

**BEAUVERIA BASSIANA - A POTENTIAL ENTOMOPATHOGEN AGAINST RICE HISPA,
DICLADISPA ARMIGERA**

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ABSTRACT: As such in search of alternative to chemical insecticides against insect pest of rice resulted in identifying white muscardine fungus, *Beauveria bassiana* an entomopathogen out of several entomogenous fungi prevalent in rice ecosystem as the potential mycoinsecticides. The fungus has 78-87% virulence against rice hispa with LC₅₀ value of 90.16. Under field situations it is required that the effectiveness of the bioagents is enhanced by combining them with compatible pesticides. A sublethal dose of a pesticide would make the insect physiologically weak which makes it much more susceptible to the attack of the entomogenous organisms. In view of this, compatibility test was conducted with 14 different commonly applied insecticides at two different doses i.e., recommended and half of the recommended dose. In small scale field trial chloropyriphos at half of the recommended dose along with *B. bassiana* was the best treatment in controlling the pest along with increased yield of the crop consecutively for two years. Similarly, in multilocal field demonstration trial conducted in hispa endemic areas of Assam viz., Sibsagar and Jorhat covering six different localities *B. bassiana* (@ 10 million spore/ml dilution) and half of the recommended dose of chloropyriphos proved superiority then the commonly applied insecticides in decreasing the pest population with increased yield of crop.

Key words: *Beauveria bassiana*, compatibility, field efficacy, rice hispa

INTRODUCTION

Rice hispa, *Dicladispa armigera* (Olivier) (Coleoptera: Chrysomelidae) is one of the major pests of rice in Assam causing 35-65% loss in yield by attacking the vegetative stage of rice (*Oryza sativa* L.) (Puzari and Hazarika, 1992). Under post flood situation, the late transplanted rice crop suffers 100% loss because of its attack (Hazarika, 1999). The farmers heavily relied on use of insecticides for its control. Due to heavy rain and its occurrence in large areas concurrently in an epizootic condition, insecticides failed to suppress the pest, even after large scale and indiscriminate application of chemical insecticides. This invited several unwanted social and environmental problems (Hazarika and Puzari, 1997; Hazarika, 1999). The pest management system in present day context demand careful and compatible integration of chemical, biological and cultural techniques in order not only to reduce environmental and ecological hazards of pesticides but to increase economical benefits. Methods and technologies have been generated for the mass production and field application of *B. bassiana* (Samsinkava, *et al.*, 1981; Rombach *et al.*, 1988a; Mazumdar *et al.*, 1994; Puzari *et al.*, 1997). It plays a significant role in rice pest management with special reference to Rice hispa because of its capacity to infect the developmental stages of hosts with varying intensity (Ferron, 1978; Fuxa, 1987; Agarwal, 1990; Hazarika and Puzari, 1995). Biocontrol agents have been studied inadequately as a component of integrated pest management system. Our earlier work (Puzari *et al.*, 2005) confirmed the compatibility of *B. bassiana* with half of the commercial concentration of some chemical viz., Alphamethrin, Cypermethrin, Deltamethrin, Quinalphos, Endosulfan, Chloropyriphos. Combined application of entomogenous fungus with compatible chemical result in synergism between the two, an approach considered promising but little studied. With these in view, small scale and pilot field trials were carried out to study the efficacy of compatible insecticide against Rice hispa which could in future be recommended as a possible combination in IPM programmes for insect pests.

MATERIALS AND METHODS**Fungus**

Beauveria bassiana was originally isolated from the infected dead cadavers collected from rice hispa endemic rice field on potato dextrose agar medium (Puzari *et al.*, 1994). The infectivity in terms of corrected mortality percentage of the isolate calculated by Abbotts formula (Abbott, 1925), 1994 was recorded 90.16% in laboratory inoculation and is superior in performance in the field to that of the recommended pesticides (Puzari and Hazarika).

Mass culture of *B. bassiana*:

To harvest large quantity of propagules, the isolate was mass cultured in polypropylene bags containing sterilized (autoclaved at 121°C for 20 min. repeated for 2 successive days) rice hull: saw dust: rice bran medium (25: 25: 100) supplemented with 2 % dextrose (Mazumdar *et al.*, 1995; Puzari *et al.*, 1997). Polypropylene bags each having capacity of 150 g containing 50 g medium was inoculated with 1ml of 1×10^7 conidia /ml suspension and incubated at $24 \pm 1^\circ \text{C}$ for 20 days. To obtain uniform growth of the fungus, the bags were mechanically shaken thoroughly after 5 days. After 21 days of incubation the bags were kept in racks at room temperature and ready for application.

Preparation of spore/ conidial suspension of *B. bassiana*:

The conidial density as obtained from the mass cultured medium (RH:SD:RB) was 39.33×10^7 conidia/ml of water. A conidial concentration 1×10^7 conidia/ml of water was used spraying by suspending 1000g of mass cultured substrate with homogenous fungal growth in 5000 ml of water. Prior to spray the mixture was filtered through single layered muslin cloth to prevent the nozzle block.

Pot tests

To determine the effective dose of compatible insecticides with *B. bassiana* six chemicals *viz.* Deltamethrin (Decis 28EC) @ 0.001%, Alphamethrin (Farsa 10 EC) @ 0.004%, Cypermethrin (Kripcord 10 EC) @ 0.003%, Endosulfan (Thiodane 35 EC) @ 0.004% Chloropyriphos (Dursban 20 EC) @ 0.125%, and Quinalphos (Ekalux 25 EC) @ 0.025% were selected on the basis of the earlier experiments (Puzari *et al.*, 2006).

The experiment was conducted in laboratory condition by growing four seedlings of 30 days old (cv. Ranjit) per plastic pots (500 ml Beaker) containing soil mixed with manure (2.5g farm yard manure) and fertilizer (0.11, 0.15, 0.04g Urea, Single Super Phosphate and Muriate of Potash respectively). Twenty laboratory reared adults 48 hr. after eclosion (starved for 6 hrs.) were released into each pot and caged with paired lantern chimney, top of which was covered with muslin cloth. The pots were inoculated with fungal propagules (1×10^7 conidia/ml) suspended over the surfactant Tween 80 (@ 0.023%) and mixed with the respective chemicals at the required concentration. Twenty ml of the suspension was sprayed on each treatment covering three replications (6.5 ml/hill with atomizer over leaf surface). Equal amount of inoculum mixed with Tween 80 was sprayed to test the efficacy of *B. bassiana* alone. Chloropyriphos @0.25% mixed with water and Tween 80 was sprayed for comparison in the respective pots. Controls were sprayed with water and Tween 80 solution. The experiment was conducted in the laboratory at room temperature ($24 \pm 1^\circ \text{C}$). Mortality records were taken daily. Dead insects were allowed to remain in cages during the time of replacement of new seedlings. Mortality (%) were adjusted with Abbott's formula and subjected to analysis of variance. For convenience in comparison the cumulative mortality data obtained on days 10 of inoculation were analyzed.

Small-scale field test

The experiment on small-scale field trial was conducted for two years in two season's *viz.*, *sali* and *ahu* season in Instruction Cum Research (ICR) farm of AAU, Jorhat, Assam in an area of 300 and 330 sq m plot respectively. The plots were subdivided into 24 small plots of $3 \times 3 \text{ m}^2$.

Preparation of seedbed and main field for *Sali rice*

Well germinated and fungicide treated (Captan @ 0.2%) seeds @ 850 g/bed were sown in puddled seed bed (10 m length x 1.25 m breadth). For each bed Cowdung 30 kg, urea 80 g, Single super phosphate 80 g and Muriate of potash 40 g were applied during the time of puddling. Water level upto saturation condition (100% pore space was filled) was maintained through irrigation.

Well rotten farmyard manure @ 10 tonnes/ha was applied during the field preparation. In addition Urea @ 88 kg/ha, single super phosphate @ 125 kg/ha and Muriate of potash @ 33 kg/ha were also applied. Half of Urea and the entire quantity of Super phosphate and Muriate of potash were applied at the time of final puddling. The remaining part of Urea was applied at the panicle initiation stage. One month old seedlings were transplanted in the main field (@ 2 to 3 seedlings per hill) at a distance of 4 to 5 cm. Standing water 5 ± 2 cm in the field was maintained from 2 – 3 days after transplanting up to 8 – 10 days before harvest. Two weeding one at 20 and another at 40 days after transplanting was done with paddy weeder. Treatment combination were same as mentioned else where except Endosulfan because of its toxic affect on aquatic animal.

Field Release of insect

In the experiment, considering the importance of rice hispa in this region of India (Hazarika and Dutta, 1991) special emphasis was given on this insect in addition to other insect pest of rice ecosystem. Since, during the year of experimentation the rice hispa was not observed in the experimental plot probably because of its endemic nature in certain major rice growing areas of Assam (Hazarika and Dutta, 1991) the insects were released on the plots (treatment wise) under mosquito prevent nylon net at economic threshold level (1 to 2 adult/hill) (Dhaliwal and Arora, 1998) after one month of transplanting @ 300 adults/plot. The population of rice hispa at both embryonic and post embryonic stages (Table 2 and 3) and the population of other insects at pre- and post-treatments were presented separately (Table 2 and 3).

Observation on the average count of eggs, larvae, pupa and adults of rice hispa were recorded on different treatments initially at 7 days after application up to 1 month at an interval of every 48 hrs. In considering the period of life cycle of rice hispa for 22 to 25 days (Mishra, 1997). In case of larvae and adults, 15 hills were selected at random and their total populations were counted early in the morning when adults did not readily fly away. For eggs, five hills were selected and total populations were counted from their individual leaves. Average population was expressed as adults/hill, larvae/hill, pupae/hill and eggs/leaf. Data from each of the treatments were subjected to analysis of variance and compared between treatments based on critical difference at 0.05 probability level.

Field Application of *Beauveria bassiana* alone and in combination with other chemicals

The prepared conidial suspension of *B. bassiana* sprayed alone and in combination with sublethal doses of chemicals viz. Alphamethrin, Cypermethrin, Deltamethrin, Quinolphos and Chloropyriphos. In all the treatments Tween 80 (polyethylene sorbitan monolourate) was added @ 0.23 ml/litre (Puzari and Hazarika, 1994). Water mixed with Tween 80 at the same rate served as control. Chloropyriphos alone at lethal dose was applied for comparison with treatments. The treatments were applied after 24 hrs. Field release of rice hispa through high-volume knapsack sprayer @ 600 litres/ha and drifting were prevented by erecting aluminium sheets during spray operation.

Preparation of field for *Ahu rice* and application of manure and fertilizer

Five ploughing followed by laddering was done for soil preparation. Laddering was done properly to retain water uniformly in the field. Well rotten FYM 10 t/ha or 15 q/bigha was applied during the initial field preparation. Fertilizer like Urea @ 88 kg/ha, Single Super Phosphate @ 125 kg/ha and Muriate of Potash @ 32 kg/ha were also applied.

The full dose of phosphate fertilizer was applied at the time of final ploughing. Half of nitrogenous fertilizer and half dose of potassic fertilizer were applied at 20 days after seed germination or after first weeding. The second application with the remaining nitrogenous and Potassic fertilizer was given 45 days after seed germination or after the second weeding. Rice seed (var. Luit) having a germination of >80% was selected and treated with Dithane M-45 (@ 2.5 g/kg of seed) for sowing. Seeds were sown in line @ 75 kg/ha.

Treatment combinations were same as the study in *in vitro* conditions.

Observation recorded

Observation of pretreatment and post treatment count of egg/leaf, larva/leaf, pupa/leaf and adult/hill was recorded. Pretreatment count was made one day before application of the treatment and post treatment count was made fifteen days after the treatments for two times at an interval of 15 days considering the life cycle of rice hispa for 22-25 days (Mishra, 1997) as well as for other insect pests present in the experimental field. Post treatment count on embryonic and post-embryonic stages was recorded as per the method described else where. The average of two post treatment count of different embryonic and post embryonic stages of rice hispa was used to calculate the percent decrease over the pretreatment count.

RESULTS AND DISCUSSION

Pot tests

Table 1 shows the efficacy of compatible combination of six insecticides together with *B. bassiana* against rice hispa (in terms of mortality percentage). Chloropyriphos at lethal dose cause highest mortality (89.60%) of rice hispa followed by Chloropyriphos at sublethal dose + *B. bassiana* (86.27%) without having any significant difference among them. Ribban *et al.*, (1983) advocated that half of the commercial dose (i.e., sublethal dose of chloropyriphos in combination with *B. bassiana* to *Ostriana rubilais* (Hbn) . The mortality percentage recorded in Quinalphos at sublethal dose + *B. bassiana* (80.38%) was next to the above two treatments. This was followed by sublethal dose of Endosulphan in combination with *B. bassiana* (74.51%) and *B. bassiana* alone (70.58%) without any significant difference. Similarly earlier study on combine effect of Chloronocotinyl insecticide Imidaclorpid and *B. bassiana* exert significant synergistic effects on mortality and mycosis of Colorado potato beetle (*Leptonitarsa decemlineata*) (Eldert *et al.*, 1991; Boucias *et al.*, 1996). Comparatively a lower percent of mortality 52.94%, 54.90% and 56.86% was recorded respectively in Alphamethrin, Deltamethrin and Cypermethrin treated pot without showing any significant difference between them. The insecticides used in the present study may induce the changes in insect behaviour which may be a potential mechanism for enhanced infection.

Small scale field trail

When mean values of two years experiments (both for *sali* and *ahu* rice) were compared a significant difference among all then treatments with almost a similar treatment of efficacy was observed in reducing the embryonic and postembryonic stages of rice hispa (Table 2 & 3). Among all the treatments, although chloropyriphos at lethal dose (0.025%) performed best, sublethal dose of Chloropyriphos (0.125%) along with *B. bassiana* (1×10^7 conidia/ml of water) was found to next to it in terms of decreasing the post embryonic stages of rice hispa without any significant difference between them.

This was followed by *B. bassiana* alone and application of sublethal dose of Alphamethrin (0.004%) along with *B. bassiana* (Table 2 & 3). Effect of *B. bassiana* alone and in combination with chemical observed in the present study is in agreement with the earlier report of Hazarika and Puzari (1995) that showed *B. bassiana* to be pathogenic to all the stages of rice hispa, including egg. Furthermore, Storch and Dill (1987) observed that larval population of *Leptinotarsa decemlineata* Say was at par in the potato (*Solanum tuberosum* L.) plots treated with insecticides and *B. bassiana*. Highest population count of egg/leaf, larva/hill and pupa/hill and infestation was recorded in the control plot where rice hispa were released alone without any biological and /or chemical treatment. The observations recorded in Cypermethrin and Deltamethrin at sublethal dose together with *B. bassiana* treated plot in terms of reducing the developmental stages of rice hispa was statistically *at par* with each other in both the crop seasons of the crop. The plot where Quinalphos was applied at sublethal dose along with *B. bassiana* was found least effective in the management of the pest.

Table 1. Efficacy of compatible combination of insecticides and *Beauveria bassiana* against rice hispa (*in vitro*)

Treatment	Mortality (%)	Corrected mortality (%)
T ₁ Control	15.00	-
T ₂ <i>B. bassiana</i> (@ x 10 ⁷ conidia/ml)	75.00	70.58 (57.15)
T ₃ Chloropyriphos (L: 0.25%)	91.16	89.60 (71.19)
T ₄ Alphamethrin (SL: 0.004%) + T ₂	60.00	52.94 (46.69)
T ₅ Cypermethrin (SL: 0.003%) + T ₂	63.33	56.86 (48.94)
T ₆ Deltamethrin (SL: 0.001%) + T ₂	61.66	54.90 (47.81)
T ₇ Quinalphos (SL: 0.025%) + T ₂	83.33	80.38 (63.71)
T ₈ Endosulfan (SL: 0.175%) + T ₂	78.33	74.51 (59.68)
T ₉ Chloropyriphos (SL: 0.125%) + T ₂	88.33	86.27 (68.25)
S.Ed (±)		2.094
CD (0.05)		4.400

Data are mean of three (3) replications
Data in the parentheses are angular transformed value

Table 2. Efficacy of *Beauveria bassiana* alone and in combination with different insecticides against rice hispa (*Sali* rice)

Treatment	Egg/leaf			Larva/hill			Pupa/hill			Adult/hill			Yield (q/ha)		
	I	II	Mean [*]	I	II	Mean [*]	I	II	Mean [*]	I	II	Mean [*]	I	II	Mean [*]
T1 : Control	3.45	5.00	4.22	3.54	2.79	3.16	1.48	1.19	1.33	8.74	4.83	6.78	46.67	44.79	45.73
T2 : <i>B. bassiana</i>	3.02	2.47	2.74	2.39	2.89	2.64	1.78	1.29	1.53	0.20	2.07	1.13	52.08	50.33	51.20
T3 : Chloropyriphos(L)	1.28	1.33	1.30	0.83	0.91	0.87	0.09	1.10	0.53	1.00	0.67	0.83	58.33	54.92	56.62
T4: Alphamethrin(SL)+T2	2.89	3.67	3.28	1.90	2.44	2.17	0.37	2.20	1.28	1.00	1.93	1.46	54.17	52.77	53.47
T5 : Cypermethrin(SL)+T2	4.08	5.47	4.77	4.30	3.10	3.70	3.17	1.27	2.22	13.80	5.93	9.86	49.75	46.57	48.16
T6: Deltamethrin(SL)+T2	1.83	6.53	4.18	2.73	3.61	3.17	1.28	1.33	1.30	1.30	2.80	2.05	52.50	50.05	51.30
T7 : Quinalphos(SL)+T2	3.56	4.40	3.98	2.11	2.53	2.32	2.11	1.06	1.58	0.48	4.40	2.44	54.17	52.71	53.44
T8: Chloropyriphos(SL)+T2	2.16	2.00	2.08	2.08	3.21	2.64	0.23	0.93	0.58	1.00	1.53	1.26	57.08	53.01	55.09
S.Ed.(±)	0.083	0.348	0.568	0.102	0.166	0.134	0.915	0.265	0.296	0.134	0.126	0.140	0.471	0.531	0.488
CD (0.05)	0.165	0.688	1.163	0.205	0.33	0.274	0.183	0.515	0.607	0.268	0.252	0.286	1.760	1.984	0.998

I= mean of 1st year, II= mean of 2nd year *Data are mean of two years experiment , L: Lethal dose, SL: Sub lethal dose

Table 3. Efficacy of *Beauveria bassiana* alone and in combination with different insecticides against rice hispa (*Ahu* Rice)

Treatment	Egg/leaf			Larva/hill			Pupa/hill			Adult/hill			Yield(q/ha)		
	I	II	Mean ^a	I	II	Mean ^a	I	II	Mean ^a	I	II	Mean ^a	I	II	Mean ^a
T1 Control	38.00	36.25	37.13	3.75	3.00	3.38	1.93	1.34	1.64	2.07	1.99	2.03	9.68	8.28	8.98
T2 <i>B.bassiana</i>	13.00	11.11	12.06	2.56	2.16	2.36	1.47	1.27	1.36	1.62	1.76	1.69	13.64	12.92	13.28
T3 Chloropyriphos(L)	8.25	6.22	7.24	1.69	1.68	1.69	0.36	0.38	0.37	0.94	0.98	0.96	21.86	22.66	22.26
T4 Alphamethrin(SL)+T2	9.67	8.25	8.96	2.40	2.34	2.37	1.38	1.18	1.28	1.47	1.58	1.53	16.91	17.05	16.98
T5 Cypemethrin(SL)+T2	11.42	9.22	10.32	2.86	2.44	2.65	1.62	1.26	1.44	1.75	1.78	1.77	11.48	12.00	11.74
T6 Deltamethrin(SL)+T2	10.66	10.28	10.47	2.16	2.16	2.16	1.42	1.32	1.37	1.56	1.41	1.49	14.68	13.86	14.27
T7 Quinalphos(SL)+T2	21.25	19.00	20.13	3.08	3.66	3.37	1.69	1.66	1.68	1.89	1.59	1.74	10.55	10.60	10.58
T8 Endosulfan(SL)+T2	11.17	10.66	10.92	2.48	2.08	2.28	1.44	1.39	1.42	1.60	1.58	3.18	13.55	14.00	13.78
T9 Chloropyriphos(SL)+T2	9.83	8.24	9.04	1.80	1.90	1.85	0.49	0.55	0.52	1.13	1.10	1.12	19.86	20.22	20.04
S.Ed(+)	1.721	1.100	1.084	0.396	0.19	0.905	0.337	0.312	0.683	0.378	0.315	0.466	1.57	1.820	1.125
CD(o.05)	3.532	2.250	2.220	0.832	0.390	1.853	0.708	0.655	1.399	0.776	0.646	0.954	4.39	4.550	2.303

= mean of 1st year, II= mean of 2nd year, L: Lethal dose, SL: Sub lethal dose, * Data are mean of two years experiment

The yield data of both the seasons for two years were compared and presented in Table 2 & Table 3. Highest mean yield of 56.62 q/ha and 22.26 q/ha in *sali* and *ahu* season respectively was recorded in Chloropyriphos (at lethal dose) treated plot. This was followed by Chloropyriphos (sublethal dose) + *B. bassiana* in *sali* (55.09 q/ha) and *ahu* (20.04% q/ha) seasons. A significant increase in yield was also observed in the treatment Quinalphos (0.025%) + *B. bassiana* (53.44 q/ha) followed by sublethal application of Deltamethrin + *B. bassiana* (51.30 q/ha) and *B. bassiana* alone (51.20 q/ha) in *sali* seasons. While in *ahu* seasons this trend of increase in yield was observed in case of the sublethal application of Alphamethrin (16.98 q/ha), followed by Deltamethrin (14.27 q/ha), Endosulfan (13.78 q/ha) and *B. bassiana* alone (13.28 q/ha). Lowest yield 45.73 q/ha and 8.98 q/ha were recorded in *sali* and *ahu* seasons respectively in the control plot where rice hispa was released alone without any chemical and/or biological treatment. Higher yield record observed in *B. bassiana* treated plot was similar with the earlier report of Hazarika and Puzari (2004) who reported higher yield of rice at various rice hispa endemic localities of Assam. They also reported that yield of rice was significantly higher in plots treated with *B. bassiana* than those treated with either Monocrotophos or neem seed oil. Similarly, Maniania (1993) also observed an increase in yield of maize (*Zea mays*L.) in the field treated with *B. bassiana*. *B. bassiana* could establish in the field and prevent the pest population to build up. Bartlett and Lefevre (1934) and Hoi et al.,(1994) showed such a capability of *B. bassiana* in controlling the European corn borer (*Ostrinia nubilis* Hbn) and the red imported fire ant (*Solenopsis invicta* Buren) in maize field and Pasteur respectively.

CONCLUSION

The present study showed encouraging result of using *B. bassiana* as an alternative management approach to curb the problem of infestation of rice hispa in major rice growing areas of Assam.

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