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RESEARCH ARTICLE

Effectiveness of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) on Parthenium weed (*Parthenium hysterophorus*) under greenhouse condition in Ethiopia

[version 1; peer review: 1 not approved]

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Abstract

Background

Parthenium is an invasive annual weed that affects crop production, rangelands, grasslands, animals, the environment, and human health. Since its introduction into Ethiopia, different management options such as uprooting, slashing, burning, plowing, and applying synthetic herbicides have been practiced. However, these options are not sustainable in weed management. However, *Zygogramma bicolorata* is known to be used in different countries. Although many countries use it under field conditions, its population-based efficacy has not been well studied in Ethiopia. This study aimed to determine the effectiveness of *Z. bicolorata* populations on parthenium plants under greenhouse conditions.

Methods

This experiment was conducted at the Ambo Agricultural Research Center. Different numbers of *Z. bicolorata* per plant were used as treatments and were laid out in a completely randomized design with three replications. The numbers of damaged leaves, eggs, and larvae were recorded daily until the leaves were completely defoliated. The collected data and the correlation between beetle population and leaf defoliation were analyzed using SAS.

Results

Open Peer Review

Approval Status

1

version 1

09 May 2024

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1. **Bhupendra Kumar**, Banaras Hindu

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The result revealed that all beetle populations caused damage to weed plants. A higher population of beetles completely defoliated the leaves within 12 days. However, lower numbers required 21 days to completely defoliate the weed. In contrast, a higher number of eggs (328.67) and larvae (272.33) were obtained from plants that received 20 pairs of beetles. The beetle population and exposure time were positively correlated, and the effectiveness of the bioagent was greater following the emergence of larvae.

Conclusions

Z. bicolorata can manage parthenium weeds, even with a lower population. However, more than 15 pairs of beetles could be suggested to manage weeds in a shorter time, and further study is needed under field conditions by considering the different agroecologies of the country.

Keywords

Beetles, Correlation, Exposure time, Larvae, Leaves defoliation



This article is included in the **Agriculture, Food and Nutrition** gateway.

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Introduction

Parthenium (*Parthenium hysterophorus* L.) is an annual herb that colonizes disturbed areas and is one of the most invasive weed species worldwide.^{1,2} It is believed that this weed is native to tropical America and is later distributed to many countries around the world. The CABI¹ distribution map indicates that weeds are reported in more than 147 countries globally. *Parthenium* weeds can adapt and grow in diverse agroecologies and soil types. These features have enhanced their distribution and adaptability across the world.^{1,3} This weed was first observed in Ethiopia at the Alemaya University of Agriculture (now Haramaya University) in 1968 and in the Dire Dawa area in 1988.^{4,5}

Parthenium weeds are known to affect crop and forage production and productivity, biodiversity, ecosystems, the environment, and animal health.^{5–7} For example, in India, the production of different crops and forage was reduced by 40 and 90%, respectively.⁸ The study showed that *parthenium* weed populations reduced plant species abundance in the Ginir District of Ethiopia.⁹

Different management options, such as manual, mechanical, cultural, chemical, and biological control, have been practiced to control *parthenium* weeds around the globe. However, it is difficult to control weeds using a single management strategy. This is because uprooting causes allergy, and cultural control leads to high weed seed production. This necessitates the use of integrated management options to reduce the impact of weeds. Between the 1970s and the 1980s, more than 250 species of herbivore arthropods were recorded on weeds in Northern America.¹⁰ Among them, the leaf-feeding beetle *Zygogramma bicolorata* was introduced to Australia, India, South Africa, and Ethiopia in 1980, 1984, 2005, and 2007, respectively. The beetle is known to be effective in different countries, feeding on weed leaves and flowers and reducing height.¹¹ The beetle effectively reduced weeds in both dry and wet seasons at 1.2 beetles/m², and is recommended as a management option for the weed in Tanzania. Similarly, five pairs of beetles per plant at an early stage in India, 15.2 + 5.43 adults, and 114.4 + 38.48 larvae/m² were suggested in Australia.^{12–14} This shows that the population of beetles and their effectiveness are highly dependent on the agro-ecology and stages of the weed plant. However, in Ethiopia, the effective beetle population has not been clearly studied. Therefore, this study was conducted to determine the effectiveness of *Z. bicolorata* beetle populations on *parthenium* plants under greenhouse conditions.

Methods

Ethics

This experiment was conducted within the appropriate ethical framework of the Ethiopian Institute of Agricultural Research, as confirmed by an institutional letter in Ref. No.: 23.11/660/2023 written on **06 October 2023**.

Raising of *parthenium* seedlings

The experiment was conducted at the Ambo Agricultural Research Center, Entomology greenhouse. *Parthenium* seeds were sown on seedling beds, and seedlings were transferred at the stage of 12 leaves to pots (18 × 20 cm dimensions) (Figure 1A). The soil used in the pot was a combination of sand, compost, and clay loam, at a ratio of 1:1:2, respectively. Seedlings of the same stage, with 18–24 leaves, were transferred to the experimental cage and used for the experiment (Figure 1B). The plants were watered daily to provide free moisture for adult bioagents, and the leaves were sprayed with



Figure 1. *Parthenium* seedlings raised (A), Seedling transferred into cage (B), and Bioagents applied on plant (C).

a sodium hypochlorite (Bleach, product of the Ghion Industrial and Chemical PLC., Addis Ababa, Ethiopia) solution to prevent disease development. Because sodium hypochlorite is the most often used disinfectant, it is a broad-spectrum disinfectant that is efficient against viruses, bacteria, fungi, and mycobacterium. The sodium hypochlorite was mixed with sterilized distilled water at 2% of the solution by using a 1-liter hand sprayer.

Rearing of *Zygogramma bicolorata*

The beetles were reared in an insect-rearing cage, which was constructed using metal and nylon mesh as a frame and cover, respectively. The size of each cage was $0.5 \times 0.5 \times 1$ m. Twenty-five pairs of beetles were released from the parthenium seedlings in the rearing cage. After two to three days of introduction and the plants were checked for oviposition, the adults were transferred to another rearing cage. The deposited eggs were monitored daily until they reached the adult stage. Within two days, pairs of emerged adults were transferred to the experimental cages (Figure 1C). Beetle rearing and experiments were conducted in a greenhouse.

Experimental treatments arrangement and data collection

A completely randomized design with three replicates was used for the experiment. Six treatments, viz., two pairs, five pairs, ten pairs, 15 pairs, and 20 pairs of *Z. bicolorata* adults per parthenium seedling, and a control without the beetle, were used. The number of leaves per plant was counted before the introduction of the beetles and at the end of the experiment. Similarly, the number of eggs deposited, hatched larvae, and damaged leaves were counted, and plant damage was measured on a scale of 0–100% daily.¹⁵

Data analysis

The collected data were checked for normal distribution prior to data analysis. Data on the number of eggs deposited, hatched larvae, and damaged leaves were analyzed by ANOVA, with the means separated by the least significant difference (LSD) using SAS version 9.4.¹⁶ Additionally, a correlation analysis was performed between the independent variable (exposure day) and the dependent variable (plant leaf damage).

Results and discussion

Number of eggs deposited per plant

Adult beetles released on parthenium plants deposited eggs from the first days of introduction. A lower egg number (71.67) was recorded from the plants treated with two pairs, while a higher egg number (328.67) was deposited from the 20 pairs used (Figure 2).

Number of larvae emerged per plant

The larvae of *Z. bicolorata* began to emerge after seven days of exposure. Similar to eggs, *Z. bicolorata* larvae were recorded on all parthenium plants treated with beetles. The data showed that the number of larvae that hatched increased with the population of beetles applied (Figure 3). The number of hatched larvae per plant treated with 20 pairs of beetles exceeds 75.89%, 57.53%, 35.13%, and 19.58% of the number of plants treated with fifteen, ten, five, and two pairs, respectively.

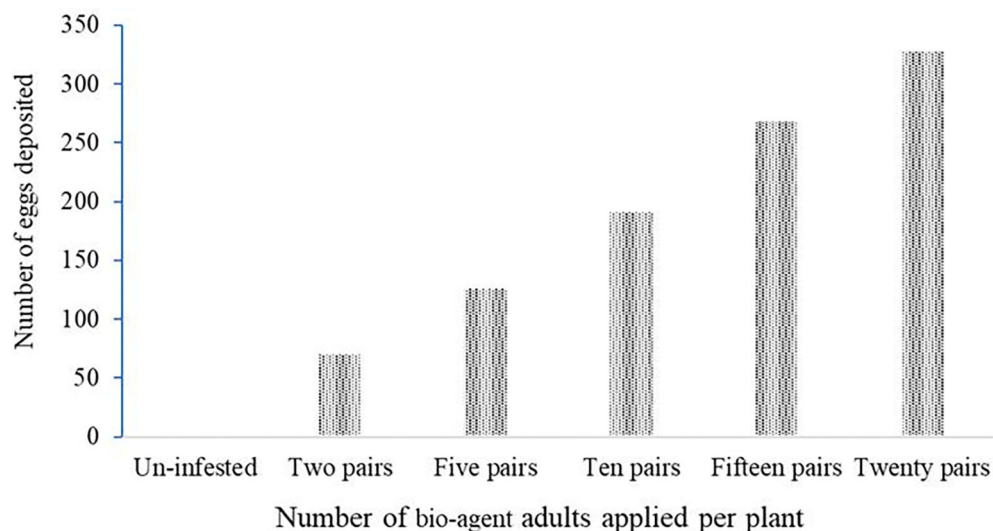


Figure 2. *Zygogramma bicolorata* eggs deposited per plant.

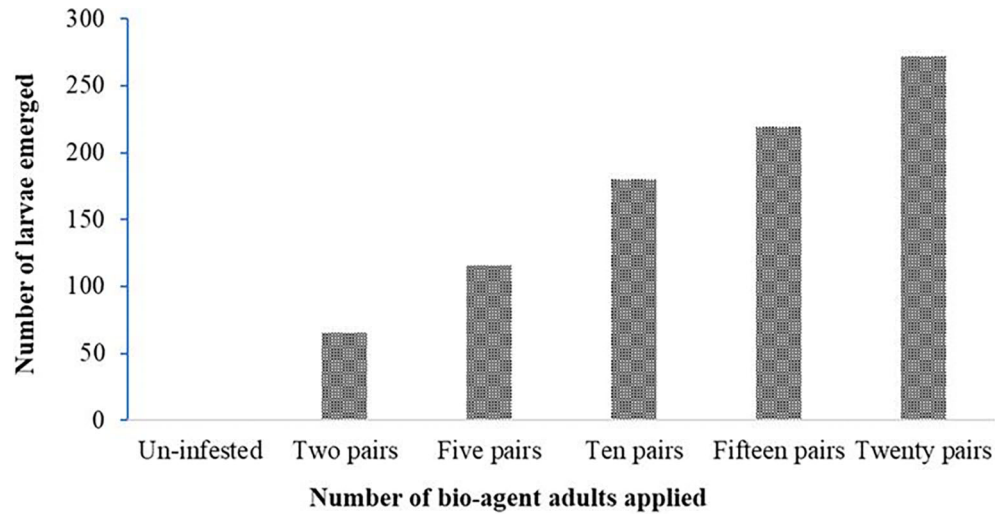


Figure 3. *Zygogramma bicolorata* hatched larvae per plant.

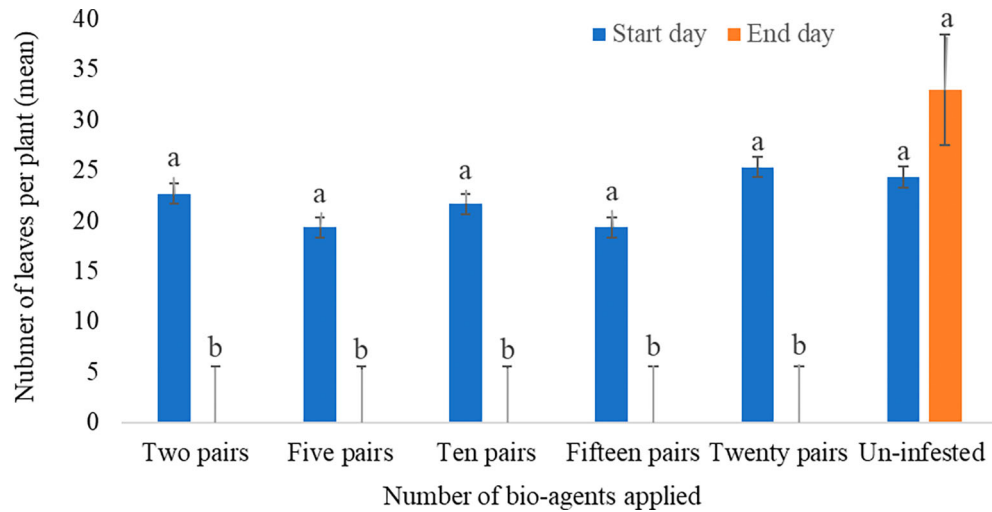


Figure 4. Number of leaves per plant at initial and final date of the experiment. Note: - indicates significant difference among population of beetles.

Effect of *Z. bicolorata* population on the leaf damage of parthenium weed

The number of leaves per plant did not vary during the initial period among the treatments used (Figure 4). However, based on the number of released beetles, leaf defoliation varied among treatments ($P < 0.001$) (Figure 5). Three days after release, the 20 pairs defoliated 53% of the leaves, which is a significant leaf defoliation, whereas no visible defoliation

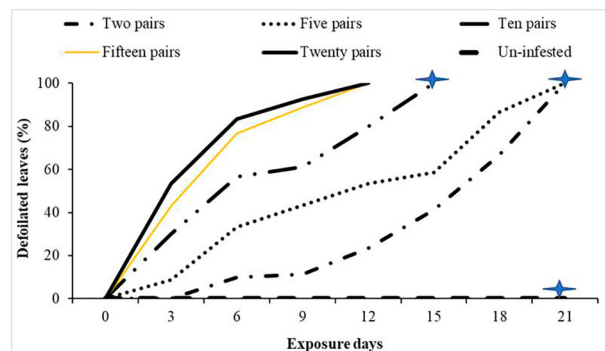


Figure 5. Parthenium leaves defoliated across exposure time (21 days).

Table 1. Relationships between bioagent populations applied and exposure time.

Pearson correlations	Population of beetles	Leaves defoliation	Date of complete leaves defoliation
Population of beetles	-		
Leaves defoliation	0.944**	-	
Date of complete leaves defoliation	-0.952*	-0.946*	-

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

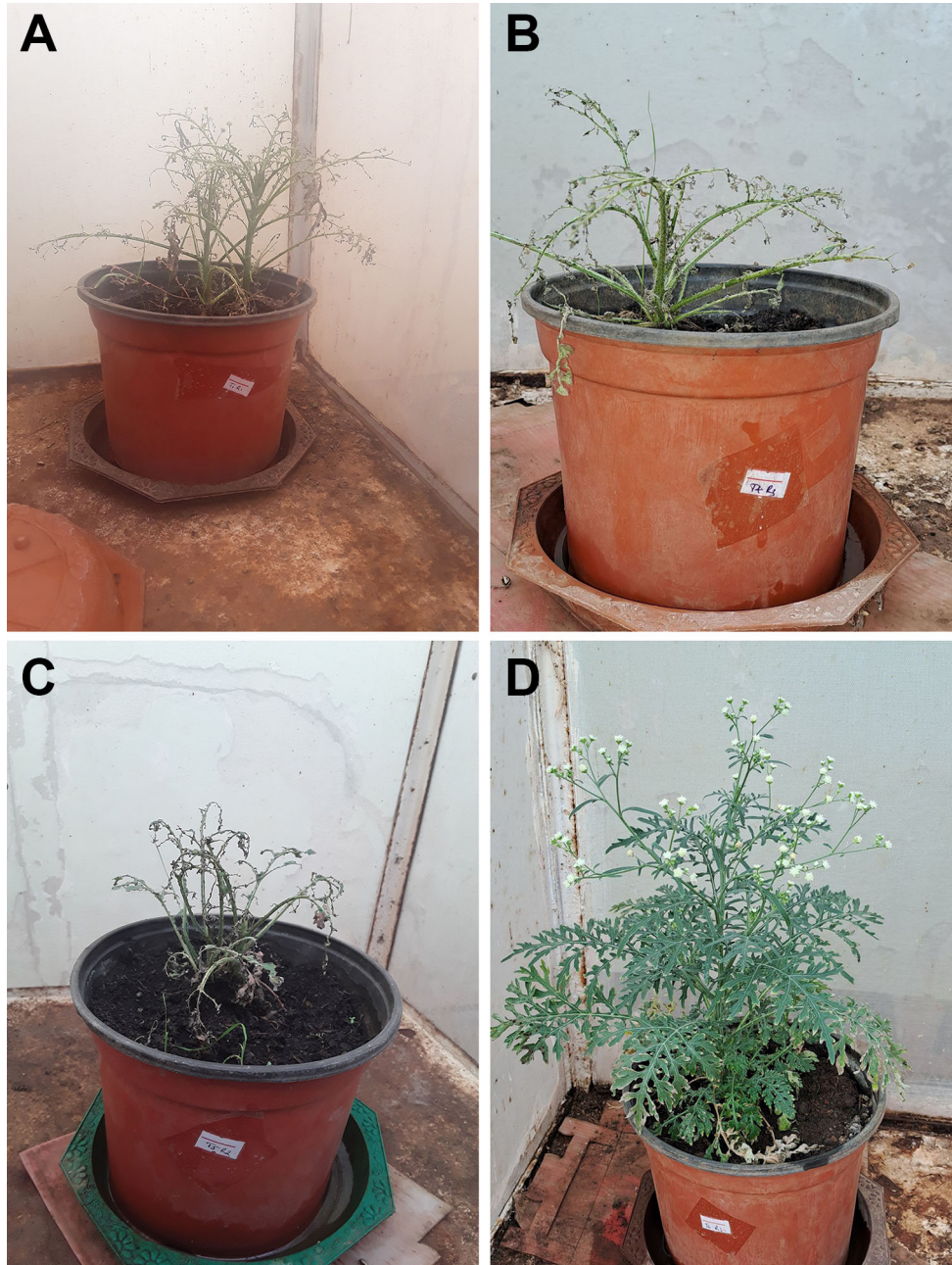


Figure 6. Parthenium leaves defoliated by bioagents (A-C), and Parthenium plant continued growth at final date of the experiment (control, bioagents not released to plant) (D).

was observed under two pairs of beetles. After six days of release, no significant leaf defoliation was recorded under the fifteen and twenty-pairs of beetles, and complete defoliation was recorded on the twelfth day. Similarly, ten pairs recorded complete leaf defoliation after 15 days of beetle release. At the first day after release, all pairs defoliated 100% of the leaves. The higher population of beetles (20 and 15 pairs) completely defoliated within 1.75 folds shorter time compared with two and five pairs of beetles. Additionally, correlation analysis indicated that the number of beetle populations was highly positively correlated with leaf defoliation ($r = 0.944$) ($p < 0.01$) (Table 1).

This study indicated that all released pairs of beetle adults defoliated the parthenium plant leaves. The shortest time (12 d) for complete leaf defoliation was recorded for 20 and 15 pairs, whereas the longest (21 d) was recorded for plants treated with two and five pairs. This implies that beetle populations per plant are important for the effective management of weeds, and increasing the population of beetles minimizes the number of days needed to achieve complete leaf defoliation. This result is in line with the results of Shabbir *et al.*'s¹⁷ and Kanagwa *et al.*'s,¹¹ who reported that beetles had defoliated leaves up to 100% and reduced weed performance within 28 days. Additionally, the defoliated leaves increased as the exposure time increased, and the beetles effectively controlled the weed and significantly reduced its vegetative and reproductive stages in Australia, India, Tanzania, and South Africa.^{11,18,19} Parthenium leaf consumption increased rapidly from 14 to 28 days by 115%, 112%, and 100% at populations of 0.4, 0.8, and 1.2 beetles per plant, respectively, in Tanzania.¹¹ In Tanzania, 1.2 beetles per plant (which was used as a higher population) caused 100% leaf defoliation after 28 days of exposure in the wet season, whereas in this experiment, two pairs per plant recorded 100% leaf defoliation within 21 days. This indicates that the beetle population is negatively correlated with the date of complete leaf defoliation in the plant. Similarly, Chandravanshi *et al.*¹⁴ reported that a higher population (five pairs) of beetles recorded more severe damage to leaves within a shorter time than lower populations (two pairs). This result agreed with that of Hasan *et al.*,¹⁹ who showed that damage from weeds was positively related to the exposure time.

After larval emergence, the leaves of weed plants were effectively defoliated. This indicates that the larvae play an important role in weed control. In contrast, the collaboration between adults and larvae ensured that the leaf of the weed was quickly eaten and growth was retarded. Similarly, Mohapatra *et al.*²⁰ reported that the larvae have the capacity to feed more on parthenium weed than adult females and recommended it as a better management option for the weed. Additionally, in Bangalore (India), larvae were fed the weed effectively and suppressed their growth and flower production, causing them to dry up completely.¹⁸ This implies that the bioagent is florivorous in nature at both the larval and adult stages and is a voracious defoliator of the weed, which enhances its susceptibility to biotic factors.^{21,22} It was understood that the plants that were not infested by the beetles continued and flourished in their growth stages, while others dried up (Figure 6A-D).

Conclusion

A higher population of *Z. bicolorata* (20 pairs of adult beetles) resulted in the production of more eggs and larvae on the treated parthenium plant. This increases the chances of effective feeding of plant leaves during the emergence of larvae. However, the use of a higher population of *Z. bicolorata* resulted in complete defoliation in a short period of time (12 days), whereas a lower population achieved the same result after 21 days. Therefore, to manage weeds with urgency before reaching the reproductive stage, using 15 or more pairs of beetles would be an advantage. This is because it achieves complete leaf defoliation within a shorter time before the plant reaches reproductive stages and minimizes its seed bank for future generations. Since this experiment was conducted in a greenhouse, the results need to be studied under field conditions to determine their establishment and effectiveness in the diverse agroecology of Ethiopia.

Ethics

This experiment was conducted within the appropriate ethical framework of the Ethiopian Institute of Agricultural Research, as confirmed by an institutional letter in Ref. No.: 23.11/660/2023 written on 06 October 2023.

Data availability statement

Underlying data

Figshare – underlying data for “Effectiveness of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) on Parthenium weed (*Parthenium hysterophorus*) under greenhouse condition in Ethiopia”, <https://doi.org/10.6084/m9.figshare.25183436.v1>.²³

This project contains following data:

- Parthenium bioagents dataset

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0)

Acknowledgement

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Version 1

Reviewer Report 22 November 2025

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Bhupendra Kumar

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In the manuscript titled "*Effectiveness of Zygommatra bicolorata Pallister (Coleoptera: Chrysomelidae) on Parthenium weed (Parthenium hysterophorus) under greenhouse condition in Ethiopia*," the authors aim to evaluate the effectiveness of *Z. bicolorata* populations on parthenium plants under greenhouse conditions. Although the topic is interesting and relevant, the manuscript requires substantial revision, particularly in its organization, methodology, and overall presentation. My specific comments and suggestions are as follows:

1. **Introduction:** The authors do not clearly state the hypotheses guiding the study. Additionally, the expected outcomes or predicted effects of *Z. bicolorata* on parthenium weed are not addressed.
2. **Materials and Methods:** Important methodological details are missing. The authors should specify the temperature, humidity, and photoperiod conditions of the greenhouse in which the beetles were reared. Information on the source and collection date of the beetles is also needed. The sample size is unclear, as is the number of mating pairs released. The duration of the experiments and the seasons during which they were conducted should likewise be clearly stated.
3. **Statistical Analysis:** The authors must indicate which statistical tests were used to assess data normality. They should also specify the type of ANOVA performed (one-way, two-way, or MANOVA) and clearly identify the dependent and independent variables. The statistical methods used to analyze percentage data should be stated, and the type of correlation analysis applied between dependent and independent variables needs clarification.
4. **Results:** The presentation of results is incomplete. ANOVA outputs (F-values, p-values, degrees of freedom) are missing. Additionally, the percentage data have not been statistically compared. Figure captions should be expanded to provide clearer explanations.
5. **Discussion:** The discussion does not adequately interpret the findings in relation to recent literature. A more thorough and updated comparison with existing studies is needed.
6. **References:** The reference list requires more recent and relevant sources to strengthen the scientific context of the study.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Entomology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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