International Journal of Agricultural Economics
2023; 8(6): 299-304
http://www.sciencepublishinggroup.com/j/ijae
doi: 10.11648/j.ijae.20230806.20
ISSN: 2575-3851 (Print); ISSN: 2575-3843 (Online)

Controlling Soybean Insect Pests and Yield Losses with Entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae*

Kito Phaviseth[1], Siviengkhek Phommalath[1, *], Thiphakone Thavonesack[2]

[1]Ministry of Agriculture and Forestry, National Agriculture and Forestry Research Institute, Vientiane Capital, Lao PDR
[2]Ministry of Education and Sports, Souphanouvong University, Laung Prabang, Lao PDR

Email address:
siviengkhek@yahoo.com (Siviengkhek Phommalath)
[*]Corresponding author

To cite this article:
Kito Phaviseth, Siviengkhek Phommalath, Thiphakone Thavonesack. (2023). Controlling Soybean Insect Pests and Yield Losses with Entomopathogenic Fungi *Beauveria bassiana* and Metarhizium anisopliae. *International Journal of Agricultural Economics*, *8*(6), 299-304. https://doi.org/10.11648/j.ijae.20230806.20

Received: November 29, 2023; **Accepted**: December 18, 2023; **Published**: December 28, 2023

Abstract: The objective of this study identifies the effective of *Beauveria bassiana* and *Metarhizium anisopliae* fungi as soybean pest control agents compared chemical in The objective of this study was to compare the effectiveness of Beauveria bassiana and Metarhizium anisopliae fungi as soybean pest control agents with chemical insecticides secticides. The experimental designed was randomized complete block design with 4 treatments: B1: Control, B2: Beauveria & Metarhizium fungi (50 g/L) weekly, B3: Beauveria & Metarhizium fungi (100 g/L) weekly and B4: Triazophos + Abamectin chemicals. The results showed the efficacy of Beauveria and Metarhizium fungi as biocontrol agents for soybean insect pests. Statistical analysis of plant height, pod number, seed weight, and pod damage revealed significant differences among treatments. Notably, Beauveria + Metarhizium at 50 g/ha significantly increased seed weight per plant ($P < 0.05$), while the 100 g/ha treatment maximized plant harvest numbers ($P < 0.05$). However, both fungal treatments exhibited no significant impact on pest population compared to the chemical control. This may be attributed to the spray application timing, coinciding with high morning sunlight, potentially affecting fungal conidia viability. Despite inconclusive pest control, the fungi-treated groups demonstrated enhanced plant growth and yield parameters, suggesting their potential as sustainable and environmentally friendly pest management alternatives. Further research is warranted to optimize application strategies and maximize their efficacy against soybean insect pests.

Keywords: Soybean, *Beauveria bassiana*, *Metarhizium anisopliae*, Biocontrol

1. Introduction

Soybean [Soybean, Glycin Max (L.) Merrill] is an important crop and one of the oldest food crops in the world. It is a plant with high nutritional value. There are many nutrients that are beneficial to health and help prevent various diseases, and can also be used in many ways [1]. Both increase income for farmers. Soybeans are becoming more important in terms of consumption such as soy milk, tofu, etc., and processing food products into raw materials for industries such as the animal feed industry, vegetable oil industry. Therefore, soybean plays an important role in the family economy and the national economy. Soybeans are now widely grown in tropical and temperate regions. They can be planted three times a year, such as before the rainy season, near the end of the rainy season, and during the dry season [2]. Soybean production by people in the Lao People's Democratic Republic (Lao PDR) currently depends on natural conditions as the basis for soybean production. This causes the yield to be uncertain in some years with high yields and in some years the yield is low. To statistical data, the soybean planting area in the country in 2020 was 1,844 hectares, 4,623 tons [3].

The issue of pest damage to soybean in Southeast Asia is a serious problem that affects the productivity and profitability of the crop. Soybean is an important source of protein, oil, and feed for humans and animals in the region. However, soybean faces many challenges from various pests, such as insects, diseases, nematodes, and weeds. These pests can reduce the quality and yield of soybean by feeding on the plant tissues, transmitting pathogens, competing for resources, and causing physiological disorders [4-7].

Some of the major insect pests that of soybean in Southeast Asia are the stem fly (Melanagromyza sojae), the tobacco caterpillar (Spodoptera litura), the Bihar hairy caterpillar (Spilosoma obliqua), the green semilooper (Chrysodeixis eriosoma), the pod borer (Helicoverpa armigera), the leaf miner (Liriomyza trifolii), the whitefly (Bemisia tabaci), the aphids (Aphis glycines and Aphis craccivora), the stinkbug (Nezara viridula), and the girdle beetle (Obereopsis brevis) [5]. These insects can cause significant damage to the soybean plant at different stages of its growth, from seedling to maturity. They can also vector viral diseases, such as the soybean mosaic virus and the yellow mosaic virus, which can further reduce the plant vigor and yield [8].

Beauveria and Metarhizium, two genera of entomopathogenic fungi, capable of infecting and killing insect pests, including those harming soybean crops. These fungi serves as biopesticides or natural enemies in the environment. They produce various toxins that help them to overcome the host's immune system and cause mortality. The effectiveness of Beauveria and Metarhizium on pest control in soybean production depends on several factors, such as the fungal strain, the pest species, the environmental conditions, the application method, and the interaction with other microbes and plants. Some studies have reported positive results of using these fungi to control soybean pests, such as the bean leaf beetle, the soybean aphid, the velvetbean caterpillar, and the soybean looper [9-12]. However, there are also challenges and limitations, such as the low persistence of the fungi in the field, the variability of their virulence, the competition with other microorganisms, and the possible negative effects on non-target organisms. Therefore, more research is needed to optimize the use of these fungi and to integrate them with other pest management strategies.

Integrated Pest-Management-IPM is associated with a change in the concept of pest control that occurred in the 1960s when the world was alerted to the dangers of inappropriate use of chemicals. The overuse of insecticides has led to many government policies to adopt integrated pest management, which considers multiple strategies to optimize the control of all types of pests [13]. From the results of the study of insect pests and non-insect pest in the soybean field using the IPM integrated pest management factor, it can be seen that there are 40 species of 6 Order insects found in the field and destroying soybean trees [14]. This study evaluated the effectiveness of using entomopathogenic fungi Beauveria and Metarhizium as biocontrol agents to manage, control, and prevent soybean pests. The aim was to reduce the yield losses and damage caused by pests, while considering the local conditions, family economy, and environmental impact. The study also seeked to enhance the quantity and quality of soybean production and encourage farmers to adopt soybean cultivation as an alternative crop.

2. Materials and Methods

This experiment was conducted in the area of the Rice and cash Crops Research Center, Vientiane Capital Lao PDR. Starting from July - September 2023. The study design was done using RCBD (Randomized Complete Block Design) with 4 treatments (B1 = Control (no use), B2 = Beauveria fungus + Metarhizium fungus at the rate of 50g/water 20 L sprayed weekly/time, B3 = Beauveria fungus + Metarhizium fungus Rate of 100g/water 20 L spray weekly/time, B4 = use chemical Triazophos rate 30 cc/water 20 L and chemical Abamectin rate 30 cc/water 20 L) and 3 replications. Soybean variety was Naphok 1.

Levels of damage to soybean plants at different ages was collected: (1) number of insect pests per plot: The number of insect pests found in each plot was observed every 7 days throughout the soybean life cycle, from germination to harvest. And (2) damage level: 5 plants/plot were observed and scored the severity or damage level of pests. This experiment followed the method of the research paper by [15, 16], which used quantitative trait loci (QTL) pyramids to improve the resistance of soybean plants to leaf chewing insects, such as bean leaf beetles and soybean looper. The experiment also used soybean defoliation charts to assess the extent of leaf damage caused by the insects.

The experiment data were analyzed using the Statistic 10 program. The analysis of variance (ANOVA) was performed to test the effects of different treatments on the following variables: plant height, number of pods per plant, seed weight per plant, 100 seed weight, number of pod damage, number of plant harvesting, weight per plot, and yield kg/ha. The least significant difference (LSD) test was used to compare the mean values of the variables between the blocks and the treatments at the 95% confidence level ($P<0.05$).

3. Results

The experiment data were analyzed using the Statistic 10 program. The analysis of variance (ANOVA) was performed to test the effects of different treatments (B1, B2, B3, and B4) on the following variables: plant height, number of pods per plant, seed weight per plant, 100 seed weight, number of pod damage, number of plant harvesting, weight per plot, and yield kg/ha. The least significant difference (LSD) test was used to compare the mean values of the variables between the treatments at the 95% confidence level ($P<0.05$) (Table 1). The table shows that there was no significant difference in plant height and 100 seed weight among the treatments. However, there was a significant difference in the other six variables. The treatment B4 had the highest mean values for number of pods per plant, weight per plot, and yield kg/ha, indicating that it was the most effective treatment for increasing soybean production. The treatment B1 had the lowest mean values for these variables, as well as for seed weight per plant and number of plant harvesting, suggesting that it was the least effective treatment. The treatment B4 also had the lowest mean value for number of pod damage, indicating that it was the most resistant to insect pests. The treatment B1 had the highest mean value for this variable, implying that it was the most susceptible to insect damage. The treatment B2 had the highest mean value for seed weight per plant, which could be a desirable trait for some markets. The treatment B3 had the highest mean value for 100 seed weight, which could also be a favorable characteristic for some consumers.

Levels of damage to soybean plants at each age of soybean: This text summarizes the data on insect damage and infestation in soybean plots treated with different methods, following the experimental design of the research paper by Ortega et al [16]. The paper used quantitative trait loci (QTL) pyramids and Bacillus thuringiensis (Bt) genes to enhance the resistance of soybean plants to leaf-chewing insects. The paper also used soybean defoliation charts to assess the extent of leaf damage caused by the insects. The data were collected at three different stages of soybean growth: 11 days, 51 days, and 107 days and the result had shown that there was a significant difference in both insect damage and number of insects among the treatments at each stage. The control plot had the highest mean values for both variables, indicating that it was the most affected by insect pests. The chemical plot had the lowest mean values for both variables, indicating that it was the most effective in reducing insect damage and infestation. The fungal plots had intermediate mean values for both variables, suggesting that they had some effect in controlling insect pests, but not as much as the chemical plot. The fungal plots also showed some variation in their effectiveness, depending on the dosage and the stage of soybean growth. The fungal plot with 50 g dosage (B2) had lower mean values for both variables than the fungal plot with 100 g dosage at 51 days and 107 days, but not at 11 days. This could imply that the lower dosage of fungi was more suitable for later stages of soybean growth, while the higher dosage of fungi was more suitable for earlier stages of soybean growth (Figures 1-3).

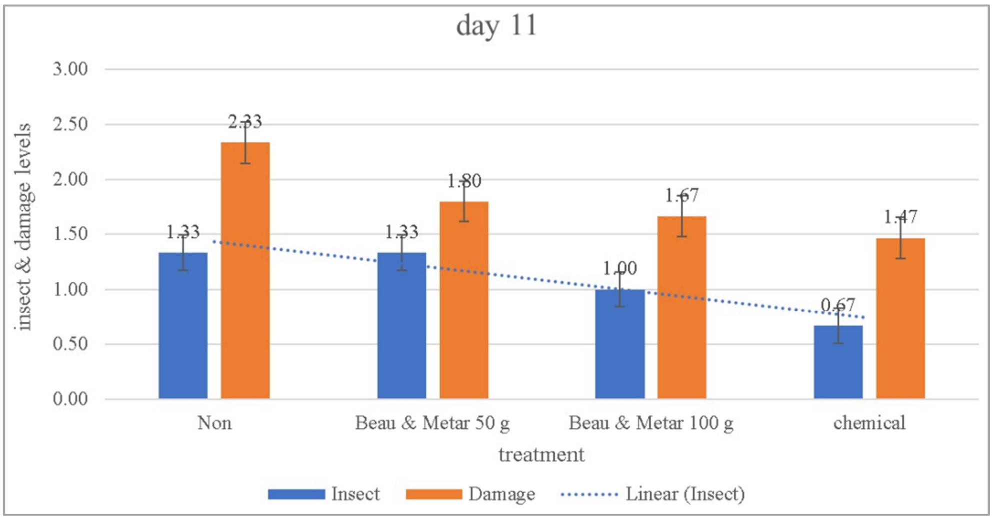

Figure 1. *Graph showing the number of insects and damage levels at 11 days of age.*

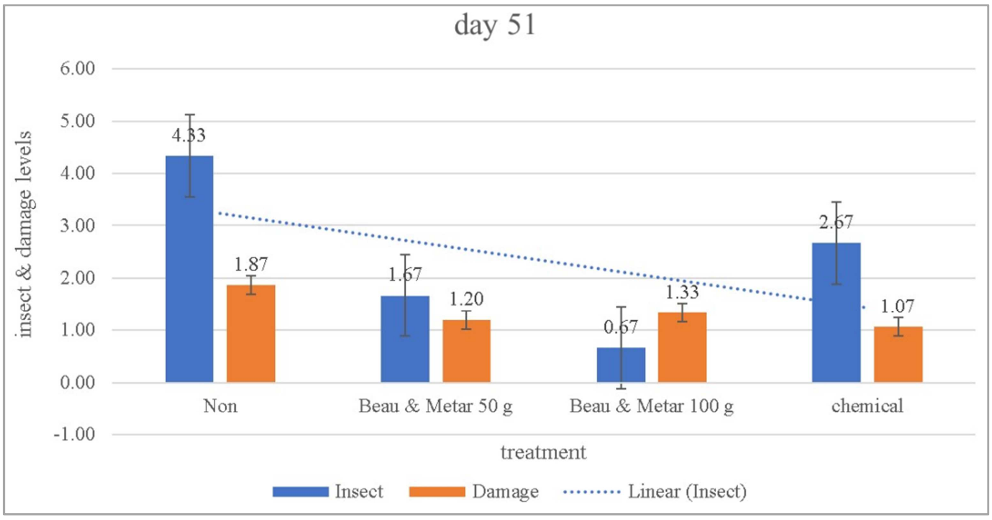

Figure 2. *Graph showing the number of insects and damage levels at 51 days of age.*

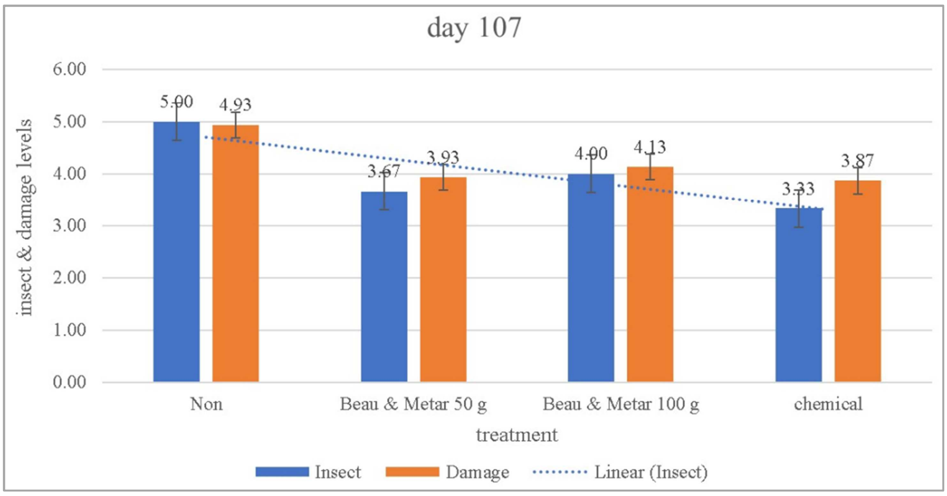

Figure 3. *Graph showing the number of insects and damage levels at 107 days of age.*

Table 1. *Plant height, Number of pods per plant, Seed weight per plant, 100 seed weight, Number of pod damage, Number of plant harvesting, Weight per plot, Yield kg/ha.*

treatment	Plant height (Cm)	Number of pod /plant	Seed weight/ plant (g)	100 seed weight (g)	Number of pod damage	Number of plant harvesting	Weight / plot (Kg)	Yield kg/ha
Control	91.7 A	101.9 C	15.0 B	12.3 A	194.0 A	159.3 A	1.3 B	1.918 B
Beauveria + Metarhizium rate of 50g/water 20L	85.0 A	139.7 B	19.5 A	12.4 A	165.3 B	144.6 AB	1.6 A	2.252 A
Beauveria + Metarhizium rate of 100g/water 20 L	85.8 A	136.0 B	17.1 AB	12.8 A	148.6 B	164.6 A	1.6 A	2.263 A
chemical Triazophos Abamectin rate 30 cc/water 20L	84.8 A	150.0 A	15.8 B	12.2 A	114.6 C	126.3 B	1.6 A	2.310 A
P-Value	Ns	**	*	Ns	**	*	**	**
CV%	6.31	3.22	7.73	10.90	9.19	7.45	3.82	3.60

Ns = no statistical difference (not significant).
** = there is a statistical difference at the confidence level greater than 99% (P<0.01).
* = there is a statistical difference at the confidence level greater than 95% (P<0.05).
abc = in the same row with the same letters there is no statistical difference.

4. Discussion

One of the main insect pests that affected soybean production in this experiment was the redbanded stink bug that causes economic damage to legumes by feeding on the fruiting structures, resulting in reduced photosynthesis, fruit and seed abortion, delayed maturity, and the flat pod disorders [6-8, 14]. The present findings suggest that the chemical control was the most effective method in reducing the damage and infestation caused by the redbanded stink bugs, as it had the lowest mean values for both variables at all stages of soybean growth. The chemical control also had the highest mean values for yield components, indicating that it had the least negative impact on soybean production.

The fungal biocontrol agents: Beauveria bassiana and Metarhizium anisopliae were less effective than the chemical control in controlling the redbanded stink bugs, but they still had some positive effects on the soybean production. Fite et al. [17] reported that B. bassiana (APPRC-9604) at 109 conidia/mL was more virulent under laboratory and field conditions by reducing larval infestations, decreasing pod damage and subsequently increasing the production yield of soybean during both cropping seasons. However, in this experiment, the fungal biocontrol agents were applied at lower dosages (50 g and 100 g per 20 L of water) and at different times (in the morning) than in the study by Fite et al [17]. These factors may have influenced the persistence and activity of the fungal conidia on the soybean plants and the insect pests. Faria et al [18] stated that the application of entomopathogenic fungi in the afternoon has many advantages such as low-insolation, low-moderate temperature, favorable moisture conditions that can enhance the activity of B. bassiana conidia. Garcia and Ignoffo [19] suggested that the wind speeds registered during the present field studies (6.5 km h-1, 3-9 on average) were sufficient to disperse conidia of M. anisopliae and B. bassiana in the soybean foliage. However, the effects of microclimatic variables (e.g., temperature, irradiance, water potentials) on populations of the entomopathogens on soybean foliage are not well understood. Conidia remaining on the boundary layer, defined as the transition zone above the leaf surface, are exposed to localized microclimate [17].

The fungal biocontrol agents showed some variation in their effectiveness, depending on the dosage and the stage of soybean growth. The fungal plot with 50 g dosage had lower mean values for the insect damage and the number of insects than the fungal plot with 100 g dosage at 51 days and 107 days, but not at 11 days. This could imply that the lower dosage of fungi was more suitable for later stages of the soybean growth, while the higher dosage of fungi was more suitable for earlier stages of the soybean growth. The fungal biocontrol agents also had higher mean values for the yield components than the control plot, indicating that they had some positive effects on soybean production, despite the higher insect damage and infestation. The fungal biocontrol agents may have other benefits, such as being environmentally friendly, enhancing the soil health, and reducing the risk of the insect resistance to chemicals [17].

5. Conclusions

In conclusion, the result showed that the chemical control was the most effective method in preventing and reducing insect damage and infestation, and increasing soybean production. The fungal biocontrol agents were less effective than the chemical control, but they still had some positive effects on soybean production. The fungal biocontrol agents also had some variation in their effectiveness, depending on the dosage and the stage of the soybean growth. The fungal biocontrol agents may have other benefits, such as being environmentally friendly, enhancing soil health, and reducing the risk of insect resistance to chemicals. The result also discussed the factors that may have influenced the effectiveness of the fungal biocontrol agents and the chemical control, such as the time of application, the wind speed, and the microclimatic variables.

ORCID

0000-0002-4504-5603 (Siviengkhek)

Acknowledgments

I would like to express my sincere gratitude and appreciation to KOPIA-Lao Center for their financial support and cooperation in this trial research. This research would not have been possible without their generous assistance and guidance. I also thank all the parties who contributed to the success of this research, such as the staff, the farmers, and the reviewers.

Conflicts of Interest

The authors declare no conflicts of interest.

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