



Biological Effects of Aqueous Plant Extracts Against *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae) Under Laboratory Conditions

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ABSTRACT

Post-harvest losses of common beans during warehouse storage are largely attributed to the Mexican bean weevil, *Zabrotes subfasciatus* Boheman, one of the most destructive pests of stored beans worldwide. Indiscriminate use of synthetic insecticides poses health risks to people and the environment. As a promising alternative, plant-derived insecticides offer a safer way to mitigate harmful effects. This study aimed to evaluate the biological effects of spraying different plant extracts against the Mexican bean weevil in stored beans under laboratory conditions. Extracts (50 g/L) were obtained by mixing 5 g of plant powder with 100 mL of distilled water. Ten sexed adults were placed with 10 g of beans in 50 mL acrylic containers. Botanical extracts were applied via airbrush in a completely randomized design with five replicates per treatment. Survival was assessed for five days post-application. At 21 days after infestation (DAI), egg counts (total, viable, nonviable) were recorded; at 37 DAI, emergence, developmental Time, and larval survival were evaluated. As a result, *A. muricata*, *L. sibiricus*, and *A. sativum* exert insecticidal effects, while *R. graveolens*, *P. barbatus*, *C. verbenacea*, *D. ambrosioides*, *L. sibiricus*, and *P. nigrum* suppress adult emergence. *Annona muricata* also prolonged developmental Time. *L. sibiricus* (6.88 live adults), *A. muricata* (7.52), and *A. sativum* (7.76) significantly reduced adult survival, while *D. ambrosioides* (11.20), *P. barbatus* (13.40), *P. nigrum* (14.00), *C. verbenacea* (15.40), *L. sibiricus* (16.00), and *R. graveolens* (17.60) reduced adult emergence. *A. muricata* prolonged development (42.90 days), whereas *D. ambrosioides* (4.60), *P. nigrum* (5.20), *P. barbatus* (7.20), *C. verbenacea* (7.20), *L. sibiricus* (8.00), *R. graveolens* (8.60), and *A. sativum* (10.00) reduced male emergence. These findings reinforce the potential of botanical extracts as sustainable alternatives to synthetic insecticides, supporting the development of eco-friendly biopesticides for safer pest management.

Keywords: alternative control, aqueous extracts, botanical insecticide, Mexican bean weevil

INTRODUCTION

Beans (*Phaseolus vulgaris* L.) are a globally significant legume valued for their economic, nutritional, social, and cultural importance (Nassary et al., 2020). Nutritionally, this crop is

a valuable source of protein and iron, as well as complex carbohydrates, dietary fiber, vitamins, and essential minerals. Moreover, beans play a critical role in ensuring food security in regions where access to animal protein is limited or

costly, serving as a key alternative protein source (Uebersax et al., 2023; Zhang et al., 2024).

Despite its socioeconomic importance, common beans suffer substantial post-harvest losses during warehouse storage, primarily due to pests (Gonçalves et al., 2015). These losses are exacerbated by the favorable climatic conditions of tropical regions for pest development, and by inadequate storage practices (Ribeiro et al., 2023).

The Mexican bean weevil, *Zabrotes subfasciatus* Boheman (Coleoptera: Chrysomelidae), is considered one of the most destructive pests of stored beans worldwide (Guzzo et al., 2018; Takaku et al., 2025). Its damage results primarily from larval penetration into the seeds, which causes weight loss and diminishes nutritional quality. Infestation also reduces grain marketability because the presence of eggs, frass, and developing larvae compromises product integrity (Hubert et al., 2018). In addition, exit holes created by emerging adults promote secondary infestations from mycotoxigenic fungi, spoilage microorganisms, and mites (Faria et al., 2023; Quintela et al., 2020). Beyond yield and quality losses, such damage often renders beans unsuitable for both human consumption and industrial processing (Mingotte et al., 2013).

Previous studies have demonstrated that plant-derived products can affect *Z. subfasciatus* through different mechanisms, including adult mortality, repellency, reduced oviposition, and suppression of progeny emergence (Gonçalves et al., 2021; Faria et al., 2023). Powders of *Piper nigrum* (L.), *Syzygium aromaticum* (L.), *Dysphania ambrosioides* (L.), and *Ruta graveolens* (L.) have shown insecticidal or repellent activity against this species. In contrast, *Annona muricata* (L.) and other *Annona* species have also demonstrated lethal and sublethal effects when used as ethanolic extracts. Essential oils from *Cordia verbenacea* (DC) and other plants have likewise been investigated for their activity against *Z. subfasciatus*. However, these studies have

predominantly evaluated plant powders, ethanolic extracts, or essential oils.

In contrast, comparatively less information is available on the biological effects of aqueous extracts applied directly to adults under standardized laboratory conditions. The use of aqueous extracts represents a distinct approach because water is a readily available and comparatively simple extraction medium, potentially facilitating preparation and application in small-scale storage systems. Nevertheless, the efficacy of aqueous preparations depends on the plant species, plant material, extraction conditions, concentration, and target pest, highlighting the need for standardized evaluations.

In the context of stored-grain pest management, botanical extracts are promising plant-derived compounds due to their broad-spectrum activity against fungi and arthropod pests (Ribeiro et al., 2023). These effects include repellency, feeding deterrence, fumigant, and contact insecticidal actions, as well as inhibition of mycelial growth and toxin production in food-deteriorating fungi (Achar et al., 2020). Over the last few decades, research on botanicals has increased considerably, rising from 1.43% of all published insecticide papers in 1980 to 21.38% in 2012 (Isman & Gieneisen, 2014). However, significant knowledge gaps remain that must be addressed to transition botanical insecticides from laboratory studies and traditional homemade uses to commercial applications (Isman, 2020). Finding new, viable sources of compounds or blends that can simultaneously act against stored-grain spoilers is crucial for their practical use in warehouses. Neotropical plant species, which have diverse secondary metabolites, represent a promising source of volatile compounds (i.e., Essential Oils) that could be applied in the phytosanitary management of both row and horticultural crops, including during pre- and post-harvest practices (Mingotte et al., 2013).

Since the mid-20th century, chemical pesticides have been the primary method for managing storage pests. However, their intensive and indiscriminate use has generated several

serious challenges, such as the development of pesticide resistance in insect populations (Machuca-Mesa et al., 2024; Shah et al., 2025), the accumulation of toxic residues in food products (Beyuo et al., 2024), and environmental contamination (Chia et al., 2024; Zhou et al., 2025). Concerns have also grown regarding the long-term impacts of synthetic pesticide use on ecosystem balance, human health, and overall food security. In addition, the rising costs of pesticide applications, particularly problematic in developing countries where storage conditions are often inadequate, have further highlighted the unsustainability of relying solely on these products. Due to these drawbacks, the search for alternative, sustainable pest control measures has become imperative. Current research emphasizes eco-friendly and economically viable strategies, such as botanical insecticides (Perzada et al., 2025), natural repellents (Nisar et al., 2022), and host plant resistance (Takaku et al., 2025), that can protect stored grains. The plant species evaluated in this study were selected based on their reported insecticidal, repellent, or bioactive properties against arthropod pests, their documented use in traditional pest management, and the availability of phytochemically diverse secondary metabolites. The selection, therefore, aimed to compare a diverse group of botanicals with previously reported biological potential (Table 1). Therefore, this study aimed to investigate the biological effects of ten aqueous plant extracts, evaluated at a single concentration, on *Z. subfasciatus* under laboratory conditions. The effects of the extracts were assessed based on adult mortality, oviposition, larval survival, F1 adult emergence, and developmental time, providing a comprehensive assessment of their potential effects on the survival and subsequent development of this important stored-bean pest.

METHODS

Zabrotes subfasciatus Rearing

The Mexican bean weevils used in the bioassays were initially obtained from a laboratory colony maintained by Professor Edson Luiz Lopes Baldin at the School of

Agriculture (FCA/UNESP). The insects were subsequently transferred to Avaré, São Paulo State, Brazil, where a new colony was established and maintained under laboratory conditions. For colony maintenance, the insects were reared in transparent glass containers (8 cm in height × 13 cm in diameter) containing insecticide-free common beans as the rearing substrate. The containers were covered with organdy fabric to provide adequate aeration while preventing insect escape. Each glass pot was filled with 300 g of common bean grains (cv. Bolinha) and approximately 400 unsexed adult insects, and was maintained in the laboratory (25 ± 2 °C, RH = 70 ± 10%, and a photoperiod of 12 L:12 D h). The grains in the pots underwent a 30-day sieving process, after which the newly emerged adults were used to infest new pots or to be incorporated into the proposed bioassays.

Obtaining Botanical Extracts

To prepare the 10 botanical extracts used in the tests, the following plant structures were collected: rue leaves [*R. graveolens*: Rutaceae], rosemary leaves [*Salvia rosmarinus* (L.) (Spenn.): Lamiaceae], Brazilian boldo leaves [*Plectranthus barbatus* (Andrews): Lamiaceae], soursop seeds [*A. muricata*: Annonaceae], black sage leaves [*C. verbenacea*: Boraginaceae], epazote leaves [*D. ambrosioides* (L.) (Mosyakin & Clemants): Amaranthaceae], honeyweed leaves [*Leonurus sibiricus* (L.): Lamiaceae], clove flowers [*S. aromaticum*: Myrtaceae], black pepper seeds [*P. nigrum*: Piperaceae], and garlic bulbs [*Allium sativum* (L.): Amaryllidaceae]. The plant materials used for extract preparation were collected from the Experimental Farm of Uneduvale in Avaré, São Paulo, Brazil (23°18'63" S, 48°95'54" W) in March 2024, during the morning. The collected samples were dried in a circulating-air oven at 40 °C for 48 h and subsequently ground using an electric knife mill. The resulting powders were then stored in dark, airtight containers, separated by species and plant part.

The extracts were prepared by mixing 5 g of powdered material with 100 mL of distilled

water using a magnetic stirrer. The resulting suspensions were kept at room temperature for 48 h to facilitate the extraction of water-soluble compounds. Afterward, the solutions were filtered through a voile fabric, yielding aqueous extracts at a concentration of 50 g L⁻¹. A concentration of 50 g L⁻¹ was selected to

standardize the aqueous preparations among plant species and provide a sufficiently concentrated botanical suspension for laboratory screening while maintaining a practical extraction procedure (Amego et al., 2023).

Table 1. Historic use of botanical extracts.

Botanical Species	Historic Use of Botanical Extracts	
	Target Insects	References
<i>Ruta graveolens</i>	<i>Anopheles stephensi</i> ; <i>Sitophilus zeamais</i>	Aivazi and Vijayan, 2010; Perera et al., 2017
<i>Salvia rosmarinus</i>	<i>S. granarius</i> ; <i>Myzus persicae</i>	Tahar et al., 2014; Billal et al., 2015
<i>Plectranthus barbatus</i>	<i>Aedes aegypti</i>	Almeida et al., 2021
<i>Annona muricata</i>	<i>Helicoverpa armigera</i> ; <i>C. maculatus</i>	Souza et al., 2017; Obembe et al., 2025
<i>Cordia verbenacea</i>	<i>O. surinamensis</i> ; <i>R. dominica</i> ; <i>S. zeamais</i>	Moreira et al., 2007
<i>Dysphania ambrosioides</i>	<i>S. zeamais</i> ; <i>C. maculatus</i>	Langsi et al., 2018; Denloye et al., 2010
<i>Leonurus sibiricus</i>	<i>Lymantria dispar</i>	Park et al., 1997
<i>Syzygium aromaticum</i>	<i>Plutella xylostella</i> ; <i>S. frugiperda</i>	Badgujar et al., 2017; Sianturi et al., 2022
<i>Piper nigrum</i>	<i>Amphimallon majale</i>	Scott et al., 2005
<i>Allium sativum</i>	<i>Tribolium castaneum</i> ; <i>C. maculatus</i>	Ahmad et al., 2024; Al-Shareefi et al., 2025

Bioassay

To initiate the study, transparent acrylic containers (50 mL; 5 cm height × 3 cm diameter with lids) were used to accommodate 10 g of bean grains. The grains were weighed on an analytical balance with 0.0001 g accuracy (Model ATY224R, Shimadzu). Subsequently, five males and five fertilized females (0–48 h old), randomly selected from the stock colony, were introduced into each container.

After the adult *Z. subfasciatus* were released into the acrylic pots, botanical extracts were applied using a professional airbrush (Model BD134K) connected to an air compressor set at 1.0 bar, delivering 1 mL of each extract per pot. Treated pots were maintained under controlled laboratory conditions (25 ± 2 °C, 70 ± 10 % RH, 12-h photoperiod). A completely randomized design was employed, with five replicates per botanical extract. Each replicate consisted of a

container containing five pairs of adult *Z. subfasciatus* (n = 50). Survival was recorded for five days following the application. Control efficiency (EC %) was calculated using Abbott's formula (Abbott, 1925). Individuals were considered dead if no antennae or leg movement was observed for more than two minutes. It is worth noting that insects of the genus *Zabrotes* exhibit thanatosis, a death feigning behavior.

Five days after spraying the botanical extracts, the adults were removed, and the total number of eggs, viable eggs, and nonviable eggs was counted at 21 days post-infestation using a stereoscopic microscope (Leica EZ4 E) (Cabral et al., 2024; Takaku et al., 2025). Grains from each plot were placed in Petri dishes (90 × 15 mm) covered with black cardboard to facilitate visualization of the eggs deposited between the grains. Viable eggs had a larval exit hole, while

nonviable eggs were intact and hyaline in appearance (Ribeiro et al., 2007).

Thirty-seven days after the initial infestation, the number of emerging insects and the developmental Time (egg to adult) were also determined by sieving the grains from each container through an appropriate mesh. The daily emergence of males and females was counted for each botanical extract until no insects emerged for five consecutive days. The weighted mean calculated by Eq. 1 was used to determine the egg-to-adult developmental Time (Cabral et al., 2024; Takaku et al., 2025).

$$\frac{\sum xf}{\sum x} \quad (1)$$

Larval survival was calculated from the number of viable eggs and the corresponding emergence rates for each botanical extract.

Data analysis

For the data analysis, we first verified normality using the Shapiro-Wilk test and homogeneity using the Levene test. When assumptions were satisfied, ANOVA was used to analyze the data, and means were compared using Tukey or Scott-Knott tests ($p < 0.05$). All statistical analyses were conducted using the R environment (R Core Team, 2026).

RESULTS

Significant differences in survival, control efficiency, adult emergence, developmental Time, and male emergence of *Z. subfasciatus* were observed among the different botanical extracts studied.

On the first and second days after spraying (DAS), only the *L. sibiricus*-based (Siberian motherwort) botanical extract differed significantly from the check (10.00 and 9.40 live insects), with means of 7.80 and 7.00 live insects, respectively, indicating its rapid insecticidal activity against *Z. subfasciatus*. In the evaluations conducted on days 3, 4, and 5 after spraying, no statistical differences were observed among the botanical extracts. After the final evaluation, the botanical extracts of *L. sibiricus* (6.88 insects), *A. muricata* (7.52 insects), and *A. sativum* (7.76 insects)

significantly reduced the number of live insects compared with the control (9.52 insects), demonstrating their potential for suppressing *Z. subfasciatus* populations under controlled conditions (Table 2). Across all evaluation days, the *L. sibiricus*-based extract consistently exhibited the highest efficacy in controlling *Z. subfasciatus* adults (Table 3).

Regarding the oviposition of *Z. subfasciatus* after spraying the different botanical extracts, no significant differences were observed in the mean number of viable eggs, nonviable eggs, or total eggs. The botanical extracts based on *D. ambrosioides* (11.20), *P. barbatus* (13.40), *P. nigrum* (14.00), *C. verbenacea* (15.40), *L. sibiricus* (16.00), and *R. graveolens* (17.60) showed a significant reduction in the number of emerged adults, reducing the population of *Z. subfasciatus* in the next generation (F1). The developmental Time of *Z. subfasciatus* varied significantly among treatments with different botanical extracts. The longest developmental period was observed with *A. muricata* (42.90 days), which differed significantly from all other treatments. In contrast, the shortest developmental Time occurred with *P. nigrum* (38.12). Intermediate values were recorded for the other species, ranging from 38.83 to 40.27 days, without significant differences from the check (39.39 days) (Table 4).

The number of emerged adults in *Z. subfasciatus* varied with the botanical extracts applied. Significant differences were observed for males, whereas no statistical difference was detected for females. The highest male adult emergence occurred in the check (14.60), *S. aromaticum* (14.60), *A. muricata* (13.40), and *S. rosmarinus* (12.80). In contrast, the extracts of *A. sativum* (10.00), *R. graveolens* (8.60), *L. sibiricus* (8.00), *C. verbenacea* (7.20), *P. barbatus* (7.20), *P. nigrum* (5.20), and *D. ambrosioides* (4.60) significantly reduced male adult emergence (Figure 1).

Larval survival of *Z. subfasciatus* was not significantly affected by the botanical extracts. Survival was lowest with *C. verbenacea* (30.52%) and highest with *S. aromaticum* (66.25%) (Figure 2).

Table 2. Mean Number ($\pm$ SE) of Adults of *Zabrotes subfasciatus* Life After Spraying the Different Botanical Extracts Under Laboratory Conditions.

Botanical Extracts	Mean of surviving insects ¹					Mean
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	
Check	10.00 $\pm$ 0.00 a	9.40 $\pm$ 0.24 ab	9.40 $\pm$ 0.24	9.40 $\pm$ 0.24	9.40 $\pm$ 0.24	9.52 $\pm$ 0.14 a
<i>Ruta graveolens</i>	9.40 $\pm$ 0.24 ab	9.20 $\pm$ 0.20 ab	9.20 $\pm$ 0.20	8.00 $\pm$ 0.84	8.00 $\pm$ 0.84	8.76 $\pm$ 0.31 ab
<i>Salvia rosmarinus</i>	10.00 $\pm$ 0.00 a	10.00 $\pm$ 0.00 a	8.20 $\pm$ 0.73	7.40 $\pm$ 0.75	7.20 $\pm$ 0.58	8.56 $\pm$ 0.19 ab
<i>Plectranthus barbatus</i>	8.80 $\pm$ 0.20 ab	8.80 $\pm$ 0.20 ab	7.80 $\pm$ 0.49	7.60 $\pm$ 0.40	7.40 $\pm$ 0.40	8.08 $\pm$ 0.12 abc
<i>Annona muricata</i>	8.80 $\pm$ 0.37 ab	7.60 $\pm$ 0.24 ab	7.40 $\pm$ 0.24	7.00 $\pm$ 0.32	6.80 $\pm$ 0.37	7.52 $\pm$ 0.14 bc
<i>Cordia verbenacea</i>	9.20 $\pm$ 0.37 ab	8.80 $\pm$ 0.58 ab	8.80 $\pm$ 0.58	8.00 $\pm$ 0.84	8.00 $\pm$ 0.84	8.56 $\pm$ 0.17 ab
<i>Dysphania ambrosioides</i>	9.60 $\pm$ 0.40 a	9.60 $\pm$ 0.40 a	8.40 $\pm$ 0.68	8.40 $\pm$ 0.68	8.40 $\pm$ 0.68	8.88 $\pm$ 0.24 ab
<i>Leonurus sibiricus</i>	7.80 $\pm$ 0.37 b	7.00 $\pm$ 0.71 b	6.80 $\pm$ 0.66	6.60 $\pm$ 0.51	6.20 $\pm$ 0.37	6.88 $\pm$ 0.29 c
<i>Syzygium aromaticum</i>	9.00 $\pm$ 0.55 ab	8.80 $\pm$ 0.58 ab	8.00 $\pm$ 0.63	8.00 $\pm$ 0.63	7.80 $\pm$ 0.66	8.32 $\pm$ 0.14 abc
<i>Piper nigrum</i>	9.80 $\pm$ 0.20 a	8.40 $\pm$ 0.51 a	8.00 $\pm$ 0.63	7.80 $\pm$ 0.73	7.80 $\pm$ 0.73	8.36 $\pm$ 0.06 abc
<i>Allium sativum</i>	8.80 $\pm$ 0.58 ab	7.80 $\pm$ 1.02 ab	7.40 $\pm$ 1.33	7.40 $\pm$ 1.33	7.40 $\pm$ 1.33	7.76 $\pm$ 0.17 bc
P	0.0019	0.0036	0.1897	0.4146	0.2000	< 0.0001

¹Mean followed by the same lower-case letter per column does not differ by the Tukey test ($p \leq 0.05$)**Table 3.** Control Efficiency (%) of Adults of *Zabrotes subfasciatus* After Spraying the Different Botanical Extracts Under Laboratory Conditions.

Botanical species	Control efficiency (%) ¹					Mean
	1 DAS	2 DAS	3 DAS	4 DAS	5 DAS	
Check	---	---	---	---	---	---
<i>Ruta graveolens</i>	6.00	2.13	2.13	14.89	14.89	7.98
<i>Salvia rosmarinus</i>	0.00	0.00	12.77	21.28	23.40	10.08
<i>Plectranthus barbatus</i>	12.00	6.38	17.02	19.15	21.28	15.13
<i>Annona muricata</i>	12.00	19.15	21.28	25.53	27.66	21.01
<i>Cordia verbenacea</i>	8.00	6.38	6.38	14.89	14.89	10.08
<i>Dysphania ambrosioides</i>	4.00	0.00	10.64	10.64	10.64	6.72
<i>Leonurus sibiricus</i>	22.00	25.53	27.66	29.79	34.04	27.73
<i>Syzygium aromaticum</i>	10.00	6.38	14.89	14.89	17.02	12.61
<i>Piper nigrum</i>	2.00	10.64	14.89	17.02	17.02	12.18
<i>Allium sativum</i>	12.00	17.02	21.28	21.28	21.28	18.49

¹Abbott's formula (Abbott, 1925) was used to calculate control efficiency

Table 4. Mean ($\pm$ SE) of Number of Viable Eggs, Unviable Eggs, Total Eggs, Adults Emergence, and Developmental Time (Days) of *Zabrotes subfasciatus* After Spraying the Different Botanical Extracts Under Laboratory Conditions.

Botanical species	Number of Viable Eggs ¹	Number of Unviable Eggs ¹	Total Number of Eggs ¹	Emerged Adults ²	Developmental Time (days) ¹
Check	51.00 $\pm$ 3.81	14.40 $\pm$ 1.81	65.40 $\pm$ 2.04	24.20 $\pm$ 1.80 a	39.39 $\pm$ 0.50 bc
<i>Ruta graveolens</i>	47.20 $\pm$ 5.54	13.80 $\pm$ 2.63	61.00 $\pm$ 7.48	17.60 $\pm$ 3.54 b	39.57 $\pm$ 0.34 bc
<i>Salvia rosmarinus</i>	52.40 $\pm$ 6.33	22.60 $\pm$ 3.87	75.00 $\pm$ 9.41	20.00 $\pm$ 4.82 a	38.83 $\pm$ 0.39 bc
<i>Plectranthus barbatus</i>	40.20 $\pm$ 6.72	16.20 $\pm$ 4.40	56.40 $\pm$ 9.76	13.40 $\pm$ 1.69 b	39.92 $\pm$ 0.26 bc
<i>Annona muricata</i>	39.80 $\pm$ 3.62	18.40 $\pm$ 2.06	58.20 $\pm$ 4.89	23.40 $\pm$ 2.58 a	42.90 $\pm$ 0.58 a
<i>Cordia verbenacea</i>	53.60 $\pm$ 7.28	24.00 $\pm$ 4.20	77.60 $\pm$ 11.07	15.40 $\pm$ 2.91 b	39.64 $\pm$ 0.36 bc
<i>Dysphania ambrosioides</i>	39.60 $\pm$ 5.05	13.80 $\pm$ 1.83	53.40 $\pm$ 5.46	11.20 $\pm$ 3.34 b	39.21 $\pm$ 0.48 bc
<i>Leonurus sibiricus</i>	27.20 $\pm$ 4.84	13.40 $\pm$ 1.21	40.60 $\pm$ 4.83	16.00 $\pm$ 4.43 b	39.47 $\pm$ 0.42 bc
<i>Syzygium aromaticum</i>	45.20 $\pm$ 4.44	21.00 $\pm$ 3.05	66.20 $\pm$ 5.95	28.40 $\pm$ 2.46 a	39.86 $\pm$ 0.22 bc
<i>Piper nigrum</i>	52.00 $\pm$ 6.57	12.00 $\pm$ 2.00	64.00 $\pm$ 7.29	14.00 $\pm$ 4.22 b	38.12 $\pm$ 0.31 c
<i>Allium sativum</i>	43.00 $\pm$ 8.50	17.80 $\pm$ 2.99	61.20 $\pm$ 11.04	21.60 $\pm$ 5.41 a	40.27 $\pm$ 0.34 b
<i>P</i>	0.0928	0.0647	0.1058	0.0263	< 0.0001

¹Mean followed by the same lower-case letter per column does not differ by the Tukey test ($p \leq 0.05$)²Mean followed by the same lower-case letter per column do not differ by the Skott-Knott test ($p \leq 0.05$). Data were transformed in $\sqrt{x + 1}$

DISCUSSION

This study's findings indicate that the evaluated botanical extracts affect the survival and biological traits of *Z. subfasciatus*, underscoring their potential for population management. These extracts can interact with the insect's body surface or respiratory system, penetrate the cuticle, and induce paralysis, mortality, or feeding inhibition.

Leonurus sibiricus, commonly called Siberian motherwort or honeyweed, has been used as a herbal medicine and as a culinary ingredient (Chua et al., 2005). Extracts of several Oriental medicinal plants were evaluated against four forest insect species and reported that extracts from *L. sibiricus* leaves exhibited both larvicidal and antifeeding activities against *Lymantria dispar* (L.) (Lepidoptera: Erebidæ) (Park et al.,

1997). These findings indicate that *L. sibiricus* contains bioactive constituents that affect insect survival and feeding behavior. In the present study, the aqueous extract of *L. sibiricus* significantly reduced the survival of adult *Z. subfasciatus*. Also, it reduced F1 adult emergence, although the magnitude of the observed effects was moderate. Our results demonstrated that this species produced the most rapid and pronounced effect on the survival and adult emergence of *Z. subfasciatus*. These effects are likely associated with the plant's diverse chemical constituents, including phenols, furanoditerpene lactones (Sayed et al., 2016), labdane diterpenes (Sayed et al., 2016), bis-spirolabdane compounds (Sayed et al., 2016), and labdane diterpenoids (Sayed et al., 2016), some of which are partially soluble in water and may contribute to insect mortality.

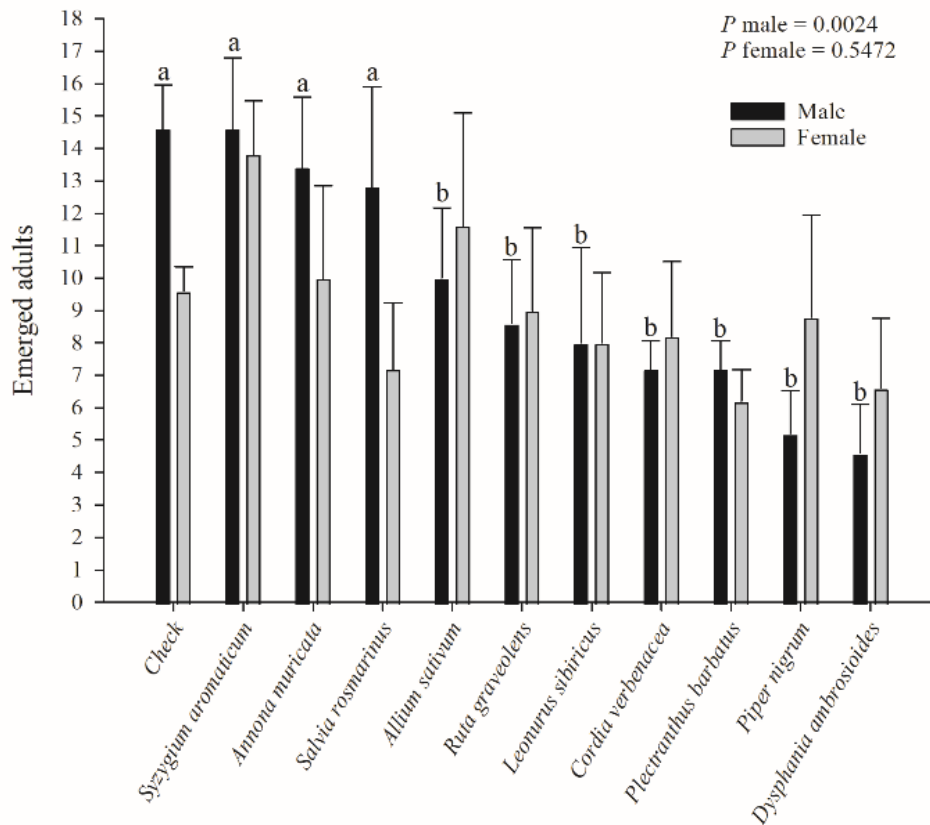


Figure 1. Mean ($\pm$ SE) of males and females of *Zabrotes subfasciatus* emerged after botanical extracts were sprayed under laboratory conditions.

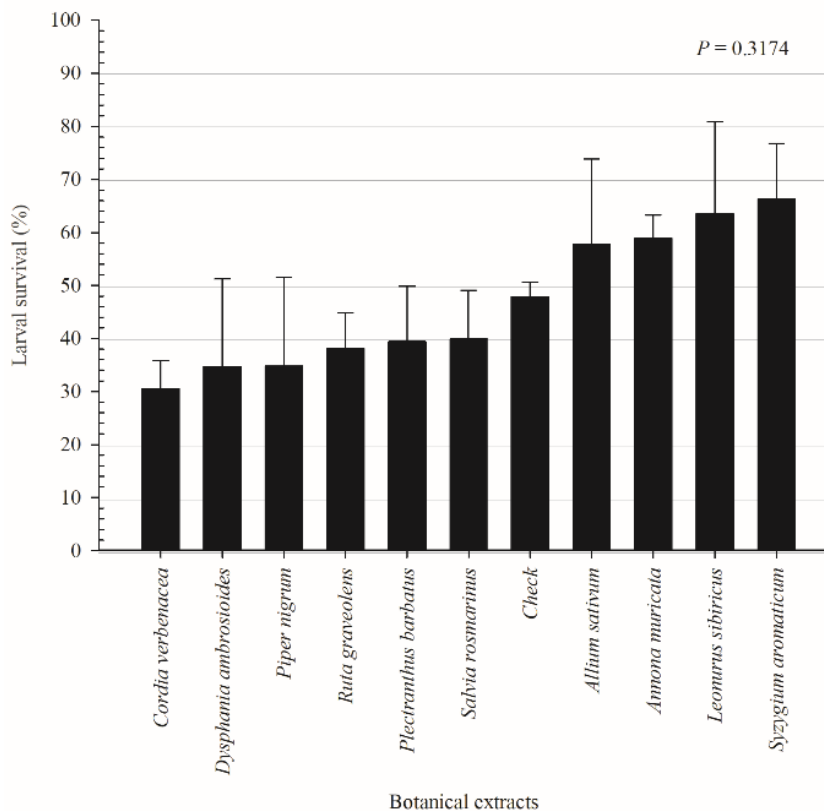


Figure 2. Mean ($\pm$ SE) of percentage of larval survival of *Zabrotes subfasciatus* after spraying with botanical extracts under laboratory conditions.

The biologically active components of *A. sativum* (garlic), which is distributed worldwide, include volatile organic sulfur compounds, polysaccharides, flavonoids, and pectin. Among these, allicin is the most powerful compound, demonstrating strong efficacy against a wide variety of pests (Cerny et al., 2013), including beetles, borers, termites, ants, whiteflies, aphids, caterpillars, armyworms, slugs, and storage pests. In a study using garlic extract diluted in acetone, 100% mortality occurred among adult cowpea weevils, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae), within four days across all tested concentrations (Al-Shareefi et al., 2025). Variations in mortality across studies may stem from differences in the extraction methods. Non-volatile constituents (e.g., alkaloids, tannins, flavonoids, phenolics, lignins, and coumarins) differ in their solubility in water and acetone, producing variable concentrations of active compounds (Ribeiro et al., 2023). Nevertheless, evaluating aqueous extracts is important because they offer a simple and accessible preparation method for small-scale producers who may not have access to organic solvents or specialized equipment.

The botanical extract from *A. muricata* (soursop or Brazilian paw-paw) also had a significant effect on survival and delayed the developmental Time of *Z. subfasciatus*. The use of *A. muricata* extracts has reportedly affected the mortality of stored-grain pests such as *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *C. maculatus* (Ribeiro et al., 2016; Obembe et al., 2025). It also damaged defoliating pests like *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) (Silva et al., 2024) and sucking pests such as *Bactericera cockerelli* Sulc (Hemiptera: Triozidae). Members of the Annonaceae family are known to contain acetogenins as their major secondary metabolites, showing biological activity against insects (Liaw et al., 2016). These compounds are present in the leaves, fruit pulp, roots, twigs, and branches, but occur predominantly in the seeds (Isman & Seffrin, 2014). Acetogenins inhibit complex I of the mitochondrial electron transport chain and the enzyme NADH (nicotinamide adenine dinucleotide) oxidase in the insect cell membrane, thereby disrupting mitochondrial electron transport and inducing apoptosis through ATP (adenosine triphosphate) depletion. (Tormo et al., 1999; Ribeiro et al., 2016). The findings highlight the significant insecticidal potential of *A. muricata*,

particularly due to acetogenins. Such attributes reinforce this plant as a promising candidate for developing sustainable botanical insecticides targeting stored-grain pests.

More than 120 phytochemical compounds have been identified in *R. graveolens* (common rue) (Oliva et al., 2003; Perera et al., 2017). In a study on *S. zeamais*, aqueous *R. graveolens* extracts contained higher levels of secondary metabolites than hexane, ethyl acetate, and methanol extracts. The compounds identified included alkaloids, saponins, flavonoids, tannins, terpenoids, anthraquinones, glycosides, coumarins, and phenols (Perera et al., 2017). These secondary metabolites were likely still present after applications were sprayed on bean grains, thereby impairing adult emergence following larval grain feeding.

Plectranthus barbatus (Indian coleus) leaves have been reported to contain secondary metabolites, including the allyl phenylpropene eugenol, the terpenoid α -pinene, and the sesquiterpene β -caryophyllene. These metabolites are toxic against the larvae of *Anopheles subpictus* Grassi (malaria mosquito), *Aedes albopictus* Skuse (Asian tiger mosquito), and *Culex tritaeniorhynchus* Giles (rice field mosquito) (Diptera: Culicidae) (Govindarajan et al., 2016). In bioassays with *Aedes aegypti* Linnaeus (Diptera: Culicidae) (yellow fever mosquito), Musau et al. (2016) evaluated the effects of *P. barbatus* leaf extracts prepared with water, acetone, and hexane on fourth-instar larvae. The authors reported no detectable LC₅₀ for the acetone extract, whereas the water and hexane extracts showed LC₅₀ values of 0.61 mg/mL and 1.053 mg/mL, respectively. Notably, the aqueous extract was approximately 1,700 times more toxic than the hexane extract. Other studies identified lectin and trypsin inhibitor proteins in *P. barbatus* extracts that inhibit larval digestive proteases and ultimately cause mortality (Napoleão et al., 2019; Almeida et al., 2021). Collectively, these findings highlight the bioactivity of *P. barbatus*, reinforcing its potential as a promising botanical resource for developing larvicidal agents.

Cordia verbenacea (Brazilian cordia) is a flowering shrub found along the Brazilian Atlantic Forest, the Brazilian coast, and low areas of the Amazon (Melo et al., 2021). This plant species has secondary metabolites with diverse pharmacological properties, including anti-inflammatory, anti-allergic, antibacterial, antiparasitic, and antitumoral effects (Michielin

et al., 2009; Martim et al., 2021; Melo et al., 2021; Pereira et al., 2021). In agriculture pests, the secondary metabolites obtained from *C. verbenacea* showed fumigant insecticidal activity, oviposition deterrence, and transgenerational effects against *C. maculatus* (Alves et al., 2015). However, hexane and ethanolic extracts from *C. verbenacea* did not cause lethal toxicity to *Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae) (sawtoothed grain beetle), *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) (lesser grain borer), or *S. zeamais* Motschulsky (maize weevil (Moreira et al., 2007).

Dysphania ambrosioides (wormseed) is well known for its diverse traditional medicinal properties and its richness in secondary metabolites. These compounds underpin its broad applications in managing plant pathogens (Zefzoufi et al., 2020) and pests (Dufera et al., 2019). Oils and extracts have been widely used in fields as foliar sprays and in storage systems as fumigants to protect food commodities from insect infestation (Pandey et al., 2014a, 2014b). In greenhouse trials, *D. ambrosioides* caused significant mortality of the citrus mealybug *Pseudococcus longispinus* Targioni Tozzetti (Hemiptera: Pseudococcidae) (Cloyd & Chiasson, 2007). The plant has also been reported to inhibit the growth and development of diamondback moth larvae, pupae, and adults, serving as a potent antifeedant against this species (Wei et al., 2015). Furthermore, the essential oil of *D. ambrosioides* has demonstrated marked biological and behavioral effects on *S. zeamais* (Chu et al., 2011; Langsi et al., 2018) and *C. maculatus* (Denloye et al., 2010). The proposed mechanisms underlying its insecticidal activity include inhibition of mitochondrial electron transport by disrupting hydrogen carriers (Liao et al., 2016), penetration of volatile compounds through the insect respiratory system leading to abnormal breathing, asphyxiation, and subsequent mortality (Paré & Tumlinson, 1999), as well as inhibition of acetylcholinesterase activity, resulting in nerve impulse blockage, paralysis, and ultimately death (Jyotshna et al., 2015).

The Piperaceae family is a rich source of bioactive phytochemicals with well-documented insecticidal properties and the most widely recognized compounds are derived from *Piper nigrum* and *P. guineense* Schum. & Thonn., and *P. tuberculatum* Jacq. Secondary metabolites from *Piper* species exhibit multiple modes of action, including contact toxicity

(Scott et al., 2005), synergistic interactions (Jensen et al., 2006), and repellent and antifeedant effects. Among these metabolites, the amide piperine is the predominant constituent in *P. nigrum* seeds and represents the compound most strongly associated with insecticidal activity. Consistent with previous findings, our study demonstrated that extracts of *P. nigrum* seeds effectively suppressed adult emergence from treated eggs. In addition, *P. nigrum* seed extracts have shown strong efficacy against several forest defoliators, including the eastern tent caterpillar *Malacosoma americanum* Fabricius, the forest tent caterpillar *Malacosoma disstria* Hübner (Lepidoptera: Lasiocampidae), the introduced pine sawfly *Diprion similis* Hartig, and the European pine sawfly *Neodiprion sertifer* Geoffroy (Hymenoptera: Diprionidae) (Scott et al., 2007).

In the context of grain storage longevity, the application of botanical extracts such as *A. sativum*, *R. graveolens*, *L. sibiricus*, *C. verbenaceae*, *P. barbatus*, *P. nigrum*, and *D. ambrosioides* can reduce the proportion of males in insect populations. These extracts may impair the reproductive success of the F1 generation, as a portion of the females will remain unfertilized. Such an imbalance reduces offspring production in the subsequent F2 generation, thereby directly limiting the population growth rate. Furthermore, the resulting reduction in effective population size leads to a loss of genetic variability, increased inbreeding, and greater susceptibility to adverse environmental factors, all of which may accelerate demographic decline and jeopardize population persistence over the medium and long term. (Engen et al., 2007; Compton and Tu, 2022).

These findings indicate that the extracts of *R. graveolens*, *P. barbatus*, *A. muricata*, *C. verbenacea*, *D. ambrosioides*, *L. sibiricus*, *P. nigrum*, and *A. sativum* have potential as botanical insecticides against *Z. subfasciatus* and could be part of an Integrated Pest Management (IPM) program. However, multiple environments and large-scale trials are required to prove the efficacy of these plant extracts in controlling pests under natural conditions.

CONCLUSIONS

A. muricata, *L. sibiricus*, and *A. sativum* significantly reduced adult survival compared with the control, while *R. graveolens*, *P.*

barbatus, *C. verbenacea*, *D. ambrosioides*, *L. sibiricus*, and *P. nigrum* effectively suppress adult emergence. Notably, *A. muricata* also prolonged developmental Time, and several plant species, including *R. graveolens*, *P. barbatus*, *C. verbenacea*, *D. ambrosioides*, *L. sibiricus*, *P. nigrum*, and *A. sativum*, significantly reduced male production, which can have profound implications on population dynamics. Collectively, these results highlight the multifaceted potential of botanical extracts to disrupt insect life-history traits at multiple levels, reinforcing their promise as sustainable alternatives to synthetic insecticides. Such outcomes provide a solid foundation for developing eco-friendly biopesticides that could be integrated into pest management programs, reducing the reliance on chemical controls and mitigating risks to human health and environmental health.

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