

Formulation of *Beauveria bassiana*, Vuill. for integrated management of rice hispa, *Dicladispa armigera* of rice

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Abstract: Formulation of *Beauveria bassiana*, for integrated management of rice hispa, *Dicladispa armigera* of rice were evaluated in the division of Entomology, Bangladesh Institute of Nuclear Agriculture, Mymensingh during 2013-2015. Investigation were carried out to mass produce *Beauveria bassiana* (Bals.) Vuill. in a locally available cheap agricultural liquid as well as solid substrates and to develop wettable powder (WP) formulation with an indigenous strain of *B. bassiana* for management of rice hispa *D. armigera* (Olivier) (Coleoptera: Chrysomelidae) of rice. Growth parameters of 12 strains of *B. bassiana* viz., radial growth, conidial density, germinability, colony forming unit (cfu) and biomass production were evaluated at 15, 20, 25 and 30°C. The strain AAU-08 was found to be superior one as compared to the other strains. Based on the infectivity test against *D. armigera* the strain AAU-08 was selected for WP formulation. For mass production of *B. bassiana*, coconut water and potato broth were the most suitable liquid media and rice husk was the most favourable solid medium with reference to sporulation and virulence against *D. armigera*. Talc based formulation of *B. bassiana* was developed for large scale field application and to improve the shelf life of the formulation in different shelved or storage conditions such as room temperature (24±1°C), refrigerated conditions (4°C) and deep freeze condition (-4°C to -6°C) which revealed that in deep freeze condition the formulation could be stored. Dose response test of *B. bassiana* against adult *D. armigera* revealed that the formulation at a concentration of 10⁷ conidia/ml was the most effective dose. Field trials conducted during aman and boro season in 2 locations of Satkhira and Comilla had demonstrated that the WP @ 1×10⁷ conidia/ml effectively controlled not only *D. armigera* but also other insect pests of rice. In laboratory test, the WP was found to be safe to natural enemies like coccinellid beetle and *Lycosa pseudoannulata*, *Apanteles* spp. and *Trichogramma japonica*. The present investigation has identified WP as a proven technology to manage not only *D. armigera* but also other major insect pests for sustainable production of rice and also as an ecofriendly agent to predator and parasitoids. From this investigation it is clear that *B. bassiana* and *T. japonica* could be utilized side by side in a given rice field, paving the for evolving an IPM programme aiming to attack borers and leaf feeders at the same time.

Key words: *Beauveria bassiana*, wettable powder (WP), *Dicladispa armigera*, *Oryza sativa*.

Introduction

Rice (*Oryza sativa* L.) is a cereal grain of the grass family (Graminae) and belongs to the genus *Oryza* under the tribe Oryzaeae. Rice is a staple food for over half of the world's population. More than 90% world's rice is produced and consumed in Asia (Sigsgaard, 2000) suggesting its dominance in the developing world. Over 100 insect species attack and damage rice crop in various growth stages (Pathak, 1968, 1977; Grist and Lever, 1969; Beevi *et al.*, 2002). Many of them often appear sporadically and do not cause economic loss (Muralidharan and Pasalu, 2006). A few species, however, do cause significant damage and are extremely important. Among the major insect pests of rice. The rice hispa *D. armigera* is one of the most important constraints to crop production globally. It is a major pest of many rice growing parts of Southern Asia more particularly India, Bangladesh, Myanmar, Indonesia, Nepal, Cambodia, China, Pakistan, Sri Lanka, Malaysia and Australia.

It attacks the vegetative stages of aman rice causing 35-65% loss in Bangladesh (Rajak *et al.*, 1986; Hazarika and Dutta, 1991) and hence it is regarded as one of the major limiting factors in rice production in Bangladesh (Hazarika and Dutta, 1991; Puzari and Hazarika, 1992). Under post flooded situation when the rice crop is transplanted late, it suffers 100% loss (Deka and Hazarika, 1999). It feeds primarily on rice. But it also feeds other crops like maize, wheat and sugarcane and several grasses.

Management of rice hispa is now a major problem among the farmers of Bangladesh who depend primarily on rice as a major crop. Due to high temperature and pest's occurrence in large areas concurrently, pesticides have been reported to fail to have produced desirable effects. In such situation farmers used pesticides at indiscriminately higher dose. Such large-scale indiscriminate application of

pesticides invites several unwanted social and environmental problems (Hazarika and Puzari, 1997; Deka and Hazarika, 1995). For instance, synthetic insecticides cause health problems to the farm operators, consumers and the environment as well (Dubey *et al.*, 2011). Pesticides are the most powerful tool available for rice pest management which is curative and reliable when pest population approaches economic threshold level. It is absolutely necessary to limit the usage of chemical pesticides to remain in the world market and sustain the competition. In India crop wise market share of pesticide usage indicate the highest use pattern to the extent of 45% in cotton followed by 22 % in rice, 9 % in vegetables, 7% in plantations, 4 % each in wheat and pulse and 9% in others. Compared with usages of chemical pesticides, biopesticides constitute around 2% in the country (David, 2008). However, the pest management system in present day context demands careful and compatible integration of chemical, biological and cultural techniques in order not only to reduce environmental and ecological hazards of pesticides but also to increase economical benefits.

The use of microbes is highly sustainable and can contribute to increase the productivity and profitability in agriculture. They are safer, economical and reliable control agents of crop pests. India is gifted with a rich biodiversity of several fungal, viral, and bacterial, protozoan and entomopathogenic nematodes of crop pests, which offer a great scope in the microbial control of crop pests. Microbial pesticides are safe to mankind and its livestock; do not pollute the environment and safe to the beneficial parasitoids and predators and generally there is no chance of development of resistance to these microbes. The different classes of biopesticides include biochemical pesticides, microbials pesticides and plant-incorporated protectants. They are usually inherently less toxic than conventional pesticides and generally affect only the target

pest and closely related organisms, in contrast to broad spectrum, conventional pesticides that may affect other organisms as well. Biopesticides are often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding pollution problems caused by the conventional pesticides. When used as a component of integrated pest management (IPM) programmes, biopesticides not only reduce damage caused by pests, but also enhance crop yield (Ware, 1994).

In India about 16 commercial preparations of *Bacillus thuringiensis*, 38 fungal formulations based on *Beauveria* and *Verticillium*, and about 45 baculovirus-based formulations of *Helicoverpa armigera* and *Spodoptera* are available (David, 2008). *Beauveria bassiana* (Ball.) Vuill. is a naturally occurring potent entomopathogenic fungus which has been successfully utilized for controlling many insect pests of rice globally. Incorporation of this agent may bring about a revolution in rice pest management in Bangladesh. Field performance of this agent is very encouraging not only in terms of the pest suppression but also in increasing yield, thereby fetching higher economic returns to the farmers compared to the conventional insecticides.

In this context, success achieved at AAU in developing a mycoinsecticide, *Beauveria bassiana* (Bats.) Vuill. for the management of rice hispa may be mentioned (Hazarika and Puzari, 1990, 1991, 1995, 1997; Hazarika *et al.*, 1998, Hazarika, 1999). However, technologies for mass production in an economical liquid medium are yet to be developed. Furthermore, formulation of the same with improved shelf life is also essential for delivery for large scale application by the farmers. Therefore the present investigation was carried out with an aim to produce a commercial formulation of *B. bassiana*.

Materials and Methods

The present investigation entitled “Formulation of *Beauveria bassiana*, Vuill. for Integrated Management of Rice Hispa, *Diadisa armigera* Olivier (Coleoptera: Chrysomelidae)” was carried out in the Division of Entomology, Bangladesh Institute of Nuclear Agriculture, Mymensingh during 2013-2015. The details of materials used and methodologies adopted in connection with various experiments are described below:

Identification of superior strains of *B. bassiana*:

Extensive field surveys were carried out in rice hispa endemic pockets in and around Satkhira and Comilla during 2013-2015 to monitor the naturally occurring entomopathogenic fungi on rice hispa and other insect pests of rice. On the basis of colony morphology and configuration of spores, the fungus was identified as *B. bassiana* and named a AAU-08 strain. Eleven strains viz. (i) BINA-5587, (ii) BINA-4517, BINA-4525, BINA-4522, BINA-4537, BINA-4535, BINA-4524, BINA-4529, AAU-08, BINA-4511, BINA-4518 and BINA-4519 of *B. bassiana* were also ordered from Bangladesh Institute of Nuclear Agriculture (BINA). In this way, out of these 12 collection the superior most strain was identified.

Isolation and pure culture: Each field collected cadaver was cut into many pieces, each measuring 0.5 to 1 mm in

length, and these pieces were then surface sterilized by 1% sodium hypochlorite solution (NaOCl_2) for 30 sec. The sterilized pieces were then transferred to PDA medium (Potato Dextrose Agar). However, each pure culture obtained from the BINA was transferred to a PDA slant by following standard methodology and was stored in a refrigerator at 4°C. All *B. bassiana* strains were sub-cultured in their respective media and maintained inside an incubator of 25°C and RH 85% for further studies.

Biological parameters of *B. bassiana* strains at different temperature: Different biological parameters of 12 strains, such as mean radial growth (mm), conidial density ($\times 10^7$ conidia/ml), germination percentage (%), colony forming unit (C.F.U in $\times 10^7$ conidia/ml) and fresh water (mg/100 ml) were studied at 15°, 20°, 25° and 30°C temperatures by culturing them in Potato Dextrose Agar (PDA) media under complete darkness.

Potato Dextrose Agar (PDA) medium: To prepare the PDA medium, 200g peeled potatoes were cut into small chips and boiled with 500 ml distilled water. Fully boiled potato extract was separated by using doubled layer muslin cloth, and 20 g of dextrose was added to the extract. In another flask, remaining 500 ml distilled water was taken and allowed agar to melt by boiling. The molten agar was stained through double layer muslin cloth and mixed with potato extract solution. The volume was made up to 1000 ml by adding distilled water and pH of the medium was maintained at 6.5. The medium was poured into culture tubes and conical flasks, plugged by non-absorbent cotton wool and then sterilized in an autoclave at 121°C (15 lb pressure per square inch) for 30 min.

Radial Growth: Inoculums of *B. bassiana* were produced by growing the fungus on PDA plates for seven days in a BOD Incubator. With the help of a 0.65 cm diameter cork borer, a piece was cut out from the actively growing region of a 7-day-old culture, and the same was placed aseptically in the center of a fresh Petri plate (9-10 cm, diameter) containing PDA medium.

Conidial Density: To determine the conidial density of different strains of *B. bassiana* the fungus was grown on PDA plates for seven days under BOD incubators maintained at different temperatures viz., 15, 20, 25 and 30°C. Spores of each strain maintained at a specific temperature were removed by scraping with a spatula, and they were suspended in 70 ml distilled water in a 200 ml test tube, which were then mixed thoroughly for 30 seconds by placing the tube on a vortex mixture.

Biomass production: In order to assess the biomass i.e. mycelia weight, mycelia mat were scraped out from the edges of an actively growing colony using an 8 mm diameter flame sterilized cork borer from each strain. Each mycelial mat was then inoculated into the conical flask (250 ml) containing the 100 ml potato broth medium.

Spore germination: The germination test of viable conidia was performed by following the method of Francisco *et al.* (2006). This test was done by spreading PDA medium uniformly on microscopic slides disinfected with 100% ethyl alcohol. Germination percentage was recorded by direct examination at 400X with a phase contrast microscope. Each slide was taken as a replicate

and mean of three replicates of each of the strains against each temperature was arranged under CRD for analysis.

Colony Forming Unit (CFU): The CFU for 12 strains was determined on PDA medium by using dilution plate technique of Pramer and Schmidt (1956). Three plates for each strain at four different temperatures were kept under incubator and arranged in CRD.

Mass rearing of rice hispa: For over 4 years, mass rearing of rice hispa has been continued in the Entomology Laboratory, Division of Entomology, Bangladesh Institute of Nuclear Agriculture, Mymensingh (Dutta and Hazarika 1992, Phukan, 2003). Large number of *D. armigera* adults, grubs and pupae were collected from infested rice fields of Satkhira and Comilla district of Bangladesh. Grubs were allowed to retunnel in to healthy rice plants inside a 60 mesh wire netting cage (65×65×90 m). Grubs developed into pupae, which later transferred into adults inside the new hosts. From the field collected pupae also adult emerged. All these adults were reared on the potted rice plants.

Bioassay of *B. bassiana* strains against *A. armigera* adults: Different *B. bassiana* strains against adults *D. armigera* were bioassayed at room temperature (30±1°C, RH 80-85%). Four seedlings of twenty-day-old (Binadhan-10) were grown in plastic pots (510 ml capacity) containing soil mixed with manure and fertilizers. Each treatment was replicated thrice in CRD. Control pots were treated with water mixed with Tween-80. Mortality of adults due to infection was recorded after 7 and 10 days of inoculation and data were subjected to ANOVA (Puzari and Hazarika, 1994).

Preparation of WP: Potato Dextrose Broth (PDB:500ml) in 1000 mL conical flask, Sterilized under autoclave at 201b pressure for 30 min, After cooling, inoculate 2 mycelia disc of *B. bassiana* under laminar flow, Incubate at room temperature (25°C) for 15 days, Collect fungal growth and blend it in the mixture for 10-15min to get a homogenous slurry, Sterilized 500gm of talc powder in hot air oven + Mixed cold sterilized talc powder with homogenous fungal slurry at 1:1 ratio, Dried under laminar flowhood for 72 hrs, Packed in sterilized polypropylene bags, Mixed well 10kg of formulation in 600 liter of water by continuous stirring, Add 138ml Tween-80 @ 0.023% in the above solution and mixed it well, and Spray by knapsack sprayer

Field test

Field efficacy of WP formulation: Based on laboratory test against *D. armigera* adult the best conidial concentration (10⁷ conidia/ml of water) of the formulation was tested under field condition during aman and boro seasons, 2013-2015 in two locations Satkhira and Comilla against all the developmental stages of rice hispa.

Statistical analysis: The data were analyzed statistically with CRD and subjected to analysis of variance (ANOVA). Before analysis the mortality data were transformed to arcsin and means were compared with Duncan Multiple Range Test (DMRT) (0.05%). From the mortality recorded, AAU-08 was adjudged as the best strain which had high virulence against the target pest, and it was selected for further studies.

Results and Discussion

The present study was conducted to develop mycoinsecticide formulation of indigenous strain of the white muscardine, *Beauveria bassiana* (Bals.) Vuill. for the management of rice hispa, *Dicladispa armigera* Olivier (Coleoptera: Chrysomelidae). The results of the investigation are presented below:

Survey of entomogenous fungi in rice ecosystem: Surveys conducted during July to November 2013, March to November 2014 in rice ecosystem of Mymensingh district a revealed occurrence of *Beauveria bassiana* on adult of rice hispa (Table 1). Infection was more prevalent on adult and fluctuated during different months.

Table 1. Month-wise natural occurrence of *B. bassiana* in rice hispa adults

Month	Adult	
	2013	2014
March	-	6.60
April	-	2.50
May	-	1.40
June	-	2.60
July	3.20	3.00
August	1.10	4.40
September	5.40	1.60
October	2.10	2.20
November	3.10	1.10

Radial growth: The experimental results showed that all the strains and temperatures were significantly different among themselves in respect of radial growth and growth rate (Table 2). At 15°C minimum mean radial growth ranged from 10.11 mm (BINA-4529) to 24.15 mm (AAU-08). The maximum radial growth of 41.24 mm followed by 40.34 mm and 38.72 mm was recorded in BINA-4519, BINA-4524 and AAU-08 strain and minimum 15.29 mm in BINA-4522 at temperature 25°C, whereas maximum growth rate of 3.78 mm/day was recorded in AAU-08.

Conidial density: The main conidial density of different strains varied significantly at different temperatures tested. Table 3 shows that conidial density was highest at temperatures 25°C (9.59×10⁷ conidia/ml) for AAU-08, which was followed at 20°C (8.55×10⁷ conidia/ml) for the same strain. Conidial density was observed minimum at 15 and 30°C for all the tested strains.

Colony forming unit (cfu), biomass production and spore germination: The man colony forming unit observed in different strains at different temperature after 14 days of incubation it was found that the maximum cfu was recorded in AAU-08. Biomass production was maximum at 25°C after 14 days of incubation. The higher germination percentage was recorded in AAU-08 strain.

Bioassay against *Dicladispa armigera*: Table 4 shows pathogenicity of a single dose 1×10⁷ spores/ml of different *B. bassiana* strains on adult *D. armigera*. All the strains showed significant difference in respect of mortality. After 7 and 10 days of inoculation (DA1), mortality varied between 5.00 to 87.55 (AAU-08) at 7 days and 40.55 to 98.23% at 10 days after treatment (DAT), respectively, it was followed by BINA-4529, which caused 82.74% and 88.15% mortality at 7 and 10 DAI, respectively.

Considering biological parameters and bioassay results, the strain AAU-08 was superior to others.

Table 2. Effect of temperatures on mean \pm SD radial growth (mm) and growth rate (mm/day) of 12 strains of *B. bassiana* after 15 days of incubation

Strain	15°C	Growth rate	20°C	Growth rate	25°C	Growth rate	30°C	Growth rate
BINA-5587	10.15 \pm 0.92	1.07	19.95 \pm 4.19	2.10	33.11 \pm 5.15	1.85	20.33 \pm 2.11	1.85
BINA-4517	12.17 \pm 1.18	1.15	16.21 \pm 0.18	1.50	22.15 \pm 4.10	3.15	31.27 \pm 4.14	2.72
BINA-4525	15.11 \pm 0.55	0.95	19.18 \pm 2.07	2.20	20.21 \pm 8.22	2.25	17.25 \pm 3.19	1.95
BINA-4522	14.25 \pm 0.11	0.99	30.15 \pm 4.21	1.90	15.29 \pm 7.52	3.51	25.19 \pm 5.21	3.01
BINA-4537	20.15 \pm 1.24	1.25	15.87 \pm 5.05	2.50	18.95 \pm 5.43	1.99	32.21 \pm 6.32	2.75
BINA-4535	17.12 \pm 1.54	2.01	35.24 \pm 3.05	2.80	19.84 \pm 4.82	2.60	39.16 \pm 7.12	2.42
BINA-4524	22.10 \pm 2.01	1.95	16.23 \pm 1.88	2.40	40.34 \pm 10.69	2.41	41.19 \pm 4.21	2.82
BINA-4529	10.11 \pm 1.88	0.98	29.13 \pm 2.00	1.90	35.55 \pm 2.51	2.85	37.22 \pm 9.10	3.01
AAU-08	24.15 \pm 0.92	2.25	20.00 \pm 1.12	2.60	38.72 \pm 6.42	3.78	11.51 \pm 8.32	1.92
BINA-4511	16.15 \pm 1.71	1.11	27.23 \pm 5.11	2.70	19.61 \pm 7.33	2.19	19.54 \pm 7.30	1.85
BINA-4518	13.17 \pm 1.85	0.88	22.18 \pm 4.51	1.90	35.51 \pm 4.45	2.36	21.44 \pm 5.28	1.20
BINA-4519	11.18 \pm 0.91	0.901	31.10 \pm 2.85	2.10	31.24 \pm 3.16	2.42	32.36 \pm 2.35	1.95
LSD _{0.05}	5.32		11.60		5.07		10.87	

Table 3. Effect of temperatures on mean \pm SD conidial density ($\times 10^7$ conidia/ml) of 12 strains of *B. bassiana* after 15 days of incubation

Strain	15°C	20°C	25°C	30°C
BINA-5587	1.54 \pm 0.21	3.11 \pm 1.14	2.25 \pm 1.25	2.11 \pm 0.22
BINA-4517	0.92 \pm 0.11	4.02 \pm 0.25	3.16 \pm 2.11	1.81 \pm 1.18
BINA-4525	0.85 \pm 1.10	5.15 \pm 0.17	5.19 \pm 1.24	0.17 \pm 0.09
BINA-4522	3.25 \pm 0.51	7.24 \pm 1.66	6.05 \pm 3.11	2.15 \pm 1.16
BINA-4537	2.11 \pm 0.42	6.17 \pm 1.91	4.59 \pm 2.09	0.97 \pm 0.15
BINA-4535	1.75 \pm 0.19	4.43 \pm 0.88	3.48 \pm 3.07	1.88 \pm 0.75
BINA-4524	0.91 \pm 1.28	5.17 \pm 0.95	7.18 \pm 1.85	2.17 \pm 0.14
BINA-4529	1.88 \pm 0.34	3.22 \pm 1.51	5.22 \pm 1.06	0.25 \pm 0.12
AAU-08	1.72 \pm 0.09	8.55 \pm 1.48	9.59 \pm 0.15	2.25 \pm 0.19
BINA-4511	0.90 \pm 1.07	2.67 \pm 0.75	4.95 \pm 1.18	0.97 \pm 1.21
BINA-4518	1.25 \pm 0.19	3.70 \pm 0.62	2.88 \pm 2.15	1.62 \pm 0.09
BINA-4519	1.37 \pm 0.15	7.23 \pm 1.72	2.74 \pm 1.21	2.54 \pm 0.12
LSD _{0.05}	0.36	0.24	1.60	0.55

Table 4. Effect of 12 strains of *B. bassiana* on adults of *D. armigera*

Strain	Mean mortality (%)	
	7 DAT	10 DAT
BINA-5587	50.50	56.14
BINA-4517	52.62	65.19
BINA-4525	61.32	72.21
BINA-4522	34.41	80.27
BINA-4537	71.28	74.31
BINA-4535	55.14	60.42
BINA-4524	65.19	55.09
BINA-4529	82.74	88.15
AAU-08	87.55	98.23
BINA-4511	35.09	51.17
BINA-4518	5.00	40.55
BINA-4519	26.16	61.28
LSD _{0.05}	4.62	6.87

Table 5. Shelf life in mean \pm SD days of *B. bassiana* maintained under different storage condition as expressed in terms of virulence and conidial density

Age of culture (days)	Room temperature (24 \pm 1°C)			Refrigerated condition (4°C)			Deep freeze (-4 $^\circ$ -6°C)		
	Corrected mortality (%)	Conidial density $\times 10^7$	% conidia dried	Corrected mortality (%)	Conidial density $\times 10^7$	% conidia dried	Corrected mortality (%)	Conidial density $\times 10^7$	% conidia dried
30	84.51 \pm 1	34.14 \pm 0.2	17.25 \pm 0.8	82.15 \pm 0.9	30.55 \pm 0.9	10.25 \pm 0.51	84.05 \pm 0.5	37.55 \pm 1.2	1.51 \pm 0.11
60	86.09 \pm 1	35.15 \pm 0.5	18.16 \pm 0.1	85.11 \pm 1.1	32.45 \pm 1.2	12.19 \pm 0.62	85.08 \pm 1.1	39.36 \pm 1.5	0.45 \pm 0.15
90	85.15 \pm 0.5	33.09 \pm 1.5	22.15 \pm 0.5	86.21 \pm 2.1	35.61 \pm 1.5	15.21 \pm 0.51	86.10 \pm 1.5	38.41 \pm 0.9	1.69 \pm 0.09
120	72.12 \pm 1.4	26.11 \pm 0.2	26.18 \pm 0.8	88.25 \pm 1.8	25.72 \pm 0.9	11.19 \pm 1.12	88.51 \pm 1.6	35.48 \pm 1.1	1.51 \pm 0.15
150	71.18 \pm 1.5	24.17 \pm 0.5	38.21 \pm 1.2	70.18 \pm 1.4	31.19 \pm 1.4	28.21 \pm 1.25	87.45 \pm 1.4	36.31 \pm 0.8	0.44 \pm 1.15
180	45.08 \pm 0.5	22.21 \pm 1.2	40.17 \pm 1.1	65.17 \pm 1.5	23.61 \pm 1.1	31.11 \pm 0.9	80.31 \pm 1.1	39.25 \pm 1.2	1.37 \pm 0.09
210	-	-	100 \pm 1.11	63.19 \pm 1.0	-	100 \pm 1.22	75.28 \pm 1.0	33.22 \pm 1.5	0.32 \pm 0.12
240	-	-	100 \pm 1.21	-	-	100 \pm 1.09	75.19 \pm 0.9	38.18 \pm 1.6	1.29 \pm 0.19
270	-	-	100 \pm 1.09	-	-	100 \pm 1.15	77.10 \pm 1.2	36.19 \pm 0.9	1.22 \pm 0.16
300	-	-	100 \pm 0.99	-	-	100 \pm 1.11	-	23.10 \pm 0.8	40.11 \pm 1.11
330	-	-	100 \pm 1.71	-	-	100 \pm 1.12	-	-	100 \pm 1.15
360	-	-	100 \pm 1.51	-	-	100 \pm 1.51	-	-	100 \pm 1.17
LSD _{0.05}			3.55		4.19	5.88		4.12	2.15

Table 6. Field efficacy of *B. bassiana* formulation against adult of *D. armigera* on rice variety Binadhan-10 (Satkhira 2014-2015)

Treatment	Pre-count (No./hill)	Post-count 7 th DOT (No./hill)	% increase or decrease (-) over pre-count	Post-count 15 th DOT (No./hill)	% increase or decrease (-) over pre-count
WP	7.06±0.38	5.16±0.25	-25.50	4.00±0.33	-40.56
Chlorpyrifos	7.00±0.39	4.88±0.21	-28.56	3.58±0.34	-44.33
Control	7.33±0.45	8.65±0.88	21.54	10.12±0.91	42.15
LSD _{0.05}		0.65		1.22	

Table 7. Field efficacy of *B. bassiana* formulation against adult of *D. armigera* on rice variety Binadhan-10 (Comilla 2014-2015)

Treatment	Pre-count (No./hill)	Post-count 7 th DOT (No./hill)	% increase or decrease (-) over pre-count	Post-count 15 th DOT (No./hill)	% increase or decrease (-) over pre-count
WP	5.81±0.24	5.45±0.25	-9.81	3.45±0.23	-38.91
Chlorpyrifos	5.72±0.24	4.85±0.26	-15.43	3.22±0.24	-39.54
Control	5.61±0.28	6.15±0.27	8.75	12.05±0.95	61.55
LSD _{0.05}		0.45		0.98	

Table 8. Effect of formulation on insect pests of rice other than rice hispa (Comilla 2014-2015)

Treatment	Brown plant hopper (No./25m ²)	Leaf roller (No./25m ²)	Stem borer (No./25m ²)	Green leaf hopper (No./25m ²)
WP	14.19±1.01	36.19±1.01	41.51±1.11	21.16±1.11
Chlorpyrifos	22.15±1.12	40.15±1.51	49.11±1.21	27.91±1.51
Control	5.51±1.05	5.08±1.11	9.15±1.09	4.19±1.12
LSD _{0.05}	1.15	0.46	1.55	0.42

Development of mycoinsecticide formulation (WP formulation): Mass culturing of *B. bassiana* strain, AAU-08 in 500 ml potato broth supplemented with 2% peptone and dextrose produced 6.67×10^9 conidia/ml after 15 DAI at 25±1°C. After incorporation of the fungal slurry (500 ml) into the carrier, talcum powder (500 gm), the final formulated product produced 6.80×10^8 conidia/gm and this formulation has been referred as WP in subsequent writing.

Shelf life study of wettable powder: The self life of WP of *B. bassiana* was significantly, different from each other of storage conditions, such as, room temperature (24±1°C), refrigerated condition (4°C) and in deep freeze condition (-4°C to -6°C) (Table 5). At room temperature, viability conidia lasted upto 180 days maintaining a strength of 22.21×10^7 conidia/ml, whereas at refrigeration the same lasted for one month more than the former having conidial strength of 23.61×10^7 conidia/ml and inside deep refrigeration, it was lasted for 300 days without affecting the conidial population.

At refrigeration, the infectivity of the viable propagules was reduced to 63.19% on 210 days of storage from that of 82.15% on 30 days and beyond 210 days the viability and infectivity of the spores were totally lost. Under deep freeze, the viability of the propagules was lasted upto 10 months showing 20% infection from that of 84.05% on 30 days and beyond which, no viability and infectivity were observed.

Field efficacy of WP formation

Location (Satkhira): In pre-treatment count of the adult population and not show any significant difference in this location and it ranged from 7.00-7.33 adult per hill (Table 6). On 7th and 15th day, the population declined to 5.16 to 4.00 and 4.88 to 3.58 adults/hill in the WP and chlorpyrifos treated plots, respectively; while in the control, it ranged between 8.65 and 10.12 adults/hill, respectively.

Location (Comilla): In pre-treatment count of the adult population did not show any significant difference in this

location and it ranged from 5.61-5.81 adult per hill (Table 7). On 7th and 15th day, the population declined to 5.46 to 3.45 and 4.85 to 3.22 adults/hill in the WP and chlorpyrifos treated plots, respectively, while in the control, it ranged between 6.15 and 12.05 adults/hill, respectively.

The population of rice leaf roller, brown planthopper, stem borer and green leaf hopper were significantly, reduced during aman seasons (2013-2015) (Table 8). It was observed that the death insect recorded in chlorpyrifos treated plot was more as compared to the other treatments. The results of the study indicated that WP as a proven technology to manage not only *D. armigera* but also other major insect pests for sustainable production of rice and also as an ecofriendly agent to predator and parasitoids. Besides from this investigation it is clear that *B. bassiana* and *T. japonica* could be utilized side by side in a given rice field, paving the for evolving an IPM programme aiming to attack borers and leaf feeders at the same time.

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