *N. rileyi*. Hence, amendments have to be made in the media identified in the present experiments to increase the sporulation of *N. rileyi* and make it remunerative as attempted by Vimala Devi *et al*. (2000).

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