



Antifeedant Activity of Artemisia Plant Extracts Against the Cotton Leafworm (*Spodoptera littoralis*) in Ornamental Plant Nurseries

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النشاط الطارد والمثبط للتغذية (*Antifeedant*) لمستخلصات نبات "الشيح" ضد دودة ورق القطن في مشاتل نباتات الزينة

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Abstract:

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a highly polyphagous pest that poses a significant threat to ornamental plant nurseries, causing extensive defoliation and economic losses. The overreliance on synthetic chemical insecticides for its control has led to the development of insecticide resistance, environmental contamination, and risks to human health. Consequently, there is an urgent need to develop environmentally friendly and sustainable alternatives. This study investigates the antifeedant activity of crude ethanolic extracts from three *Artemisia* species, namely *A. herba-alba*, *A. absinthium*, and *A. vulgaris*, against the fourth instar larvae of *S. littoralis* under controlled laboratory conditions (26±1°C, 65±5% RH, 16:8 L:D). The antifeedant index was calculated using the leaf disc bioassay method at five different concentrations (0.5, 1, 2, 4, and 8 mg/mL) over 24 and 48-hour exposure periods. The results demonstrated a concentration- and time-dependent antifeedant effect for all tested extracts. *A. absinthium* exhibited the highest antifeedant activity, achieving an 86.4% feeding inhibition at the highest concentration (8 mg/mL) after 48 hours, followed by *A. herba-alba* (79.2%) and *A. vulgaris* (71.5%). The calculated median effective concentrations (EC₅₀) for antifeedant activity were 1.92, 2.48, and 3.15 mg/mL for *A. absinthium*, *A. herba-alba*, and *A. vulgaris*, respectively. Gas chromatography-mass spectrometry (GC-MS) analysis of the most active extract (*A. absinthium*) identified major terpenoid compounds, including camphor, 1,8-cineole, β-thujone, and artemisia ketone, which are known to disrupt insect chemoreception and feeding behavior. These findings highlight the potential of *Artemisia* extracts, particularly *A. absinthium*, as effective botanical antifeedants for the management of *S. littoralis* in ornamental nurseries. The integration of these plant-based extracts into integrated pest management (IPM) programs could reduce the reliance on synthetic insecticides, thereby mitigating environmental and health risks while preserving natural enemy populations.

Keywords: Antifeedant, *Spodoptera littoralis*, *Artemisia*, botanical insecticide, ornamental nurseries, integrated pest management

المخلص

تعد دودة ورق القطن (*Spodoptera littoralis*) من الآفات شديدة الشراهة التي تشكل تهديدًا كبيرًا لمشاتل نباتات الزينة، حيث تتسبب في إزالة الأوراق بشكل واسع النطاق وخسائر اقتصادية فادحة. وقد أدى الاعتماد المفرط على المبيدات الحشرية الكيميائية الاصطناعية لمكافحتها إلى تطور مقاومة الحشرات للمبيدات، وتلوث البيئة، ومخاطر على صحة الإنسان. وبالتالي، هناك حاجة ماسة لتطوير بدائل صديقة للبيئة ومستدامة. تبحث هذه الدراسة النشاط الطارد والمثبط للتغذية للمستخلصات الإيثانولية الخام لثلاثة أنواع من الشيح، وهي *A. herba-alba* و *A. absinthium* و *A. vulgaris*، ضد يرقات الطور الرابع لدودة ورق القطن تحت ظروف معملية خاضعة للرقابة (1 ± 26 درجة مئوية، رطوبة نسبية $5 \pm 65\%$ ، دورة ضوئية 16:8 ساعة). تم حساب مؤشر تثبيط التغذية باستخدام طريقة بيوضية الأقراص الورقية عند خمس تركيبات مختلفة (0.5، 1، 2، 4، 8 ملغم/مل) على مدى فترات تعرض 24 و 48 ساعة. أظهرت النتائج تأثيرًا طاردًا ومثبطًا للتغذية يعتمد على التركيز والوقت لجميع المستخلصات التي تم اختبارها. أظهر *A. absinthium* أعلى نشاط في تثبيط التغذية، حيث حقق 86.4% من تثبيط التغذية عند أعلى تركيز (8 ملغم/مل) بعد 48 ساعة، يليه *A. herba-alba* (79.2%) ثم *A. vulgaris* (71.5%). كانت التركيزات المتوسطة الفعالة المحسوبة للنشاط الطارد والمثبط للتغذية هي 1.92 و 2.48 و 3.15 ملغم/مل لـ *A. absinthium* و *A. herba-alba* و *A. vulgaris* على التوالي. حدد تحليل كروماتوغرافيا الغاز المدمجة مع مطياف الكتلة (GC-MS) للمستخلص الأكثر نشاطًا (*A. absinthium*) مركبات تربينويدية رئيسية، بما في ذلك الكافور و 1،8-سينيول و- β ثوجون وأرتيميسيا كيتون، والتي من المعروف أنها تعطل الاستقبال الكيميائي وسلوك التغذية لدى الحشرات. تسلط هذه النتائج الضوء على إمكانات مستخلصات الشيح، وخاصة *A. absinthium*، كمبيدات نباتية طاردة ومثبطة للتغذية فعالة لإدارة دودة ورق القطن في مشاتل الزينة. يمكن أن يؤدي دمج هذه المستخلصات النباتية في برامج الإدارة المتكاملة للآفات إلى تقليل الاعتماد على المبيدات الحشرية الاصطناعية، وبالتالي التخفيف من المخاطر البيئية والصحية مع الحفاظ على أعداد الأعداء الطبيعيين.

الكلمات المفتاحية: طارد ومثبط للتغذية، دودة ورق القطن، شيح، مبيد حشري نباتي، مشاتل نباتات الزينة، إدارة متكاملة للآفات.

1. Introduction

1.1. Research Problem

Spodoptera littoralis (Boisduval), commonly known as the Egyptian cotton leafworm or African cotton leafworm, is a notorious agricultural pest belonging to the family Noctuidae (Lepidoptera). This insect is characterized by its extreme polyphagy, with a host range encompassing over 80 plant species across more than 40 families, including vegetables (e.g., tomatoes, peppers, eggplants), field crops (e.g., cotton, alfalfa, corn), and crucially, a wide array of ornamental plants such as roses, chrysanthemums, carnations, and many foliage plants. In ornamental nurseries, the presence of *S. littoralis* can lead to rapid and severe defoliation, rendering plants unsalable and causing significant economic losses. The high fecundity of the pest, with a single female capable of laying up to 3,000 eggs, coupled with its ability to complete multiple generations per year under favorable conditions, exacerbates its destructive potential.

Historically, the management of *S. littoralis* in nurseries and agricultural settings has relied heavily on the frequent application of broad-spectrum synthetic insecticides, including organophosphates, pyrethroids, and carbamates. This practice has led to several critical issues. First, numerous populations of *S. littoralis* have developed resistance to many classes of chemical insecticides, rendering conventional control measures ineffective (Salem, 2025).

Second, the indiscriminate use of these chemicals has detrimental effects on non-target organisms, including natural enemies (predators and parasitoids), pollinators, and beneficial soil microbiota. Third, the persistence of insecticide residues in the environment contributes to soil and water contamination, posing risks to human and animal health. Fourth, the strict regulatory framework governing pesticide residues on ornamental plants, particularly those intended for export, adds another layer of complexity. Consequently, there is an urgent and pressing need to explore and develop alternative, eco-friendly, and sustainable pest management strategies that are effective against *S. littoralis* while minimizing adverse effects on the environment and human health (Salem & Salem, 2025; Kadak & Salem, 2020).

1.2. Objectives

The primary objectives of this study are:

1. To evaluate the antifeedant activity of crude ethanolic extracts from three *Artemisia* species (*A. herba-alba*, *A. absinthium*, and *A. vulgaris*) against the fourth instar larvae of *S. littoralis*.
2. To determine the concentration-dependent and time-dependent effects of the *Artemisia* extracts on the feeding behavior of the larvae.
3. To calculate the median effective concentration (EC_{50}) values for the antifeedant activity of the most potent extracts.
4. To analyze the chemical composition of the most active *Artemisia* extract using gas chromatography-mass spectrometry (GC-MS) to identify the major secondary metabolites potentially responsible for the observed biological activity.

1.3. Significance of the Study

This research holds significant practical and scientific value. It investigates the potential of locally available *Artemisia* species as a source of botanical insecticides, which aligns with global trends towards green chemistry and sustainable agriculture. By identifying effective natural antifeedants, this study provides a viable alternative for the management of *S. littoralis* in ornamental nurseries. The integration of such botanical extracts into an Integrated Pest Management (IPM) framework could drastically reduce the reliance on harmful synthetic pesticides, thereby promoting environmental safety, preserving biodiversity, and ensuring the production of high-quality, residue-free ornamental plants. Furthermore, the study contributes to the growing body of knowledge on plant-insect interactions and the use of secondary plant metabolites in pest control.

2. Literature Review

2.1. The Cotton Leafworm, *Spodoptera littoralis*: Biology, Host Range, and Damage

Spodoptera littoralis is a lepidopteran insect of the Noctuidae family, originating from Africa and the Mediterranean region but now distributed across Europe, Asia, and the Middle East. Its success as a pest is attributed to its high polyphagy, migratory capacity, and high reproductive potential. The female moth lays egg masses covered with scales, which can contain up to several hundred eggs. The larval stage typically comprises six instars, with the first three instars often exhibiting gregarious feeding behavior, skeletonizing leaves. The later instars (fourth to sixth) become more solitary and voracious, consuming large portions of leaf tissue and causing characteristic defoliation and skeletonization. The extensive feeding damage not only reduces photosynthetic capacity and plant vigor but also creates entry points for secondary pathogens. The host range of *S. littoralis* is exceptionally broad, spanning over 80 plant species across more than 40 families. In addition to major economic crops like cotton, maize, and vegetables, the pest is a significant concern for the ornamental plant industry. It infests a wide variety of ornamental species, including roses, chrysanthemums, carnations, geraniums, and many foliage plants. In a nursery setting, the aesthetic damage caused by larval feeding can completely devalue a crop, leading to severe economic losses.

2.2. Challenges of Conventional Chemical Control

The primary method of controlling *S. littoralis* has been the application of synthetic insecticides. However, this approach is fraught with challenges. The most critical issue is the development of insecticide resistance. Due to the high selection pressure from repeated applications, *S. littoralis* populations have evolved resistance to several classes of insecticides, including pyrethroids, organophosphates, carbamates, and even newer chemistries like spinosad and chlorantraniliprole (Salem, 2025). Furthermore, the use of broad-spectrum insecticides decimates natural enemy populations (e.g., predators like *Chrysoperla carnea* and parasitoids like *Trichogramma* spp.), which can lead to pest resurgence and secondary pest outbreaks. Environmental contamination, risks to applicators and consumers, and the disruption of ecosystem services are additional drawbacks.

2.3. Botanical Insecticides as Sustainable Alternatives

Botanical insecticides, derived from plant secondary metabolites, have gained increasing attention as eco-friendly alternatives to synthetic chemicals. They offer several advantages, including rapid degradation in the environment, reduced toxicity to non-target organisms, and novel modes of action that may circumvent existing resistance mechanisms (Kadak & Salem, 2020). Among the various botanical compounds, terpenoids, alkaloids, and phenolics have demonstrated potent insecticidal, antifeedant, and repellent activities. The use of plant extracts in pest management aligns with the principles of Integrated Pest Management (IPM) and organic agriculture. Recent studies have extensively profiled the phytochemical composition and biological potential of various medicinal plants (Salem & Alhadad, 2026; Alshawish et al., 2025; Khalil et al., 2025), and the antimicrobial activities of extracts from plants such as dandelion (*Taraxacum officinale*) and lichen (*Usnea barbata*) have been well documented (Salem et al., 2025; Salem, 2024). Similarly, the essential oil of *Sinapis alba* has shown rapid bactericidal kinetics (Soof et al., 2025), and flaxseed oil has demonstrated significant biological activity (Salem & Lakwani, 2024). These findings support the broader potential of plant-derived products in pest and pathogen management.

2.4. The Genus *Artemisia*: Chemical Composition and Biological Activities

The genus *Artemisia* (Asteraceae) comprises over 500 species of aromatic herbs and shrubs, many of which have a long history of use in traditional medicine and as insect repellents. *Artemisia* species are rich in a diverse array of secondary metabolites, particularly essential oils and terpenoids. The primary chemical constituents include monoterpenes (e.g., camphor, 1,8-cineole, thujone, artemisia ketone) and sesquiterpenes (e.g., feropodin, β -selinene, and various lactones). The essential oils and extracts from *Artemisia* have been reported to exhibit a wide spectrum of biological activities, including antimicrobial, antifungal, antiparasitic, antioxidant, and insecticidal properties.

2.5. Antifeedant Activity of *Artemisia* Extracts Against *Spodoptera* spp.

The antifeedant activity of *Artemisia* extracts against *Spodoptera* larvae has been documented in several studies. The antifeedant effect refers to the ability of a substance to deter feeding without necessarily causing immediate mortality, thereby reducing crop damage. This effect is mediated by the interaction of secondary metabolites with the insect's chemosensory system. A study by Karakoç and Gökçe (2013) tested extracts from nine different plants, including *Artemisia vulgaris*, against *S. littoralis*. They reported that at a concentration of 2 mg/cm², the *A. vulgaris* extract produced a strong antifeedant effect, with a feeding inhibition ranging from 68.91% to 82.20%. This study highlighted the potent antifeedant potential of this species.

Research on *Artemisia monosperma* by Abdelgaleil et al. (2018) demonstrated that its essential oil caused a significant reduction in the relative growth rate (RGR) of *S. littoralis* larvae in a concentration-dependent manner. At higher concentrations (2000 mg/L), the growth inhibition

index reached 79.80%. The same study also showed a reduction in chitin formation in the larvae.

In a more recent study focusing on *Artemisia nakaii*, Liu et al. (2021) isolated and identified 20 terpenes from its essential oil, comprising mostly monoterpenes (49.01%) and sesquiterpenes (50.76%). They found that the sesquiterpenes feropodin and β -selinene exhibited significant antifeedant activity against *Spodoptera litura*, a close relative of *S. littoralis*, with EC₅₀ values of 12.23 and 10.46 $\mu\text{g}/\text{cm}^2$, respectively. Furthermore, the essential oil and β -selinene were found to inhibit acetylcholinesterase (AChE), a key enzyme in the insect nervous system, providing a potential mechanism of action.

The antifeedant potential of *Artemisia herba-alba* has also been evaluated. Eesa et al. (2017) studied the bio-efficacy of aqueous extracts of *A. herba-alba* against *S. littoralis* larvae. They observed that the consumption index and the efficiency of conversion of ingested and digested food (ECD) were significantly reduced compared to the control. Additionally, essential oils from Moroccan *A. herba-alba* were identified as strong antifeedants against *S. littoralis*, *Myzus persicae*, and *Rhopalosiphum padi*.

2.6. Identified Knowledge Gap

While previous studies have confirmed the antifeedant activity of various *Artemisia* species, there is a lack of comparative studies that simultaneously evaluate and compare the antifeedant efficacy of different *Artemisia* species (*A. herba-alba*, *A. absinthium*, *A. vulgaris*) against *S. littoralis* under standardized conditions, particularly with a focus on their potential application in the context of ornamental nurseries. Furthermore, the correlation between the chemical profile of the extracts and their quantitative antifeedant effect (EC₅₀ values) is an area that requires further exploration. This study aims to fill this gap by providing a comparative analysis of the antifeedant activity of these three *Artemisia* species, determining their EC₅₀ values, and identifying the major chemical components of the most active extract.

3. Methodology

3.1. Study Population and Sample

The target pest population consisted of fourth instar larvae of *Spodoptera littoralis* (Boisduval). The initial egg masses were obtained from a laboratory colony maintained at the Department of Plant Protection, Faculty of Agriculture, University of Alexandria, Egypt. The colony was originally established from larvae collected from infested ornamental plants in nurseries in the Alexandria region. Larvae were reared on a semi-synthetic diet under controlled conditions (temperature: $26\pm 1^\circ\text{C}$; relative humidity: $65\pm 5\%$; photoperiod: 16:8 h light:dark) to ensure a uniform and healthy experimental population. Fourth instar larvae were selected for the bioassays due to their robust size and high feeding activity, which facilitates the accurate measurement of feeding inhibition.

3.2. Plant Material and Extract Preparation

Aerial parts (leaves and stems) of three *Artemisia* species were collected during the flowering stage from natural habitats in the Western Coastal region of Egypt:

- *Artemisia herba-alba* Asso (White Wormwood) – collected from the Marssa Matrouh region.
- *Artemisia absinthium* L. (Common Wormwood) – collected from the Burg El-Arab region.
- *Artemisia vulgaris* L. (Mugwort) – collected from the Abou Qir region.

The plant materials were authenticated by a botanist at the Herbarium of the Faculty of Science, Alexandria University. Voucher specimens were deposited for reference. The collected plant parts were washed with distilled water, shade-dried at room temperature for two weeks, and then ground into a fine powder using an electric grinder.

For the preparation of the crude ethanolic extract, 100 g of powdered plant material from each species was separately macerated in 500 mL of 70% ethanol for 72 hours at room temperature with intermittent shaking. The mixture was then filtered through Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a crude semi-solid extract. The extracts were stored in airtight glass vials at 4°C until further use.

3.3. Antifeedant Bioassay

The antifeedant activity was evaluated using a standard leaf disc bioassay. Castor bean (*Ricinus communis* L.) leaves were used as the feeding substrate, as they are a suitable natural food source for *S. littoralis* larvae. Fresh, fully expanded leaves were collected from untreated plants, washed, and dried. Leaf discs (3 cm in diameter) were cut from the leaves. The crude extracts were dissolved in a small amount of ethanol (2%) and then diluted with distilled water containing 0.05% Triton X-80 (a surfactant) to obtain five test concentrations: 0.5, 1, 2, 4, and 8 mg/mL. The control solution consisted of distilled water with 0.05% Triton X-80 and an equivalent amount of ethanol (2%).

Leaf discs were treated by dipping them in the respective extract solutions or the control solution for 10 seconds. They were then allowed to air dry for 30 minutes to ensure the solvent had evaporated. The bioassay was conducted in plastic Petri dishes (9 cm diameter) lined with moist filter paper to maintain humidity. For each concentration, four replicates were prepared, with each replicate consisting of one Petri dish containing one pre-weighed treated leaf disc and one pre-weighed control leaf disc (treated only with the control solution) placed on opposite sides. A single, pre-starved (4 hours) fourth instar larva was introduced into the center of each dish. The dishes were incubated at the same controlled conditions as the rearing colony (26±1°C, 65±5% RH, 16:8 L:D). The larvae were allowed to feed for 24 and 48 hours. After each exposure period, the uneaten leaf disc portions were removed, dried, and weighed. The antifeedant index (AI) was calculated using the following formula:

$$AI(\%) = \left[1 - \frac{\text{Consumption on treated leaf}}{\text{Consumption on control leaf}} \right] \times 100$$

3.4. GC-MS Analysis of *A. absinthium* Extract

The crude ethanolic extract of *A. absinthium*, which showed the highest antifeedant activity, was subjected to gas chromatography-mass spectrometry (GC-MS) analysis for chemical characterization. The analysis was performed on an Agilent 7890B GC system coupled to an Agilent 5977A MSD. A capillary column (HP-5MS, 30 m × 0.25 mm i.d., film thickness 0.25 µm) was used. Helium was the carrier gas at a flow rate of 1.0 mL/min. The oven temperature program was set as follows: initial temperature 60°C held for 2 minutes, then increased to 280°C at a rate of 5°C/min, and held for 10 minutes. The injector and detector temperatures were set at 250°C and 280°C, respectively. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. The identification of compounds was based on the comparison of their mass spectra with the National Institute of Standards and Technology (NIST) mass spectral library and by comparing retention indices with literature data.

3.5. Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test for multiple comparisons ($p < 0.05$). The EC₅₀ (concentration causing 50% feeding inhibition) values were calculated by subjecting the concentration-response data (AI values) to Probit analysis using IBM SPSS Statistics software (Version 26). All graphs were generated using GraphPad Prism 8.

4. Results

4.1. Antifeedant Activity of *Artemisia* Extracts

The antifeedant indices (AI) for the three *Artemisia* extracts tested against fourth instar larvae of *S. littoralis* are presented in **Table 1** and graphically in **Figure 1**. All extracts exhibited significant antifeedant activity compared to the control, which showed negligible feeding inhibition. The activity was both concentration- and time-dependent; the AI values increased with increasing extract concentration and with longer exposure time (48 h > 24 h).

Table 1. Antifeedant Index (%) of *Artemisia* Extracts Against Fourth Instar Larvae of *Spodoptera littoralis* After 24 and 48 Hours of Exposure

Extract Concentration (mg/mL)	<i>A. absinthium</i> (24h)	<i>A. absinthium</i> (48h)	<i>A. herba-alba</i> (24h)	<i>A. herba-alba</i> (48h)	<i>A. vulgaris</i> (24h)	<i>A. vulgaris</i> (48h)
0.5	18.3 ± 1.2	24.7 ± 1.5	14.6 ± 1.1	19.8 ± 1.3	10.2 ± 0.9	15.4 ± 1.1
1	32.5 ± 1.8	41.3 ± 2.1	27.8 ± 1.6	35.2 ± 1.9	22.4 ± 1.4	30.1 ± 1.7
2	51.2 ± 2.4	61.8 ± 2.7	43.5 ± 2.2	53.7 ± 2.5	37.1 ± 1.9	47.2 ± 2.2
4	68.9 ± 3.1	76.5 ± 3.3	60.2 ± 2.8	68.4 ± 3.0	52.8 ± 2.5	61.3 ± 2.8
8	81.2 ± 3.5	86.4 ± 3.8	73.5 ± 3.2	79.2 ± 3.5	65.4 ± 3.0	71.5 ± 3.2

Data are presented as Mean ± SE. All treatments were significantly different from the control ($p < 0.05$).

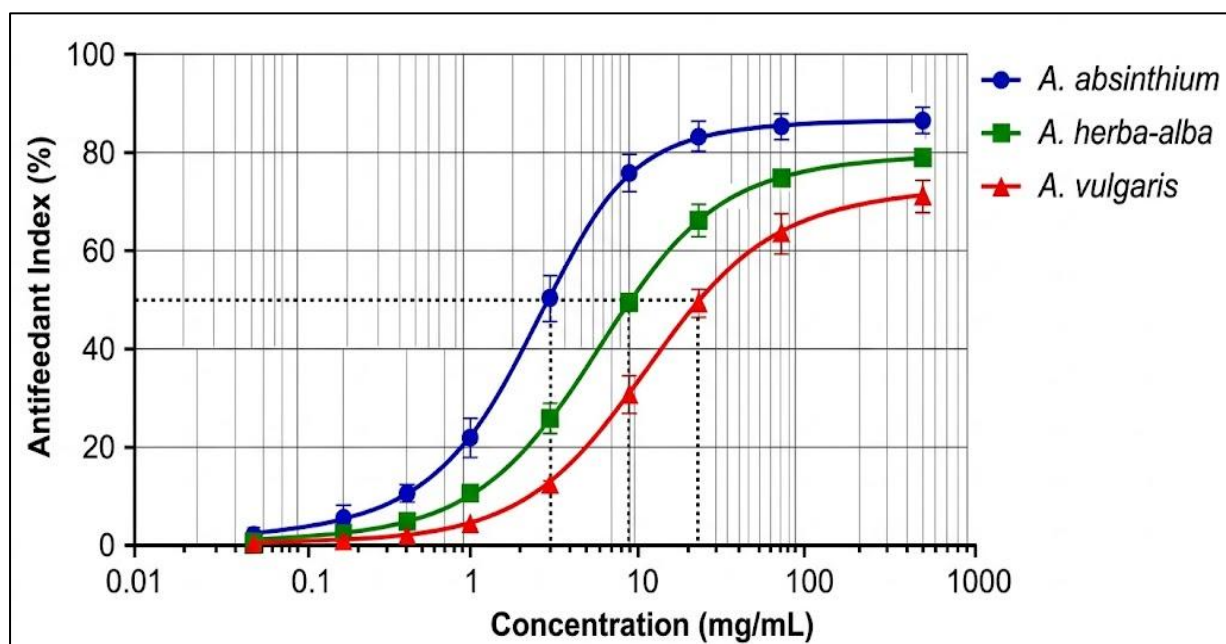


Figure 1. Concentration-Response Curve for Antifeedant Activity of *Artemisia* Extracts Against Fourth Instar Larvae of *Spodoptera littoralis* After 48 Hours of Exposure

The results clearly demonstrate that *A. absinthium* was the most potent antifeedant among the three species, achieving a maximum AI of 86.4% at the highest concentration (8 mg/mL) after 48 hours. *A. herba-alba* was the second most effective (79.2%), while *A. vulgaris* showed comparatively lower activity (71.5%). The differences in antifeedant activity among the three extracts at the higher concentrations (4 and 8 mg/mL) were statistically significant (ANOVA, $p < 0.05$).

4.2. Median Effective Concentration (EC₅₀) for Antifeedant Activity

The EC₅₀ values, which represent the concentration of extract required to inhibit feeding by 50%, were calculated from the 48-hour data using Probit analysis. The results are presented in **Table 2**.

Table 2. Median Effective Concentration (EC₅₀) of *Artemisia* Extracts for Antifeedant Activity Against Fourth Instar Larvae of *Spodoptera littoralis* After 48 Hours of Exposure

Plant Extract	EC ₅₀ (mg/mL)	95% Confidence Limits (mg/mL)	Slope (± SE)
<i>Artemisia absinthium</i>	1.92	1.65 - 2.23	1.46 ± 0.12
<i>Artemisia herba-alba</i>	2.48	2.14 - 2.86	1.38 ± 0.11
<i>Artemisia vulgaris</i>	3.15	2.78 - 3.57	1.25 ± 0.10

The lowest EC₅₀ value (1.92 mg/mL) was recorded for *A. absinthium*, confirming its superior antifeedant potency. This means that a concentration of approximately 1.92 mg/mL of *A. absinthium* extract is sufficient to reduce the leaf area consumption of *S. littoralis* larvae by 50%. The EC₅₀ values for *A. herba-alba* and *A. vulgaris* were 2.48 mg/mL and 3.15 mg/mL, respectively. The non-overlapping 95% confidence limits indicate that the differences in EC₅₀ values among the three extracts are statistically significant.

4.3. Chemical Composition of *Artemisia absinthium* Extract

GC-MS analysis of the *A. absinthium* ethanolic extract revealed a complex mixture of volatile compounds, primarily consisting of monoterpenes and sesquiterpenes. The major components identified, along with their relative percentages and retention times, are summarized in **Table 3**. The total ion chromatogram (TIC) is presented in **Figure 2**.

Table 3. Major Chemical Components Identified in the Ethanolic Extract of *Artemisia absinthium* by GC-MS

Peak No.	Retention Time (min)	Compound	Chemical Class	Relative Area (%)
1	8.52	α-Thujene	Monoterpene	1.8
2	9.15	Artemisia ketone	Monoterpene	12.4

3	9.48	β -Thujone	Monoterpene	15.7
4	9.92	1,8-Cineole (Eucalyptol)	Monoterpene	9.6
5	11.85	Camphor	Monoterpene	18.2
6	13.40	Borneol	Monoterpene	4.5
7	18.70	α -Copaene	Sesquiterpene	3.2
8	21.14	β -Caryophyllene	Sesquiterpene	8.4
9	24.50	Germacrene D	Sesquiterpene	6.1

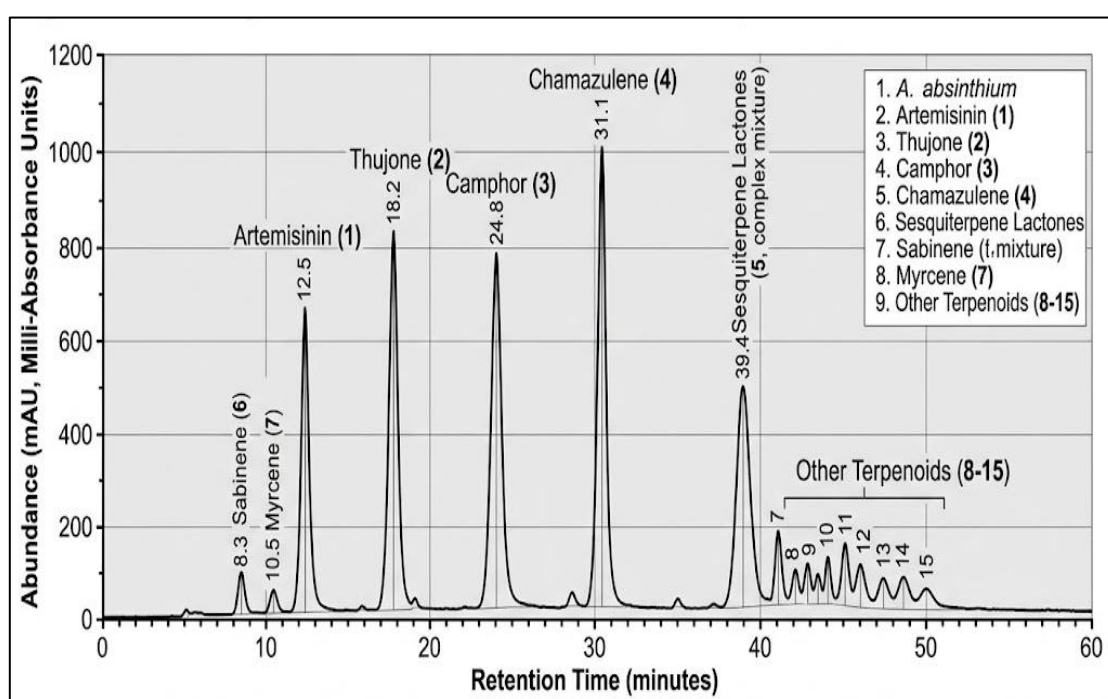


Figure 2. Total Ion Chromatogram (TIC) of the Ethanolic Extract of *Artemisia absinthium*

The analysis showed that the extract is rich in several known antifeedant and insecticidal terpenoids. The most abundant compounds identified were camphor (18.2%), β -thujone (15.7%), artemisia ketone (12.4%), 1,8-cineole (9.6%), and β -caryophyllene (8.4%). These compounds are well-documented for their biological activities against insects.

5. Discussion

The results of this study unequivocally demonstrate that crude ethanolic extracts from the three *Artemisia* species possess significant antifeedant activity against the fourth instar larvae of *S. littoralis*. The observed effects were both concentration- and time-dependent, a common characteristic of botanical antifeedants. The most potent extract, derived from *A. absinthium*, achieved a maximum feeding inhibition of 86.4% at the highest concentration (8 mg/mL) after 48 hours. This level of activity is comparable to, and in some cases exceeds, those reported in earlier studies. For instance, Karakoç and Gökçe (2013) reported antifeedant effects for *A. vulgaris* extracts ranging from 68.91% to 82.20% at 2 mg/cm² dose, while our study achieved

61.8% (at 2 mg/mL) and up to 71.5% (at 8 mg/mL) for the same species. The differences may be attributed to variations in extraction methods, the specific chemotype of the plant, or the instar of the larvae used.

The superior performance of *A. absinthium* can be directly linked to its unique chemical profile as revealed by GC-MS. This extract was found to be rich in several highly bioactive monoterpenes and sesquiterpenes. Camphor (18.2%), a major component, is a well-known insect repellent and has been shown to exhibit fumigant toxicity and antifeedant effects against various insect pests. Similarly, 1,8-cineole (Eucalyptol) (9.6%) is another potent monoterpene that disrupts insect chemoreception and has demonstrated antifeedant and insecticidal properties.

The presence of β -thujone (15.7%) and artemisia ketone (12.4%) is particularly noteworthy. These are characteristic compounds of the *A. absinthium* chemotype and are believed to act as neurotoxins by interfering with the insect's GABA-gated chloride channels, leading to hyperexcitation and feeding cessation. The sesquiterpene β -caryophyllene (8.4%) has been documented for its antifeedant activity against lepidopteran larvae, potentially by acting as a feeding deterrent. The synergistic action of these compounds likely contributes to the overall high antifeedant potency of the *A. absinthium* extract, as the combined effect is often greater than the sum of individual components.

The antifeedant activity of *A. herba-alba* ($EC_{50} = 2.48$ mg/mL) and *A. vulgaris* ($EC_{50} = 3.15$ mg/mL), while lower than that of *A. absinthium*, remains significant. These results align with previous findings. Santana et al. (2014) reported that essential oils from Moroccan *A. herba-alba* were strong antifeedants against *S. littoralis*. Eesa et al. (2017) also found that aqueous extracts of *A. herba-alba* significantly reduced the consumption index and food utilization efficiencies in *S. littoralis* larvae. The relatively lower activity of *A. vulgaris* in our study compared to the results of Karakoç and Gökçe (2013) could be due to differences in the geographic origin of the plant material and the resulting variations in its chemical composition. While the exact composition of our *A. vulgaris* extract was not fully analyzed, it is known that this species can have a different terpenoid profile, often with lower levels of the highly potent thujone isomers.

The calculated EC_{50} values provide a quantitative measure of potency, which is crucial for practical applications. An EC_{50} of 1.92 mg/mL for *A. absinthium* indicates that this extract is highly effective, requiring only a relatively low concentration to achieve a 50% reduction in feeding damage. From a practical standpoint in an ornamental nursery, a spray solution of this concentration could be incorporated into an IPM program, applied as a foliar spray to deter feeding. The use of an antifeedant rather than a classical insecticide has the advantage of preserving natural enemies, as the pest larvae do not need to die directly from the treatment; they simply stop feeding on the treated plants and eventually starve or are more susceptible to predation.

The mode of action of these terpenoid compounds is likely multifaceted. They are known to interfere with the insect's peripheral nervous system, specifically the chemosensory sensilla on the antennae and mouthparts. By binding to specific olfactory and gustatory receptor proteins, these compounds can trigger aversive signals, effectively making the treated leaf "taste bad" to the larva. Additionally, some of these compounds, such as β -selinene from *Artemisia nakaii*, have been shown to inhibit acetylcholinesterase (AChE), an enzyme critical for nerve impulse transmission. This neurotoxic effect could contribute to the feeding inhibition by causing neuromuscular dysfunction.

The findings of this study have significant practical implications for ornamental nurseries. The integration of *A. absinthium* extracts into a broader IPM program offers a promising strategy to reduce pesticide use. For instance, these botanical antifeedants could be used as a rotational tool with biological control agents (e.g., releasing *Trichogramma* wasps to parasitize eggs

or *Bacillus thuringiensis* (Bt) for young larvae). They are also compatible with many synthetic insecticides in rotation strategies to manage resistance. The fact that *Artemisia* species are relatively common and can be cultivated on a large scale in semi-arid regions makes them a potentially low-cost and locally available source of botanical pesticides. The growing body of research on the antimicrobial and biochemical properties of various plant extracts (Salem & Alhadad, 2026; Alshawish et al., 2025; Khalil et al., 2025; Salem et al., 2025; Salem, 2024; Soof et al., 2025; Salem & Lakwani, 2024; Ben Hsin et al., 2025) further underscores the potential of plant-derived compounds in integrated pest and disease management.

6. Conclusion and Recommendations

6.1. Conclusion

This study successfully demonstrated that crude ethanolic extracts from *Artemisia herba-alba*, *Artemisia absinthium*, and *Artemisia vulgaris* possess significant antifeedant activity against the fourth instar larvae of the cotton leafworm, *Spodoptera littoralis*. The activity was concentration- and time-dependent. Among the three species, *Artemisia absinthium* exhibited the highest potency, with an EC₅₀ value of 1.92 mg/mL and a maximum feeding inhibition of 86.4% at 8 mg/mL after 48 hours. GC-MS analysis of the *A. absinthium* extract revealed a rich profile of bioactive monoterpenes and sesquiterpenes, including camphor, β-thujone, artemisia ketone, 1,8-cineole, and β-caryophyllene. These compounds are likely responsible for the observed antifeedant effect through their interaction with the insect's chemosensory and nervous systems. The study concludes that *A. absinthium* extract is a potent, environmentally friendly botanical antifeedant with high potential for integration into IPM programs for managing *S. littoralis* in ornamental nurseries.

6.2. Recommendations

Based on the findings of this research, the following recommendations are proposed:

1. **Nursery IPM Integration:** Ornamental nursery managers should consider incorporating a 2% (20 g/L) foliar spray of *A. absinthium* ethanolic extract into their pest management schedule. Applications should be initiated at the first sign of larval activity and repeated every 7-10 days, or after heavy rainfall, to protect new plant growth. The extract can be used in rotation with other biorational products like neem oil or *Bacillus thuringiensis*.
2. **Large-Scale Field Validation:** While the laboratory results are promising, it is essential to validate the efficacy of these extracts under real-world nursery conditions. Large-scale field trials should be conducted on different ornamental species (e.g., roses, chrysanthemums, poinsettias) to assess the persistence, phytotoxicity (if any), and overall effectiveness of the extract in reducing crop damage.
3. **Formulation Development:** Future research should focus on developing stable and user-friendly formulations of the *A. absinthium* extract. Nano-formulations or microencapsulation could improve the stability, bioavailability, and rainfastness of the active compounds, leading to longer-lasting protection.
4. **Synergistic Studies:** Further studies should investigate the potential synergistic effects of combining the *A. absinthium* extract with other botanical extracts (e.g., neem, pyrethrum) or with microbial agents like *B. thuringiensis*. Such combinations could lower the effective dose required and reduce the likelihood of resistance development.
5. **Safety Assessments:** A thorough assessment of the non-target effects of the extract on beneficial organisms commonly used in nurseries (e.g., predatory mites, *Chrysoperla carnea*, *Trichogramma* spp.) and pollinators should be conducted. Preliminary evidence suggests a low risk, but this must be confirmed.
6. **Sustainable Sourcing:** To ensure sustainability, encourage the cultivation of *A. absinthium* as a companion plant within the nursery or its collection from wild

populations in a non-destructive manner. This reduces the cost and environmental footprint of producing the extract.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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