

# INSECTICIDE ACTIVITY OF *Beauveria bassiana* AND CASTOR BEAN OIL AGAINST *Plutella xylostella* UNDER GREENHOUSE

## ATIVIDADE INSETICIDA DE *Beauveria bassiana* E ÓLEO DE MAMONA SOBRE *Plutella xylostella* EM CASA TELADA

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**ABSTRACT:** The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), one of the principal pests of brassicas, can reduce productivity and thus cause losses for brassica farmers. Alternative controls, such as biological agents and plant extracts, may be used to reduce insect populations, either alone or in conjunction with pest management programs. The objective was to evaluate the insecticidal activity of *Beauveria bassiana* (Bals.) Vuill. and castor bean oil and mixtures of both components against the diamondback moth. To do so, we separately used castor bean oil (at 2% concentration), the isolate ESALQ-447 and a commercial formulation (Boveril<sup>®</sup> WP), and a mixture of castor bean oil with the isolate and the *B. bassiana* product formulation, totaling six treatments with a control. Assays were carried out under greenhouse with the respective treatments sprayed on cabbage plants infested with four second instar larvae of *P. xylostella*. The evaluated parameters were larval mortality and pupal and larval viability. All treatments reduced larval viability in relation to the control, however, only the ESALQ-447 isolate or a mixture of the isolate with castor bean oil reduced pupal viability, significantly reducing the pest population levels in the next generation. Castor bean oil mixed with *B. bassiana*, however, does not augment pest mortality.

**KEYWORDS:** Phytosanitary management. *Ricinus communis*. Microbial control. Plant insecticides. Diamondback moth.

### INTRODUCTION

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the principal pests of brassicas, capable of reducing leaf area, devaluing products, slowing growth and causing plant death if left uncontrolled (MONNERAT et al., 2004). Among the factors that favor the occurrence and consequent infestation of the diamondback moth, its elevated biotic potential is considered relevant since the cycle takes approximately 16 days and each females deposits about 140 eggs (temperature of 25°C and relative humidity of 70%) (TORRES et al., 2006; BOIÇA JUNIOR et al., 2011).

This pest can be controlled through biological agents such as the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill., that is virulent to *P. xylostella* (SILVA et al., 2003; RONDELLI et al., 2011) and is effective at reducing the pest population and increasing cabbage production (GODONOU et al., 2009). This fungus is compatible with *Trichogramma atopovirilia* Oatman & Platner (Hymenoptera: Trichogrammatidae) (POLANCZYK et al., 2010), a

parasitoid of diamondback moth eggs (PRATISSOLI et al., 2008).

Plant insecticides provide another form of control by producing compounds toxic to herbivores. These compounds can be extracted from tissue and used to control pest insect (WIESBROOK, 2004; BOIÇA JÚNIOR et al., 2013). The castor bean plant, *Ricinus communis* L., is one of these plants and its seed-extracted oil, (at a concentration of 3%) caused 44% mortality in *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) (BESTETE et al., 2011). Additionally, the mixture of castor bean oil and *B. bassiana* showed a synergistic effect on the control of diamondback moth (RONDELLI et al., 2011).

Synthetic chemical products are widely used to control *P. xylostella*, with pyrethroids and organophosphorous compounds being the most studied groups (CASTELO BRANCO; MEDEIROS, 2001; MONNERAT et al., 2004; ABRO et al., 2013). However, the bacteria *Bacillus thuringiensis* Berliner is also used to control this pest (MEDEIROS et al., 2006). Nevertheless, there are known cases of diamondback moth populations being resistant to some products, including those formulated with *B. thuringiensis* (CASTELO

BRANCO et al., 2001; CASTELO BRANCO et al., 2003; RIBEIRO et al., 2012). The importance of controlling diamondback moths with other agents has thus been observed, such as with parasitoids of the larvae *Oomyzus sokolowskii* Kurdjumov (Hymenoptera: Eulophidae) (SANTOS JR. et al., 2006) and eggs *T. atopovirilia* (PRATISSOLI et al., 2008), since these biological agents can implement integrated management programs of *P. xylostella*.

Moreover, the combination of chemical products with biological agents may be an option for efficiently managing this pest. For example, the insecticide deltamethrin is compatible with *B. bassiana* at the recommended product concentrations (ALVES et al., 1998). However, some insecticides are incompatible with entomopathogenic fungi, such as methamidophos, endosulfan, diflubenzuron, thiodicarb and methyl parathion, which affect or reduce the natural infection of *Nomuraea rileyi* (Farlow) Samson on the velvetbean caterpillar (BARBOSA et al., 1997).

Thus, the objective of this study was to evaluate the insecticidal activity of the entomopathogenic fungus *B. Bassiana* and castor bean oil and a mixture of the oil with the fungus *B. Bassiana* for the control of diamondback moth under greenhouse.

## MATERIAL AND METHODS

The experiment was conducted at the Center for Scientific and Technological Development in Phytosanitary Management of Pests and Diseases (NUDEMAFI), at the Center for Agricultural Sciences, Federal University of Espírito Santo (CCA/UFES), in Alegre, Espírito Santo State, Brazil.

### Attainment and rearing of *P. xylostella*.

The insects used were part of a stock maintained by the Entomology Laboratory at NUDEMAFI, originating from planted brassicas in the municipality of Alegre, Espírito Santo State, Brazil, and raised in collard green leaves, *Brassica oleracea* L. var. *acephala*, in accordance with the methodology developed by Barros and Vendramin (1999).

### Attainment, production, and revigoration of *B. bassiana*.

The standard isolate ESALQ-447 and commercial formulation Boveril® WP, which contains isolate ESALQ-PL63 were evaluated. For the bioassay, the isolate ESALQ-447 was reproduced on 9.5 x 1.5 cm (diameter and height)

Petri dishes containing a culture medium of potato-dextrose-agar, with added yeast and antibiotic (PDAY+A). In all phases of research, fungal development occurred in a climatized chamber at a temperature of  $26 \pm 1$  °C, and relative humidity of  $70 \pm 10\%$  and 12 hour photophase.

The reactivation of the virulence of the isolate ESALQ-447 was carried out on the coffee borer beetle, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), which was originally grown at the Entomology Laboratory at NUDEMAFI following Leite et al. (2003). The viability of the conidia was quantified by the germination method (SILVA et al., 2003), which were considered viable upon showing a germination rate above 90%.

### Attainment and extraction of castor bean oil.

Mature fruits of the castor bean plant were collected in the municipality of Alegre, Espírito Santo State, Brazil, and exposed to sun until completely dried and had shed their seeds before extracting their oil by cold pressing and filtering with the aid of a sieve with a mesh of 35. The oil was stored in an airtight glass container and kept at  $26 \pm 2$  °C and a 14 hour photophase.

### Bioassay.

The experiment was conducted in a greenhouse in the municipality of Alegre, Espírito Santo State, Brazil ( $20^{\circ} 45' 50''$  S  $41^{\circ} 31' 58''$  W, altitude of 254 meters). During the experiment, the temperature inside the greenhouse varied between 23 and 40 °C and the relative humidity varied from 33 to 80%.

Cabbage seedlings were transferred to pots with a capacity of 2.4 liters, containing growth substrate (soil, sand and manure). When plants were 40 days old, they were infected with four second instar diamondback moth larvae.

Suspensions were prepared at a concentration of  $5 \times 10^6$  conidia/mL of the ESALQ-447 isolate and formulated Boveril® WP, using sterilized distilled water (SDW) and Tween® 80 adhesive spreader (S) at 0.05% (SDW+S). Castor bean oil was used 70 days after extraction at a concentration of 2%, diluted in SDW+S. The treatments were as follows: ESALQ-447 isolate; Boveril® WP formulation; castor bean oil; ESALQ-447 isolate with added 2% castor bean oil; and Boveril® WP formulation with added 2% castor bean oil. The mixture of the fungus with castor bean oil was made one hour before application. SDW+S was used as the control.

Plants containing insects were sprayed with a treatment to the point of runoff using a manual sprayer (Guarany®) with a 1.25 liter volume. Spraying was done in the final part of the afternoon to avoid exposing the conidia to solar radiation and low humidity until the following morning, providing favorable environmental conditions for the development of the fungus.

Larval mortality was evaluated on the third, fifth, and eighth day after application. Larval viability evaluations were conducted during pupal stage evaluations. The pupae were collected in a 6.0 x 2.0 cm (diameter and height) germination box and maintained in a climatized chamber at a temperature of 26 ± 1 °C, relative humidity 70 ± 10% and 12 hour photophase to evaluate pupal viability through the emergence of adults.

To assess the number of larvae per plant, a completely randomized design was used with treatments in a 6x3 (treatments x evaluation periods) split-plot, while the parameters of pupal and larval

viability were evaluated with a completely random design. Twenty repetitions were used with each containing four insects. The data were analyzed by an analysis of variance (ANOVA) and the measurements compared using the Tukey test at 5% probability.

**RESULTS AND DISCUSSION**

The results showed an interaction between the treatments and evaluation periods (F<sub>10, 323</sub> = 2.04; P = 0.0294). On the third day after the application there was no difference in the number of larvae per plant among all treatments. Nevertheless, on the fifth and eighth days after treatment, application differences were only observed between the control and the rest of the treatments, with the efficiency of the control on the fifth day varying between 25.4 and 35.8% and on the eighth varying between 29.0 and 51.6% (Table 1).

**Table 1.** Mortality of second instar larvae of *Plutella xylostella* caused by the fungus *Beauveria bassiana* (5 x 10<sup>6</sup> conidia/mL), castor bean oil (2%) and a mixture of *B. bassiana* and castor bean oil, at three, five, and eight days after application.

Treatment <sup>1</sup>	3 DAA <sup>2</sup>		5 DAA		8 DAA		Statistics, GL = 2, 323; F <sup>P</sup>
	N <sup>3</sup>	E <sup>4</sup> (%)	N	E (%)	N	E (%)	
Control	3.55 ± 0.11 Aa	–	3.35 ± 0.15 Aa	–	3.10 ± 0.16 Aa	–	1.48 <sup>=0.2258</sup>
Castor bean oil	2.95 ± 0.18 Aa	16.9%	2.50 ± 0.18 ABb	25.4%	2.20 ± 0.25 Bb	29.0%	4.15 <sup>=0.0162</sup>
ESALQ-447	3.10 ± 0.18 Aa	12.7%	2.40 ± 0.20 Bb	28.0%	1.60 ± 0.22 Cb	48.4%	16.41 <sup>&lt;0.0001</sup>
Boveril® WP	3.00 ± 0.21 Aa	15.5%	2.50 ± 0.20 Ab	25.4%	1.50 ± 0.24 Bb	51.6%	16.99 <sup>&lt;0.0001</sup>
ESALQ-447 + Castor bean oil	3.15 ± 0.18 Aa	11.3%	2.15 ± 0.17 Bb	35.8%	1.60 ± 0.21 Bb	48.4%	17.98 <sup>&lt;0.0001</sup>
Boveril® WP + Castor bean oil	3.10 ± 0.18 Aa	12.7%	2.20 ± 0.16 Bb	34.3%	1.50 ± 0.21 Cb	51.6%	18.74 <sup>&lt;0.0001</sup>
Statistics, GL = 5, 323; F <sup>P</sup>	1.32 <sup>=0.2537</sup>	–	5.50 <sup>&lt;0.0001</sup>	–	11.82 <sup>&lt;0.0001</sup>	–	–

<sup>1</sup>Means (± SE) (n = 20 repetitions) followed by the same upper case letter, in the row, and lower case, in the column, were not different from each other under the Tukey test at 5% probability. <sup>2</sup>DAA = Days after application. <sup>3</sup>N = Number of larvae per plant (N = 4 larvae). <sup>4</sup>E = Control efficiency calculated with the formula from Henderson and Tilton (1955).

Thus, the association between *B. bassiana* (Boveril® WP or the isolate ESALQ-447) mixed with castor bean oil did not result in increased pest mortality relative to the application of fungus in the greenhouse. However, laboratory tests revealed synergism in the association between Boveril® WP (3 x 10<sup>5</sup> conidia/mL) + castor bean oil (2%, 70 days of storage) in their effect on *P. xylostella*, where a larval mortality of 78% was observed (RONDELLI

et al., 2011). The differences between these results could be related to environmental differences, because the environmental conditions in which these experiments were conducted were not always favorable to fungal development, as the temperature varied between 23 and 40 °C and the relative humidity varied between 33 and 80%.

The use of castor bean oil 70 days after it was extracted could also bias its efficiency against

*P. xylostella* because Rondelli et al. (2011) verified that the insecticide activity of this oil decreases with time, since oil stored for 70 days caused a 24.4% larval mortality in *P. xylostella*, whereas oil used only 24 hours after extraction caused a mortality of 53.9%.

The number of larvae in the control did not differ in the days after treatment application, indicating that the environmental conditions and the plant were adequate for the pest. However, in the ESALQ-447 and Boveril® WP + castor bean oil treatments this difference occurred in all days after treatment application. However, the number of larvae per plant in the ESALQ-447 + castor bean oil treatment differed only on the fifth day after application, while in treatments with castor bean oil and Boveril® WP this difference only occurred on the 8th day of the evaluation (Table 1). These results show the not acute toxic effect of the fungus as well as castor bean oil against diamondback moth. Even so, in the field these treatments would reduce the population levels during breeding and consequently that of subsequent populations. Additionally, the plant extracts can have a sublethal effect (TORRES; BARROS; OLIVEIRA, 2001; VASCONCELOS; GONDIM JÚNIOR; BARROS, 2006).

Castor bean oil does not possess ricin (SEVERINO, 2005) and 80.5% of it is composed of ricinoleic acid (COSTA et al., 2004), which is likely the substance toxic to diamondback moth, since ricinoleic acid of the castor bean oil added to rabbit feed inhibited reproduction (vitellogenesis) of the mite *Rhipicephalus sanguineus* (Latreille) (Acari: Ixodidae) that parasitizes rabbits (ARNOSTI et al.,

2011). Additionally, castor bean oil is viscous and could make locomotion and feeding difficult for the larvae, thus increasing pest mortality.

The efficiency of the isolate ESALQ-447 (at a concentration of  $5 \times 10^6$  conidia/mL) against *P. xylostella* larva on the eighth day after treatment application was 48.4%. Silva et al. (2003), studying the isolate ESALQ-447 against *P. xylostella*, observed a control of 68.0 and 86% of larva at concentrations of  $10^7$  and  $10^8$  conidia/mL. This indicates that higher fungus concentration can cause higher pest mortality. In the field at Benin, with daily temperatures between 21 and 31 °C and relative humidity between 66 and 99%, the isolate Bba5653 of *B. bassiana* at a concentration of  $10^8$  conidia/mL controlled *P. xylostella*, causing lower numbers of larvae per plant and increased the average weight of cabbage heads by approximately 300% (GODONOU et al., 2009).

For the larval viability parameter, there was a difference observed only in the control relative to the rest of the treatments, varying from 37.5 to 55% (Table 2). Larval viability of 77.5% was observed in the control, as similarly reported by Torres, Barros e Oliveira (2001) in a laboratory study. The isolate ESALQ-447 and the formulation Boveril® WP caused larval viability at 40 and 37.5%, respectively, while Oliveira et al. (2008) in the laboratory observed larval viability of 76.7 and 56.7% for *Diatraea saccharalis* F. (Lepidoptera: Crambidae) treated with the isolate ESALQ-447 of *B. bassiana* at concentrations of  $10^4$  and  $10^5$  conidia/mL, respectively.

**Table 2.** Larval and pupal viability of *Plutella xylostella* after spraying plants with the fungus *Beauveria bassiana* ( $5 \times 10^6$  conidia/mL), castor bean oil (2%) and association of the mixture of *B. bassiana* with castor bean oil.

Treatment	Larval viability (%) <sup>1</sup>	Pupal viability (%) <sup>1</sup>
Control	77.5 ± 4.01 a	93.8 ± 2.89 a
Castor bean oil	55.0 ± 6.18 b	94.9 ± 3.88 a
ESALQ-447	40.0 ± 5.56 b	60.8 ± 9.37 c
Boveril® WP	37.5 ± 5.88 b	91.7 ± 6.45 ab
ESALQ-447 + Castor bean oil	40.0 ± 5.26 b	67.6 ± 8.63 bc
Boveril® WP + Castor bean oil	37.5 ± 5.29 b	95.1 ± 3.43 a
Statistics	F <sub>5, 114</sub> = 8.68; P < 0.0001	F <sub>5, 99</sub> = 6.32; P < 0.0001

<sup>1</sup>Means (± SE) (n = 20 repetitions) followed by the same letter in the column did not differ between themselves using the Tukey test at 5% probability.

The association of *B. Bassiana* mixed with castor bean oil did not result reduced larval viability in relation to the fungus alone. However, the castor bean oil resulted in a larval viability of 55%.

For the pupal viability parameter, only the ESALQ-447 (60.8%) and ESALQ-447 + castor

bean oil (67.6%) treatments differed from the control (93.8%) (Table 2). However, Oliveira et al. (2008), did not find interference between in same strain of *B. bassiana* at a concentration of  $10^5$  conidia/mL against the pupal viability of *D. saccharalis* in the laboratory, even though this

parameter was 60%. However, aqueous extracts of the plants *Melia azedarach* L. and *Laurus nobillis* L. caused pupal viability of *P. xylostella* at 0 and 10%, respectively (TORRES; BARROS; OLIVEIRA, 2001).

## CONCLUSIONS

The isolate ESALQ-447 of *B. bassiana* (at a concentration of  $5 \times 10^6$  conidia/mL) and the mixture with castor bean oil (at a concentration of 2% and at 70 days in storage) reduced larval viability to 40% and pupal viability to 60.8 and 67.6%, respectively of the diamondback moth under greenhouse, significantly diminishing the population level of the pest in the following generation.

The use of castor bean oil at a concentration of 2% after 70 days in storage mixed with *B. bassiana* at a concentration of  $5 \times 10^6$  conidia/mL did not increase diamondback moth mortality in the greenhouse.

Castor bean oil (at a concentration of 2% and 70 days in storage) reduced larval viability of the diamondback moth to 55%, but did not reduce the pupal viability under greenhouse.

Based on pupal viability, it is concluded that the isolate ESALQ-447 is most efficient on the diamondback moth, both when used in isolation and mixed with castor oil. Nevertheless, due to the low availability of commercial formulations of entomopathogenic fungi in Brazil, Boveril® WP is considered to be useful for managing *P. xylostella*.

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**RESUMO:** A traça-das-crucíferas, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), uma das principais pragas das brássicas, pode ocasionar redução na produtividade e consequentemente prejuízos aos produtores rurais destas culturas. A busca por alternativas de controle, como o uso do controle biológico e extratos de plantas são métodos de redução populacional de insetos que podem ser usados isoladamente ou associados em programas de manejo fitossanitário. Dessa forma, o objetivo foi avaliar a atividade inseticida de *Beauveria bassiana* (Bals.) Vuill., do óleo de mamona e a associação destes, visando ao controle da traça-das-crucíferas. Para isso, foram utilizados isoladamente o óleo de mamona (na concentração de 2%), o isolado ESALQ-447 e um formulado comercial (Boveril® WP) e a mistura do óleo de mamona com o respectivo isolado e com o produto formulado de *B. bassiana*, totalizando assim seis tratamentos com a testemunha. Os ensaios foram realizados em casa telada, onde plantas de repolho infestadas com quatro larvas de segundo ínstar de *P. xylostella* foram pulverizadas com os respectivos tratamentos mencionados. Os parâmetros avaliados foram mortalidade larval, viabilidade larval e pupal. Todos os tratamentos reduziram a viabilidade larval em relação à testemunha, no entanto apenas o isolado ESALQ-447 ou a sua associação com o óleo de mamona reduziram a viabilidade pupal, diminuindo significativamente o nível populacional da praga na próxima geração. Contudo, o óleo de mamona misturado com *B. bassiana* não aumenta a mortalidade da praga.

**PALAVRAS-CHAVE:** Manejo fitossanitário. *Ricinus communis*. Controle microbiano. Inseticidas vegetais. Traça-das-crucíferas.

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