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ORIGINAL ARTICLE

Development of a bioinsecticide derived from dehydrated neem (*Azadirachta indica a. juss*) leaf extract and green coconut husk liquid (*Cocos nucifera L.*)

Desenvolvimento de um bioinseticida derivado do extrato de folhas desidratadas de nim (*Azadirachta indica a. juss*) e do líquido da casca verde do coco (*Cocos nucifera L.*)

Eleonora Osana Farina Sartori[1], Carlúcio Roberto Alves[2], Luis de França Camboim Neto[2], Sacha Aubrey Alves Rodrigues Santos[3], José Ferreira Nunes[2], Cristiane Clemente de Mello Salgueiro[2], Antonio Eufrásio Vieira-Neto[4]

1. Secretaria de Educação, Governo do Estado do Rio Grande do Sul, Veranópolis, RS, Brasil. 2. Universidade Estadual do Ceará (UECE), Fortaleza, CE, Brasil. 3. Núcleo de Biologia Experimental, Universidade de Fortaleza (UNIFOR), Fortaleza, CE, Brasil. 4. Secretaria Municipal de Educação, Prefeitura de Fortaleza (PMF), Fortaleza, CE, Brasil.

Abstract

Objective: to develop and evaluate the efficacy of a bioinsecticide formulated from the aqueous extract of dehydrated neem leaves (*Azadirachta* indica), supplemented with green coconut husk liquid (LCCV) as an adjuvant. **Method:** The insecticide formulation was evaluated for its effectiveness against sucking insects *Aphis gossypii* and *Dactylopius coccus* in lemon and eggplant crops, alongside evaluations of foliar sensitivity. Bioinsecticide formulations composed of 10, 20, 50, 80, and 90% neem extract combined with 90, 80, 50, 20, and 10% LCCV, respectively, were sprayed onto infested plants. Seven days after application, leaf integrity, phytotoxicity, and pest mortality rates were recorded. **Results**: The bioinsecticide exhibited strong inhibitory effects on insect gastrointestinal function, impairing development, growth, fertility, fecundity, and ultimately inducing mortality. The formulation containing 90% neem extract and 10% LCCV demonstrated the highest efficacy. Chemical characterization revealed higher protein content in the pure neem extract relative to the combined formulation, reflecting the influence of tannins, phenolics, and secondary metabolites from LCCV. Soluble carbohydrate levels decreased in the mixed bioinsecticide; however, a substantial amount was retained, indicating preserved biological potential. Infrared spectroscopy confirmed the chemical profiles of the extracts and suggested the formation of a new compound. **Conclusion**: Overall, the integration of neem extract with LCCV yielded a bioinsecticide with satisfactory insecticidal activity, supporting its potential as a biotechnological tool for the sustainable management of agricultural pests.

Keywords: bioinsecticide; agricultural pests; *Azadirachta* indica; green coconut husk liquid.

Resumo

Objetivo: desenvolver e avaliar a eficácia de um bioinseticida formulado a partir do extrato aquoso de folhas desidratadas de nim (*Azadirachta* indica), suplementado com líquido da casca do coco verde (LCCV) como adjuvante. **Método**: A formulação inseticida foi avaliada quanto à sua eficácia contra os insetos sugadores *Aphis gossypii* e *Dactylopius coccus* em culturas de limão e berinjela, juntamente com a avaliação da sensibilidade foliar. Formulações do bioinseticida, compostas por 10, 20, 50, 80 e 90% de extrato de nim combinados, respectivamente, com 90, 80, 50, 20 e 10% de LCCV, foram pulverizadas sobre plantas infestadas. Sete dias após a aplicação, foram avaliadas a integridade foliar, a fitotoxicidade e as taxas de mortalidade dos insetos. Resultados: O bioinseticida apresentou forte efeito inibitório sobre a função gastrointestinal dos insetos, comprometendo o desenvolvimento, o crescimento, a fertilidade, a fecundidade e, por fim, induzindo a mortalidade. A formulação contendo 90% de extrato de nim e 10% de LCCV demonstrou a maior eficácia. A caracterização química revelou maior teor de proteínas no extrato puro de nim em comparação à formulação combinada, refletindo a influência de taninos, compostos fenólicos e metabólitos secundários provenientes do LCCV. Os níveis de carboidratos solúveis diminuíram no bioinseticida misto; entretanto, uma quantidade substancial foi mantida, indicando a preservação do potencial biológico. A espectroscopia no infravermelho confirmou os perfis químicos dos extratos e sugeriu a formação de um novo composto. **Conclusão:** A integração do extrato de nim com o LCCV resultou em um bioinseticida com atividade inseticida satisfatória, sustentando seu potencial como ferramenta biotecnológica para o manejo sustentável de pragas agrícolas.

Palavras chave: bioinseticida; pragas agrícolas; *Azadirachta* indica; líquido da casca do coco verde.

INTRODUCTION

The control of agricultural pests still relies predominantly on synthetic chemical insecticides, which, despite their effectiveness, have raised growing concerns regarding environmental contamination, selection of resistant pest populations, and adverse effects on non-target organisms, including pollinators and natural enemies[1,2,3]. These limitations have intensified the global search for safer and more sustainable pest management strategies. In this context, botanical insecticides have gained considerable attention due to their biodegradability, reduced ecotoxicological impact, and compatibility with integrated pest management programs. Among economically relevant sap-sucking insects, aphids (Aphis gossypii) and scale insects (Dactylopius coccus) stand out because of their high reproductive capacity, rapid colonization of host plants, and ability to transmit phytopathogens[5]. By feeding directly from the phloem, these pests induce physiological stress, impair photosynthesis, promote chlorosis and tissue deformation, and favor the development of sooty

Correspondence: Antonio Eufrásio Vieira-Neto. Av. dos Expedicionários, 3910, Jardim América, Fortaleza - CE, 60410-446. E-mail: aevneto@gmail.com
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mold, ultimately leading to significant yield losses[6,7].

Plant-derived extracts rich in secondary metabolites represent a promising alternative for the control of sap-sucking pests8. *Azadirachta indica a. juss* (neem), a tropical species widely cultivated in several regions of the world, is one of the most extensively studied botanical insecticides[9,10]. Neem tissues contain a complex mixture of limonoids, among which azadirachtin, salannin, nimbin, and meliantriol are the most biologically active[11,12]. These compounds exhibit multiple modes of action, including antifeedant effects, disruption of molting and metamorphosis, inhibition of oviposition, and impairment of insect growth and reproduction[13]. Although neem seeds are the richest source of these metabolites, neem leaves also present relevant insecticidal activity and offer practical advantages related to accessibility, renewability, and lower production costs, making them attractive for large-scale and low-input formulations[8,11].

The effectiveness of botanical insecticides, however, is strongly influenced by formulation strategies[14]. The use of adjuvants capable of improving extract stability, adhesion to leaf surfaces, spreading, and bioavailability of active compounds can substantially enhance insecticidal performance[15]. In this regard, liquid obtained from green coconut husks (LCCV) emerges as a promising and underexplored resource. Generated in large volumes as a by-product of coconut processing, particularly in tropical countries such as Brazil, green coconut husk residues pose an environmental challenge when inadequately disposed of 16. Chemically, this liquid fraction is rich in soluble carbohydrates, minerals, polyphenols, and tannins, compounds known for their antimicrobial, antioxidant, and protein-binding properties, which may contribute to biological activity or synergistic effects when combined with plant extracts[17].

Cocos nucifera L. is widely cultivated in Brazil, especially in the Northeast region, where coconut water consumption and industrial processing have increased markedly in recent decades18. This expansion has intensified the accumulation of husk residues in urban and rural environments[19]. Studies have shown that the green coconut husk contains high concentrations of hydrolyzable and condensed tannins, as well as fermentable sugars and inorganic ions, which have supported its use in applications ranging from effluent remediation to biotechnological processes[20]. Physicochemical characterization of LCCV typically reveals elevated electrical conductivity and slightly acidic pH, characteristics that may limit its direct agronomic application but allow its use as a diluted component or functional additive in formulated products, provided appropriate adjustments are made18. Therefore, the objective of this study was to develop and evaluate a bioinsecticide formulated from the aqueous extract of dehydrated neem (*Azadirachta* indica) leaves combined with green coconut husk liquid (*Cocos nucifera L.*), assessing its physicochemical characteristics, insecticidal efficacy against *Aphis gossypii* and Dactylopius coccus, and potential effects on plant tissues.

MATERIAL AND METHODS

Bioassays were conducted from February 2016 to March 2017 to assess the response of aphid (Homoptera) and scale insect (Hemiptera: Diaspididae) populations in lemon and eggplant crops under controlled greenhouse conditions[21].

Reagents

Analytical-grade reagents used for chemical analyses included sulfuric acid (PA), 5% phenol solution, and trichloroacetic acid with 10% acetone, provided by the University of Fortaleza (UNIFOR)[22].

Equipment

Chemical and physicochemical analyses were performed using a full-spectrum UV-Vis spectrophotometer (NanoDrop ND-1000), a fixed-wavelength UV-Vis spectrophotometer (Nanovue Plus, λ = 280 nm), an FTIR spectroscope with ATR detector, and an Ultraspec spectrophotometer[23].

Green Coconut Husk Liquid Extraction (LCCV)

LCCV was obtained from mechanically processed green coconut husks at EMAPE (Maracanaú, CE). After hammer-mill fragmentation and five-roller pressing, two fractions were separated: fiber and liquid. LCCV represented approximately 30% of the initial biomass and exhibited a dark-brown coloration with phenolic compounds, fermentable sugars, cations, anions, and high COD and BOD levels. Samples were refrigerated at 5 °C and subsequently characterized at UNIFOR for pH and UV-Vis spectral properties, focusing on protein quantification[24].

Extract of Dehydrated Neem Leaves

Azadirachta indica leaves were collected in March 2016 in the metropolitan region of Fortaleza (CE). Branches were pruned at night, and leaves were shade-dried for six days, yielding a brittle dehydrated material that was manually separated from stems and ground into a fine powder. To prepare the extract, 150 g of powdered leaves were immersed in 1 L of water (pH 8.2; 20.6 °C) under agitation for 24 h, following an adapted protocol. The suspension was sieved and filtered to avoid residues capable of obstructing sprayers, then refrigerated.

Preparation of Bioinsecticide Solutions

Five formulations (S1–S5) were prepared by combining neem extract and LCCV at proportions ranging from 10–90% (v/v). Solutions were mixed sequentially (neem first, followed by LCCV), labeled, and color-coded for subsequent greenhouse testing. Based on preliminary screening, S1 (90% neem extract + 10% LCCV) was selected as the optimal bioinsecticide. Control solutions were prepared for comparative assays: SX (10% LCCV + 90% water) and SY (90% neem extract + 10% water). These were evaluated in vivo alongside S123. The formulation scheme is presented in Table 1[25].

Table 1. Experimental design of bioinsecticide formulations (Neen+LCCV; Only Neen; Only LCCV).

Solutions	Proportions	Quantities	
S1 - Orange Ribbon	10% of Nim 90% of LCCV	5 ml of Nim	45 ml - LCCV
S2 - Lilac Ribbon	20% of Nim 80% of LCCV	10 ml of Nim	40 ml - LCCV
S3 – Red Ribbon	50% of Nim 50% of LCCV	25 ml of Nim	25 ml - LCCV
S4 - Green Ribbon	80% of Nim 20% of LCCV	40 ml of Nim	10 ml - LCCV
S5 - Blue Ribbon	90% of Nim 10% of LCCV	45 ml of Nim	5 ml - LCCV
Isolated Compounds			
S1 – Orange Ribbon	10%Lccv + 90% Neen		
Sx - Blue Ribbon	10%Lccv + 90% Water		
Sy - Pink Ribbon	90% Neen + 10% Water		

Source: author

Evaluation of Bioinsecticide Activity

Experiments were conducted in a greenhouse in Eusébio (CE) using lemon (Citrus × limon) and eggplant (Solanum melongena) plants. Prior to treatment, pest populations were quantified. Each formulation was applied using a 1-L manual sprayer at ~30 mL per branch, directed to the abaxial leaf surfaces, with three replicates per treatment. After seven days, treated leaves were collected and examined at the FATENE Biochemistry Laboratory for leaf integrity, desiccation, pest behavior, and mortality under a digital stereomicroscope[26].

The second in vivo assay was performed on citrus trees (lemon and orange) in Caucaia (CE). The optimal formulation (S1) and control insecticides were applied in three replicates using a 1-L sprayer. Approximately 30 mL per branch was administered to the abaxial surfaces. After twelve days, plants were revisited for population reassessment and efficacy analysis[26].

Partial Chemical Characterization of the Bioinsecticide: Determination of Total Protein Content and Soluble Carbohydrates

Protein quantification in neem extract and in the combined bioinsecticide followed the Bradford colorimetric protocol. A standard curve using bovine serum albumin (BSA) was generated in the NanoDrop software. Samples underwent TCA precipitation (10% acetone), incubation at −20 °C for 24 h, centrifugation, and analysis of the protein-rich supernatant. Protein concentrations were calculated based on absorbance at 595 nm[26].

Soluble carbohydrates were quantified using the phenol–sulfuric acid (Dubois) method. A standard glucose curve (10–70 µg/mL) was prepared. Samples were reacted with concentrated sulfuric acid and 5% phenol, incubated for 20 min, and read at 490 nm using an Ultraspec spectrophotometer. Analyses were conducted in triplicate to determine total carbohydrate content[24].

Fourier-Transform Infrared Spectroscopy (FTIR–ATR)

FTIR-ATR spectra were acquired to identify functional groups and molecular signatures of neem extract and the neem–LCCV formulation. The spectral region (600–3400 cm^{-1}) was analyzed to determine vibrational modes indicative of major chemical constituents[25].

RESULTS

The aqueous extract of dehydrated *Azadirachta* indica leaves exhibited physicochemical characteristics consistent with a concentrated plant-derived bioactive matrix, including a translucent olive-green coloration, low viscosity, and absence of particulate matter following sequential filtration. The yield obtained, approximately 1 L of extract per 150 g of powdered leaves, indicates efficient solubilization of both polar and amphiphilic metabolites.

During the first bioassay series, the five formulations (S1–S5) containing graded proportions of Nim and LCCV demonstrated distinct patterns of foliar tolerance and entomotoxicity in lemon and eggplant plants. Although all treatments exhibited robust activity against aphids and scale insects, the physiological responses of plant tissues were markedly species-dependent. Eggplant leaves, featuring a pilose adaxial surface and elevated epidermal permeability, manifested pronounced symptoms of phytotoxic stress, including darkening, wilting, marginal necrosis, and laminar curvature, suggesting that their morphological and anatomical features facilitated excessive penetration or retention of active constituents (Figure 1).

Among the tested formulations, S5, composed of 90% neem extract and 10% LCCV, showed the highest insecticidal efficacy (Figure 2). This formulation promoted complete mortality of both *Aphis gossypii* and Dactylopius coccus. In addition, plants treated with S5 maintained leaf structural integrity, as evidenced by stereomicroscopic analysis, which revealed preserved epidermal and mesophyll tissues without visible signs of phytotoxicity.

In comparison with the control treatments, the LCCV–water formulation (SX) resulted in only partial suppression of insect populations, whereas the neem–water formulation (SY) produced high, but not complete, mortality. Overall, intermediate formulations containing lower proportions of neem extract exhibited reduced insecticidal effectiveness when compared with S5.

Figure 1. Leaf sensitivity and pest mortality with the use of S1 bioinsecticide solution (10% Neem + 90% LCCV); (A) S1 (orange ribbon) against Limoeiro leaves: before sprinkling; (B) S1 (orange ribbon) against Limoeiro leaves: after sprinkling; (C) S1 (orange ribbon) against eggplant leaves: before sprinkling; (D) S1 (orange ribbon) against eggplant leaves: after sprinkling.

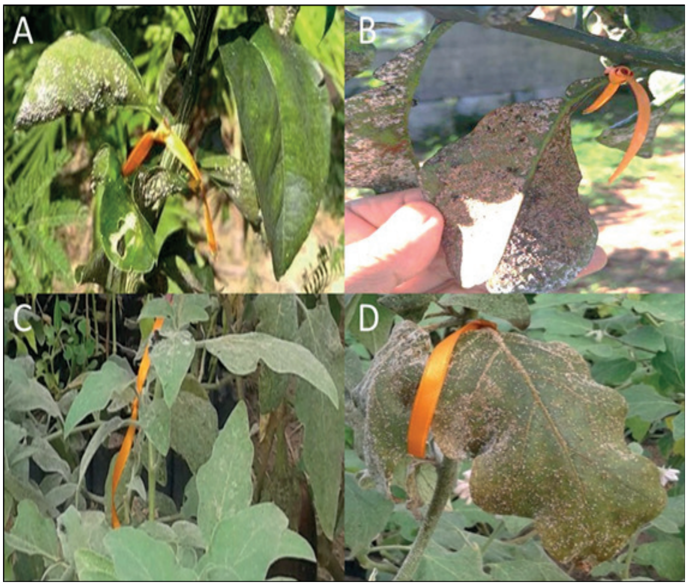

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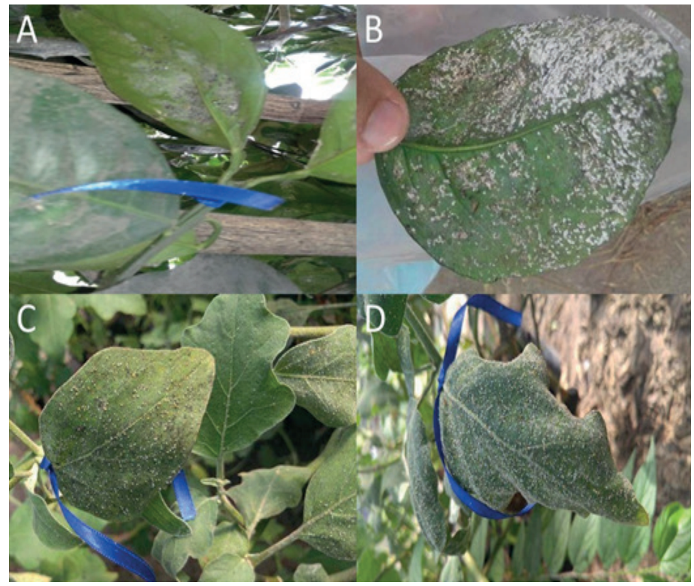
Figure 2. Leaf sensitivity and pest mortality with the use of bioinsecticidal solution S5 (90% neem + 10% LCCV): (A) S5 (orange ribbon) against Limoeiro leaves before sprinkling; (B) S5 (orange ribbon) against Limoeiro leaves after sprinkling; (C) S5 (orange ribbon) against eggplant leaves before sprinkling; (D) S5 (orange ribbon) against eggplant leaves after sprinkling.



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Comparative analyses of oxidative behavior demonstrated that the extract obtained from dehydrated neem leaves exhibited faster oxidative darkening than the extract prepared from fresh macerated leaves (Table 2). The addition of green coconut husk liquid (LCCV) to the dehydrated-leaf extract reduced the rate of oxidative darkening, although oxidation was not completely suppressed.

Table 2. Evaluation of leaves after seven days of bioinsecticide application.

Jars	Lemon plant assay	Eggplant assay
S1 – Orange Ribbon (10% Neem + 90% LCCV)	Controlled pests ++++ brittle	Controlled pests Wilted and very darkened appearance at the bottom and on top and brittle appearance
S2 – Lilac Ribbon	Controlled pests	Controlled pests
(20% Neem + 80% LCCV)	+++ Brittle	Withered and darkened above and below
S3- Red Ribbon	Controlled pests	Controlled pests
(50% Neem + 50% LCCV)	++ brittle	Slightly wilted and darkened
S4- Green Ribbon	Controlled pests	Controlled pests
(80% Neem + 20% LCCV)	+ brittle	Minimally wilted and darkened
S5- Blue Ribbon	Controlled pests	Controlled pests
(90% Neem + 10% LCCV)	Normal appearance	Minimally wilted and darkened

Source: author

Leaf-injury assessments indicated that the S5 formulation was agronomically compatible with lemon plants, showing low damage scores. In contrast, eggplant plants consistently exhibited high levels of leaf injury, which justified their exclusion from subsequent bioassays.

aled a clear relationship between neem concentration and insect mortality. The S5 formulation and the neem–water control (SY) achieved approximately 95% suppression, whereas the LCCV–water control (SX) resulted in only 50% mortality. Intermediate formulations produced proportional mortality rates ranging from 50% to 85%, depending on the neem extract concentration (Table 3).

Table 3. Mortality of aphids and mealybugs treated with different concentrations of the bioinsecticide.

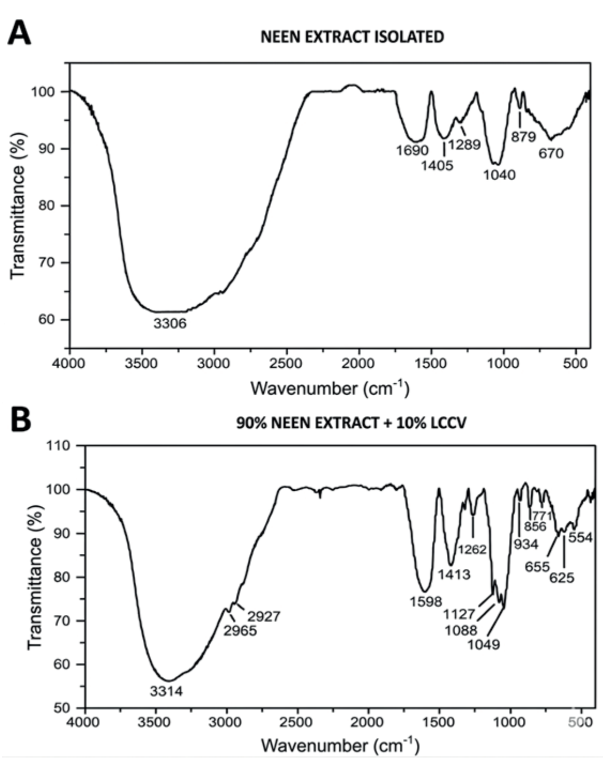
Bionseticide	% of dead pests (average)
Isolated component 1 (Neem + water)	95
Isolated component 2 (LCCV + water)	50
10% of Nim + 90% of LCCV	50
20% of Nim + 90% of LCCV	60
50% of Nim + 90% of LCCV	70
80% of Nim + 90% of LCCV	85
90% of Nim + 90% of LCCV	95

Source: author

Chemical analyses revealed that the bioinsecticide formulations exhibited a slightly acidic pH, approximately 6,2. Protein quantification using the Bradford method showed higher protein content in the pure neem extract compared to the neem–LCCV mixture. Additionally, a reduction in soluble carbohydrate concentrations was observed in the composite formulation, although measurable residual levels remained.

FTIR spectroscopy confirmed the presence of characteristic absorption bands corresponding to C–O, N–H, and C=O functional groups typically associated with neem-derived metabolites. In the neem–LCCV formulation, subtle spectral shifts were detected, suggesting chemical interactions between components of the two extracts (Fig. 3).

Figure 3. Infrared spectrum (FTIR): (A) Neen extract; (B) bioinsecticide developed from the formulation Neen+LCCV.



DISCUSSION

The results obtained in this study highlight the relevance of formulation design as a central element in the performance of botanical insecticides. Rather than acting solely through the intrinsic toxicity of plant-derived metabolites, the bioinsecticide evaluated here demonstrated that physicochemical interactions among formulation components, plant tissues, and target insects play a decisive role in determining biological efficacy and agronomic safety. The integrated analysis of insect mortality, plant responses, oxidative behavior, and chemical characterization reveals a coherent pattern in which neem-derived limonoids, formulation matrix properties, and crop-specific traits interact to shape pest control outcomes. This perspective supports interpreting the findings not as isolated biological effects, but as the result of coordinated mechanisms operating at biochemical, physiological, and agronomic levels.

Within this formulation framework, green coconut husk liquid (LCCV), characterized by its dark-brown coloration and high organic load, was incorporated as a functional coadjuvant rather than as a primary insecticidal agent. Its role was strategically defined to enhance spray adhesion to plant surfaces and to mitigate lixiviation losses under conditions simulating field application. Organic matrices rich in polyphenolic compounds have been increasingly explored in botanical pesticide formulations due to their ability to promote retention and prolong the persistence of active metabolites on the phylloplane, without contributing directly to insect toxicity27. This function is particularly relevant for aqueous neem-based extracts, which, despite their biological potency, are inherently susceptible to rapid runoff and environmental degradation after application28. By improving formulation stability and surface interaction, LCCV likely contributed to the enhanced and more consistent insecticidal performance observed across treatments, reinforcing the importance of formulation engineering in maximizing the agronomic effectiveness of plant-derived bioinsecticides.

This formulation approach is consistent with current trends in biopesticide development, which prioritize the association of plant-derived bioactive compounds with biodegradable carriers or adjuvants capable of optimizing deposition, spreading, and interaction with plant and insect cuticles29. Rather than intensifying toxicity, these systems aim to prolong the residence time of active metabolites on the target surface, thereby improving efficacy while maintaining low environmental impact[30]. The use of agroindustrial residues such as LCCV further reinforces this approach by aligning pest control technologies with the principles of the circular economy and sustainable resource use.

Plant-specific responses to the formulations also highlight the importance of leaf surface characteristics in determining agronomic compatibility. Lemon leaves, which present a smoother epidermal surface, a well-developed cuticular layer, and lower surface hydrophilicity, exhibited minimal injury even when exposed to formulations containing high concentrations of neem extract. It contrasts with the higher sensitivity observed in other plant species and suggests that cuticular thickness and surface morphology play a decisive role in regulating extract penetration and associated stress responses.

Collectively, these findings indicate that the interaction between botanical formulations and plant tissues is not solely dependent on extract composition, but is strongly modulated by leaf architecture and physicochemical properties. Understanding these interactions is essential for the rational design of bioinsecticides that are both biologically effective against target pests and agronomically safe for specific crops, reinforcing the need for crop-oriented evaluation during formulation development.

The outstanding efficacy observed for the S5 formulation is primarily associated with its elevated proportion of neem extract, which ensures a higher availability of azadirachtin and related limonoids31. These compounds are well known for their multifaceted interference with insect physiology, acting simultaneously on developmental, digestive, and reproductive processes32. By disrupting molting cycles, inhibiting chitin biosynthesis, and compromising nutrient assimilation, neem-derived metabolites exert cumulative stress that ultimately leads to mortality, particularly in hemipteran pests whose feeding strategy depends on sustained phloem extraction[33]. The complete suppression of both target species obtained with S5 underscores the importance of maintaining sufficiently high concentrations of these bioactive constituents to achieve consistent biological outcomes.

Importantly, the greater performance of S5 compared with the neem–water control indicates that the presence of LCCV enhances formulation efficiency without contributing direct insecticidal toxicity. The absence of substantial mortality in the LCCV–water treatment supports its role as a functional adjuvant rather than an active toxicant. Through improved adhesion to leaf surfaces, increased retention after application, and possible modulation of compound solubility, LCCV likely facilitates prolonged exposure of insects to neem metabolites. This indirect contribution aligns with previous reports describing azadirachtin-rich extracts as potent regulators of hemipteran feeding behavior and development, while also highlighting the relevance of formulation design in maximizing the efficacy of botanical insecticides. Together, these findings reinforce the potential of optimized neem-based formulations as sustainable alternatives for the management of sap-sucking pests.

The faster oxidative darkening observed in the extract prepared from dehydrated neem leaves is likely a consequence of structural and biochemical changes induced during the dehydration and milling processes34. Cellular desiccation disrupts membrane integrity and promotes the release and exposure of chlorophyll derivatives, phenolic compounds, and other redox-active molecules that are normally compartmentalized within intact tissues[35]. Once exposed, these constituents become more susceptible to oxidation, particularly under light, resulting in accelerated pigment degradation and color darkening[36].

The incorporation of green coconut husk liquid partially moderated this oxidative process, suggesting a modulatory rather than fully protective effect. LCCV contains a complex mixture of phenolics, tannins, and inorganic ions that may influence redox dynamics by scavenging reactive species, chelating pro-oxidant metals, or altering the microenvironment in which photo-oxidative reactions occur. Such interactions can slow, but not entirely prevent, pigment oxidation, which explains the incomplete suppression of darkening observed in the composite extract. This behavior is consistent with photochemical models describing chlorophyll degradation pathways and pigment instability under light exposure, where oxidation progresses through multiple, competing reactions influenced by matrix composition and light intensity [35,36].

The agronomic compatibility of the S5 formulation with lemon plants demonstrates that elevated concentrations of neem extract can be applied without causing relevant phytotoxic effects in this crop, supporting its practical feasibility under agricultural conditions. In contrast, the recurrent leaf injury observed in eggplant reveals a marked species-specific sensitivity to the formulation, underscoring the need to consider crop selectivity during the development and recommendation of botanical insecticides. Such differential responses are likely associated with variations in leaf surface characteristics, cuticular composition, and metabolic tolerance among plant species.

The clear dose–response relationship observed across treatments further confirms that insecticidal performance is closely linked to the concentration of neem-derived limonoids, particularly azadirachtin37. Increasing neem content resulted in progressively higher mortality rates, reflecting the targeted action of these compounds on key physiological systems of insects, including endocrine signaling, digestive efficiency, and reproductive capacity. Additionally, the gradual escalation of mortality across formulations supports the notion that botanical insecticides operate through cumulative and multi-site physiological disruption, such as growth retardation, feeding inhibition, and reduced fecundity, rather than through rapid neurotoxic mechanisms typical of many synthetic insecticides. This mode of action not only explains the observed mortality patterns but also highlights the potential of neem-based formulations as sustainable tools for pest management with a reduced risk of acute toxicity and resistance development [8,38].

The slightly acidic pH measured in the formulations creates a chemical environment that is conducive to the stability and solubility of neem limonoids, which may partially explain the sustained biological activity observed for the bioinsecticide 39,40. Limonoid compounds are known to be sensitive to extreme pH conditions, and maintenance of a mildly acidic range likely favors their preservation and functional availability after application[41].

The lower protein content detected in the neem–LCCV formulation is consistent with the well-documented affinity of tannins and phenolic acids present in LCCV for protein molecules. Through hydrogen bonding and hydrophobic interactions, these compounds can promote protein complexation and precipitation, thereby reducing apparent protein solubility in the mixed extract. This interaction does not necessarily imply a loss of biological function but rather reflects a reorganization of macromolecular associations within the formulation.

A similar trend was observed for soluble carbohydrates, whose reduced levels suggest chemical interactions with LCCV constituents that may influence matrix organization and extract stability. Importantly, the persistence of measurable carbohydrate fractions indicates that the formulation maintains sufficient structural coherence and potential for synergistic biological effects. The FTIR spectral shifts provide further evidence of intermolecular interactions between neem-derived metabolites and LCCV components, supporting the formation of new complexed structures. Such physicochemical synergism is likely to influence compound delivery, surface retention, and bioavailability, reinforcing the relevance of formulation chemistry in enhancing the performance of complex botanical bioinsecticides [26,42].

Taken together, the evidence indicates that the Neem–LCCV formulation represents a strategically balanced system in which biological activity and formulation chemistry are mutually reinforcing. The enhanced insecticidal performance observed with higher neem concentrations, combined with the modulatory role of LCCV on adhesion, stability, and molecular interactions, underscores the importance of matrix engineering in botanical pesticide development. At the same time, the crop-dependent phytotoxic responses and the observed physicochemical adjustments within the formulation emphasize that effectiveness cannot be dissociated from selectivity and environmental compatibility. By integrating insect physiology, plant surface characteristics, and formulation chemistry, this study contributes to a more nuanced understanding of how complex botanical systems can be optimized for sustainable pest management, reinforcing their potential as viable alternatives to conventional synthetic insecticides.

A temporal gap between the experimental phase and manuscript submission should be acknowledged as a limitation of this study. This delay was due to unforeseen interruptions in the research process. Despite this, all experimental data were systematically recorded, securely preserved, and later analyzed following consistent and rigorous methodological procedures. Given the stability of the evaluated parameters and the controlled experimental conditions, this interval does not compromise the validity of the results. Nonetheless, it should be taken into account when considering the temporal context of the findings.

CONCLUSION

The integration of dehydrated *Azadirachta* indica leaf extract with green coconut husk liquid (LCCV) proved to be an effective and sustainable strategy for the development of a botanical bioinsecticide. The formulation combined the insecticidal activity of neem limonoids with the physicochemical properties of LCCV, resulting in enhanced adhesion, persistence, and biological efficacy against hemipteran pests, while maintaining agronomic compatibility with selected crops. In addition to its high insecticidal performance, the Neem–LCCV bioinsecticide aligns with principles of sustainable agriculture by employing renewable, biodegradable components and a multimechanistic mode of action that reduces reliance on neurotoxic synthetic pesticides. The physicochemical interactions promoted by leaf dehydration and LCCV incorporation further contributed to formulation stability and bioavailability. Overall, these findings support the Neem–LCCV formulation as a promising, scalable alternative for integrated pest management and environmentally responsible crop protection.

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