Field efficacy of entomopathogenic fungi *Beauveria bassiana* (Balsamo.) for the management of mungbean insect pests

M S Y I Bayu* and Y Prayogo*

*1*Indonesian Legumes and Tuber Crops Research Institute, Jl. Raya Kendalpayak KM 08, Po. Box 66, Malang 65101, East Java, Indonesia

Email : santi4_nov@yahoo.co.id

**Abstract.** In order to reduce the use of insecticide, the application of *Beauveria bassiana* may be an alternative control. The objective of this study was to evaluate the efficacy of *B. bassiana* for controlling mungbean pest. The experiment was conducted in Ngale Research Station from February to May 2017, using randomized block design, seven treatments, four replicates. The treatments were frequency of application; P1= six times, P2= five times, P3= four times, P4= three times, P5= once, P6= full protection using chemical insecticide, and P7= no protection. Application of *B. bassiana* four to six times can suppress the population of *Empoasca* sp., *Riptortus linearis*, and *Maruca testulalis*, but did not significantly different with the application of chemical insecticide. Based on the seed weight, application of *B. bassiana* six times (659.7 g/plot) led to significantly high as compare with the application of chemical insecticide (374 g/plot). Application of *B. bassiana* tended to be secure to natural enemies, especially *Coccinella* sp., *Oxyopes javanus*, and *Paederus fuscipes*. Both of those predators were not found on the application of chemical insecticide. Hence, *B. bassiana* can be recommended as a biological agent in integrated pest management component on mungbean because of effective and environmentally friendly.

**Keywords**: Efficacy, fungi, mungbean insect pests

1. **Introduction**

Mungbean, *Vigna radiata* (L.) Wilczek, is an important food crop that widely distributed all over the world including tropical and subtropical areas in which south and southeast Asia become the main grown region [1]. Mungbean is easily adopted into multiple cropping systems in the drier and warmer climates because of its short life cycle and most preferred by the farmer due to high protein content [2], [3].

Mungbean is prone to a large number of insect pests that attack mungbean from the early growth up to harvesting time. The infestation by an array of insect pest cause significant yield loss and some of them can destroy seeds in storage. The insect pests that attack mungbean can be classified based on their appearance in the field. They are stem borer, foliage feeders, foliage sucker, pod sucker, pod borer, and pod feeders. According to [4], stem borer, thrips, and pod borer are the most important pest on mungbean. In Indonesia, the major pest on mungbean were dominated by foliage feeder (*Longitarsus suturellus* Duftschmid) and pod borer (*Maruca testulalis* Geyer) that have been considered to be highly responsible for yield losses up to 80% [5].

Recently, more-selective and less-polluting products tend to be used in modern agriculture and farmers have been minimized the use of insecticide to manage the pest population [6].

---

Content from this work may be used under the terms of the Creative Commons Attribution 3.0 licence. Any further distribution of this work must maintain attribution to the author(s) and the title of the work, journal citation and DOI. Published under licence by IOP Publishing Ltd
Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin is a control agent that infected insect body by contact instead ingestion and widely distributed in nature in wide host range. Moreover, *B. bassiana* known to be safety to non-target organism such as predator and parasitoid and its can be easily cultured on potato dextrose agar (PDA) medium in the laboratory [7]. *B. bassiana* has been evaluated in the laboratory and applied in a field to control numerous of insect pests [8]-[11].

Most study explained the efficacy of *B. bassiana* under laboratory condition. *B. bassiana* is ovisidal for egg of green stinks bug (*Nezara viridula* Linnaeus) which showed the high potential to inhibit the hatchability of egg up to 96% [8]. Moreover, the number of lettuce aphid (*Nasonovia ribisnigri* Mosley) nymph especially fourth instar was significantly affected by the *B. bassiana* in which the mortality increased with increasing the density of conidia [12]. In the two-spotted spider mites, *Tetranychus urticae* Koch, *B. bassiana* known to have a high virulence, but it was shown an opposite trend on the predatory mites in which no pathogenicity of the *B. bassiana* [7]. The laboratory results may be cannot accurately reflect the ecological aspect in the field. Hence, field trials are necessary to confirm the laboratory findings to field conditions, especially for *M. testulalis*, the most damaged of mungbean pest.

In term of integrated pest management (IPM) programs, evaluating of the compatibility of applying *B. bassiana* and natural enemies to control pest of mungbean become a critical issue. In order to improve the utilization of entomopathogenic fungi against insect pest, all factors that contribute to the side effects of entomopathogenic fungi on natural enemies need to be understood [13]. Based on this point of view, the objectives of this study were to evaluate the efficacy of the entomopathogenic fungi *B. bassiana* to control mungbean pest in terms of the level of insect population suppression and seed weight.

2. Materials and Methods

The experiment was conducted in Ngale Experimental Station, Ngawi, East Java, Indonesia, from February to May 2017. The experiment was arranged using randomized block design with seven treatments and four replicates. The treatments were frequency of application of *B. bassiana*; P1= six times, P2= five times, P3= four times, P4= three times, P5= once, P6= full protection using chemical insecticide, and P7= no protection.

2.1 Experimental site

Mungbean Vima-1 was planted on a plot of 4 m x 5 m with the spacing of 40 cm x 15 cm, 2 seeds/hole. Urea 37.5 kg / ha; SP36 73.5 kg / ha; KCl 37.5 kg/ha, and manure 4 kg /ha were applied before planting. Weeding was done manually on 14 and 28 days after planting (DAP). Chemical fungicide was applied to avoid leaf disease and soil borne fungus.

2.2 Entomopathogenic fungi

Isolates of *B. bassiana* was originally collected from Laboratory of Biopesticide of ILETRI then was cultured on PDA. At 21 days after inoculation (DAI), the conidium was taken by using fine brush dipped into the water then was counted by using *haemocytometer* to obtain conidia with $10^7$/ml density. The suspension of conidia was added 2 ml/1000 ml of Tween 80 then be shaken for 30 minute before applied into the plant. *B. Bassiana* suspension was applied on the entire surface of plant especially on pod section with the spray volume of 400-600 l/ha.

2.3 Sampling

The observation was made on 10 sample/treatment randomly on pest species and population at 39 and 60 DAP, species and population of natural enemies at 39 dan 60 (DAP), pod damage intensity, the number of pod/plant, the number of empty pod/plant, and seed weight. The diversity of species and population of pest and natural enemies was collected by using sweep net. This kind of trap was used to catch all active insects around the plant. This method was done as many as five times in a single swing. The observation on 39 DAP was aimed to inventory the arthropod on vegetative phase and
observation on 60 DAP was aimed to inventory the arthropod on reproductive phase. All arthropod obtained through the sweep net were inserted on the bottle and be brought into the laboratory to be identified based on morphological character then grouped on each family [14], [15].

2.4 Statistical analysis

The analysis was done with one-way ANOVA (SPSS version 22). Means associated with the treatment for each variable was separated using the Tukey’s HSD test when significant values were obtained.

3. Result and Discussion

3.1 Pest species and population

Analysis of variance showed that frequency of application of \textit{B. bassiana} did not significantly influence the population of foliage sucker in case of \textit{Empoasca} sp. The number of \textit{Empoascoa} sp. found on mungbean field ranged from 1-12 individuals/plot. The population of \textit{Empoasca} sp. was not significantly different between the applications of \textit{B. bassiana} and chemical insecticide. However, the population of \textit{Empoasca} sp. led to significantly higher (12.35 individuals/plant) on plot with no application of \textit{B. bassiana} neither chemical insecticide than that on other treatments (Figure 1).

![Figure 1](https://example.com/figure1.png)

\textbf{Figure 1}. Efficacy of biopesticide \textit{B. bassiana} for the management of foliage sucker, \textit{Empoasca} sp. on mungbean.
Figure 2. Efficacy of biopesticide *B. bassiana* for the management of pod borer, *M. testulalis* on mungbean.

Figure 2 showed the pod damaged intensity caused by pod borer, *M. testulalis* that was significantly different between each treatment. The highest intensity of pod damaged was found on plot without any application of chemical insecticide or biopesticide, which is 39.7%. An application of chemical insecticide showed the lower intensity of pod damaged (10.5%), did not significantly different as compare with an application of *B. bassiana* six times (10.8%) and five times (10.9%).

In case of pod sucking bugs, *R. linearis*, an application of chemical insecticide led to significantly suppress the population of this major pest as compare with an application of *B. bassiana* (Figure 3). The average number of *R. linearis* found on plot with application of chemical insecticide was 1 individual. However, the population found on plot with no treatment was high (8 individuals/plot). An application of *B. bassiana* with different frequency of application did not significantly different in the population of *R. linearis* which ranged from 2-3 individuals/plot.

Figure 3. Efficacy of biopesticide *B. bassiana* for the management of brown stink bugs, *R. linearis* on mungbean.

3.2 Yield component

The number of empty pod was observed as an effect of pod sucking bugs attack on mungbean that was significantly different between each treatment (Figure 4). The result showed that an application of *B. bassiana* six times reduced the number of empty pod and it seems no different with an application of
chemical insecticide that was only 1 pod/plot. The highest number of empty pod was found on plot with no application of chemical insecticide and biopesticide (2.3 pod/plot).

![Number of empty pod caused by R. linearis on mungben field applied by B. bassiana](image)

**Figure 4.** Number of empty pod caused by *R. linearis* on mungben field applied by *B. bassiana*

Analysis of variance showed that seed weight was significantly affected by the treatments, ranged from 338.3 – 659.7 g (Figure 5). The highest seed weight was found on the treatment of six times application of *B. bassiana* (659.7 g), followed by the treatment of five times application of *B. bassiana*. Application of *B. bassiana* four times during the plant growth showed no significantly different in seed weight as compare with three times application and once. A lower seed weight was found on plot with no application of *B. bassiana* neither chemical insecticide (338.3 g) and showed no significantly different with plot in which chemical insecticide applied (374.0 g).

![Seed weight of mungbean on each treatment](image)

**Figure 5.** Seed weight of mungbean on each treatment.
3.3 Effect of biopesticide B. bassiana on the survival of predator

The highest population of predator was found on P7 in which no both chemical insecticide and biopesticide applied during the growth season. A total of 17 individuals were found on P7, consists of Coccinella sp. Oxyopes sp. and Paederus sp. However, the application of chemical insecticide resulted in low population and diversity of predator in which only 1 individual of Paederus sp. founded (Table 1).

| Treatment | Predator population (individuals/plot) |
|-----------|---------------------------------------|
|           | Coccinella sp. | Oxyopes sp. | Paederus sp. |
| P1 (6x application of B. bassiana) | 2,50 | 1,50 | 1,50 |
| P2 (5x application of B. bassiana) | 3,25 | 1,50 | 0,00 |
| P3 (4x application of B. bassiana) | 2,75 | 0,00 | 4,25 |
| P4 (3x application of B. bassiana) | 0,00 | 2,25 | 3,25 |
| P5 (1x application of B. bassiana) | 2,50 | 1,25 | 5,50 |
| P6 (6x application of chemical insecticide) | 0,00 | 0,00 | 1,25 |
| P7 (no protection) | 5,50 | 3,50 | 7,25 |

Predators found on mungbean field are those who inhabit plant canopy and soil surface. Canopy-dwelling predator was those from the order of Coleoptera, Coccinella sp. and Paederus sp.. Moreover, the species from order Araneae, Oxyopes sp. was also found on mungbean field. These predators usually prey on pest who inhabit plant canopy such as Aphid sp., B. tabaci, Empoasca sp., and pest from the order of Hemiptera.

4. Discussion

Entomopathogenic fungi as microbial pesticide play an important role in sustainable food crop production by providing successful pest management. This present study indicated that B. bassiana significantly reduce the population of insect pests and the damaged level of mungbean pod. Moreover, The use of B. bassiana is safe to the predators and effectively increased the yield as compared with the application of chemical insecticide and without any treatments.

In case of insect pest existence, the population of Empoasca sp. found on mungbean was less and it shows no significantly different between the treatments. The possible reason is that the conidia may not be able to penetrate the cuticle of Empoasca sp. even though in abundance colony. This phenomenon occurred maybe due to the presence of protein on their integument that plays as a barrier for conidia penetration [16]. Another reason is that Empoasca sp. did not develop their resistance against chemical insecticide yet thus the application of chemical can suppress their population in field.

The major destructive pest on mungbean namely Maruca sp., feed on flowers, buds, and pods by webbing them [17]. Tight webbing produced by larva of Maruca sp. can protect them selves from pesticide exposure. The application of B. bassiana six and five times during the growth stage was more effective than once to four times application, but it shows same effective with the application of chemical insecticide. Based on the number of adult R. linearis, the application of chemical insecticide was more effective than the application of B. bassiana from once to six times. However, six and five times application of B. bassiana are better than other frequencies of application. The abundance colony of B. bassiana can be produced when it applied frequently.
According to [18], the distribution of entomopathogenic fungi occurs horizontally in which the more conidia produced makes the greater distribution of the pathogens and the epizootion on insect body will occur faster than that on less population. Even though the intensity of pod damage was less, the control of *Maruca* sp. by using chemical insecticide is less favorable as compared with the control by using biopesticide. The application of chemical insecticide support an evolution of resistance against chemical and require high cost for traditional farmers in developing country [19], [20]. According to [21], *B. bassiana* endophyte-inoculated common bean plants effective to suppress the population of leaf mining fly larvae, *Liriomyza huidobrensis*, resulting in less pupation and reducing the adult emergence from pupae developing on inoculated plants.

In general, the population of insect pest observed in this study showed no significant different between those applied by *B. bassiana* and chemical insecticide. This may be due to the time of application was not favorable for *B. bassiana* conidia to develop. We applied *B. bassiana* by foliar spray in the morning when the sunlight almost full. It may influence the persistence of *B. bassiana* on the plant because conidia are not protected from UV radiation. The application of entomopathogenic fungi in the afternoon has many advantages such as low-insolation, low-moderate temperature, favorable moisture conditions in which can enhances the activity of *B. bassiana* conidia [22].

The field efficacy of *B. bassiana* toward various insect pests depends on several factors, some of them are related to the behavior of the insect host in its natural habitat [23]. Soil is known to be a natural habitat of *B. bassiana* and the persistence of this fungal on soil is high [24]. Most of the arthropod dwells in the soil, especially larvae and pupae of lepidopteran and coleopteran. The application of entomopathogenic fungi by foliar spray caused an infection on their body. Although the adults of lepidopteran or other arthropod such as *R. linearis* inhabit plant canopy and feed on plant foliage, they can be seen crawling on the soil surface where the fungal already colonize. It makes them to be possibly contaminated by the fungal conidia [9]. Furthermore, foliar applications of *B. bassiana* against insect pest consistently produce moderate to high level of inoculation and infection, but the unfavorable environmental conditions in the plant canopy may inhibit the development of the conidia thus they cannot penetrate insect body [25].

The application of *B. bassiana* tended to be safe to non-target organism such as predator. This finding is in line with the previous study reported by [26] that the entomopathogenic fungi did not affect the survival rate of *Oxyopes javanus* under laboratory condition. However, some researchers reported that predatory mites namely *Neoseiulus californicus*, *Phytoseiulus persimilis*, and *Amblyseius swirskii* could be infected by *B. bassiana* when exposed to dry residue under laboratory condition [27], [28]. Although *B. bassiana* cause infection on these predatory mites, but the mortality was low (<30%) and showed no different to the control treatment over a 12-day period [28].

Based on the seed weight, the application of *B. bassiana* led to significantly high as compare with other treatment. Although the population of some insect pest showed similar response between application of *B. bassiana* and application of chemical insecticide, the seed weight on the plot with six times application of *B. bassiana* was high. Presumably, insect pest founded on mungbean plot with six times application of *B. bassiana* did not attack the leaf as well as pod but only colonize the plant without any other act.

5. Conclusion
In conclusion, the findings of these field trials are important, especially for mungbean’s farmers. *B. bassiana* showed the potential as an alternative to the currently control of insect pest. *B. bassiana* provide safer and more acceptable alternatives than conventional control method due to low-risk pesticide and low cost. Application of *B. bassiana* tended to be secure to natural enemies, especially *Coccinella* sp., *O. javanus*, and *Paederus fuscipes*.

References
[1] Chadha M L 2010 Short duration mungbean: a new success in south Asia. Asian Vegetable
Research and Development Center. Asia-Pacific Association of Agricultural Research Institutions. Bangkok, Thailand pp 1-4

[2] Yaqub M, Mahmood T, Akhtar M, Iqbal M M and Ali S 2010 Induction of mungbean \textit{(Vigna radiata} (L.) Wilczek\textit{)} as a grain legume in the annual rice-wheat double cropping system \textit{Pakistan Journal of Botany} 42 3125–3135

[3] Ratnasekhera D and Subhashi A P T 2015 Morpho-physiological response of selected mungbean (\textit{Vigna radiata} L.) Sri Lanka genotypes to drought stress \textit{Journal of Agriculture Search} 2(3) 162-166

[4] Hossain M A, Zaman M S and Alam M J 2011 Management of flower thrips and pod borers in mungbean, \textit{Vigna radiata} L. \textit{Bangladesh Journal of Life Science} 23(2) 79-86

[5] Indiati S W 2007 The control of pod borer on mungbean \textit{Journal of Agriculture Research and Information} 11(2) 138-147

[6] Siegwart M, Graillot B, Lopez C B, Besse S, Bardin M, Nicot P C and Lopez-Ferber M 2015 Resistance to bio-insecticides or how to enhance their sustainability: a review. Frontier in Plant Science 6 381 doi: 10.3389/fpls.2015.00381

[7] Wu S, Gao Y, Zhang Y, Wang E, Xu X and Lei Z 2014 An Entomopathogenic Strain of \textit{Beauveria bassiana} against \textit{Frankliniella occidentalis} with no Detrimental Effect on the Predatory Mite \textit{Neoseiulus barkeri}. Evidence from Laboratory Bioassay and Scanning Electron Microscopic Observation \textit{PLoS ONE} 9 (1): e84732. doi:10.1371/journal.pone.0084732

[8] Prayogo Y 2013 Pathogenicity of \textit{Beauveria bassiana} Bals. Vuill. (Deuteromycotina: Hyphomycetes) on various stages of eggs and nymphs of the green stink bug (\textit{Nezara viridula} L.).\textit{Journal of Tropical Pest and Disease} 13 (1) 75-86

[9] Reddy G V P, Zhihua Z and Richard A H 2014 Laboratory and field efficacy of entomopathogenic fungi for the management of the sweetpotato weevil, \textit{Cylas formicarius} (Coleoptera: Brentidae) \textit{Journal of Invertebrate Pathology} 122 10–15 doi: http://dx.doi.org/10.1016/j.jip.2014.07.009

[10] Lopes R B, Laumann R A, Blassioli-Moraes M C, Borges M and Faria M 2015 The fungistatic and fungicidal effects of volatiles from metathoracic glands of soybean-attacking stink bugs (Heteroptera: Pentatomidae) on the entomopathogen \textit{Beauveria bassiana} \textit{Journal of Invertebrate Pathology} 132 77–85

[11] Wakil W and Schmitt T 2015 Field trials on the efficacy of \textit{Beauveria bassiana}, diatomaceous earth and Imidacloprid for the protection of wheat grains from four major stored grain insect pests \textit{Journal of Stored Products Research} 64 160-167

[12] Shrestha G, Enkegaard E and Steenberg T 2015 Laboratory and semi-field evaluation of \textit{Beauveria bassiana} (Ascomycota: Hypocreales) against the lettuce aphid, \textit{Nasonovia ribisnigri} (Hemiptera: Aphididae) \textit{Biological Control} 85 37–45

[13] Alavo T B C, Sermann H and Bochow H 2002 Biocontrol of aphids using \textit{Verticillium lecanii} in greenhouse: Factors reducing the effectiveness of the entomopathogenic fungus \textit{Archives of Phytopathology and Plant Protection} 34 407-424

[14] Norton G, Taylor M, Thiele K and Pickering J 2000 Identification guide to insect orders. The University of Queensland

[15] Schell S and Latchininsky A 2007 Insect identification. University of Wyoming

[16] Wu S, Xie H, Li M, Xu X and Lei Z 2016 Highly virulent \textit{Beauveria bassiana} strains against the two-spotted spider mite, \textit{Tetranychus urticae}, show no pathogenicity against five phytoseiid mite species \textit{Experimental Applied Acarology} 70 421–435 DOI 10.1007/s10493-016-0090-x

[17] Grigolli J F J, Laurencila A L F and Avia C J 2015 Field efficacy of chemical pesticides against \textit{Maruca vitrata} Fabricius (Lepidoptera: Crambidae) infesting soybean in Brazil \textit{AJPS} 6(7) 537-544

[18] Kreutz J, Zimmermann G and Vaupel O 2004 Horizontal transmission of the
entomopathogenic fungus *Beauveria bassiana* among the spruce bark beetle *Ips typographus* (Coleoptera: Scolytidae) in the laboratory and under field conditions *Biocontrol Science and Technology* **14** 837–848 doi: http://dx.doi.org/10.1088/1074-3583/14/4/003

[19] Kym A 2006 Transgenic Crops, EU Precaution, and Developing Countries. *International Journal of Technology and Globalization* **2** 65-80

[20] Oparaek and Mbonu A 2006 The Potential for Controlling *Maruca vitrata* Fab. and *Clavigralla tomentosicollis* Stal. Using Different Concentrations and Spraying Schedules of *Syzigium aromaticum* (L.) Merr and Perr on Cowpea Plants *Journal of Plant Sciences* **1** 132-137 http://dx.doi.org/10.3923/jps.2006.132.137

[21] Akutse K S, Maniania N K, Fiaboe K K M, van den Berg J and Ekesi S 2013 Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae) *Fungal Ecology* **6** 293–301

[22] Faria M, Hotchkiss J H, Hajek A E and Wraight S P 2010 Debilitation in conidia of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* and implications with respect to viability determinations and mycopesticide quality assessments *Journal of Invertebrate and Pathology* **105** 74–83

[23] Gindin G, Levski S, Glazer I and Soroker V 2006 Evaluation of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* against the red palm weevil *Rhynchophorus ferrugineus* *Phytoparasitica* **34** 370–379

[24] Ansari M A, Brownbridge M, Shah F A, Butt T M 2008 Efficacy of entomopathogenic fungi against soil-dwelling life stages of western flower thrips, *Frankliniella occidentalis*, in plant-growing media *Entomologia Experimentalis et Applicata* **127** 80–87 doi: http://dx.doi.org/10.1111/j.1570-7458.2008.00674.x

[25] Wraight S P and Ramos M E 2015 Delayed efficacy of *Beauveria bassiana* foliar spray applications against Colorado potato beetle: Impacts of number and timing of applications on larval and next-generation adult populations *Biological Control* **83** 51-67

[26] Bayu M S Y I and Prayogo Y 2013 The influence of the application of entomopathogenic fungi *Lecanicillium lecanii* on the survival of *Oxyopes javanus* Proceeding of National Conference of Research on Legumes and Tuber Crops pp 673-679

[27] Numa Vergel S J, Bustos R A, Rodriguez C D and Cantor R F 2011 Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae* *Biological Control* **57** 143–149

[28] Midthassels, A, Leather S R, Wright D J and Baxter I H 2016 Compatibility of *Amblyseius swirskii* with *Beauveria bassiana*: two potentially complimentary biocontrol agents *Biological Control* **61** 437–447 DOI 10.1007/s10526-016-9718-3