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Effect of Nostalgist BL® on Biology of *Acanthoscelides obtectus'* (Say) (Coleoptera: Bruchidae)

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ABSTRACT

Acanthoscelides obtectus, which is a pest of stored products, completes its larval development in legumes. It is among economically important species in our country since it reduces the germination value and nutritional value of seeds. As a result of understanding the effects of entomopathogenic fungi on pests in recent years, researchers have also focused on fungi in fighting pests. These fungi, which are sold as bioinsecticides in the market, are generally preferred by producers who want to engage in organic or environmentally sensitive agriculture. Although there are a large number of sources in literature on the mortality rates of *Beauveria bassiana*'s *A. obtectus*, studies on the effects on the biology of the pest, the number of eggs it lays and the number of adults emerging from beans after the application are limited. In this study, the effects of different concentrations of *Beauveria bassiana* (Nostalgist BL®) on the mortality rates of *A. obtectus* adults, the number of eggs they lay and the number and percentages of adults emerging from bean seeds were evaluated with dipping and spraying method. It was found that as the concentration of *B. bassiana*, which was applied at concentrations of 0.1%, 0.2% and 0.4% increased, the mortality rates on the adults of the pest increased and the number of eggs they laid and the number of adults emerging from bean seeds decreased. It can be said that different doses of *B. bassiana* are quite effective on the mortality rates of *A. obtectus* adults, the number of eggs they lay, and especially the number of adults emerged from beans.

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Key words
***Acanthoscelides obtectus*, Biologic control, *Beauveria bassiana*, Number of egg, Insect physiology, Pest**

INTRODUCTION

Legumes are very similar to animal proteins in terms of their nutritional properties (Emekçi and Ferizli, 2000). They are kept in warehouses before they are delivered to buyers and in this process, they are very vulnerable to the threat of pests. *Acanthoscelides obtectus* Say, 1831 (Coleoptera: Bruchidae) is the most important pest which causes the highest loss to legumes (Emekçi *et al*., 2015). These pests lay their eggs on grains in fields and the contaminated grains are transported to post-harvest warehouses. Larvae feed and develop in seeds. When they mature, they emerge by making holes in seeds. With this damage on stored products, they cause many undesirable consequences such as decrease in germination power and weight of the seed, decrease in protein value and therefore decrease in market value (Rinku *et al*., 2018). One of the most used methods in fighting stored product pests is chemical methods. Insecticide use is preferred due to its ease of application, faster results and cheaper cost (Çolak *et al*., 2018). However, biological methods have come to the fore in recent years because pests develop resistance against insecticides and are highly harmful to humans and the environment (Khan et. al., 2018). Biological control is the method of taking predatory insects, parasitoids or fungi under control (Shaheen *et al*., 2016; Sugandi and Awaknavar, 2017). Especially bioinsecticides draw the attention of manufacturers since they do not leave environmental residues and are harmless to human and environmental health (Öztürk *et al*., 2015; Şahin and Karaca, 2019; Atanasova and Vasilev, 2020). Entomopathogenic fungi can infect pests through the skin, trachea, digestion, respiration or other openings (Keskin *et al*., 2019; Altınok and Altınok, 2020; Kidanu and Hagos, 2020). In recent years, biological control studies in the field of fighting pests with fungi have gained momentum (Castrillo *et al*., 2011; Khashaveh *et al*., 2011; Hirsch and Reineke, 2014). Studies conducted with *Beauveria bassiana* 1912 (Bals. Criv) Vuillemin (Hypocreales: Cordycipitaceae) show that this fungus is very effective on pests (Ferron and Robert, 1975; Balcı and Durmuşoğlu, 2020;

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Abbreviations
A.obtectus, Acanthoscelides obtectus; B. bassiana, Beauveria bassiana; B. tenella, Beauveria tenella; L. muscarium, Lecaniallium muscarium; I. farinose, Isaria farinosa; L. decemlineata, Leptinotorsa decemlineata; sec, Second; mm, Milimeter; mL, Milliliter; S.E., Standard Eror; D, Dipping method; S, Spraying method; CD, Control Group of Dipping method; CS, Control Group of Spraying method.

Gad *et al*., 2020; Laib *et al*., 2020; Özdemir *et al*., 2020). In a study they conducted, Padin *et al*. (2002) found that grain weight loss decreased in beans treated with *B. bassiana*. In a study they evaluated the effects of 12 native isolates on *Callosobruchus maculatus* Fabricius, 1775 (Coleoptera: Bruchidae), Cherry *et al*. (2005) found *B. bassiana* was effective. Mahdneshin *et al*. (2011) found that mortality rates increased as the dose applied to *C. maculatus* adults increased with dipping method, Bayrak *et al*. (2015) found that mortality rates increased in direct proportion to the dose increase on *A. obtectus* and that mortality rate increased up to 98.8% on day 14. In their study they conducted on *A. obtectus* adults with *Lecanicillium muscarium* (Hypocreales: Cordycipitaceae) and *Isaria farinosa* Holmsk. Fr. 1832 (Hypocreales : Cordycipitaceae) isolates, Altınok and Altınok (2020) found mortality rates for *L. muscarium* as 81.5% on day 9 and for *I. farinosa* isolate as 83.3% on day 11. The objective of biological control is to reduce or completely destroy the pest population with another organism, such as a parasitoid, predator insect or fungus. Although there are a large number of sources in literature on the mortality rates of *B. bassiana*'s *A. obtectus*, studies on the effects on the biology of the pest, the number of eggs it lays and the number of adults emerging from beans after the application are limited. It is also important to know the biology/physiology of the pest with biological control. In this study, the aim is to determine the number and percentage of adults emerging from the eggs laid by *A. obtectus* and from the beans after bioinsecticide application and after mortality rates are found. Knowing the physiology of the pest and how it will react to applied bioinsecticides in a biological control study to be carried out with bioinsecticides will contribute to the fight against stored products.

MATERIALS AND METHODS

This study deals with the effect of Nostalgist on a warehouse pest *Acanthoscelides obtectus* adults. Nostalgist BL® (Agrobest Group) is a microbial insecticide containing spore and micelle particles of entomopathogenic fungus *Beauveria bassiana.* Different concentrations viz., 0.1%, 0.2% and 0.4% of Nostalgist were prepared in sterile distilled water.

A. obtectus cultures, which have been available in our laboratory since 2015, were reared in 1 litre jars half filled with beans (500 g) sterilized by keeping them in a deep freezer (Profilo 6600) for 48 h to ensure they were not previously parasitized by any insect. The pest was exposed to different concentrations of Nostalgist by dipping and spraying methods.

In the dipping method, 10 *A. obtectus* adults (3 - 4 days old) from the main cultures were taken into Petri dishes (90 mm) after they were kept in 0.1%, 0.2% and 0.4% *B. bassiana* solutions for 5 sec and 10 sterilized dry beans were put so that they could ovulate.

In spraying trials, 10 adult insects were put in Petri dishes containing blotting paper moistened with sterile distilled water. Approximately 2 mL 0.1%, 0.2% and 0.4% *B. bassiana* solution was sprayed on 10 insects from a distance of 20 cm with a spraying bottle. 10 pieces of sterilized dry bean seeds were put in Petri dishes for egg laying.

For both methods experiments were done in 3 groups with 3 repetitions (3 × 3 × 10 = 90 insects). Total of 360 insects were used in each method. A total of 720 insects were used for both methods. The Petri dishes of both spraying group and dipping group were incubated at 27±2°C, 60 ± 5 relative humidity and continuous dark conditions. All Petri dishes were checked at the same hour every day and the dead insects were recorded.

The eggs laid by the adults on the Petri dishes and bean surface were counted under microscope (Leica EZ4). The beans taken in clean Petri dishes were incubated under the laboratory conditions until hatching of eggs. Petri dishes were checked every day for the number of adult insects. The number of adults divided by the number of eggs and the percentages of the number of adults emerged were determined.

SPSS 22.0 (Statistical Package for Social Sciences) was used in the analysis of data obtained. Abbott's formula was used in calculating insect mortality rates and the percentage of mortality rates was calculated according to the formula. Abbott's formula was introduced by Abbott, (1925) as given below:

$$P= [(C - T) / C] \times 100$$

where P is estimated % insect rate killed by entomopathogenic fungi; C is % of insects that survived in the control group after application and T is % of insects that survived after fungi application.

Normality test of the data was conducted with Shapiro-Wilk test and it was found that the groups were normally distributed ($p > 0.05$). Variance analysis (ANOVA) was used to compare the groups and TUKEY HSD multiple comparison test was used to find out the differences between means, after the applications, it was found from which day mortality rates began to differ statistically. Independent t-test between two groups was used for paired comparisons in the study. Level of significance was taken as 0.05 for all statistical comparisons in the study.

RESULTS

Table I shows the effect of different concentrations of *B. bassiana* on *A. obtectus*'s mortality rate, the number of eggs laid and the number of adult insects emerged from beans. It was found that in dipping method the maximum mortality was achieved on day 7 with 0.1% concentration (72.26%) and on day 5 with 0.2% and 0.4% concentrations (82.23%, 86.03%, respectively) (df= 6, F= 12.652; df= 7, F= 17.879, respectively). With spraying method, the highest mortality rates at 0.1% and 0.2% concentrations occurred on day 7 (50.8%, 65.37%, respectively) (df= 12, F= 34.611; df= 8, F= 23.7619, respectively) (Table II). With 0.4% concentration of spraying method, a statistical difference was found after day 5 and the highest mortality rate was found on day 9 (55.56%) (df= 8, F= 23.497). As can be seen in Table I, adults continued to live until day 9 at 0.1% concentration with dipping method and until day 13 at 0.1% concentration with spraying method (Table II). When compared with the control group, it was found that especially 0.1% concentration of spraying method did not differ much from the control group in terms of adult life. Although the insects in the control group survived until day 17 (not shown in table) this was limited to 13 days at 0.1% concentration and 9 days at 0.2% and 0.4% concentrations in the spraying group. When concentrations of 0.1%, 0.2% and 0.4% were evaluated daily, they were found to differ from the control group starting from day 2 in both methods (Tables I, II and Fig. 1). For example, when groups of 0.1%, 0.2% and 0.4% were compared in dipping and spraying methods, they were found to differ from the control group starting from the first day in the dipping group and starting from the second day in the spraying group (df= 3, F= 4.280).

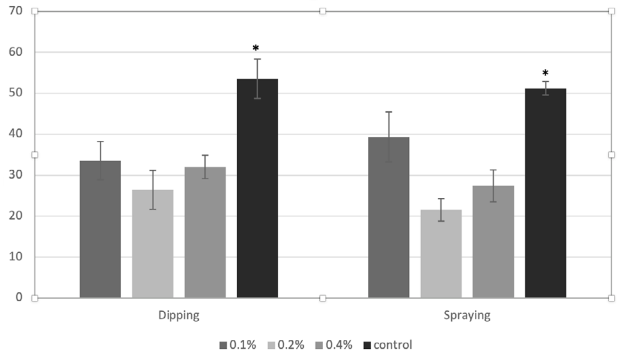

Fig. 1. The number of eggs adults left on Petri and bean surface after different concentrations of *Beauveria bassiana* were applied to *Acanthoscelides obtectus* adults with dipping and spraying method. There is no statistical difference in those with the same sign (*), p>0.05.

When the number of eggs laid was examined, it was found that the number of eggs laid decreased with increasing dose (F= 19.518, df= 3, p= 0.079). Although all other groups were found to be statistically different than the control group (F= 12.143, df= 3, p= 0.001), no significant difference was found between concentrations of 0.1%, 0.2% and 0.4% (F= 4.000, p= 0.094).

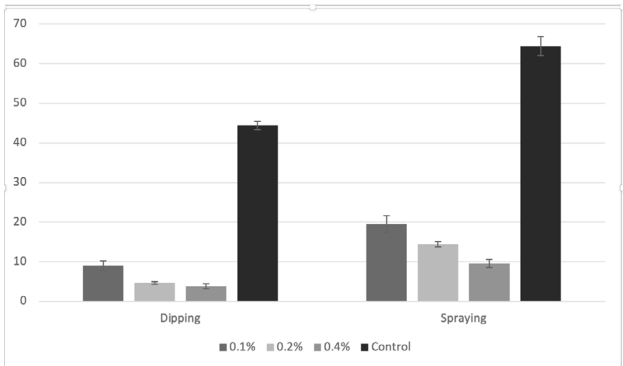

Fig. 2. The number of adults emerged from beans after different concentrations of *Beauveria bassiana* were applied to *Acanthoscelides obtectus* adults with dipping and spraying method.

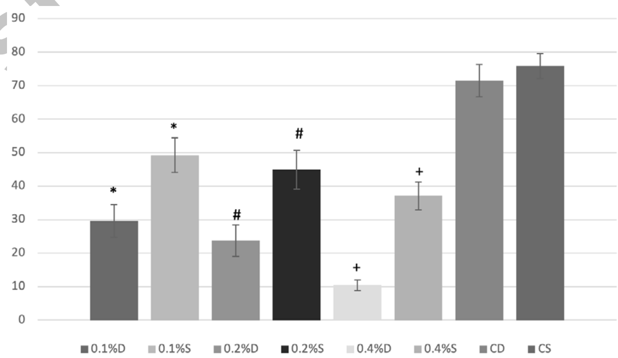

Fig. 3. The percentages of adults based on the number of eggs they lay, after different concentrations of *Beauveria bassiana* were applied to *Acanthoscelides obtectus* adults with dipping and spraying method. **There are statistical difference between same concentrations (same signs), p>0.05. D: Dipping method, S: Spraying method, CD: Control Group of Dipping method, CS: Control Group of Spraying method.

As can be seen in Figure 2, there is statistical difference between the dipping method and spraying method, especially in terms of the number of adults emerged. The number of adults emerged from bean seeds was found to decrease in dipping method depending on the dose increase and it was found to be significantly different from the control group (F= 121.626, df= 3, p < 0.001). Considering the percentages of the number of adults that emerged, it was observed that the percentage decreased with the dose

Table I. Mortality (Mean ± SEM) of *Acanthoscelides obtectus* adults against different concentrations of *Beauveria bassiana* (*B.b*) with dipping method (%).

Conc. of *B.b*	Days *#									
	1	2	3	4	5	6	7	8	9	p
0.1%	11.94± 5.55 Aa	2.22± 2.22 Aa	29.78± 5.91 Aa	24.93± 8.95 Aa	22.71± 10.16 Aa	40.60± 9.34 Aa	72.26± 10.32 Ba	48.91± 15.99 Ba	55.56± 17.56 Ba	P<0.001
0.2%	23.33± 5.27 Aa	44.44± 7.47 Ab	38.76± 1015 Aa	64.59± 10.80 Ab	82.23± 11.06 Bb	11.11± 11.11 Cb	22.22± 14.69 Ab	—	—	P<0.001
0.4%	21.39± 3.79 Aa	30.56± 7.56 Ab	33.60± 9.34 Aa	8.33± 8.33 Bc	86.03± 5.72 Cb	16.41± 10.87 Ab	28.62± 14.33 Ab	44.44± 17.56 Aa	—	P=0.006
Control	2.22± 2.22 Ab	0.00± 0.00 Ac	3.33± 1.66 Ab	2.22± 1.46 Ad	1.11± 1.11 Ac	3.33± 1.66 Ac	3.33± 2.35 Ac	3.33± 2.35 Ab	3.33± 1.66 Aa	

S.E., Standard error; * There is no statistical difference between the values shown in capital letters on the same row (p > 0.05); # there is no statistical difference between the values shown in lower case letters on the same column (p > 0.05).

Table II. Mortality (Mean ± SEM) of *Acanthoscelides obtectus* adults against different concentrations of *Beauveria bassiana* (*B.b*) with spraying method (%)*

Conc. of *B.b*	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	p
0.1%	5.56± 3.37 Aa	0.00± 0.00 Aa	15.68± 5.28 Aa	16.88± 5.20 Aa	23.79± 7.39 Aa	15.96± 8.26 Aa	50.80± 8.48 Ba	48.27± 12.31 Aa	30.63± 12.20 Aa	35.28± 14.60 Aa	22.22± 14.69 Aa	8.89± 8.89 Aa	44.44± 17.56 Aa	P= 0.038
0.2%	3.33± 1.66 Aa	14.44± 6.03 Bb	27.52± 6.08 Ba	20.97± 9.14 Ba	51.44± 10.85 Ba	51.56± 13.50 Bb	65.37± 14.27 Ca	40.73± 16.45 Ba	11.11± 11.11 Bb	–	–	–	–	P= 0.004
0.4%	8.89± 1.11 Aa	20.00± 7.63 Ab	19.72± 8.90 Aa	5.56± 5.56 Ab	41.10± 9.40 Ba	44.33± 12.79 Bb	40.89± 13.49 Ba	53.16± 14.65 Ba	55.56± 17.56 Ba	–	–	–	–	P= 0.05
Control	0.00± 0.00 Aa	0.00± 0.00 Ac	3.33± 2.35 Ab	7.77± 2.22 Ac	7.77± 3.33 Ab	4.44± 1.75 Ac	8.88± 2.60 Ab	5.55± 2.42 Ab	4.44± 1.75 Ab	4.44± 2.42 Ab	5.55± 1.75 Ab	3.33± 1.66 Aa	8.88± 3.51 Ab	

For statistical symbols, see Table I.

increase in the dipping method and it was different from the control group (Fig. 3) (F= 38.602, df= 3, p < 0.001). In trials with spraying method, although not as effective as dipping method, the number of eggs emerged from beans was to decrease in parallel with the dose increase and it was found to be significantly different from the control group (F=38.604, df= 3, p < 0.001). When the percentages of the number of adults emerged were examined, it was also found that the all groups was statistically different from the control group (Fig. 3) (F=12.292, df= 3, p < 0.001). When the dipping and spraying methods were compared in terms of the number and percentages of adults emerged and from beans, statistically significant difference was found in all doses (F= 4.500, p= 0.001; F= 4.722, p= 0.015). It can be said that dipping method was more effective than the spraying method in all groups.

DISCUSSION

When the damage to the environment and humans caused by chemical control is considered, studies conducted with fungi and other biological control agents have come to the forefront in recent years. Many researchers have also focused on the fight on warehouse pests with fungi. In our country, producers have started to prefer alternative methods in chemical fight due to reasons such as the resistance developed by pests against chemicals and the consumers turning to products made with organic agriculture (Güven *et al*., 2015).

It was found in the present study that the most effective result was obtained with 0.2% and 0.4% dipping method and that spraying method was not as effective as dipping method. This study showed that as the concentration of applied *B. bassiana* increases, it had a positive effect on the mortality rate of *A. obtectus.* At the same time the decrease in the number of eggs and the number of adults emerging from beans may affected the egg laying physiology of *A. obtectus* adults.

In literature, there are a large number of studies on the effects of warehouse pests on mortality rates and their results are in parallel with the results of the present study. Sevim *et al*. (2015) examined the effects of bacteria and fungi on *A. obtectus* and *C. maculatus* and found that fungi had higher killing effect than bacteria and the highest mycosis rate was seen in *B. bassiana* with 90%. Bello *et al*. (2006) found that aqueous fungal solution was much more effective on *A. obtectus* than a mixture with diatomaceous earth-dry fungus. In a study they conducted with *A. obtectus* and *B. bassiana,* Crespo *et al*. (2002) found that 4 × 10^6 conidia/mL concentration increased mortality rate starting from day 7.

Rodriguez-Gonzalez *et al*. (2017) found that *B. bassiana* had a good inhibition effect on eggs and larvae with 82% in *A. obtectus*. These fungi can be considered as a highly effective tool for controlling the immature stages of these species. In Rodriguez-Gonzalez *et al*. (2017) study, *B. bassiana* was applied directly on eggs and larvae and the number of eggs laid and the number of *A. obtectus* adults emerging from beans after application of *B. bassiana* were found. According to the results of present study, the number of eggs laid by pests trying to survive after fungus application and especially the number of adults emerging from beans were statistically different than those of the control group (Figs. 1 and 2). Therefore, in addition to being effective on mortality rates, it can be seen that fungi are effective on egg production, oviposition, developmental and hatching physiology. This brings to mind that the pest fighting with fungus allocates almost all its sources to immune mechanism and fight with fungus.

In parallel with the present study, there are many studies which show that as concentration of *B. bassiana* and other entomopathogenic fungi increases, mortality rates also increase. In a study Özdemir *et al*. (2020) conducted on *C. maculatus* with two entomopathogenic fungi *Metarhizium anisopliae* (Metchnikoff) Sorokin 1883 (Hypocreales: Clavicipitaceae) and *B. bassiana* found that mortality rates increased as the concentration of the solutions increased. In a study Gad *et al*. (2020) examined the effects of different concentration solutions of *Trichoderma harzianum* Rifai 1969 (Hypocreales: Hypocreaceae) on *A. obtectus* found that mortality rates of 2×10^7 conidia/mL solution was 93.88% at the end of day 7. Ferron and Robert (1975) found that *A. obtectus*'s mortality rates increased as the concentration of fungus increased. They found that mortality rates also increased as concentration increased between 5×10^6 and 1×10^9 conidia/mL and that they were sensitive against *A. obtectus*'s *B. bassiana, B. tenella, M. anisopliae* and *Paecilomyces fumosoroseus* Samson 1974 (Eurotiales: Trichocomaceae) spores. Laib *et al*. (2020) found that different concentrations of *Isaria fumosorosea* Wize 1904 (Hypocreales: Clavicipitaceae) (1 × 10^7, 1 × 10^6 and 1 × 10^5 conidia/mL) were effective on *A. obtectus* and *Locusta migrotoria* Linnaeus 1758 (Orthoptera: Acrididae) and mortality increased as concentration increased, while the most effective suspension was 1 × 10^7 conidia/mL with 80% mortality rate. In the present study, it was found that mortality rate increased as fungus concentration increased and the highest mortality rate occurred on day 5 with 0.4% concentration as 86.03%.

Researchers conduct studies on not only *B. bassiana,* but also with a large number of bioinsecticides that can fight pests. In a study they conducted with *Myzus persicae* Sulzer 1776 (Hemiptera: Aphididae), Şahin and Karaca (2019) analyzed various commercially sold bioinsecticides and as a result of this study, they found the mortality rate of Nostalgist® as 47% at the end of day 7 in Petri trials and as 53.70% in pot trials. In a study they examined the effects of a large number of commercially sold bioinsecticides on potato beetle *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae), Öztürk *et al*. (2015) found mortality rate as 82.1% in second stage larvae and as 33.3% in adult stage by using Nostalgist® with spraying method. In a study conducted with *L. decemlineata,* Atanasova and Vasilev (2020) found the mortality rates of larvae and adults on day 5 with 0.2% Naturalis® (*B. bassiana*) as 78.4% and 68.4% and found that 100% of both larvae and adults died on day 7. In this current study, the mortality rate in the dipping method in the same concentration was found to be 82.23% on day with 5, and it is similar to the work of Atanasova and Vasilev (2020).

CONCLUSION

According to non-official data, approximately 297 tons of pesticides were used in the control of stored product pests in 1998 in Turkey (Emekçi and Ferizli, 2000). The harms of chemical control on the environment and humans and public awareness on this are increasing each day. Therefore, in fighting pests, researchers and producers are in a search for alternative fighting methods which harm the environment less or which do not harm the environment at all. Stored products have great contributions to the economy of our country, as in developing countries, since they are rich in nutrients and can be produced in abundance. For these reasons, it is important that products are stored in warehouses in a less costly and more healthily way after harvest. In order for products to be stored safely in warehouses after harvest, it is necessary to carry out the necessary maintenance and repairs of warehouses in addition to fight against pests that can breed and reproduce in warehouses. As a result, when the mortality rates of different concentrations of *B.*

bassiana tested in laboratory environment on the adults of stored product pest bean seed, the number of eggs they lay, the number and percentages of adults emerging from beans is considered, promising results have been shown in controlling this pest. It can be said that different doses of *B. bassiana* are highly effective on the mortality rates of *A. obtectus* adults, the number of eggs they lay and especially the number of adults that emerge from beans. While the increase in the mortality rates of 0.2% and 0.4% doses of fungi after application is an expected result, the significant decrease in the number of eggs laid and the number and percentages of adults emerging from beans show that the harm in warehouses to a minimum, considering that this pest reproduces many times a year. It can be said that *B. bassiana* has a potential of supportive use in integrated control methods as a biological control agent against pests. In addition, according to the results of this article, it can be said that *B. bassiana* affects the *A. obtectus*' egg laying and adult emergence physiology, perhaps mating behavior. For future studies, in addition to comparative trials on other bioinsecticides sold in the market, their effect on the number of eggs laid and the number of adults to be hatched from these eggs are among topics to be researched. In addition to studies conducted in laboratory, studies should be carried out on the effect of biological control studies in the field on the beans to be stored.

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Statement of conflict of interest

The author have declared no conflict of interest.

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