Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(5): 1538-1545

Received: 13-07-2017
Accepted: 14-08-2017

Rohini Sugandi
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad - 580005, Krishinagar, Karnataka, India

Awaknavar JS
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad - 580005, Krishinagar, Karnataka, India

Correspondence
Rohini Sugandi
Department of Agricultural Entomology, University of Agricultural Sciences, Dharwad - 580005, Krishinagar, Karnataka, India

Preliminary lab studies on the use of *Beauveria bassiana* (Balsamo) Vuillemin against *Callosobruchus chinensis* (Fabricius) (Coleoptera: Bruchidae) under different temperature and relative humidity regimes

Rohini Sugandi and Awaknavar JS

Abstract
Laboratory experiments were carried out in order to assess the insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin against *Callosobruchus chinensis* (Coleoptera: Curculionidae). Two fungal preparations *viz*., a conidia and a conidial suspension were compared, dry conidial bioassays were carried out on 250 g of greengram, in glass jars maintained at 28±2°C, and 70% RH. At higher doses ($3.5x10^8$ conidia/g), *B. bassiana* isolate-1 produced a higher percentage of mortality up to 82.00 percent whereas, Bb-2 recorded 72.50 percent mortality at $2.25x10^8$ conidia/g at 10 DAT. Immersion with conidial suspension bioassays were carried out, at higher concentrations of Bb-1 *i.e*, 2×10^8 and 2×10^7 recorded 86.67 percent mortality at 10 DAT. At the same concentration level Bb-2 recorded 80.00 percent mortality. In all the experiments, mortality increased with increase in dose rates and exposure time. Bb-1 showed lowest LC_{50} value i.e., $1.347x10^8$ conidia/g at 9 DAT. Bb-1 performed well when compared to Bb-2. Bioassay with standardised dose of fungus has been carried out with different temperature and relative humidity regimes *viz*., 20, 25, 30 and 35 °C and four relative humidity (RH) levels, 30, 50, 70 and 90%. The organoleptic study magnificently demonstrate that there was no significant difference between green gram when treated with entomopathogen regarding all organoleptic characteristics after twelve months of storage. For commercial purposes the application of admixtures of dry conidia of entomopathogenic fungi in food grains is advised. The results of the present work suggest that, *B. bassiana* can be used to manage *C. chinensis* in storage.

Keywords: *Beauveria bassiana*, *Callosobruchus chinensis*, temperature and relative humidity, organoleptic

Introduction
The most worldwide pulse grain damaging bruchid pest is the pulse beetle, *Callosobruchus chinensis*, which infests pods in the fields as well as in the storage. As a pest of whole grain, causes both quantitative and qualitative damage to stored grains.

Residual insecticides are the most common agents for protection of stored products against stored products pests. They have several negative properties such as mammal toxicity, residues on grain, as well as increased resistance of pests [1], the increased public awareness and concern for environmental safety has directed research to the development of alternative control strategies [2, 3]. The use of entomogenous fungi, *Beauveria bassiana* to manage the storage pests offers the opportunity of using living organisms that are natural enemies of the target pests to maintain pest population well below the economic threshold level. *B. bassiana* is registered by the U.S. Environmental Protection Agency (EPA) for a wide range of insect control applications [4]. First investigation by [5] followed by [6-9] opined that *B. bassiana* is a potential microbial control agent against some stored product pests. Recently Boverosil®, Mycotrol® ES, Mycotrol® 22WP, Naturalis® SC registered formulations of *B. bassiana* are commercially available for use in storage facilities.

As little work has been done until now relating to the above research in India, it will be quite helpful for assessing *B. bassiana* against storage pests like *C. chinensis*, and to evolve an effective tool for pest management through economic and eco-safe technology.

Materials and Methods

The experiments were carried out during 2011-13 in the laboratory of the department of Agricultural Entomology, University of Agricultural Sciences (UAS), Dharwad. Dharwad, a district head quarter in North Karnataka is located at 150 26' North latitude, 750 07' East Longitude and at an altitude of 731.8 m above mean sea level (MSL). It is lying in North transitional zone (Zone-8) and receives an annual rainfall of 860 mm distributed all over the year. The temperature and relative humidity range from 11 to 37^0C and 40 to 85 percent respectively.

Insect rearing

Maintenance of stock culture: To ensure continuous supply of insects for undertaking bioassay tests with *B. bassiana*, the target insects were cultured under laboratory condition.

Pulse beetle: Stock cultures of the *C. chinensis* were initiated by collecting adult insects from the infested green gram grains from University farm house. *C. chinensis* were identified by the key[10] and 50 adult insects were released in to glass jars of capacity of 1 litre containing healthy green gram grains and covered with muslin cloth fastened by rubber band to prevent escape of insects and to ensure proper ventilation. Newly emerged adult insects (males and females) were used in the experiments. All the jars were kept at a laboratory temperature that fluctuated between 20 to 27 °C and RH 70-80 per cent.

Pure culture: Pure cultures of the pests were developed by infesting insect free host grains with freshly emerged ten pairs selected from the stock culture, were allowed to mate in glass bottles of one litre capacity. Mouths of such containers were covered with muslin cloths. One week after release, all adults were removed from the containers and placed separately for the development till completion of life cycle. This procedure was followed at weekly intervals up to four to five weeks to obtain all the stages of populations of all insects separately.

In vitro production of fungal pathogens

Spore harvesting: One litre of distilled water and 39 g Potato Dextrose Agar (PDA) were boiled in a conical flask until completely dissolved. This solution was autoclaved at 121 °C for 30 minutes prior to use. The spores of the fungus were inoculated (aseptically) on PDA broth medium and kept for incubation at room temperature, 26 ±1 °C. After 5 days, mycelium developed on broth culture was filtered through Waltsman No.1 filter paper and mycelium collected on filter paper was dried aseptically at room temperature. This was used for the bioassay study. The method used by Abdul and Sohail (2007) [11] was followed.

Fungal preparations

The *B. bassiana* isolate used during the experiment

Sl. No.	Fungal pathogen	Isolates	Conidia/ml or g
1	*Beauveria bassiana*	Dharwad isolate-1	2×10^8
2		Bangalore isolate-2	1×10^8

Mass culturing of the fungus: Hundred grams of crushed rice grains were taken in 500ml saline bottle, 100ml of distilled water containing yeast (1%) was added to this flask, thoroughly mixed and plugged with cotton. After soaking for 6 hrs, bottles were sterilized at 15 psi pressure and 121°C for 20 minutes. After cooling, the flasks containing media were inoculated with the fungal spores obtained from PDA plates using sterilized needle under aseptic conditions. Inoculated media was incubated at 26 ±1 °C temperature. Conidia were harvested after 25 days.

Formula for assessment of conidial strength using haemocytometer

$$\text{Concentration (Spores / ml)} = \frac{\text{Number of spores from 80 cells}}{80} \times 400 \times 0.1 \times 1000 \times DF$$

Wherein,

DF - Dilution factor

Bioassay has been carried out by following two methods.

Bioassay

Grain admixuring with conidia: Desired concentrations of all isolates of *B. bassiana* conidia were prepared and mixed with 250 g of green gram in a glass jar and shaken gently for 2-5 min to ensure a thorough admixture with the grains. The jars and their contents were then allowed to settle for 10 min. Thirty pulse beetles were taken from the stock culture and placed in the jars. The jars were covered with muslin cloth held with rubber bands and kept at 26±1°C and 70 to 90 percent RH. Each treatment replicated thrice in completely randomized design. The mortality of the adult insects were recorded after 6, 7, 8, 9 and 10 days and all dead adults were removed after each count and were transferred to humid chamber (petri plate layered with cotton and moistened tissue paper), incubated until sporulation occurred. Mortality due to *B. bassiana* was then confirmed by microscopic examination of hyphae and spores on the surface of the cadaver.

Immersion bioassay with conidial suspension: The spore suspension was vortexed properly to get uniform distribution of spores. Stock culture was prepared by dissolving 1 g of dried conidia in 9 ml of sterile water. One ml of spore suspension from stock was transferred to nine ml of sterile water (dilution blank) with the help of sterilized pipette and likewise further dilutions were made as and when required from 10^2 to 10^8 and spore concentrations were determined using doubled ruled Neaubauer's Haemocytometer after serial dilutions for definite concentration of spores/ml under a phase contrast microscope [12].

Desired concentrations of the isolates of *B. bassiana* (Bb-1 and Bb-2) conidial suspensions ($2x10^8$, $2x10^7$, $2x10^6$, $2x10^5$, $2x10^4$, $2x10^3$ and $2x10^2$ spores/ml) were prepared and each insect was dipped in the inoculum for 20-30 seconds and then placed in petri dishes to dry. After drying, the treated insects were carefully transferred to bottles (0.25 L capacity) containing 50 g of healthy green gram for each treatment. For each treatment 3 replicates were used in completely randomized design. The control insects were dipped in the distilled water [11, 13]. The mortality of pulse beetle was recorded each day starting from day 5^{th} to 10^{th} used to get the mortality range between 20-80 per cent.

The data pertaining to the laboratory observations were analysed in completely randomized design (CRD) and the percentage values were converted to arcsine values. The LC_{50} was determined by probit analysis using the software package SPSS 16.0. The mean values of the experiments were separated using Duncan's Multiple Range Test (DMRT) [14].

Maintenance of required temperatures and relative humidity levels: The following temperatures levels 20, 25, 30 and 35 °C and relative humidity (RH) 30, 50, 70 and 90% were selected for the investigation, was done by using various saturated salt solutions [15] in the air tight desiccators. Constant temperatures were maintained in the BOD incubator with a fluctuation of ± 0.5 ^{0}C.

The standardised doses of *B. bassiana* was mixed with 100g of healthy greengram. After thoroughly mixing with the conidia, thirty adult insects of *C. chinensis* were introduced into the petri dishes. The top of the petri dish was covered with muslin cloth and tied with rubber band. Locally made iron ring of suitable height was made to rest on petri dish, so that a portion of it would remain above the level of chemical solution used to maintain the required relative humidity. The inner wall of the containers of the saturated salt solution was smeared with petroleum jelly to prevent the rinse of the chemical solution. A time of one week was allowed for equilibration of the humidity inside the containers, used all temperatures, before starting the experiment. The percent mortality of *C. chinensis* was recorded after 7, 8, 9 and 10 days after the treatment in different temperature 20, 25, 30 and 35^{0}C at 30, 50, 70 and 90 percent relative humidity levels. After each observation dead insects were removed and counted. The cause of death was confirmed by placing all the dead insects on moist filter papers in petri dishes in humid chamber to facilitate fungal sporulation. The number of insects that expressed mycosis was noted. For each treatment 2 replicates were used in factorial completely randomized design.

Organoleptic evaluation for *B. bassiana* on the grains: *B. bassiana* was mixed thoroughly @ three percent with greengram on weight/weight basis. The treated and untreated grains were packed in polyethylene pouches heat sealed and maintained at room temperature of 25±2°C and 70±5 percent RH for 12 months duration. Samples were drawn at monthly interval, cleaned and cooked along with untreated and analysed for various parameters.

The studies were conducted at room temperature. Two replications were maintained for each treatment. Sensory evaluation of the grains was carried out by using 9 point hedonic scale (Table 6) employing a panel of ten trained judges drawn from staff and students of the Department of Food Science and Nutrition, College of Rural Home Science, UASD. Judges were instructed individually to evaluate the cooked samples without consulting each other. Observations on organoleptic characters like appearance, colour, texture, taste, flavour and overall acceptability at monthly interval were recorded and further analysed statistically (Factorial CRD).

Results and Discussion

Adult mortality of *C. chinensis* due to Bb-1 in greengram admixed with conidia

Cumulative mortality of adult insects increased with an increase in the concentration and exposure period. At 7 DAT, mortality increased drastically, at the highest concentration of 3.5 x10^8 conidia/g, 82.00 percent of the adults were killed in period of 10 days after infection and was significantly superior over all other treatments (Table 1).

Adult mortality of *C. chinensis* due to Bb-2 in greengram admixed with conidia

Mortality of the adult insects commenced after 5 days of treatment, lower concentrations of the fungus could not inflict mortality. The treatment T_7 proved to be consistently superior to other treatments at all intervals of observations. Both Bb-1 and Bb-2 were on par with each other (Table 2). Results obtained with the application of *B. bassiana* was in agreement with many previous workers [16-24, 8, 7, 4, 11] who reported *B. bassiana* was highly effective against the major stored grain insect pests.

Adult mortality of *C. chinensis* due to Bb-1 in immersion bioassay

Cumulative mortality of beetle was maximum at higher concentrations and decreased with decrease in concentrations (6, 7, 8 and 9 DAT). T_1 recorded highest percent mortality at all intervals of observations (Table 3).

Adult mortality of *C. chinensis* due to Bb-2 in immersion bioassay

All the treatments recorded differential mortality rate at different interval of observations. At the highest concentration of 2x10^8 conidia/ml, 10.00, 33.33, 56.67 and 70.00 percent of the adults were killed in period at 6, 7, 8 and 9 days after infection and was significantly superior over all other treatment concentrations. 10 DAT, the higher mortality was observed in T_1 and T_2 (80.00%) (Table 4).

Adult beetles showed 86.67 percent mortality with 2×10^7 and 2×10^8 conidia/ml with isolate, Bb-1. Whereas, Bb-2 recorded 80 percent with the same conidial load, both at 10 DAT, the difference between the two isolates being very small. Present findings agrees with Cherry *et al*. (2005) [9] they stated that different isolates from *B. bassiana* can provide good control of *C. maculatus* by immersion bioassay. Adults of *C. maculatus* recorded LT_{50} values in suspensions with highest concentrations (2.3 × 10^7 conidia ml^{-1}) were 6.63 days with *B. bassiana* in immersion bioassay [25].

Estimation of median lethal concentration (LC_{50}) of *B. bassiana* to the adult of *C. chinensis* at 9 DAT

At 9 DAT, lower LC_{50} was recorded in Bb-1 isolate (1.347x10^8 conidia/g) compared to 1.587 x10^{8}conidia/g in Bb-2 (Table 5). Several researchers worked on the LC_{50} for stored grain pests includes Chrrey *et al*. (2005) [9] recorded LC_{50} on day 9 post-treatment were 9.10 × 10^4 and 7.10 × 10^5 conidia ml^{-1} for *B. bassiana* and *M. anisopliae* against *Callosobruchus maculates.* Again, the LC_{50} values on day 9 post-treatment were 3.17 x 10^6 and 6.08 x 10^7 conidia ml^{-1} for *C. maculatus* and *S.granarium*, when treated with *B. bassiana* respectively [25]. As time progress, cumulative mortality for both the isolates increased but at differing rates. Our results demonstrate that *B. bassiana* is effective against *C. chinensis* and highly influenced by several factors such as the exposure interval, the target species, the dosage and especially, the characteristics of the fungal preparation.

At 30 ^{0}C at 90% RH an isolate Bb-1 recorded 83.00 percent mortality followed by 25 ^{0}C at 90% RH (75.00%) and 25 ^{0}C at 70% RH (64.33%). However, isolate Bb-2 recorded 91.00, 70.00 and 63.00 percent mortality at same regimes of temperature and relative humidity at 10 DAT (Fig 1). Several authors have also reported humidity-independent infection of stored product insects by entomopathogenic fungi [5, 26, 27]. A high level of mortality was observed in *C. serratus* at 26±2 ^{0}C and 65 to 70 percent RH [20]. Entomopathogenic fungi, germinate at relative humidity above 90 percent [28] although, it has been shown that fungi work also at lower humidity levels, probably due to favourable microclimates surrounding

the hosts [29, 23].

It's widely believed that entomopathogenic fungi are not only most efficacious in high moisture conditions, but that they are not at all effective with low moisture. However, many studies have demonstrated that this is often not the case, ambient humidity has been found to have little impact on *B. bassiana* efficacy in some insect systems [30, 5]. The researchers have reported that the longevity of *B. bassiana* conidia is best when dry [31, 23, 32]. Stored grains are however hygroscopic and it is therefore necessary that during storage, humidity and moisture content of the grains are kept low to avoid spoilage.

Samples treated with *B. bassiana*, and the samples without *B. bassiana*, were scored between like very much (8) to liked extremely (9) and they did not differ significantly in twelve months storage period (Fig. 2). On the whole, green gram received higher scores (9-like extremely) during storage up to twelve months with better sensory scores. For commercial purposes the application of admixtures of dry conidia of entomopathogenic fungi in food grains is more advantageous. As such there are no reviews pertaining to different organoleptic charecteristics to entomopathogen on stored grain pests is conserned. This study is first of its kind to investigate and hence there is no reviews to discuss. The cooked green gram treated with entomopathogen was found acceptable when subjected to sensory evaluation.

Table 1: Effect of *Beauveria bassiana* Bb-1 isolate conidia admixed with grains on *Callosobruchus chinensis* adults

Concentration (Conidia/g)**		Percent mortality					
		5 DAT*	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
T_1	0.2 x 10^8	0.00 (0.91) ^{b}	0.00 (0.91) ^{e}	10.33 (18.73) ^{g}	15.00 (22.78) ^{f}	20.00 (26.55) ^{g}	25.00 (29.99) ^{f}
T_2	1 x 10^8	0.00 (0.91) ^{b}	0.00 (0.91) ^{e}	18.66 (25.58) ^{f}	26.00 (30.64) ^{e}	37.00 (37.45) ^{f}	42.00 (40.38) ^{e}
T_3	1.5 x 10^8	0.00 (0.91) ^{b}	2.20 (8.52) ^{d}	25.00 (29.99) ^{e}	42.00 (40.38) ^{d}	50.00 (44.98) ^{e}	58.00 (49.58) ^{d}
T_4	2 x 10^8	0.00 (0.91) ^{b}	3.33 (10.50) ^{c}	32.00 (34.44) ^{d}	50.00 (44.98) ^{d}	55.00 (47.85) ^{d}	64.00 (53.11) ^{c}
T_5	2.5 x 10^8	0.00 (0.91) ^{b}	4.00 (11.53) ^{c}	40.00 (39.21) ^{c}	55.00 (47.85) ^{c}	64.00 (53.11) ^{c}	75.00 (59.98) ^{b}
T_6	3 x 10^8	3.33 (10.51) ^{a}	6.00 (14.14) ^{b}	49.00 (44.41) ^{b}	59.00 (50.17) ^{b}	68.00 (55.53) ^{b}	77.00 (61.32) ^{b}
T_7	3.5 x 10^8	3.33 (10.51) ^{a}	8.00 (16.40) ^{a}	52.00 (46.13) ^{a}	63.00 (52.51) ^{a}	73.00 (58.67) ^{a}	82.00 (64.87) ^{a}
T_8	Untreated control	0.00 (0.91) ^{b}	0.00 (0.91) ^{e}	0.00 (0.91) ^{h}	2.00 (8.13) ^{g}	4.43 (12.15) ^{h}	8.00 (16.40) ^{g}
S.Em±		0.11	0.24	0.44	0.37	0.29	0.37
CD @ 1%		0.32	0.71	1.29	1.09	0.84	1.09

*DAT= Days after treatment

** Grams of each concentration was mixed with 250 g of grains

Figures in parentheses are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01%)

Table 2: Effect of *Beauveria bassiana* Bb-2 isolate conidia admixed with grains on *Callosobruchus chinensis* adults

Concentration (Conidia/g)**		Percent mortality					
		5 DAT*	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
T_1	0.75 x 10^8	0.00 (0.91) ^{c}	1.00 (5.74) ^{e}	8.00 (16.42) ^{g}	20.00 (26.54) ^{g}	28.00 (31.93) ^{e}	38.00 (38.04) ^{e}
T_2	1 x 10^8	0.00 (0.91) ^{c}	1.00 (5.74) ^{e}	11.00 (19.35) ^{f}	25.00 (29.98) ^{f}	36.50 (37.15) ^{d}	41.00 (39.80) ^{de}
T_3	1.25 x 10^8	0.00 (0.91) ^{c}	2.20 (8.51) ^{d}	14.33 (22.23) ^{e}	29.00 (33.20) ^{e}	39.00 (38.63) ^{d}	44.00 (41.54) ^{d}
T_4	1.5 x 10^8	1.10 (6.01) ^{b}	4.00 (11.53) ^{c}	19.33 (26.07) ^{d}	37.00 (37.45) ^{d}	46.00 (42.69) ^{c}	51.33 (45.74) ^{c}
T_5	1.75 x 10^8	1.10 (6.01) ^{b}	4.33 (12.01) ^{bc}	28.66 (32.35) ^{c}	46.33 (42.88) ^{c}	54.00 (47.28) ^{b}	61.50 (51.63) ^{b}
T_6	2.00 x 10^8	2.00 (8.13) ^{a}	5.00 (12.87) ^{b}	36.66 (37.25) ^{b}	50.33 (45.17) ^{b}	56.33 (48.62) ^{b}	65.11 (53.77) ^{b}
T_7	2.25 x 10^8	2.00 (8.14) ^{a}	8.00 (16.42) ^{a}	48.00 (43.84) ^{a}	55.00 (47.85) ^{a}	64.33 (53.31) ^{a}	72.50 (58.35) ^{a}
T_8	Untreated control	0.00 (0.91) ^{c}	0.00 (0.91) ^{f}	2.00 (8.75) ^{h}	4.00 (11.52) ^{h}	6.00 (13.67) ^{f}	7.00 15.13) ^{f}
S.Em±		0.17	0.18	0.23	0.44	0.37	0.37
CD @ 1%		0.50	0.52	0.68	1.29	1.09	1.09

*DAT= Days after treatment

** Grams of each concentration was mixed with 250 g of grains

Figures in parentheses are arc sine transformed values

Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01%)

Table 3: Effect of *Beauveria bassiana* Bb-1 isolate conidial suspension on *Callosobruchus chinensis* adults

Concentration (Conidia/ml) **		Percent mortality					
		5 DAT*	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
T_1	2 x 10^8	10.00 (18.43) [a]	20.00 (26.55) [a]	46.67 (43.07) [a]	63.33 (52.71) [a]	76.67 (61.09) [a]	86.67 (68.56) [a]
T_2	2 x 10^7	6.67 (14.96) [b]	13.33 (21.41) [b]	40.00 (39.22) [b]	60.00 (50.75) [b]	73.33 (58.89) [b]	86.67 (68.56) [a]
T_3	2 x 10^6	6.67 (14.96) [b]	13.33 (21.41) [b]	36.67 (37.25) [c]	56.67 (48.81) [c]	66.67 (54.71) [c]	76.67 (61.09) [b]
T_4	2 x 10^5	3.33 (10.50) [c]	10.00 (18.37) [c]	30.00 (33.20) [d]	43.33 (41.15) [d]	53.33 (46.89) [d]	60.00 (50.75) [c]
T_5	2 x 10^4	0.00 (0.91) [d]	3.33 (10.52) [d]	13.33 (21.41) [e]	20.00 (26.55) [e]	30.00 (33.19) [e]	36.67 (37.25) [d]
T_6	2 x 10^3	0.00 (0.91) [d]	3.33 (10.52) [d]	10.00 (18.37) [f]	20.00 (26.54) [e]	26.67 (31.08) [f]	33.33 (35.25) [e]
T_7	2 x 10^2	0.00 (0.91) [d]	0.00 (0.91) [e]	6.67 (14.96) [g]	10.00 (18.41) [f]	13.33 (21.41) [g]	16.67 (24.09) [f]
T_8	Control	0.00 (0.91) [d]	0.00 (0.91) [e]	0.00 (0.91) [h]	2.00 (8.13)[g]	4.00 (11.53) h	7.00 (15.34)[g]
S.Em±		0.11	0.90	0.15	0.14	0.15	0.24
CD @ 1%		0.32	0.26	0.44	0.41	0.45	0.70

*DAT= Days after treatment
** Grams of each concentration was mixed with 250 g of greengram
Figures in parentheses are arc sine transformed values
Means in the columns followed by the same alphabet do not differ significantly by DMRT (P=0.01%)

Table 4: Effect of *Beauveria bassiana* Bb-2 isolate conidial suspension on *C. chinensis* adults

Concentration (Conidia/ml) **		Percent mortality					
		5 DAT*	6 DAT	7 DAT	8 DAT	9 DAT	10 DAT
T_1	2 x 10^8	6.67 (14.96) [a]	10.00 (18.43) [a]	33.33 (35.25) [a]	56.67 (48.81) [a]	70.00 (56.77) [a]	80.00 (63.41) [a]
T_2	2 x 10^7	3.33 (10.52) [b]	6.67 (14.96) [b]	30.00 (33.20) [b]	50.00 (44.98) [b]	66.67 (54.71) [a]	80.00 (63.41) [a]
T_3	2 x 10^6	3.33 (10.43) [b]	6.67 (14.96) [b]	20.00 (26.55) [c]	40.00 (39.21) [c]	50.00 (44.98) [b]	73.33 (58.89) [b]
T_4	2 x 10^5	0.00 (0.91) [c]	3.33 (10.43) [c]	10.00 (18.43) [d]	20.00 (26.55) [d]	30.00 (33.20) [c]	50.00 (44.98) [c]
T_5	2 x 10^4	0.00 (0.91) [c]	3.33 (10.43) [c]	10.00 (18.43) [d]	20.00 (26.55) [d]	20.00 (26.55) [d]	26.67 (31.08) [d]
T_6	2 x 10^3	0.00 (0.91) [c]	0.00 (0.91) [d]	3.33 (10.52) [e]	6.67 (14.96) [e]	13.33 (21.41) [e]	20.00 (26.55) [e]
T_7	2 x 10^2	0.00 (0.91) [c]	0.00 (0.91) [d]	3.33 (10.52) [e]	6.67 (14.96) [e]	10.00 (18.43) [f]	13.33 (21.41) [f]
T_8	Control	0.00 (0.91) [c]	0.00 (0.91) [d]	2.00 (8.13) [f]	3.00 (9.97) [f]	6.00 (14.17) [g]	7.00 (15.34) [g]
S.Em±		0.18	0.25	0.18	0.40	0.44	0.36
CD @ 1%		0.53	0.75	0.53	1.19	1.30	1.06

*DAT= Days after treatment
** Grams of each concentration was mixed with 250 g of greengram
Figures in parentheses are arc sine transformed values
Means in the columns followed by the same alphabet do not differ significantly

Table 5: Median lethal concentration (LC_{50}) of *Beauveria bassiana* to the adult of *Callosobruchus chinensis* at 9 DAT

Isolates	LC_{50} (conidia/g)	Fiducial limits of LC_{50}		LC_{95} (conidia/g)	Slope	X^2 -Value
		Lower limit	Upper limit			
Bb -1	1.347 x 10^8	0.887 x 10^8	1.919 x 10^8	33.631 x 10^8	1.177	1.375
Bb -2	1.587 x 10^8	1.276 x 10^8	2.206 x 10^8	11.529 x 10^8	1.910	0.366

LC_{50} = Concentration calculated to give 50 percent mortality.

Table 6: Score card for the organoleptic evaluation of cooked green gram (9 point hedonic scale)

Quality	Score
Liked extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor Dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

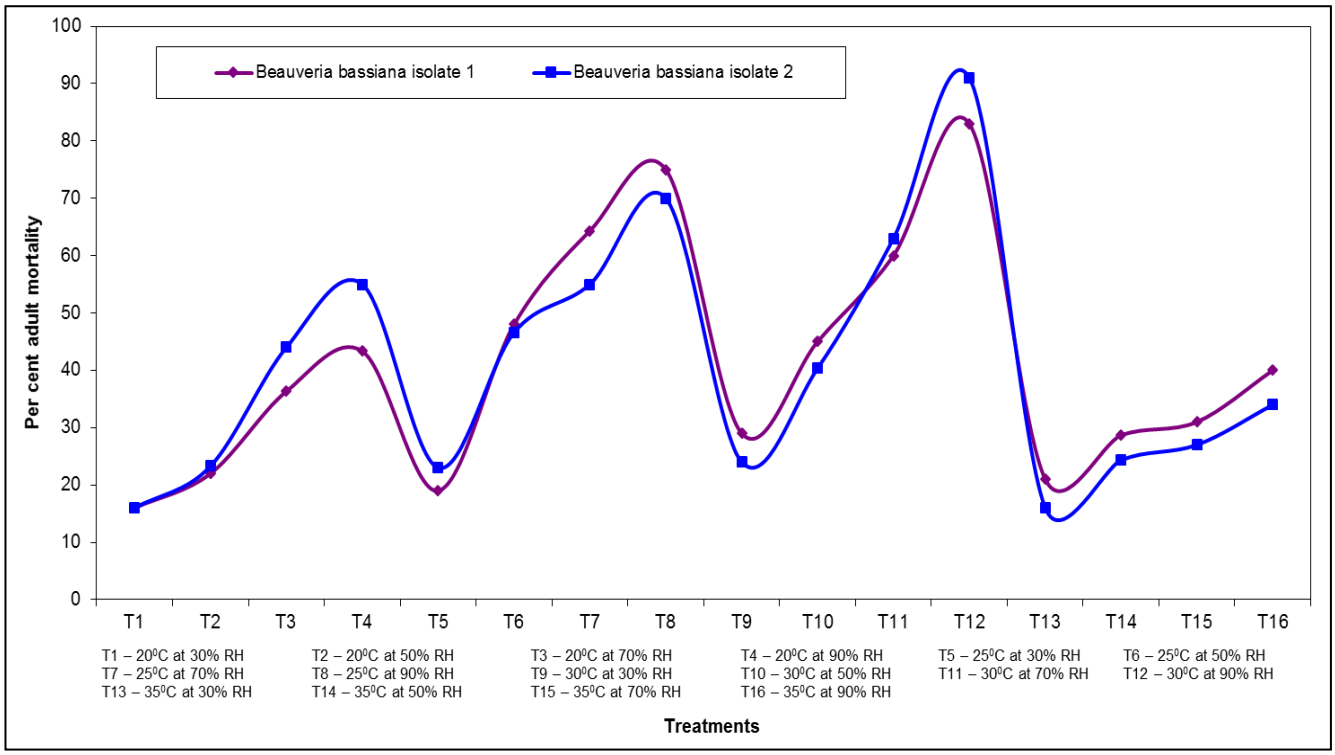

Fig 1: Effect of temperature and relative humidity on mortality of *Callosobruchus chinensis* in greengram admixtured with isolates of *Beauveria bassiana*

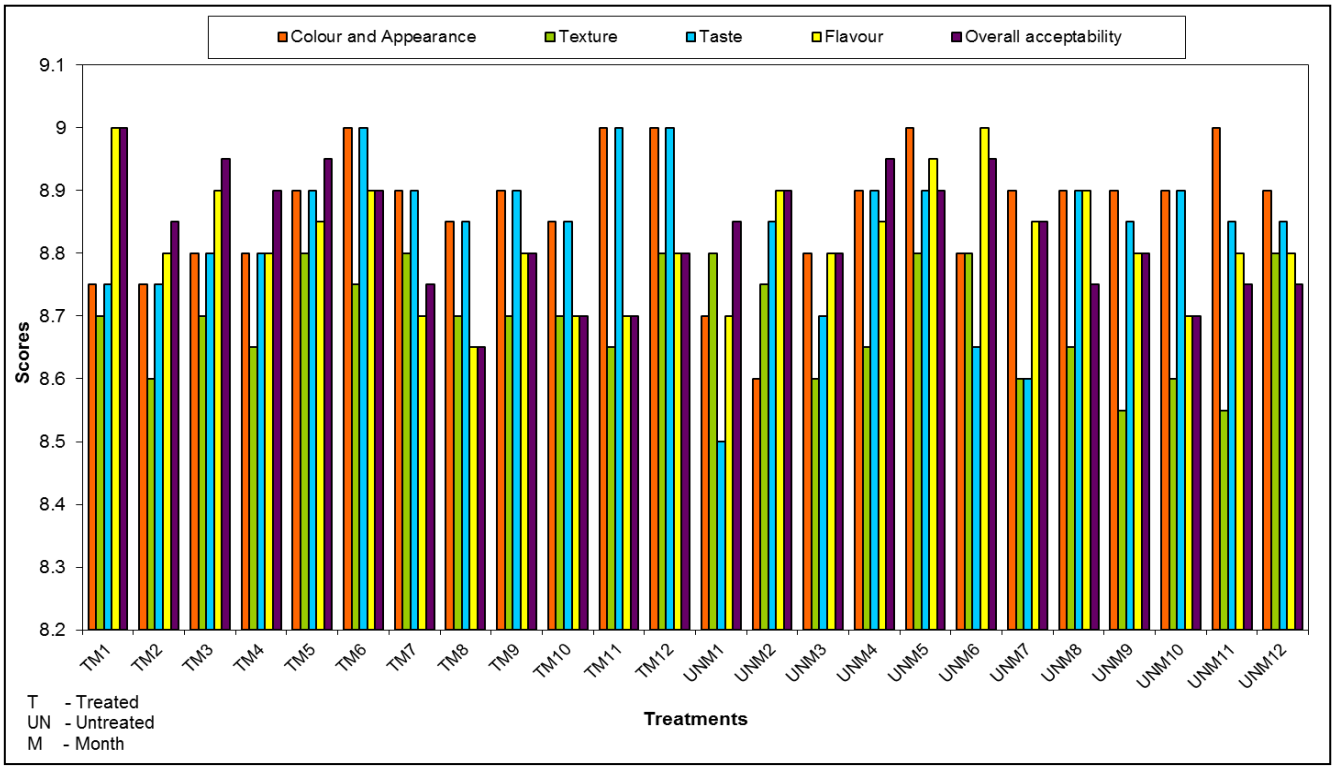

Fig 2: Mean sensory scores of greengram when admixed with *Beauveria bassiana*

References

1. Arthur FH. Grain protectants: current status and prospects for the future. Journal of Stored Product Research. 1996; 32:293-302.
2. Inglis GD, Johnson DL, Goettel MS. Field and laboratory evaluation of two conidial batches of *Beauveria bassiana* (Balsamo) Vullemin against grasshoppers. The Canadian Entomologist. 1997; 129:171-186.
3. Lomer CJ, Bateman RP, Johnson DL, Langewald J, Thomas M. Biological control of locusts and grasshoppers. Annual Review of Entomology. 2001; 46:667-702.
4. Lord JC. Desiccant dust synergize the Effect of *Beauveria bassiana* (Hyphomycetes: Moniliales) on stored grain beetles. Journal Economic Entomology. 2001; 94(2):367-372.
5. Ferron P. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of *Acanthosclelides obtectus* (Col: Bruchidae). Entomophaga. 1977; 22:393-396.
6. Searle T, Doberski J. An investigation of the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. as a potential biological control agent for *Oryzaephilus surinamensis*. Journal of Stored Product Research. 1984; 20:17-23.
7. Dal Bello G, Padin S, Lastrab CL, Fabrizio M. Laboratory evaluation of chemical biological control of rice weevil, *Sitophilus oryzae* L. in stored grain. Journal of Stored Product Research. 20013; 7(1):77-84.
8. Padin SB, Dal-Bello G, Fabrizio M. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtecus* in stored durum wheat and beans treated with *Beauveria bassiana*. Journal of Stored Product Research. 2002; 38(1):69-74.
9. Cherry AJ, Abalo P, Hell K. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuilleum and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. Journal of Stored Product Research. 2005; 41:295-309.
10. Raina AK. *Callasobruchus* sp. infesting stored pulses (grain legume) in India and their comparative study of biology. Indian Journal Entomology. 1970; 32(4):87-89.
11. Abdul RD, Sohail A. Evaluation of fungus *Beauveria bassiana* (Bals.) infectivity to the larger grain borer *Prostephanus truncatus* (Horn.). Pakistan Entomology. 2007; 29(2):77-82.
12. Hall RA. A bioassay for the pathogenicity of *Verticillium lecanii* conidiospores on the aphid, *Macrosiphonella sanborni*. Journal of Invertibrate Pathology. 1976; 27:41-48.
13. Mahdneshin Z, Hassan Safaralizadah M, Ghosh Y. Study on the efficiency of iranian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin against *Rhyzopertha dominica* F. (Coleptera: Bostrichidae). Journal of Biological Sciences. 2009; 9(2):170-174.
14. Gomez KA, Gomez AA. Statistical procedures for agricultural research. John Wiley and sons, New York, 1984, 680.
15. Winston PW, Bates DH. Saturated salt solutions for biological research. Ecology. 1960; 41(1):232-236.
16. Masuka AJ, Manjonjo V. Laboratory screening of Metarhizium flavoviride and Beauveria bassiana for the control of Mecostibus pinivorus, a Punis patula defoliator in Zimbabwe. Journal of Applied Science in Southern Africa. 1996; 2:91-95.
17. Padin SB, Bello GM, Vasicek AL, Dal-Bello G. Bioinsecticide potential of entomopathogenic fungi for stored grain pests. Revista de la Facultad de Agronomia Universidad de Buenos Aires. 1996; 15(1):1-7.
18. Padin SB, Dal-Bello G, Vasicek AL. Pathogenicity of *Beauveria bassiana* for adults of *Tribolium castaneum* (Coleoptera, Tenebrionidae) in stored-grains. Entomophaga. 1997; 42(4):569-574.
19. Moino A, Alves SB, Pereira RM. Efficacy of *Beauveria bassiana* (Balsamo) Vuillemin isolates for control of stored grain pests. Journal of Applied Entomology. 1998; 122:301-305.
20. Ekesi S, Egwurube EA, Akpa AD, Onu I. Laboratory evaluation of the entomopathogenic fungus, *Metarhizium anisopliae* for the control of the groundnut bruchid, *Caryedon serratus* on groundnut. Journal of Stored Product Research. 2001; 37:313-321.
21. Stathers T. Entomopathogenic fungi in grain storage-any lessons for Europe from elsewhere? In: Zdarkova E, Lukas J, Hubert J. (Eds.), Proceedings of the 2nd meeting of WG 4 of COST Action 842, Prague, 2002, 100-109.
22. Wakefield M, Cox PD, Wildey KB, Price NR, Moore D, Bell BA. The use of entomopathogenic fungi for stored product insect and mite control- further progress in the ''Mycopest project''. In: Proc. 2nd meeting of Working Group 4: Biocontrol of Artropod Pests in the Strored Products. Ed. Zdarkova E, Lukas J, Hubert J, Action 842, Prague, 2002, 110-115.
23. Akbar W, Lord JC, Nechols JR, Howard RW. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology. 2004; 97:273-280.
24. Kassaye A. Susceptibility of the rice weevil, *Sitophilus oryzae* (Coleoptera: Curculionidae) to native entomopathogenic fungal isolates. M. Sc. Thesis, Addis Ababa Univ. 2010, 51.
25. Shams G, Safaralizadeh MH, Imani S, Shojai M, Aramideh S. A laboratory assessment of the potential of the entomopathogenic fungi *Beauveria bassiana* (Beauvarin®) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). African Journal of Microbiology Research. 20115; 10:1192-1196.
26. Rodrigues C, Pratissoli D. Pathogenicity of *Beauveria brongniartii* (Sacc.) Petch. and *Metarhizium anisopliae* (Mols.) Sorok. and its effect on corn weevil and bean beetle. Annals of the Entomology Society of Brazil. 1990; 19:301-306.
27. Adane K, Moore D, Archer SA. Preliminary studies on the use of *Beauveria bassiana* to control *Sitophilus zeamais* (Coleoptera: Curculionidae) in the Laboratory. Journal of Stored Product Research. 1996; 32(2):105-113.
28. Roberts DW, Campbell AS. Stability of entomopathogenic fungi. Miscellaneous publications of the entomological society of America. 1977; 10(3):19-76.
29. Ramoska WA. The influence of relative humidity on *Beauveria bassiana* infectivity and replication in the chinch bug, *Blissus leucopterus.* Journal of Invertebrate Pathology. 1984; 43:389-394.

30. Moore GE. Pathogenicity of three entomogenous fungi to southern pine beetle at various temperatures and humidities. Environmental Entomology. 1973; 2:54-57.
31. Wraight SP, Jackson MA, de Kock SL. Production, stabilization and formulation of fungal biocontrol agents. In: Fungi as Biocontrol Agents. Ed. Butt TM, Jackson C. Magan N. CAB International, Wallingford, UK, 2001, 253-286.
32. Lord JC. From metchnikof to monsanto and beyond: The path of microbial control. Journal of Invertebrate Pathology. 2005; 89:19-29.