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Toxicity Efficacy of Methanolic Leaf Extracts of Neem (*Azadirachta indica*) and Basil (*Ocimum basilicum*) Against Cotton Aphid (*Aphis gossypii*) on Pepper Crop (Capsicum annuum)

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الفاعلية السمية للمستخلصات الميثانولية لأوراق نبات النيم والريحان ضد حشرة المن (*Aphis gossypii*) على محصول الفلفل

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Abstract:

The cotton aphid, Aphis gossypii Glover (Hemiptera: Aphididae), is a significant pest affecting pepper crops worldwide, causing substantial yield losses through direct feeding damage and virus transmission. The increasing concerns over synthetic pesticide resistance and environmental contamination have intensified the search for sustainable, eco-friendly alternatives. This study evaluated the insecticidal efficacy of methanolic leaf extracts from two medicinal plants—neem (Azadirachta indica A. Juss.) and basil (Ocimum basilicum L.)—against A. gossypii under controlled laboratory conditions. Methanolic extraction was performed using the Soxhlet apparatus, and five concentrations (0.5%, 1.0%, 2.0%, 4.0%, and 8.0% w/v) of each extract were tested against adult aphids using the leaf-dip bioassay method. Mortality was recorded at 12, 24, 48, and 72 hours post-treatment, with distilled water serving as the negative control. The results demonstrated that both plant extracts exhibited significant concentration-dependent aphicidal activity. Neem extract showed superior toxicity, achieving 96.4% mortality at the highest concentration (8.0%) after 72 hours, with an LC_{50} value of 1.82% w/v. Basil extract exhibited moderate efficacy, reaching 78.3% mortality at the same concentration, with an LC_{50} value of 3.56% w/v. Basil extract, however, demonstrated faster initial action, with 24-hour mortality rates of 42.5% at 8.0% compared to 34.7% for neem at the same concentration and time point. Statistical analysis using one-way ANOVA followed by Tukey's HSD test revealed significant differences among treatments ($p < 0.001$). These findings suggest that both neem and basil methanolic leaf extracts possess promising aphicidal properties, with neem demonstrating greater overall efficacy while basil offers more rapid initial knockdown effects. The study supports the potential integration of these botanical extracts into sustainable integrated pest management (IPM) programs for pepper cultivation, reducing reliance on synthetic chemical pesticides and promoting environmentally responsible agricultural practices.

Keywords: Aphis gossypii, Azadirachta indica, Ocimum basilicum, methanolic extract, botanical insecticide, pepper, integrated pest management, LC50.

الملخص

تُعد حشرة المن القطني Aphis gossypii (Hemiptera: Aphididae) آفة رئيسية تصيب محاصيل الفلفل حول العالم، مسببة خسائر كبيرة في المحصول من خلال التغذية المباشرة ونقل الفيروسات. أدت المخاوف المتزايدة بشأن مقاومة المبيدات الاصطناعية والتلوث البيئي إلى تكثيف البحث عن بدائل مستدامة صديقة للبيئة. قيمت هذه الدراسة الفعالية الإبادة الحشرية للمستخلصات الميثانولية لأوراق نباتين طبيين—النيم (Azadirachta indica A. Juss.) والريحان—(Ocimum basilicum L.) ضد حشرة A. gossypiiتحت ظروف معملية محكمة. تم إجراء الاستخلاص الميثانولي باستخدام جهاز Soxhlet ، واختبرت خمسة تركيزات (0.5، 1.0، 2.0، 4.0، و8.0% وزن/حجم) من كل مستخلص ضد حشرات المن البالغة باستخدام طريقة غمس الأوراق الحيوية. سُجلت نسب الموت بعد 12 و24 و48 و72 ساعة من المعاملة، واستخدم الماء المقطر كمجموعة ضابطة سالبة. أظهرت النتائج أن كلا المستخلصين النباتيين أظهرا نشاطاً إبادياً معنوياً مرتبطاً بالتركيز. أظهر مستخلص النيم سمية متفوقة، محققاً نسبة موت 96.4% عند أعلى تركيز (8.0%) بعد 72 ساعة، بقيمة LC_{50} قدرها 1.82% وزن/حجم. أظهر مستخلص الريحان فعالية متوسطة، محققاً نسبة موت 78.3% عند نفس التركيز، بقيمة LC_{50} قدرها 3.56% وزن/حجم. ومع ذلك، أظهر مستخلص الريحان تأثيراً أولياً أسرع، حيث بلغت نسب الموت بعد 24 ساعة 42.5% عند تركيز 8.0% مقارنة بـ 34.7% لمستخلص النيم عند نفس التركيز والنقطة الزمنية. كشف التحليل الإحصائي باستخدام ANOVA أحادي الاتجاه متبوعاً باختبار Tukey HSD عن فروق معنوية بين المعاملات ($p < 0.001$). تشير هذه النتائج إلى أن كلا المستخلصين الميثانوليين لأوراق النيم والريحان يمتلكان خصائص إبادة حشرية واعدة، حيث أظهر النيم فعالية كلية أعلى بينما قدم الريحان تأثيرات إسقاط أولية أسرع. تدعم الدراسة إمكانية دمج هذه المستخلصات النباتية في برامج الإدارة المتكاملة للآفات المستدامة لزراعة الفلفل، مما يقلل الاعتماد على المبيدات الكيميائية الاصطناعية ويعزز الممارسات الزراعية المسؤولة بيئياً.

الكلمات المفتاحية: *Aphis gossypii*، *Azadirachta indica*، *Ocimum basilicum*، مستخلص ميثانولي، مبيد حشري نباتي، فلفل، إدارة متكاملة للآفات، *LC50*.

1.1. Introduction

1.1 Problem Statement

Pepper (*Capsicum annuum* L.) is one of the most economically important vegetable crops cultivated globally, valued for its nutritional content, distinctive flavor, and culinary versatility. However, pepper production faces persistent threats from various arthropod pests, among which aphids constitute a particularly damaging group. The cotton aphid, Aphis gossypii Glover (Hemiptera: Aphididae), is a polyphagous pest that inflicts severe damage on pepper crops through direct feeding on phloem sap, causing leaf curling, stunted growth, and reduced photosynthetic efficiency, as well as indirect damage through the transmission of plant viruses such as Cucumber Mosaic Virus (CMV) and Potyviruses.

The conventional approach to aphid management has relied heavily on synthetic chemical insecticides. However, this strategy has led to several critical problems. First, A. gossypii has demonstrated remarkable ability to develop resistance to multiple insecticide classes, including organophosphates, carbamates, pyrethroids, and neonicotinoids. Second, broad-spectrum synthetic insecticides pose significant risks to non-target organisms, including natural enemies such as parasitoids and predators that contribute to biological control. Third, pesticide residues in food products and the environment raise serious concerns for human health and ecosystem sustainability. The growing challenge of insecticide resistance and environmental impact caused by synthetic pesticides necessitates the exploration of sustainable, eco-friendly alternatives. Plant-derived secondary metabolites have shown significant bioactivity, with research consistently highlighting their potential in pest management and antimicrobial applications (Salem & Lakwani, 2024; Salem & Salem, 2025; Shouran et al, 2026).

In response to these challenges, integrated pest management (IPM) strategies have been developed, emphasizing the use of biorational products that are effective against target pests while minimizing adverse effects on beneficial organisms and the environment. Among biorational approaches, botanical insecticides derived from plants with known insecticidal properties have gained considerable attention. Plants produce a diverse array of secondary metabolites, including alkaloids, terpenoids, phenolics, and flavonoids, which have evolved as defense mechanisms against herbivorous insects. These natural compounds often exhibit multiple modes of action, potentially reducing the likelihood of resistance development compared to synthetic pesticides with single target sites.

Among the vast repository of insecticidal plants, neem (Azadirachta indica A. Juss., family Meliaceae) stands as one of the most extensively studied and documented botanical insecticides. The tree, native to the Indian subcontinent, produces azadirachtin—a complex tetranortriterpenoid—along with other active compounds including salannin, nimbin, and meliantriol. These compounds exhibit a wide range of biological activities against insects, including antifeedant effects, growth regulation, oviposition deterrence, and direct toxicity. Basil (Ocimum basilicum L., family Lamiaceae), a culinary herb widely cultivated globally, produces essential oils rich in monoterpenes and phenylpropanoids such as estragole (methyl chavicol), linalool, eugenol, and methyl cinnamate. Recent studies have demonstrated the insecticidal potential of basil essential oils and extracts against various pest insects.

1.2 Research Objectives

This study was conducted with the following specific objectives:

1. To prepare and characterize methanolic leaf extracts from A. indica and O. basilicum using standardized extraction procedures.
2. To evaluate the concentration-dependent toxicity of both plant extracts against adult A. gossypii under controlled laboratory conditions.
3. To determine the median lethal concentrations (LC_{50}) for each extract at different exposure times.
4. To compare the relative efficacy of neem and basil extracts in terms of overall mortality and speed of action.
5. To assess the potential of these botanical extracts as sustainable alternatives to synthetic insecticides for aphid management in pepper cultivation.

1.3 Significance of the Study

The findings of this research hold practical significance for multiple stakeholders. For pepper growers, the identification of effective botanical insecticides provides viable alternatives that may reduce production costs associated with synthetic pesticides while minimizing health risks to applicators and consumers. For agricultural extension services and IPM practitioners, the comparative data on neem and basil efficacy can inform evidence-based recommendations for aphid management strategies. From an environmental perspective, the adoption of botanical insecticides contributes to reduced chemical load in agroecosystems, preservation of natural enemy populations, and decreased risk of pesticide resistance development. Furthermore, the utilization of locally available plant materials such as neem and basil supports the development of accessible, low-cost pest management solutions for smallholder farmers in developing regions.

2. Literature Review

2.1 Aphis gossypii: Biology, Damage, and Economic Impact

Aphis gossypii, commonly known as the cotton aphid or melon aphid, is a small, soft-bodied insect belonging to the family Aphididae within the order Hemiptera. The species exhibits a cosmopolitan distribution and is recognized as one of the most economically significant aphid pests worldwide. Adult females typically measure 1.0 to 1.5 mm in length and may be yellow, green, or dark green in color, with dark cornicles (siphunculi) and cauda. The life cycle of A. gossypii is characterized by telescoping generations, wherein females give birth to live nymphs through parthenogenetic viviparity under favorable conditions, allowing rapid population increase. A single female can produce up to 80-100 offspring within her lifetime, and under optimal temperatures (25-30°C), population doubling time can be as short as 2-3 days.

Figure (1): Aphis gossypii Glover (Hemiptera: Aphididae),

The pest inflicts damage on pepper crops through multiple mechanisms. Direct feeding damage results from the insertion of stylets into phloem tissues to extract sap, leading to nutrient depletion, reduced plant vigor, leaf curling and distortion, stunted growth, and in severe infestations, plant death. The excretion of honeydew, a sugar-rich liquid, promotes the growth of sooty mold fungi on leaf surfaces, which impairs photosynthesis and reduces fruit quality. Perhaps more significantly, A. gossypii serves as an efficient vector for numerous plant viruses, including Cucumber Mosaic Virus (CMV), Zucchini Yellow Mosaic Virus (ZYMV), and Papaya Ringspot Virus (PRSV), causing secondary disease impacts that often exceed the direct damage from feeding.

Economic losses attributed to A. gossypii infestations in pepper production can be substantial. In greenhouse pepper crops, uncontrolled aphid populations can cause yield reductions ranging from 30% to 100%, depending on infestation severity, timing, and virus presence. In Argentina, where greenhouse pepper production is economically important, A. gossypii and Myzus persicae are considered key pests requiring intensive management interventions.

2.2 Conventional Management and Challenges of Synthetic Insecticides

The predominant approach to A. gossypii control has involved the application of synthetic chemical insecticides from various classes, including organophosphates (e.g., malathion, dimethoate), carbamates (e.g., carbaryl, methomyl), pyrethroids (e.g., cypermethrin, deltamethrin), and neonicotinoids (e.g., imidacloprid, acetamiprid). However, this reliance on synthetic chemistry has created multiple interconnected problems.

First, A. gossypii has demonstrated exceptional capacity for insecticide resistance development. Mechanisms of resistance include target site insensitivity (such as kdr mutations in sodium channels conferring pyrethroid resistance), enhanced metabolic detoxification through elevated activities of esterases, glutathione S-transferases, and cytochrome P450 monooxygenases, and reduced cuticular penetration. The widespread and often indiscriminate use of insecticides has selected for resistant populations across many agricultural regions, rendering previously effective products obsolete.

Second, the broad-spectrum activity of many synthetic insecticides results in substantial non-target effects. Natural enemies of aphids, including parasitoid wasps (e.g., Aphidius colemani, Lysiphlebus testaceipes), predatory ladybeetles (Coccinellidae), lacewings (Chrysopidae), and hoverflies (Syrphidae), are highly susceptible to direct toxicity from insecticide applications. The elimination of these biological control agents often triggers pest resurgence, wherein the target pest population rebounds more rapidly than natural enemy populations, necessitating further insecticide applications in an escalating cycle.

Third, environmental contamination from pesticide runoff and spray drift affects non-target ecosystems, including soil microorganisms, aquatic organisms, and pollinators. Additionally, pesticide residues on harvested produce raise food safety concerns and may affect international trade through violation of maximum residue limits (MRLs) established by importing countries.

2.3 Integrated Pest Management and Biorational Approaches

In response to the limitations of conventional chemical control, integrated pest management (IPM) has emerged as a comprehensive approach combining multiple compatible tactics to maintain pest populations below economically damaging thresholds while minimizing environmental and health risks. IPM strategies for aphid management incorporate cultural controls (crop rotation, sanitation, resistant varieties), biological controls (conservation and augmentation of natural enemies), behavioral controls (monitoring and threshold-based decision-making), and judicious use of biorational pesticides when necessary.

Biorational pesticides—products derived from natural sources with selective activity and minimal environmental persistence—represent a key component of modern IPM programs. Among biorational options, botanical insecticides derived from plant secondary metabolites have received extensive research attention due to their diverse modes of action, rapid environmental degradation, and generally lower toxicity to non-target organisms compared to synthetic counterparts.

2.4 Neem (Azadirachta indica) as a Botanical Insecticide

Azadirachta indica, commonly known as neem, is a fast-growing evergreen tree belonging to the family Meliaceae, native to the Indian subcontinent but now widely naturalized in tropical and subtropical regions worldwide. The insecticidal properties of neem have been recognized for centuries in traditional agriculture, with various plant parts—seeds, leaves, bark, and oil—used for pest management.

Figure (2): Neem (Azadirachta indica)

The principal active ingredient in neem is azadirachtin, a complex limonoid tetranortriterpenoid comprising approximately 0.2-0.4% of neem seed kernel weight. Azadirachtin exhibits multiple modes of action against insects, including: (1) antifeedant activity, deterring feeding through interaction with chemoreceptors; (2) growth regulation, disrupting ecdysteroid and juvenile hormone signaling to interfere with molting and metamorphosis; (3) oviposition deterrence, reducing egg-laying on treated surfaces; (4) direct toxicity, causing mortality through neurotoxic and cytotoxic effects; and (5) sublethal effects, including reduced fecundity and egg viability. Additional neem compounds with insecticidal activity include salannin, nimbin, nimbidin, and meliantriol.

The efficacy of neem-based formulations against A. gossypii has been documented in multiple studies. Castresana and Puhl (2021) evaluated botanical formulations in greenhouse pepper crops and reported that neem oil-containing treatments recorded lower aphid numbers compared to other treatments and the absolute control. Similarly, Ebrahimi et al. (2013) investigated essential oils of A. indica, Eucalyptus camaldulensis, and Laurus nobilis against A. gossypii, finding that A. indica possessed the highest lethal activity with an LC_{50} value of 1.96 μL L^{-1} air. Singh et al. (2012) examined repellency properties of leaf extracts from A. indica, Eucalyptus globulus, and O. basilicum, reporting that A. indica extract exhibited the highest repellency at 99.0% after 24 hours.

Furthermore, research by Esparza Díaz et al. (2010) compared four neem extracts for azadirachtin concentration, insecticide efficacy, and phytotoxicity against A. gossypii, concluding that the methanolic extract exhibited the highest insecticidal potential with 0.2 mg azadirachtin. The superior performance of methanolic extraction likely reflects the high solubility of azadirachtin and related limonoids in polar organic solvents.

2.5 Basil (Ocimum basilicum) as a Botanical Insecticide

Ocimum basilicum L., commonly known as sweet basil, is an aromatic herb belonging to the family Lamiaceae, cultivated worldwide for culinary, medicinal, and ornamental purposes. The plant produces a complex mixture of essential oils dominated by phenylpropanoids and monoterpenes, with chemotype variations depending on genetic background and environmental conditions. Major constituents include estragole (methyl chavicol), linalool, eugenol, methyl cinnamate, and 1,8-cineole.

Figure (3): Basil (Ocimum basilicum)

The insecticidal properties of basil have been attributed primarily to the monoterpene and phenylpropanoid components of its essential oil. These compounds interact with insect nervous systems through multiple mechanisms, including acetylcholinesterase inhibition, octopamine receptor antagonism, and GABA-gated chloride channel modulation, leading to hyperexcitation, paralysis, and mortality.

Studies evaluating basil against A. gossypii have demonstrated significant insecticidal potential. El-Shourbagy et al. (2023) investigated the biochemical and insecticidal efficacy of basil essential oil against cotton aphids, reporting an LC_{50} value indicating moderate potency, with basil being approximately 4.44 times less potent than clove essential oil. However, basil essential oil exhibited significant effects on digestive enzyme activities, reducing trehalase activity and altering protease and lipase profiles, contributing to overall toxicity.

Research by Singh et al. (2012) on repellency properties of leaf extracts from A. indica, Eucalyptus globulus, and O. basilicum demonstrated that basil extract showed moderate repellency (91.0% after 24 hours) compared to neem (99.0%). A study by Murz (2021) examining the effect of basil leaf extract on A. gossypii mortality found an LC_{50} value of 2.196% w/v after 24 hours exposure, with the highest concentration (2.4%) achieving 98% mortality by 24 hours. These findings confirm the potential of basil extracts as effective aphicidal agents, though with somewhat lower potency compared to neem.

2.6 Methanolic Extraction: Rationale and Methodology

The choice of extraction solvent critically influences the composition and biological activity of plant extracts. Methanol, a polar organic solvent, is particularly effective for extracting a broad spectrum of secondary metabolites, including alkaloids, flavonoids, tannins, terpenoids, saponins, and phenolic compounds. Compared to non-polar solvents such as hexane or dichloromethane, methanol extracts tend to exhibit higher overall biological activity due to the wider range of compounds solubilized.

The Soxhlet extraction method, employed in this study, offers several advantages for preparing plant extracts for bioassays. This continuous extraction technique involves repeated cycles of solvent evaporation and condensation, allowing exhaustive extraction of soluble compounds from plant material. The method ensures high extraction efficiency, reproducibility, and the ability to process relatively large quantities of plant material. Temperature control during Soxhlet extraction (maintained below solvent boiling point) minimizes thermal degradation of heat-sensitive bioactive compounds.

3. Methodology

3.1 Study Site and Period

The research was conducted at the Plant Protection Laboratory, Faculty of Agriculture, during the period from March to September 2025. Laboratory conditions were maintained at 25 ± 2°C, 65 ± 5% relative humidity, and a 16:8 hour (light:dark) photoperiod.

3.2 Plant Material Collection and Authentication

Fresh, healthy, mature leaves of Azadirachta indica A. Juss. (neem) and Ocimum basilicum L. (sweet basil) were collected from the university botanical garden. Plant specimens were authenticated by a qualified botanist, and voucher specimens were deposited in the university herbarium (accession numbers: NI-2025-042 for neem, OB-2025-089 for basil). Leaves were thoroughly washed with distilled water to remove surface contaminants, shade-dried at room temperature (25-28°C) for 14 days until constant weight, and ground into fine powder using an electric grinder. The powdered material was stored in airtight containers at 4°C until extraction.

3.3 Methanolic Extraction

Methanolic extraction was performed using the Soxhlet extraction method. For each plant species, 100 g of dried leaf powder was placed in a cellulose thimble and inserted into a Soxhlet extractor. Methanol (500 mL, 99.8% purity) was used as the extraction solvent. The extraction process was carried out at 65°C for 8 hours, completing approximately 15-20 siphoning cycles. The resulting methanolic extract was concentrated under reduced pressure using a rotary evaporator at 45°C to remove the solvent. The crude extract was then transferred to pre-weighed glass vials and dried completely in a vacuum desiccator. The dried extract was weighed to calculate extraction yield (percentage of dry plant material extracted), then re-dissolved in methanol to prepare stock solutions at known concentrations. Stock solutions were stored at 4°C in amber glass vials to prevent photodegradation.

3.4 Insect Rearing

Aphis gossypii colonies were established from specimens collected from infested pepper plants (Capsicum annuum L.) grown in a greenhouse free from pesticide application. Identification was confirmed using taxonomic keys. Colonies were maintained on potted pepper plants (variety ‘California Wonder’) in insect-rearing cages (60 × 60 × 90 cm) under controlled environmental conditions (25 ± 2°C, 65 ± 5% RH, 16:8 L:D photoperiod). Fresh, uninfested pepper plants were introduced weekly to maintain colony vigor. Only adult apterous females of uniform size and age (3-5 days old) were used for bioassays.

3.5 Experimental Design

The experiment employed a completely randomized design (CRD) with factorial arrangement. Factors included: (1) plant extract type (neem extract, basil extract, and distilled water control); (2) extract concentration (0.5%, 1.0%, 2.0%, 4.0%, and 8.0% w/v, plus 0% control); and (3) exposure time (12, 24, 48, and 72 hours). Each treatment combination was replicated five times, with each replicate consisting of 10 adult aphids, for a total sample size of 650 aphids (3 extracts × 6 concentrations × 4 times × 5 replicates × 10 aphids).

3.6 Leaf-Dip Bioassay

The leaf-dip bioassay method, adapted from standard protocols recommended by the Insecticide Resistance Action Committee (IRAC), was employed to evaluate extract toxicity. Fresh, uninfested pepper leaves were collected from greenhouse-grown plants, surface-sterilized with 0.1% sodium hypochlorite solution for 30 seconds, rinsed three times with distilled water, and air-dried. Leaf discs (4 cm diameter) were excised using a sterile cork borer. For each concentration, leaf discs were immersed in the respective extract solution for 10 seconds with gentle agitation, then placed on absorbent paper to remove excess solution. Control leaf discs were immersed in distilled water containing 0.1% Tween-80 (surfactant). Treated leaf discs were air-dried for 30 minutes at room temperature to evaporate residual solvent.

Each leaf disc was placed adaxial side up in a sterile plastic Petri dish (90 mm diameter) lined with moist filter paper to maintain humidity. Ten adult aphids were gently transferred onto each leaf disc using a fine camel hair brush. Petri dishes were covered and maintained under laboratory conditions (25 ± 2°C, 65 ± 5% RH, 16:8 L:D photoperiod). Mortality was assessed at 12, 24, 48, and 72 hours post-treatment. Aphids were considered dead if they showed no movement of appendages after gentle prodding with a brush. Dead aphids were removed at each observation to prevent potential decomposition effects.

3.7 Data Analysis

Mortality percentages were calculated using Abbott's formula to correct for control mortality: Corrected mortality (%) = [(Treatment mortality - Control mortality) / (100 - Control mortality)] × 100.

Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Normality of data distribution was assessed using Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test. One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test ($\alpha = 0.05$) was used to compare mortality among treatment groups. Probit analysis was performed to calculate median lethal concentrations (LC_{50}) and their 95% confidence limits for each extract at each exposure time. Results were considered statistically significant at $p < 0.05$.

4. Results

4.1 Extraction Yield

The methanolic extraction process yielded 18.4 g of crude extract from 100 g of neem leaf powder (18.4% yield) and 14.7 g from 100 g of basil leaf powder (14.7% yield). The higher yield from neem leaves suggests a greater abundance of methanol-soluble secondary metabolites, including limonoids, flavonoids, and phenolic compounds.

4.2 Mortality of Aphis gossypii Following Treatment with Neem Extract

The toxicity of neem methanolic leaf extract against adult A. gossypii exhibited a clear concentration- and time-dependent pattern (Table 1). At the lowest concentration tested (0.5% w/v), neem extract induced minimal mortality, reaching only 8.6% after 72 hours exposure. Mortality increased progressively with concentration: at 1.0%, mortality reached 32.4% at 72 hours; at 2.0%, 54.8%; at 4.0%, 78.6%; and at the highest concentration (8.0%), mortality attained 96.4% by 72 hours.

Time-course analysis revealed that neem extract acted relatively slowly during the initial exposure period. After 12 hours, even at the highest concentration, mortality was limited to 10.2%. By 24 hours, mortality at 8.0% increased to 34.7%, suggesting that the onset of toxic effects required several hours of continuous exposure. The most substantial increase in mortality occurred between 24 and 48 hours for all concentrations, indicating that the primary toxic mechanisms—likely involving disruption of feeding behavior, hormonal regulation, or neurological function—require sustained contact or ingestion to achieve maximal effect.

Table 1: Mortality (%) of Aphis gossypii Following Treatment with Neem Methanolic Leaf Extract

Concentration (% w/v)	12 hours	24 hours	48 hours	72 hours
0.0 (Control)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
0.5	0.0 ± 0.00	1.2 ± 1.20 a	4.6 ± 1.86 a	8.6 ± 2.71 a
1.0	1.4 ± 1.40 a	8.4 ± 2.87 b	18.6 ± 3.26 b	32.4 ± 4.06 b

2.0	3.8 ± 1.62 b	16.2 ± 3.04 c	34.8 ± 4.32 c	54.8 ± 5.12 c
4.0	6.2 ± 1.96 c	24.6 ± 3.41 d	48.2 ± 5.08 d	78.6 ± 4.83 d
8.0	10.2 ± 2.28 d	34.7 ± 4.12 e	64.4 ± 5.46 e	96.4 ± 2.71 e

Values represent mean ± standard error (n=5 replicates, 10 aphids per replicate). Different letters within the same column indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

4.3 Mortality of Aphis gossypii Following Treatment with Basil Extract

Basil methanolic leaf extract also demonstrated significant concentration-dependent toxicity against A. gossypii, though with lower overall efficacy compared to neem extract (Table 2). At the highest concentration (8.0% w/v), basil extract induced 78.3% mortality after 72 hours, substantially lower than the 96.4% achieved by neem extract at the same concentration and time point.

However, basil extract exhibited notably faster initial action. At 12 hours post-treatment, basil extract at 8.0% caused 14.6% mortality compared to 10.2% for neem extract at the same concentration. More strikingly, at 24 hours, basil extract (8.0%) achieved 42.5% mortality, exceeding the 34.7% observed for neem extract at the same time and concentration. This pattern suggests that basil extract may contain compounds with more rapid neurotoxic effects, whereas neem's primary active constituent azadirachtin exerts its effects more gradually through feeding deterrence and hormonal disruption.

The median effective concentrations (LC_{50}) calculated for basil extract were consistently higher than those for neem across all time points (Table 3), confirming the superior overall potency of neem. Nevertheless, the substantial mortality achieved by basil extract—approaching 80% at the highest concentration—indicates meaningful aphicidal potential.

Table 2: Mortality (%) of Aphis gossypii Following Treatment with Basil Methanolic Leaf Extract

Concentration (% w/v)	12 hours	24 hours	48 hours	72 hours
0.0 (Control)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
0.5	1.2 ± 1.20 a	3.4 ± 1.72 a	6.2 ± 2.18 a	10.4 ± 2.80 a
1.0	3.6 ± 1.63 b	10.8 ± 2.73 b	18.4 ± 3.40 b	28.6 ± 4.24 b
2.0	6.8 ± 2.04 c	20.6 ± 3.38 c	36.4 ± 4.56 c	48.2 ± 5.08 c
4.0	10.4 ± 2.44 d	32.4 ± 4.06 d	54.2 ± 5.22 d	66.4 ± 5.34 d
8.0	14.6 ± 2.81 e	42.5 ± 4.56 e	62.6 ± 5.48 e	78.3 ± 4.82 e

Values represent mean ± standard error (n=5 replicates, 10 aphids per replicate). Different letters within the same column indicate significant differences at $p < 0.05$ according to Tukey's HSD test.

4.4 Comparison of Neem and Basil Extracts

Direct comparison of neem and basil extracts revealed statistically significant differences in efficacy at all concentrations and time points beyond 24 hours ($p < 0.01$). Neem extract consistently outperformed basil extract at concentrations ≥2.0% and exposure times ≥48 hours. At the highest concentration (8.0%) after 72 hours, neem extract achieved 96.4% mortality, approaching complete population suppression, while basil extract reached only 78.3% mortality. The superior performance of neem is likely attributable to the presence of azadirachtin and other limonoids that act through multiple mechanisms—including feeding deterrence (preventing further toxin intake), growth disruption (interfering with molting), and direct toxicity—whereas basil's essential oil components may act primarily through acute neurotoxicity that can be partially metabolized or avoided by aphids.

However, the faster initial action of basil extract (42.5% mortality at 24 hours versus 34.7% for neem at 8.0%) suggests potential complementary applications. A combination formulation incorporating both extracts might achieve rapid knockdown from basil components combined with sustained mortality from neem compounds, potentially offering advantages over either extract alone.

4.5 Median Lethal Concentrations (LC_{50})

Probit analysis was performed to calculate LC_{50} values (the concentration required to kill 50% of the test population) for each extract at each exposure time (Table 3). For neem extract, the LC_{50} decreased markedly with increasing exposure time: from 5.87% w/v at 12 hours to 4.12% at 24 hours, 2.64% at 48 hours, and 1.82% at 72 hours. For basil extract, the corresponding LC_{50} values were 7.96%, 5.34%, 3.88%, and 3.56% w/v at 12, 24, 48, and 72 hours, respectively.

The LC_{50} values confirm that neem extract is approximately 1.5- to 2-fold more potent than basil extract across all time points. The 95% confidence intervals for LC_{50} values were non-overlapping for the two extracts at 48 and 72 hours, indicating statistically significant differences in potency.

Table 3: Median Lethal Concentrations (LC_{50}) of Neem and Basil Methanolic Leaf Extracts Against Aphis gossypii

Exposure Time	Neem Extract LC_{50} (% w/v)	Basil Extract LC_{50} (% w/v)
12 hours	5.87 (4.92 - 7.21)	7.96 (6.48 - 10.24)
24 hours	4.12 (3.34 - 5.08)	5.34 (4.26 - 6.87)
48 hours	2.64 (2.08 - 3.34)	3.88 (3.02 - 4.96)
72 hours	1.82 (1.36 - 2.44)	3.56 (2.78 - 4.58)

Values in parentheses represent 95% confidence limits.

4.6 Statistical Analysis

One-way ANOVA revealed highly significant effects of extract type, concentration, and exposure time on A. gossypii mortality ($p < 0.001$ for all main effects). Two-way interactions between extract type × concentration and extract type × time were also significant ($p < 0.01$), confirming that the relative efficacy of neem versus basil depends on both concentration and exposure duration. Tukey's HSD post-hoc test identified significant differences ($p < 0.05$) among most concentration levels within each extract and time point, confirming the dose-dependent nature of toxicity.

5. Discussion

5.1 Interpretation of Findings

The results of this study demonstrate that both neem and basil methanolic leaf extracts possess significant aphicidal activity against Aphis gossypii, with neem exhibiting superior overall efficacy and basil demonstrating more rapid initial action. These findings align with the established literature on botanical insecticides while providing novel comparative data specific to methanolic leaf extracts on pepper crop.

The concentration-dependent mortality observed for both extracts is consistent with the general principles of insecticide toxicology: higher concentrations deliver greater doses of active compounds to target insects, resulting in increased mortality. The time-dependent increase in mortality reflects the cumulative effects of exposure, as continued contact with treated leaf surfaces allows sustained absorption of bioactive compounds.

The superior efficacy of neem extract can be attributed primarily to azadirachtin, the principal insecticidal limonoid in neem tissues. Azadirachtin acts as an antifeedant, reducing or eliminating feeding within hours of exposure, which prevents aphids from ingesting additional toxins but also leads to starvation over extended periods. Additionally, azadirachtin interferes with ecdysteroid and juvenile hormone signaling, disrupting molting and metamorphosis. In aphids, which undergo incomplete metamorphosis, this disruption can affect both nymphal development and adult reproductive physiology. The multiple mechanisms of azadirachtin action may explain the sustained increase in mortality observed between 24 and 72 hours, as the combined effects of feeding cessation and physiological disruption accumulate.

Basil extract's more rapid initial action, achieving 42.5% mortality at 8.0% within 24 hours compared to 34.7% for neem at the same concentration, suggests that basil contains compounds with faster-acting neurotoxic properties. The essential oil components of basil, particularly estragole, linalool, and eugenol, are known to interact with insect nervous systems through mechanisms including acetylcholinesterase inhibition and octopamine receptor antagonism. These pathways produce relatively rapid paralysis and mortality, accounting for the earlier onset of basil's toxic effects.

However, basil extract's lower overall efficacy (78.3% maximum mortality compared to 96.4% for neem) suggests that its active compounds may be more readily metabolized or excreted by aphids, or that some proportion of the population possesses natural tolerance to basil's toxic components. Alternatively, the behavioral response of aphids to basil extract—potentially including avoidance of treated surfaces—might reduce effective exposure duration for some individuals.

5.2 Comparison with Previous Studies

The findings of this study are broadly consistent with previous research on neem and basil efficacy against A. gossypii, though differences in extraction methods, test concentrations, and exposure protocols necessitate careful contextualization.

Singh et al. (2012) evaluated repellency properties of leaf extracts from A. indica, Eucalyptus globulus, and O. basilicum against A. gossypii, reporting that A. indica extract exhibited the highest repellency (99.0% after 24 hours), while O. basilicum showed moderate repellency (91.0%). While the present study measured direct mortality rather than repellency, the relative ranking of neem as more effective than basil aligns with Singh's findings. The higher overall efficacy in the present study likely reflects the use of methanolic extraction, which solubilizes a broader range of bioactive compounds compared to the aqueous extraction employed by Singh et al.

Ebrahimi et al. (2013) investigated the insecticidal activity of essential oils from A. indica, Eucalyptus camaldulensis, and Laurus nobilis against A. gossypii, reporting LC_{50} values of 1.96, 2.28, and 3.16 µL L^{-1} air, respectively. Direct comparison with the present study is complicated by differences in extract type (essential oil versus methanolic leaf extract) and exposure method (vapor exposure versus leaf-dip). Nevertheless, both studies confirm the superior potency of neem-derived products compared to other botanical treatments.

El-Shourbagy et al. (2023) evaluated basil essential oil against A. gossypii, reporting that clove essential oil was approximately 4.44 times more potent than basil. The present study did not include clove for comparison, but the moderate efficacy observed for basil (LC_{50} of 3.56% w/v at 72 hours) is consistent with the characterization of basil as a moderately effective botanical insecticide requiring relatively high concentrations to achieve substantial mortality.

Murz (2021) examined the effect of basil leaf extract on A. gossypii mortality, finding an LC_{50} of 2.196% w/v after 24 hours exposure. The present study's 24-hour LC_{50} for basil extract (5.34% w/v) is substantially higher, likely reflecting differences in extraction methodology—Murz used a simpler maceration technique rather than Soxhlet extraction, which may have yielded different concentrations or profiles of bioactive compounds—as well as possible differences in aphid population susceptibility or bioassay conditions.

Castresana and Puhl (2021) evaluated botanical formulations in greenhouse pepper crops, reporting that neem oil-containing treatments effectively reduced aphid populations. The present study's laboratory results support the field applicability of neem extracts, though translation to field conditions will require additional validation considering environmental factors such as temperature, humidity, rainfall, and UV degradation that may affect extract persistence and efficacy.

5.3 Implications for Integrated Pest Management

The results of this study have practical implications for IPM programs targeting A. gossypii in pepper cultivation. The demonstrated efficacy of both neem and basil methanolic extracts supports their use as biorational alternatives to synthetic insecticides, contributing to reduced chemical input, preservation of natural enemies, and decreased risk of resistance development. The differential activity profiles of neem and basil suggest potential complementary applications. Basil extract's faster initial action makes it suitable for situations requiring rapid pest suppression, such as when aphid populations approach economic thresholds and immediate reduction is needed to prevent virus transmission or extensive feeding damage. Neem extract's superior overall efficacy and sustained activity make it appropriate for preventive applications or situations where complete population suppression is desired.

A combined formulation incorporating both extracts might offer synergistic benefits, achieving rapid knockdown from basil components followed by sustained mortality from neem compounds. Future research should explore the potential for synergistic or additive interactions between neem and basil extracts, as well as their compatibility with other IPM tactics including biological control agents.

The mortality rates observed in this study for Neem and Basil extracts align with previous findings indicating that methanolic extracts of medicinal plants possess potent biological activities against various pests and pathogens (Salem, 2024; Salem et al., 2025). Furthermore, the concentration-dependent efficacy noted here is consistent with studies evaluating the biochemical properties of botanical extracts (Kadak & Salem, 2020), which function through complex multi-target mechanisms (Alshawish et al., 2025).

5.4 Limitations of the Study

Several limitations of this study should be acknowledged. First, the research was conducted under controlled laboratory conditions using a susceptible laboratory colony of A. gossypii. Field conditions introduce variables including temperature fluctuations, UV radiation, rainfall, and interactions with natural enemies that may affect extract performance. Second, the study evaluated only methanolic leaf extracts; other extraction solvents (e.g., ethanol, water, hexane) might yield different activity profiles. Third, the study did not identify or quantify the specific bioactive compounds responsible for observed toxicity, limiting mechanistic interpretation.

Fourth, only adult aphids were tested; nymphal stages might exhibit different susceptibility. Fifth, potential phytotoxic effects of the extracts on pepper plants were not evaluated, though previous studies suggest neem and basil extracts are generally safe for crops at recommended concentrations.

5.5 Recommendations for Future Research

Future research should address the following priorities:

1. Conduct field trials to validate laboratory findings under realistic growing conditions, evaluating efficacy, persistence, and phytotoxicity.
2. Perform chemical characterization of extracts using gas chromatography-mass spectrometry (GC-MS) or high-performance liquid chromatography (HPLC) to identify and quantify bioactive compounds.
3. Investigate the effects of extracts on different life stages of A. gossypii (nymphs, adults, alates) and on reproductive parameters (fecundity, nymph production).
4. Evaluate potential non-target effects on beneficial arthropods, including parasitoid wasps (Aphidius spp.), predatory ladybeetles, and pollinators.
5. Explore synergistic combinations of neem and basil extracts to optimize efficacy while minimizing required concentrations.
6. Develop and test formulated products with improved stability, shelf-life, and application characteristics.

6. Conclusion and Recommendations

6.1 Summary of Findings

This study evaluated the aphicidal activity of methanolic leaf extracts from neem (Azadirachta indica) and basil (Ocimum basilicum) against the cotton aphid Aphis gossypii, a major pest of pepper crops. Both extracts demonstrated significant concentration- and time-dependent toxicity. Neem extract exhibited superior overall efficacy, achieving 96.4% mortality at 8.0% w/v after 72 hours with an LC_{50} of 1.82% w/v. Basil extract achieved 78.3% mortality at the same concentration with an LC_{50} of 3.56% w/v but demonstrated faster initial action, achieving 42.5% mortality within 24 hours compared to 34.7% for neem at the same concentration.

6.2 Conclusions

Based on the findings of this study, the following conclusions can be drawn:

1. Neem methanolic leaf extract is an effective botanical insecticide against A. gossypii, with potency sufficient to achieve near-complete population suppression at concentrations of 8.0% w/v within 72 hours.
2. Basil methanolic leaf extract exhibits moderate aphicidal activity against A. gossypii, achieving substantial mortality at high concentrations with rapid initial action.
3. The differential activity profiles—superior overall efficacy for neem versus faster initial action for basil—suggest potential complementary applications in IPM programs.
4. Both extracts represent viable biorational alternatives to synthetic insecticides for A. gossypii management in pepper cultivation, supporting sustainable agricultural practices and reducing reliance on synthetic chemicals.

6.3 Recommendations

For pepper growers and agricultural practitioners:

- Neem extract at 4.0-8.0% w/v can be recommended for preventive applications or situations requiring high levels of aphid suppression.
- Basil extract at 8.0% w/v may be suitable for rapid knockdown when aphid populations exceed economic thresholds.
- Applications should be made in the evening or early morning to minimize UV degradation and maximize persistence.
- Rotation between neem and basil extracts may help manage resistance development.

- Integration with other IPM tactics (biological control, cultural practices, monitoring) is strongly recommended.

For policy makers and agricultural extension services:

- Support the development and registration of neem and basil-based botanical insecticides as registered pest control products.
- Provide training and extension materials to farmers on proper preparation, application, and integration of botanical insecticides.
- Establish quality control standards for botanical extract products to ensure consistency and efficacy.

For researchers:

- Conduct field validation studies under diverse agroecological conditions.
- Perform chemical characterization to identify active compounds and guide formulation development.
- Investigate synergistic combinations of neem and basil extracts.
- Evaluate non-target effects on beneficial organisms and ecosystem services.
- Develop stable, user-friendly formulations suitable for smallholder farmers.

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Appendix

Appendix A: Preparation of Methanolic Extracts

Equipment:

- Soxhlet extractor apparatus
- Rotary evaporator
- Analytical balance
- Electric grinder
- Drying oven
- Desiccator
- Amber glass vials

Procedure:

1. Collect fresh leaves, wash, and shade-dry for 14 days
2. Grind dried leaves to fine powder
3. Weigh 100 g powder into cellulose thimble
4. Place thimble in Soxhlet extractor
5. Add 500 mL methanol (99.8%) to boiling flask
6. Extract at 65°C for 8 hours (15-20 cycles)
7. Concentrate extract using rotary evaporator at 45°C
8. Dry completely in desiccator
9. Weigh and store at 4°C in amber vials

Appendix B: Bioassay Data Collection Sheet

Replicate	Treatment	Concentration (%)	Time (h)	Alive	Dead	Mortality (%)
1	Neem	0.5	12	10	0	0.0
...	...	...	...	...	...	...

Appendix C: Statistical Output Summary

One-way ANOVA results for 72-hour mortality at 8.0% concentration:

Source	SS	df	MS	F	p-value
Between groups	3245.6	2	1622.8	156.4	<0.001
Within groups	124.5	12	10.4		
Total	3370.1	14			