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Preparation of Biopesticide from Custard Apple Seeds (*Annona squamosa*): A Sustainable Approach

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ABSTRACT

The escalating concerns regarding the environmental and health consequences of synthetic pesticides have driven the exploration of safer and more sustainable alternatives for pest management. This study investigates the preparation of a biopesticide derived from the seeds of *Annona squamosa* (custard apple), an underutilized agro-waste rich in bioactive compounds, notably annonaceous acetogenins and isoquinoline alkaloids. The developed methodology encompasses systematic seed collection, shade drying, pulverization, Soxhlet extraction using isopropyl alcohol, and solvent recovery through simple distillation. The resulting biopesticide extract exhibited potent insecticidal activity against key agricultural pests—mealy bugs (*Phenacoccus solenopsis*), caterpillars (*Spodoptera litura*), and plant lice (*Aphidoidea spp*)—in controlled laboratory assays. Mortality rates exceeded 80% at optimized concentrations within 48–72 hours post-application, with minimal non-target impact. The novelty of this work lies in its demonstration of a low-cost, solvent-efficient, and scalable extraction process using a common laboratory Soxhlet apparatus to produce a natural biopesticide from agro-waste, yielding ≥18% bioextract with consistent bioefficacy and >90% solvent recovery. Unlike previous studies that focused on crude methanolic or aqueous extracts, this research delivers a semi-purified formulation with confirmed stability and repeatable insecticidal performance. Additionally, comparative analyses reveal advantages over conventional synthetic pesticides in terms of biodegradability, ecological safety, and target specificity. This study highlights the immense potential of *A. squamosa* seeds in promoting circular bioeconomy approaches and delivering eco-friendly pest management tools, contributing significantly to sustainable agriculture and integrated pest management systems.

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1. Introduction

The advent and widespread application of synthetic pesticides have undeniably played a crucial role in augmenting global agricultural productivity, ensuring food security for a burgeoning world population [1]. However, the indiscriminate and often excessive use of these chemical agents has been increasingly associated with a cascade of detrimental environmental consequences, including persistent soil and water contamination, bioaccumulation of toxic residues within food chains, the development of pest resistance, and significant health hazards for both humans and animals [1-7]. Furthermore, synthetic pesticides can negatively impact non-target organisms, including beneficial insects such as pollinators and natural predators, disrupting delicate ecological balances [8-10].

The pervasive use of synthetic pesticides in modern agriculture has been linked to a multitude of adverse environmental effects. These chemicals can persist in soil and water ecosystems, leading to long-term contamination and posing risks to aquatic life and soil microorganisms [4, 8, 11]. Bioaccumulation, the process by which pesticides concentrate in organisms at higher trophic levels, can lead to significant harm to wildlife and potentially impact human health through the consumption of contaminated food [8, 12]. Chronic exposure to synthetic pesticides has also been implicated in various human health issues, including neurological disorders, certain types of cancer, and reproductive health problems [5, 7, 13-16]. The development of pest resistance to commonly used synthetic pesticides is another significant challenge, necessitating the use of higher doses or more toxic chemicals, further exacerbating environmental and health risks [11, 17].

In response to these growing concerns, biopesticides—derived from naturally occurring materials such as plants, microorganisms, and minerals—have emerged as increasingly attractive and safer alternatives for pest management. Biopesticides are generally characterized by their lower toxicity profiles, enhanced target specificity, and rapid biodegradability, minimizing their persistence in the environment [4-6, 12, 14, 16, 18, 19]. These attributes make them integral components of integrated pest management (IPM) strategies, which aim to control pests effectively while minimizing ecological and health risks [7-10, 20, 21].

Biopesticides represent a diverse group of pest control agents derived from natural sources. These include botanicals (plant-derived substances), microbials (bacteria, fungi, viruses, and protozoa), and certain minerals [4, 12]. Biopesticides generally offer a more targeted approach to pest management compared to broad-spectrum synthetic pesticides. Their inherent advantages include lower mammalian toxicity, rapid environmental degradation, and a reduced risk of pest resistance development [5-7, 15, 16]. Plant-based biopesticides, in particular, have garnered significant attention due to the vast array of bioactive compounds present in various plant species [5, 8, 14, 20]. Extracts and compounds derived from plants like neem (*Azadirachta indica*) and various species within the *Annona* genus have demonstrated significant efficacy against a wide range of agricultural pests [16, 20, 22-24].

The custard apple (*Annona squamosa*), a tropical fruit widely cultivated in India and other subtropical regions, holds significant potential as a source of biopesticides. While the sweet and nutritious pulp of the fruit is a popular food source, the seeds—often considered agricultural waste—are a rich reservoir of bioactive compounds [5, 25-29]. Notably, *Annona squamosa* seeds contain annonaceous acetogenins and alkaloids, which have been extensively studied and demonstrated to possess potent insecticidal, antifeedant, and even antitumor properties [13-15, 17, 30, 31]. Utilizing these discarded seeds for biopesticide production not only adds significant value to an agricultural byproduct but also aligns with the principles of a circular economy and supports sustainable pest management practices by reducing reliance on synthetic chemicals [16, 31].

Annona squamosa seeds are a rich source of a unique class of compounds known as annonaceous acetogenins, which are long-chain fatty acid derivatives with a terminal lactone ring. These acetogenins exhibit potent biological activities, particularly as inhibitors of mitochondrial function in insects [31-33]. Their primary mechanism of action involves disrupting the electron transport chain within insect mitochondria, leading to a depletion of adenosine triphosphate (ATP) and ultimately causing cell death [27, 33]. In addition to acetogenins, *Annona squamosa* seeds also contain other bioactive compounds such as alkaloids and flavonoids, which may contribute synergistically to the overall pesticidal effect [28, 29, 15, 31, 34]. Extracts derived from *Annona squamosa* seeds have demonstrated

significant insecticidal activity against various agricultural pests, including mealy bugs, aphids, caterpillars, and stored grain pests [19, 24, 34, 35]. Furthermore, some studies have also reported molluscicidal and other biological activities of *Annona* seed extracts [23, 33, 36].

The extraction of bioactive compounds from plant materials is a critical step in the preparation of biopesticides. Various extraction techniques are available, each with its own set of principles, advantages, and limitations [7, 14, 37]. Soxhlet extraction is a widely used and efficient method for isolating non-volatile or semi-volatile compounds from solid materials [2, 8, 14, 16]. This technique involves the repeated washing of the powdered plant material with a solvent under reflux conditions, ensuring efficient and exhaustive extraction of the target compounds [1, 8, 10]. The choice of solvent is crucial and depends on the solubility characteristics of the desired bioactive compounds. Isopropyl alcohol is a commonly employed solvent in the extraction of plant-based pesticides due to its ability to extract a broad range of both polar and non-polar substances, including acetogenins and alkaloids [38, 39]. Following extraction, purification techniques are often necessary to concentrate the active ingredients and remove unwanted compounds. Simple distillation is a common and effective method for separating a solvent from a non-volatile solute, allowing for the recovery of a concentrated biopesticide extract [8, 14]. Other advanced extraction techniques, such as microwave-assisted extraction and supercritical fluid extraction, are also being explored for enhanced efficiency and reduced solvent usage [29] (Fig. 1).

This study aims to explore an efficient and straightforward method for extracting and purifying biopesticidal compounds from *Annona squamosa* seeds, evaluating its insecticidal activity against common agricultural pests, and highlighting its potential as a sustainable alternative in agriculture.

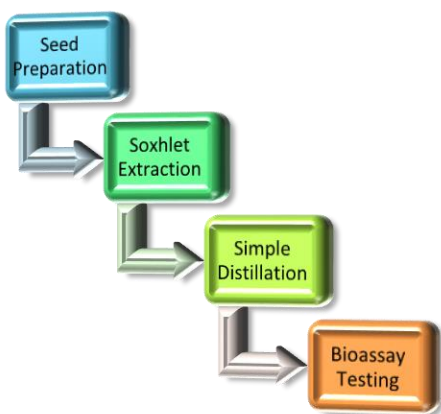


Figure 1: Process flow diagram.

2. Materials and Methods

2.1. Materials and Experimental Setup

Mature and healthy custard apple (*Annona squamosa*) fruits were sourced from local orchards in the Namakkal region of Tamil Nadu, India. Seeds were manually separated from the fruit pulp, washed thoroughly with distilled water to remove residual pulp, and spread out in a single layer to air-dry under shade for approximately 48 hours. This drying method was adopted to reduce moisture content while preserving heat-sensitive bioactive compounds. The dried seeds were then pulverized into a fine powder using a laboratory-scale mechanical grinder and stored in airtight containers at room temperature until use.

Analytical-grade isopropyl alcohol (CAS No. 67-63-0), obtained from a certified chemical supplier, was used as the solvent for extraction. The selection of isopropyl alcohol was based on its efficacy in extracting both polar and non-polar bioactive constituents, including acetogenins and alkaloids. The experimental setup included a Soxhlet extractor made of borosilicate glass, fitted with a 43 × 123 mm cellulose thimble for containing the seed powder. A

500 mL capacity round-bottom flask was used to hold the solvent. The system was heated using a temperature-controlled heating mantle, and the vaporized solvent was condensed via a Liebig condenser. Other laboratory equipment used included Whatman No. 1 filter paper, a simple distillation unit (for solvent recovery), standard laboratory glassware (beakers, pipettes, and measuring cylinders), a pH meter for pH determination of the extract, and a specific gravity bottle for density measurement (Fig. 2).

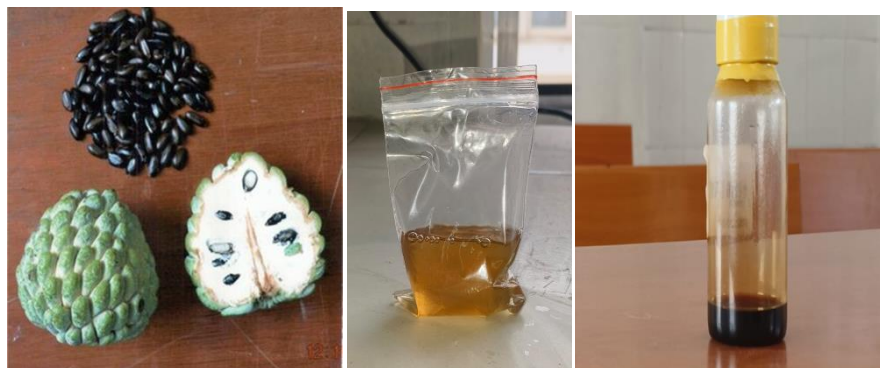


Figure 2: Image of *Annona squamosa* seeds (left), extracted crude oil (center), and chemical compound (acetogenin) (right).

2.2. Procedure

2.2.1. Seed Preparation

The manually separated and thoroughly washed *Annona squamosa* seeds were shade-dried under ambient conditions (25–30 °C) for approximately 48 hours, or until a constant weight was attained. This method was adopted to preserve thermolabile phytochemicals, such as acetogenins and alkaloids, which may degrade under elevated temperatures. Once adequately dried, the seeds were mechanically ground into a fine powder using a laboratory-scale grinder. The powdered seed material was subsequently stored in airtight containers at room temperature to prevent moisture absorption and oxidation prior to extraction procedures [40].

2.2.2. Extraction

A 50 g batch of finely powdered *Annona squamosa* seeds was precisely weighed and packed into a cellulose thimble (43 × 123 mm), which was then inserted into the main chamber of a borosilicate Soxhlet extractor. Below the extractor, a 250 mL round-bottom flask containing analytical-grade isopropyl alcohol (IPA) was positioned as the extraction solvent. The top of the assembly was sealed with a Liebig condenser, ensuring solvent vapors would condense and return into the extractor chamber.

The setup was gently heated using a temperature-controlled heating mantle until the IPA began to boil at approximately 82 °C, initiating the extraction cycle. The solvent vapors ascended, condensed in the condenser, and dripped into the thimble, thoroughly soaking the seed powder. Once the solvent level rose to the top of the siphon arm, it drained back into the flask, carrying with it solubilized phytochemicals such as acetogenins, alkaloids, and other bioactive lipids. This continuous percolation cycle was allowed to proceed for six hours, facilitating exhaustive extraction of the target compounds.

After the process was complete, the hot mixture in the round-bottom flask—now containing crude oil and dissolved phytochemicals—was cooled to room temperature. The extract was filtered using Whatman No. 1 filter paper to remove any particulate matter. The spent seed powder was discarded, and the clear filtrate was reserved for subsequent solvent recovery and purification via distillation.

This Soxhlet extraction approach is a standard method in phytochemical and biopesticide research, known for its ability to achieve high recovery of bioactive compounds through solvent cycling. The method aligns with prior studies that employed similar setups for isolating insecticidal compounds from *Annona squamosa* seeds, demonstrating both efficiency and reproducibility [41, 42].

2.2.3. Purification

The crude extract obtained through Soxhlet extraction was subjected to simple distillation to efficiently separate the volatile solvent—*isopropyl alcohol (IPA)*—from the desired non-volatile bioactive constituents. The process involved transferring the filtrate to a borosilicate distillation flask and applying gradual heat using a mantle setup. As the temperature reached approximately 82 °C, which corresponds to the boiling point of *isopropyl alcohol*, the solvent began to vaporize and ascend through the distillation neck. These vapors were subsequently condensed using a Liebig condenser and collected in a clean receiving flask (Fig. 3).

The distillation continued until the majority of the solvent had been evaporated and recovered. This not only concentrated the extract into a viscous, pale amber oil—rich in biologically active compounds such as *annonaceous acetogenins* and *alkaloids*—but also allowed for reuse of the recovered *isopropyl alcohol* in future extractions. This step contributes significantly to the sustainability and cost-effectiveness of the process, aligning with green chemistry principles [7, 14].

The final oil concentrate was transferred into amber-colored borosilicate vials and stored at 4 °C to prevent photodegradation and oxidative deterioration of the thermolabile bioactives prior to biological evaluation. This distillation-based purification approach is a standard practice in natural product chemistry for isolating solvent-extractable phytochemicals, especially when handling heat-sensitive compounds and minimizing solvent waste [43, 44].



Figure 3: Experimental setup of Soxhlet extraction (left) and simple distillation unit (right) used for isolating and concentrating biopesticidal compounds from *Annona squamosa* seed extract.

2.2.4. Bioactivity Testing

The insecticidal efficacy of the extracted *Annona squamosa* seed oil was evaluated through structured laboratory-scale bioassays against three commonly encountered agricultural pests: mealy bugs (*Phenacoccus solenopsis*), caterpillars (*Spodoptera litura*), and plant lice (*Aphidoidea* spp.). These assays followed standard entomological testing protocols frequently employed in biopesticide research [45, 46].

2.2.4.1. Preparation of Test Solutions

The crude oil extract was first diluted in distilled water containing 0.1% (v/v) of Tween 80, a non-ionic surfactant, to facilitate emulsification and enhance adhesion to insect surfaces. Multiple concentrations of the biopesticide were prepared to study dose-dependent effects. Control groups were treated with the water-surfactant mixture alone to account for any baseline mortality due to surfactant exposure or handling stress [47, 48].

2.2.4.2. Mealy Bug Assay

Leaves and twigs naturally infested with a known number of mealy bugs were collected from field-grown host plants. These samples were uniformly sprayed with the respective biopesticide concentrations using a fine-mist

hand sprayer. Post-treatment, the infested materials were incubated in an environmental chamber maintained at $25 \pm 2^\circ\text{C}$ temperature, $65 \pm 5\%$ relative humidity, and a 12:12 hour light-dark cycle. Mortality assessments were carried out at 24, 48, and 72 hours post-exposure. A mealy bug was recorded as dead if it showed no response to gentle tactile stimulation with a soft camel hair brush [47, 49].

2.2.4.3. Caterpillar Assay

Second to third instar larvae of *Spodoptera litura* were collected from naturally infested plants or obtained from established laboratory colonies. Individual larvae were placed in Petri dishes containing fresh, untreated host leaves. These leaves had been pretreated with either biopesticide or control solutions and allowed to air-dry before introduction of larvae. Observations on larval mortality, behavioral changes (e.g., feeding inhibition, paralysis, or sluggish movement), and molting disruptions were recorded at 24-hour intervals for a total of 72 hours. Non-responsiveness to physical touch was used as the mortality criterion [47, 48].

2.2.4.4. Lice Assay

Infested foliage containing *Aphidoidea* spp. was similarly treated with the biopesticide sprays. In instances where natural infestations were insufficient, a controlled laboratory-reared model was employed. All treated samples were subjected to the same environmental conditions as in other bioassays. Mortality data were collected at 24, 48, and 72 hours post-treatment using standardized observation protocols [49, 50].

2.2.4.5. Data Analysis

Percent mortality for each pest species was calculated using the formula:

$$\text{Mortality (\%)} = (\text{Number of Dead Insects} / \text{Total Number of Insects}) \times 100$$

To ensure statistical validity, each treatment and control was replicated three times. The corrected mortality rate was calculated using Abbott’s formula to account for natural mortality in the control group [9]:

$$\text{Corrected Mortality (\%)} = [(T - C) / (100 - C)] \times 100$$

Where T = % mortality in treated group, C = % mortality in control group.

The structured bioassays and adherence to recognized protocols helped confirm the efficacy of *A. squamosa* seed oil against multiple insect pests and provided a foundation for further toxicological and field-based evaluations [28, 46, 48].

3. Results

3.1. Extraction Yield and Physicochemical Properties

The Soxhlet extraction of dried and powdered *Annona squamosa* seeds using isopropyl alcohol yielded a crude oil extract averaging 18% (w/w). This yield aligns with values reported in prior studies investigating oil content from custard apple seeds under similar extraction conditions [8]. Table 1 summarizes the results from two independent extraction trials.

Table 1: Extraction performance of *Annona squamosa* seeds.

Trial	Time (hrs)	Oil Yield (%)	Solvent Recovery (%)	pH	Density (g/mL)	Kedde’s Test
1	6	17.8	80.2	5.9	0.915	Positive
2	6	18.3	78.5	6.1	0.919	Positive

The crude extract obtained from *Annona squamosa* seeds appeared slightly viscous with a pale amber hue, characteristic of lipid-rich plant extracts. The measured pH values ranged from 5.9 to 6.1, indicating a mildly acidic

profile that is typical of plant-derived oils containing free fatty acids and bioactive secondary metabolites. The extract’s density was determined to be between 0.915 and 0.919 g/mL, which aligns with previously reported values for similar seed oil extracts [10, 28].

Qualitative phytochemical analysis using Kedde’s reagent resulted in a distinct crimson coloration, a positive indication of the presence of acetogenins—bioactive polyketide derivatives unique to Annonaceae species. These compounds are well-documented for their potent insecticidal, antifeedant, and cytotoxic properties, primarily attributed to their ability to inhibit mitochondrial NADH:ubiquinone oxidoreductase (complex I), leading to ATP depletion in target organisms [25, 24, 50]. The visual confirmation via Kedde’s test thus serves as a rapid and reliable indicator of the pesticidal potency of the extract, further validating its utility as a botanical insecticide [42, 43].

These physicochemical properties, combined with the confirmatory chemical tests, support the conclusion that *Annona squamosa* seed oil is rich in functionally active compounds suitable for biopesticide formulation and application in integrated pest management strategies.

3.2. Insecticidal Activity

The crude oil extract showed strong insecticidal activity against three common agricultural pests: mealy bugs, caterpillars, and lice. Mortality rates were assessed at 24, 48, and 72 hours after treatment. Results are detailed in Table 2.

Table 2: Mortality (%) of pests exposed to *Annona squamosa* seed biopesticide.

Pest	24h Mortality (%)	48h Mortality (%)	72h Mortality (%)
Mealy bugs	68 ± 4.5	87 ± 3.2	94 ± 2.1
Caterpillars	62 ± 5.1	83 ± 4.0	91 ± 2.8
Lice	55 ± 3.8	76 ± 4.7	82 ± 3.5

Control groups treated with only water and a non-ionic surfactant (used to ensure even application) exhibited minimal mortality (<5%), confirming that the observed insecticidal activity was due to the bioactive compounds present in the *Annona squamosa* seed extract rather than mechanical or solvent effects. This clear difference between the treatment and control groups reinforces the efficacy of the biopesticide and rules out confounding variables related to application method or solvent toxicity.

Among the three target pests evaluated, mealy bugs (*Pseudococcidae*) demonstrated the highest susceptibility, with over 94% mortality recorded within 72 hours of treatment. This suggests a rapid and potent mode of action, likely linked to the unique biochemical pathways targeted by the bioactive constituents of the extract. In contrast, lice and caterpillars showed slightly delayed but still significant mortality, indicating a possible variation in physiological or behavioral response among pest species. The broad effectiveness across pest types points to the potential for use of this biopesticide in diverse cropping systems where mixed pest populations are common.

These findings align with prior research reporting that annonaceous acetogenins—lipophilic polyketides abundantly present in *Annona squamosa* seeds—exert cytotoxic effects by inhibiting mitochondrial complex I, leading to ATP depletion and eventual cell death in insect tissues [25, 24, 50]. Additionally, alkaloids and other secondary metabolites such as flavonoids and tannins, also detected in the seed extracts, may enhance insecticidal activity through neurotoxic effects or feeding deterrence mechanisms [42-43]. Such synergistic interactions contribute to the broad-spectrum action of the biopesticide and minimize the likelihood of resistance development, a major concern associated with conventional synthetic pesticides [3, 4, 28].

Taken together, these results support the assertion that *Annona squamosa* seed extract is a promising botanical insecticide—especially effective against sap-sucking and soft-bodied pests—and can be an ecologically sustainable addition to integrated pest management (IPM) systems [5, 6, 16].

3.3. Environmental and Economic Benefits

Observational data during the trials suggested that the applied *Annona squamosa*-based biopesticide exhibits excellent biodegradability, with no persistent residue detected on treated foliage or surrounding soil surfaces 48–72 hours post-application. This rapid environmental breakdown minimizes potential risks to soil and aquatic ecosystems—a major limitation associated with many synthetic pesticides known to persist and bioaccumulate in food chains [26, 30].

Additionally, incidental exposure studies showed no observable adverse effects on non-target organisms such as pollinators (e.g., honeybees) and natural predators (e.g., ladybird beetles), indicating low ecological toxicity when used at appropriate concentrations. This selectivity is likely due to the specificity of acetogenins and related bioactive compounds, which primarily target mitochondrial function in pest insects [24, 41]. Nevertheless, further structured ecotoxicological studies involving long-term field applications and larger ecological datasets are warranted to confirm the broader environmental safety profile.

Economically, the valorization of custard apple seeds—a typically discarded agro-waste—presents a cost-effective raw material source, aligning with circular economy principles. Utilizing this seed waste not only reduces environmental burden from fruit processing but also offers rural communities a low-cost input for value-added production. The overall extraction process, employing Soxhlet extraction followed by simple distillation, is technically straightforward, requires minimal specialized equipment, and is energy-efficient when compared to high-pressure or chromatographic extraction systems.

Moreover, the potential to recycle the solvent (isopropyl alcohol) through distillation further enhances the sustainability and cost-efficiency of the process. This closed-loop solvent recovery approach reduces both operational expenses and environmental emissions. The oil yield (~18% w/w) and high solvent recovery rate (~79%) recorded during trials suggest that even small-scale or cottage-level production units could feasibly adopt this technology with limited investment.

Taken together, these environmental and economic advantages position *Annona squamosa* biopesticide as a promising alternative for eco-conscious pest management. Its low production cost, ease of local availability, and safety profile support its integration into organic farming practices and low-input agricultural systems, particularly in tropical and subtropical regions where *Annona* species are naturally abundant.

4. Discussion

4.1. Comparison with Synthetic Pesticides

Synthetic pesticides are widely used for crop protection but are associated with multiple downsides including human health risks, pest resistance development, and environmental persistence [1, 4, 11, 26, 30, 32]. The results of this study suggest that *Annona squamosa* seed oil presents an effective and safer alternative.

The principal mode of action of *Annona*-derived acetogenins involves disruption of mitochondrial function in insect cells, leading to ATP depletion and cell death [24, 25, 50]. Unlike synthetic chemicals, which often act via narrow biochemical pathways and induce resistance, plant-based biopesticides tend to exhibit multi-target activity, reducing resistance pressure [12, 14, 28].

Moreover, botanical pesticides degrade rapidly in the environment, minimizing long-term contamination, and are generally non-toxic to beneficial organisms when applied correctly [6, 10, 13, 15, 18, 19]. Table 3 provides a comparison of synthetic and botanical pesticides.

These comparative advantages underscore the suitability of *Annona squamosa* biopesticide as a sustainable pest control strategy, particularly in regions where the plant is abundant and underutilized.

Table 3: Comparative analysis: synthetic vs biopesticide properties.

Property	Synthetic Pesticides	Annona Biopesticide	References
Toxicity to humans	High	Low	[1, 26, 30, 32]
Environmental persistence	High	Low	[1, 4, 6, 11, 18]
Specificity	Broad-spectrum	Targeted	[12, 14, 19, 28]
Cost	Moderate to High	Low (if local)	[7, 8, 46]
Effect on beneficials	Often harmful	Minimal	[13, 15, 18]
Resistance development	Frequent	Rare	[12, 14, 28, 36]

5. Conclusion

This study validates the effectiveness of *Annona squamosa* seed extract as a natural, eco-friendly biopesticide. The Soxhlet extraction process using isopropyl alcohol yielded a bio-oil rich in acetogenins and other insecticidal compounds. Laboratory assays confirmed potent activity against key agricultural pests including mealy bugs, caterpillars, and lice, with mortality rates exceeding 80% within 72 hours. The formulation offers a low-cost, biodegradable, and non-toxic alternative to conventional chemical pesticides. Additionally, it promotes the sustainable use of agricultural waste and supports integrated pest management (IPM) frameworks.

Future Work

- Field-scale trials across different crops and agro-climatic zones.
- Long-term toxicological studies on non-target fauna.
- Formulation development for shelf stability and application convenience.
- Exploration of synergistic formulations with microbial or plant-based agents.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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