

Development and Validation of an Eco-Friendly Neem-Based Biopesticide (*Azadirachta indica*) for Sustainable Agricultural Applications

Shabib Al Rashdi

National University of Science and Technology

Lakkimsetty Nageswara Rao

lnrao1978@gmail.com

American University of Ras Al Khaimah

Houriya Waleed Ali Al Shehhi

National University of Science & Technology

Alshima Ahmed Al-Mujaini

National University of Science and Technology

Fatma Waheed Albalushi

National University of Science and Technology

Said Hamed Rashid

National University of Science and Technology

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Abstract

This study presents a comprehensive investigation is thorough research on the production of green biopesticides using Neem (*Azadirachta indica*) leaves and Custard apple (*Annona squamosa*) seeds waste as a substitute for synthetic pesticides and present alternative solutions to environmental pollution caused by synthetic pesticides. The extraction of neem leaves was done through both hot and cold maceration process, and the extraction of custard apple seeds was done in Soxhlet extraction using solvents such as ethanol, ethyl acetate, hexane and acetone. Analysis of neem extracts by High-Performance Liquid Chromatography (HPLC) showed that azadirachtin was the predominant bioactive compound and Gas Chromatography-Mass Spectrometry (GC-MS) of the custard apple seed oil formed that squalene, oleic acid, stearic acid, and palmitic acid were the main compounds. Neem extracts were also attained by cold ethanol extraction which gave chemically stable extracts and hexane gave custard apple seed oil at about 5% w/w with a solvent recovery of more than 90%. It was shown by laboratory tests that up to 80% of the pest population could be reduced, and there were no phytotoxic effects or any other detrimental effect on the soil pH or the well-being of microbes. This data shows that agricultural waste can be turned into high-efficacy low-toxicity biopesticides, which would promote the principles of the circular economy and sustainable pest control.

1. Introduction

The widespread application of synthetic chemical pesticides in contemporary farming has created some significant environmental and health risks that comprise soil erosion, water and food pollution, pest resistance to pesticides and the adverse impacts of non-target organisms. These issues explain why there is an extreme need to develop safer and more sustainable approaches to pest management. Natural biopesticides made of plants and agro-residues have received growing interest because they are biodegradable, environmentally friendly and not very toxic (Abubakar et al.2023). The leaves of neem (*Azadirachta indica*) and the waste of custard apples are some of these resources and contain bioactive compounds (azadirachtin, squalene, and fatty acids), which have high insecticidal, antifungal, and antimicrobial activities (Adusei and Azupio 2022; Al-Kathiri et al. 2025).

This study is dedicated to the creation of a biopesticide that is environmentally friendly based on neem leaves and underused waste of custard apples in the form of seed as a sustainable source of bioactive compounds. Maceration and Soxhlet techniques were used as extraction methods, and the advanced analytical techniques were used to identify and characterize the most important phytochemicals and determine the efficiency of solvents, including High-Performance Liquid Chromatography (HPLC) and Gas Chromatography -Mass Spectrometry (GC-MS). The study also covers the increasing demand of pest control products that are environmentally friendly as well as in the agricultural areas that are arid where overuse of pesticides may cause a further depletion of scarce water resources and jeopardize food security (Al Rashdi et al. 2026).

The laboratory level study was used to carry out and involved preparation of the raw materials, extraction, chemical characterization and the concept design of the process. The analyses of the neem extracts were performed using HPLC, whereas the characterization of the custard apple seed extracts was examined using GC-MS to identify the presence of the compounds of pesticides. Overall, this study shows that there is a sustainable process of transforming agricultural waste into biodegradable pest control products, which will aid in integrated pest management and alternatives that is cheap and environmentally friendly to farmers (Chopra et al. 2025).

2. Materials and Methods

It describes the methodology framework which is employed in the design and testing of the biopesticides which are eco-friendly and are based on the leaves of neem and the seed waste of custard apple. These botanical resources are renewable and have bioactive compounds like azadirachtin, nimbin, squalene, and other fatty acids that have great insecticidal effects and antimicrobial effects (Dhakad et al. 2025; FAO 2020). The methodology combines both extraction methods, chemical characterization and process modelling to determine the viability of producing sustainable biopesticides. The focus is on environmental sustainability, effective use of agricultural leftovers, and possibilities of large-scale production of plant-based pest control products.

Azadirachta indica (neem) leaf bioactive compounds were extracted in a two-stage process with aqueous solvents and organic solvents; this would help to obtain maximum recovery of the thermolabile compound like azadirachtin. Washed fresh leaves were dried by air and pounded into fine powder. The water-soluble compounds were extracted by hot water (60 to 70°C, 3–4 hrs.) and cold water (48 to 72hrs.) with ethanol and ethyl acetate and obtained moderately polar and non-polar bio actives respectively. Vacuum filtration, reduced pressure (< 40 °C) concentration, vacuum oven, or freeze-drying, followed by desiccator storage were used to prepare extracts before chemical characterization (Fernandes et al. 2023; Foka 2023).

Custard apple seed bioactive oil was extracted to investigate the polarity of the solvent used on yield and composition. Seeds were shade-dried and ground to 250 to 500 µm. The extracts were carried out using Soxhlet (hexane with non-polar oils and acetone with moderately polar compounds) and 6 to 8 hours were carried out with continuous cycling. Reduced pressure (< 50°C) was used to remove solvents, and crude oil was stored at 4 ° C. The characterization of extracts was done using GC-MS, FTIR and HPLC, which have offered a reliable means of using custard apple seed waste as a green source of biopesticides.

3. Data Interpretation and Collection

3.1 HPLC Results for Neem

The bioactive components of neem leaf extract were identified, and their concentration was determined using High-Performance Liquid Chromatography (HPLC). In agreement with what has been reported in the literature, azadirachtin was identified as the major compound that has a retention time of 2.971 min (Ghosh 2014). Other peaks showed the existence of other phytochemicals in lower concentrations, which showed the complex phytochemical composition of neem leaves. The azadirachtin was the primary bioactive compound, the peak of which was prominent in the chromatogram of 2.971 minutes, and the results were presented in Table 1.

Table 1
HPLC Chromatogram Results for
Neem Extract

Retention Time (min)	Peak Area
2.103	17,612
2.522	200,492
2.971	5,481,744

3.2 GC-MS Results for Custard Apple Seed Oil

Analysis of custard apple seed oil using GC-MS analysis the key bioactive compounds, such as squalene (triterpene with anti-inflammatory properties, insecticidal properties, antioxidant properties), oleic acid (unsaturated fatty acid also with anti-inflammatory activities), stearic acid (saturated fatty acid and viscosity-stabilizing effects), palmitic acid (anti-microbial activity and surfactant like activity) (Ghoshal and Sandal 2024). These compounds highlight the fact that the extract has multifunctional potential in agriculture and pharmaceutical use.

3.3 Performance Indicators Recorded

Process performance Indicators used in the evaluation of performance and extract quality included yield (g extract/g dry weight), solvent evaporation rates, filtration ease, color clarity and bioactive compound concentration (mg/mL) assessed by HPLC or GC-MS, with the best performance being demonstrated by cold ethanol maceration in preserving azadirachtin in neem leaves, and hexane Soxhlet extraction of custard apple seeds with the highest level of oil yield and minimal thermal degradation and solvent recovery (Isman 2006).

3.4 Methodological Framework for Neem and Custard Apple Bioactive Extraction

The process flow was designed as a way of extracting and analyzing neem leaves and custard apple seeds. Biomass was shade-dried, ground to 250–500 µm, and cold ethanol macerated (neem) and cold hexane Soxhlet extracted (custard apple seeds) and optimized in terms of temperature, solvent to solid ratio, and time. Solvents were recrystallized (> 90%), and extracts were dried and stored at 4 °C. The quantity of bioactive compounds was measured by the methods of HPLC and GC -MS, and custard apple

seeds produced an oil (w/w) content of approximately 5%. The technical feasibility of the process, scalability, and effectiveness in mixing, heating, and solvent recycling were validated using simulations and it is possible to support the sustainable production of bio-pesticidal extracts. It starts with the sourcing and drying of biomass, then reduction of particle sizes and sieving to get a homogenous product to extract. The cold ethanol maceration of neem leaves is done to extract bioactive compounds, where the extract is then gathered by rotary evaporation. Simultaneously, custard apple seeds are exposed to solvent based extraction, solvent recovery, drying and storing of the extracted oil. The extracts obtained because of both the sources then undergo bioactive compound validation and chemical characterization to determine and quantify the major constituents of pesticides (Kamanga et al. 2025). This organized work process offers a systematic and reproducible mode of changing the agricultural biomass into sustainable bio-pesticidal products. The section describes the experimental, simulation and analytical methods that were employed to come up with sustainable bio-pesticides made from neem and custard apples. Soxhlet and maceration weighs were used to extract and GC–MS and HPLC were used to verify the presence of azadirachtin and squalene as the main bioactive. The experimental findings indicated that ethanol and hexane were the best solvents regarding yield and retention of compounds. This combination forms a scientifically proven and ecologically friendly system of industrial-level manufacturing of plant-based bio-pesticides (Khan and Patel 2023).

3.5. Validation of Design, Implementation, And Testing

The growing necessity of farming methods that are sustainable has penetrated the creation of eco-friendly alternatives to synthetic pesticides. This chapter provides the conceptualization, development and validation of a neem based biopesticide to be used in controlling the soil-borne pests without impairing the integrity of the ecosystem. The design focused on utilization of neem leaf waste instead of commercially produced seeds, compatibility to the environment and positive work with beneficial insects and soil microbiota, as well as the accessibility and cost-effectiveness of the technology to smallholder farmers. Extraction, formulation, chemical analysis, biological assessment, and simulation were some of the methodologies employed, and attention was paid to the conservation of soil and plant health by balancing effective control of pests by research with sustainable development of the agricultural sector.

This framework was implemented in the implementation phase to suggest a working biopesticide prototype by extracting the biopesticide in a systematic manner and optimizing it. Hot water extraction was a low-cost, small-scale procedure, and cold solvent extraction using ethyl acetate and ethanol was more useful in preserving thermolabile compounds and increasing phytochemical diversity. Refinements in the processes- such as pre-drying leaves, pH, bi-stage filtration, optimized solvent to powder ratios, enhanced yield, purity of the extract, chemical stability and microorganism safety. Difficulties of variable leaf moisture, rapid spoilage, clogging filtration, loss of solvent and odor of residual solvent were solved by standardized drying, refrigeration, finer filtration, sealed extraction, and airing of post-evaporation. All these steps guaranteed a successful, safe and scaled neem leaf bio-pesticide that could be applied to a larger scale in the agricultural field (Tiwari et al. 2011).

The filtered neem extracts were pH adjusted and refrigerated to come up with a prototype biopesticide that could be applied manually in the soil. Optimization of extract concentration, chemical stability and shelf life was done by laboratory testing. HPLC analytical validation was used to identify the concentration of azadirachtin and the related compounds, whereas the molecular identity of several bioactive components was confirmed with the help of GC–MS. The nature of chromatograms was that they had clear peaks that were taken to represent the target compounds which guaranteed reproducibility and chemical integrity of the formulation. Regulated laboratory experiments on plants and insects showed a maximum of 80 percent reduction in the population of pests without causing any detrimental effect on the development of the plants, their leaves, and the health of the soil. The presence of azadirachtin, nimbin and salannin as the main bioactive constituents were verified by the HPLC retention times (2.971 and 6.360⁻¹ min) and the GC–MS mass spectra confirmed the presence of azadirachtin, nimbin, and salannin as primary bioactive constituents (Koul and Walia 2009).

Environmental tests ensured that the formulation did not change the pH and the microbial communities in the soil, which indicates its compatibility with regenerative and organic agriculture and demonstrates its ability to comply with the UN SDGs 12 (Responsible Consumption) and 15 (Life on Land). Although there are positive laboratory outcomes, additional research will be required to measure field performance under variegated crops and climate, scale up shelf-life research and determine economic viability of large-scale production. All in all, the neem-based biopesticide was shown to have a high bioactive content, pest control, and safety to the environment, and as such, there is a scientifically proven basis in the future to scale-up and employ the biopesticide in actual agricultural applications. Figures 1 & 2 shows the HPLC chromatogram of bioactive compounds from neem leaves and GC/MS chromatogram shows multiple bioactive components in the sample.

4. Results and Discussion

The detailed discussion of the results of the experiment in terms of chemical composition, extraction efficiency, biological efficacy and environmental compatibility. Comparative evaluation, literature confirmation and graphic analysis were part of data interpretation to measure reliability and practical importance.

4.1 Extraction Efficiency Analysis

The recovery differed depending on method and selection of solvent and the yield of neem leaf using hot water maceration was 3.2% with a low level of azadirachtin and nimbin whereas cold solvent extraction by using ethyl acetate/ethanol yielded a higher % of 7.6 and storage of thermolabile compounds such as azadirachtin, salannin, and nimbin (Martínez et al. 2013).

4.2 Chemical Characterization via HPLC and GC–MS

HPLC analysis indicated a strong peak of azadirachtin at 2.971 min, which indicates the preservation of chemicals were shown in Fig. 4). The effectiveness and reproducibility of the extraction protocols were

also confirmed by GC-MS analysis which showed squalene, oleic acid, and palmitic acid as the key bioactive compounds of the extract was shown in Fig. 3 (Michel et al. 2023).

4.3 Biological Efficacy

Effective impacts of pesticidal action against aphids, rootworms, and fungi of soil were confirmed by use of laboratory assays shown in Fig. 5 (Neelendra Singh Verma et al. 2023). Growing plants with the formulation had better leaf vitality than the controls, which highlights the efficacy and safety of the formulation.

4.4 Soil and Environmental Compatibility

The analysis of soils revealed that the pH level did not decrease, and the number of microbial colonies remained constant, which proved that the biopesticide has an eco-friendly and soil-compatible profile (Shaik et al. 2026; Singh and Singh 2005).

4.5 Implementation Problems and Enhancements.

Dual-stage filtration, pH correction and optimized refrigerated storage were used to address operational problems such as solvent evaporation, microbial spoilage and filter plugging that greatly improved extract clarity and shelf life was shown in Fig. 6.

4.6 Statistical Evaluation

Variation in extraction yield was statistically effective ($p < 0.05$), whereas variation in pest reduction was minimum ($SD < 2\%$), indicating reliability of the results were shown in Fig. 7.

4.7 Competing Evaluation of Commercially available Pesticides.

The neem leaf-based biopesticide was also observed to be less damaging to the environment, safer to selective insects, and cheaper, although it had a shorter shelf life (1014 days versus 6090 days), than synthetic pesticides. Maximized cold and hot extraction techniques produced high levels of bioactive components such as azadirachtin, nimbin and salannin, which were determined by HPLC and GC-MS. Laboratory tests recorded increase up to 80% reduction in pest populations without negative impacts on plant health, soil pH or microbial communities. The stability and practicality were improved with process improvements including pH and refrigerated storage, whereas the chemical integrity, biological efficacy, extraction efficiency, and optimization of operations are demonstrated. The results taken together have provided a strong and validated case in support of neem-based biopesticides as a highly promising and efficient, environmentally friendly, and scalable alternative means of maintaining sustainable low-cost pest control (Tiwari et al. 2011). A qualitative assessment showed that neem extract, though slower in action, posed fewer health and environmental risks compared to synthetic pesticides. Cost analysis also revealed affordability for smallholder farmers was listed in Table 2.

Table 2
Neem Biopesticide vs. Synthetic Pesticides

Parameter	Neem Biopesticide	Synthetic Pesticide
Environmental Impact	Low	High
Cost per Liter	~ 0.5 OMR	~ 1.8 OMR
Safety to Beneficial Insects	High	Low
Shelf Life (with additives)	10–14 days	60–90 days

5. Conclusions

This paper illustrates how effective use of neem (*Azadirachta indica*) leaves and Custard apple (*Annona squamosa*) seed waste has been to formulate sustainable biopesticides that can replace synthetic pesticides due to their eco-friendly nature. Ideal cold and hot extracts with high chemical stability, which were verified by using HPLC and GC-MS, contained azadirachtin, nimbin, salannin, squalene, oleic acid, stearic acid, and palmitic acid, as the result of optimized cold and hot extraction methods using solvents like ethanol and hexane. Lab tests revealed a maximum depletion of 80 per cent of pest populations without any phytotoxic activity or other negative effects on the soil pH and the well-being of microorganisms. This research paper provides a low-cost, scalable methodology of turning agricultural waste into useful and environmentally safe biopesticides that would facilitate sustainable agriculture and circle economy concepts.

Declarations

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Authors' Contributions

Shabib Al Rashdi: Material preparation, data collection, Investigation; Laboratory Supervision

Lakkimsetty Nageswara Rao: Data analysis and compilation, Draft preparation, Writing – Review & Editing, Final Manuscript Review

Houriya Waleed Ali Al Shehhi: Experimental study and data validation

Alshima Ahmed Al-Mujaini: conception and design

Fatma Waheed Albalushi: Material preparation, data collection

Said Hamed Rashid: Conceptualization; Methodology

Ethical Approval

This article does not contain any studies involving human participants or animals performed by any of the authors.

Consent to Participate: Not applicable.

Consent to Publish

All authors consent to the publication of this manuscript and approve its submission to the journal.

Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Figures

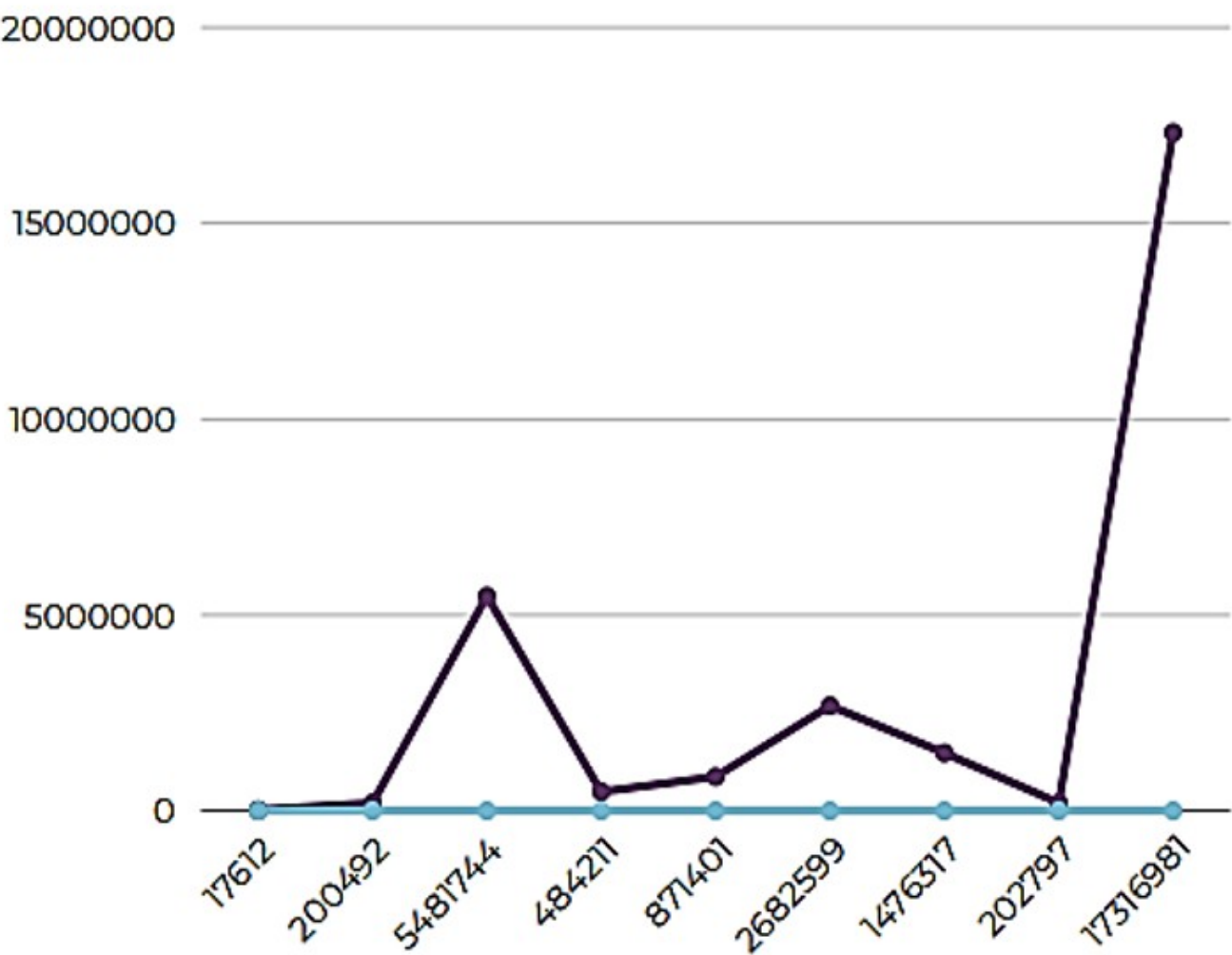


Figure 1

HPLC chromatogram of bioactive compounds from neem leaves.

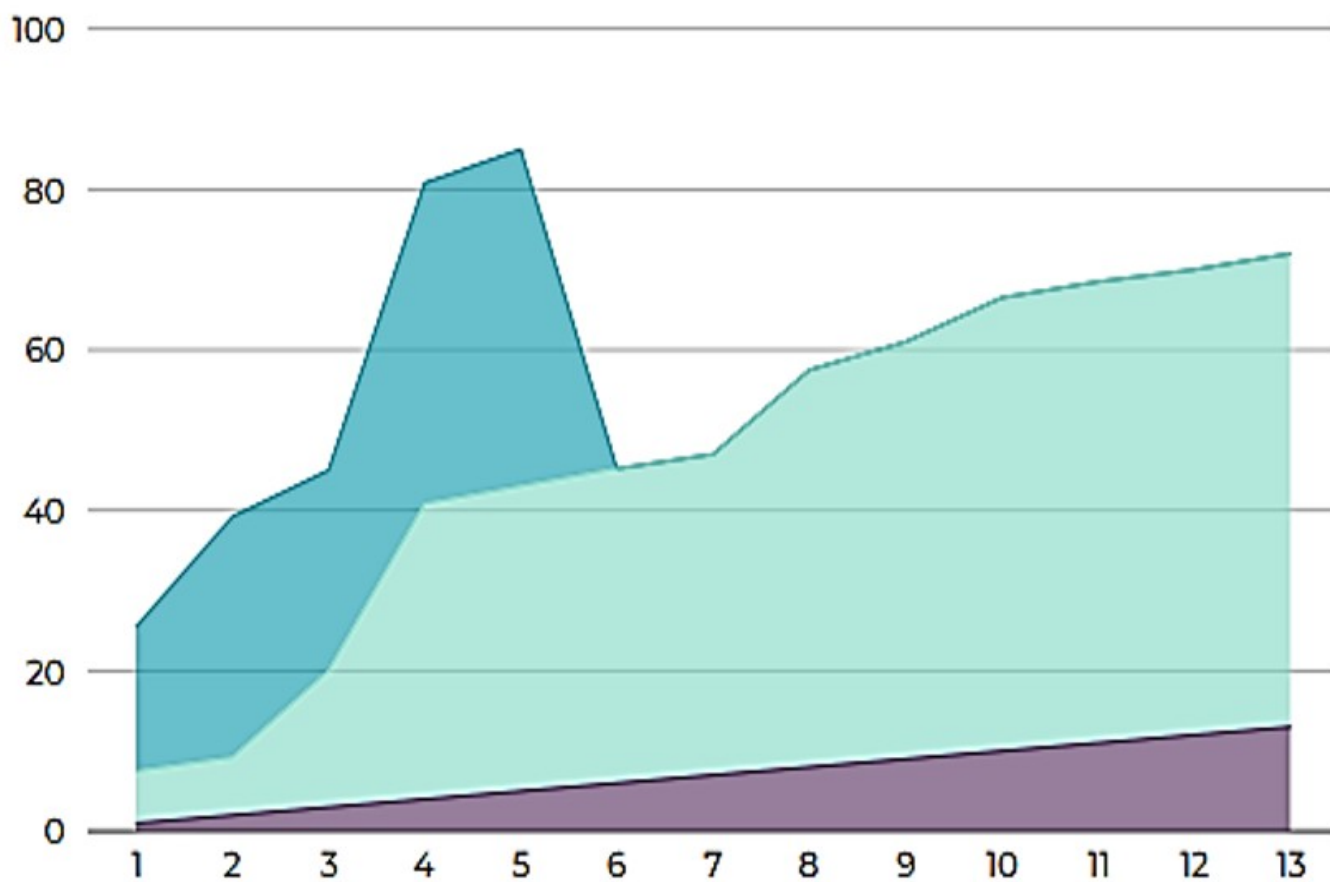


Figure 2

GC/MS chromatogram shows multiple bioactive components in the sample

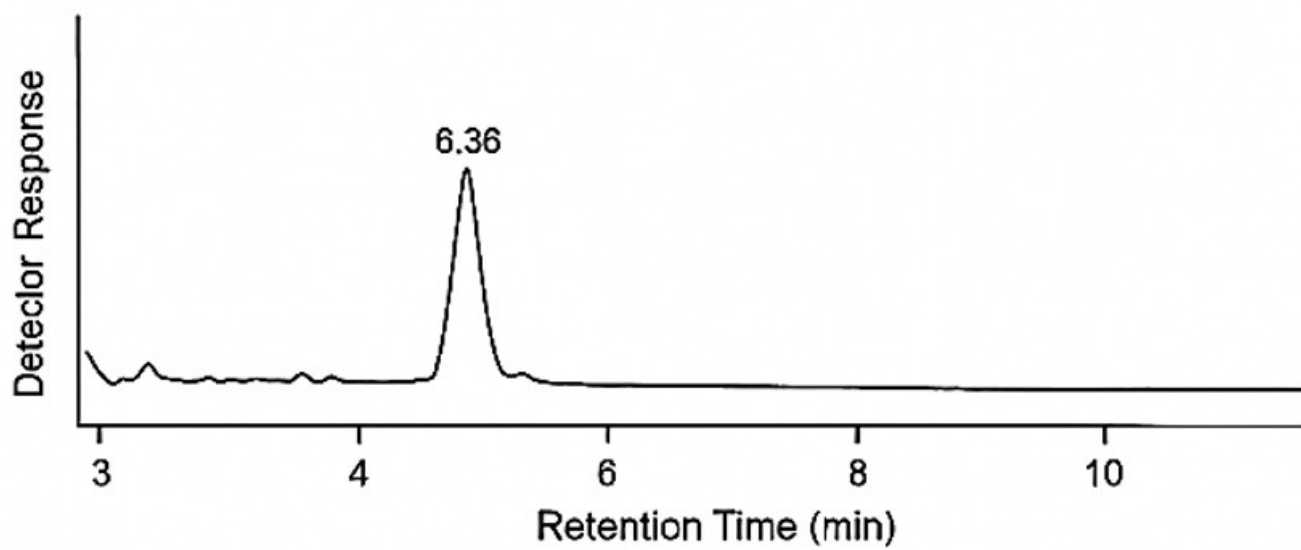
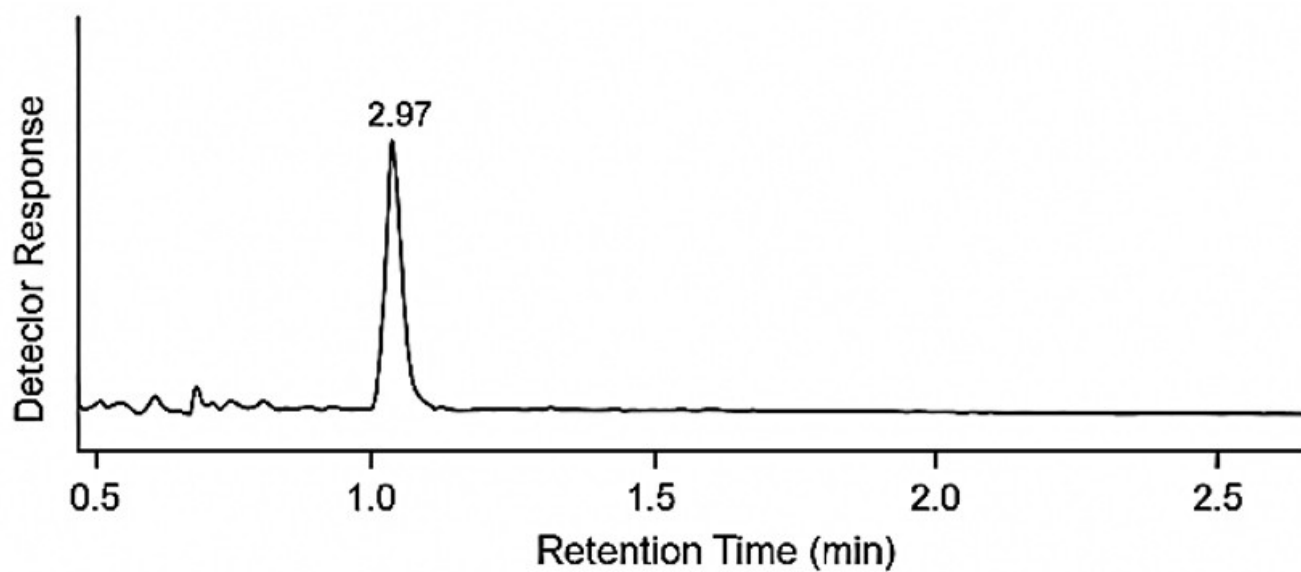


Figure 3

(Chromatograms) validate the extraction process (Martinez et al.,2013)

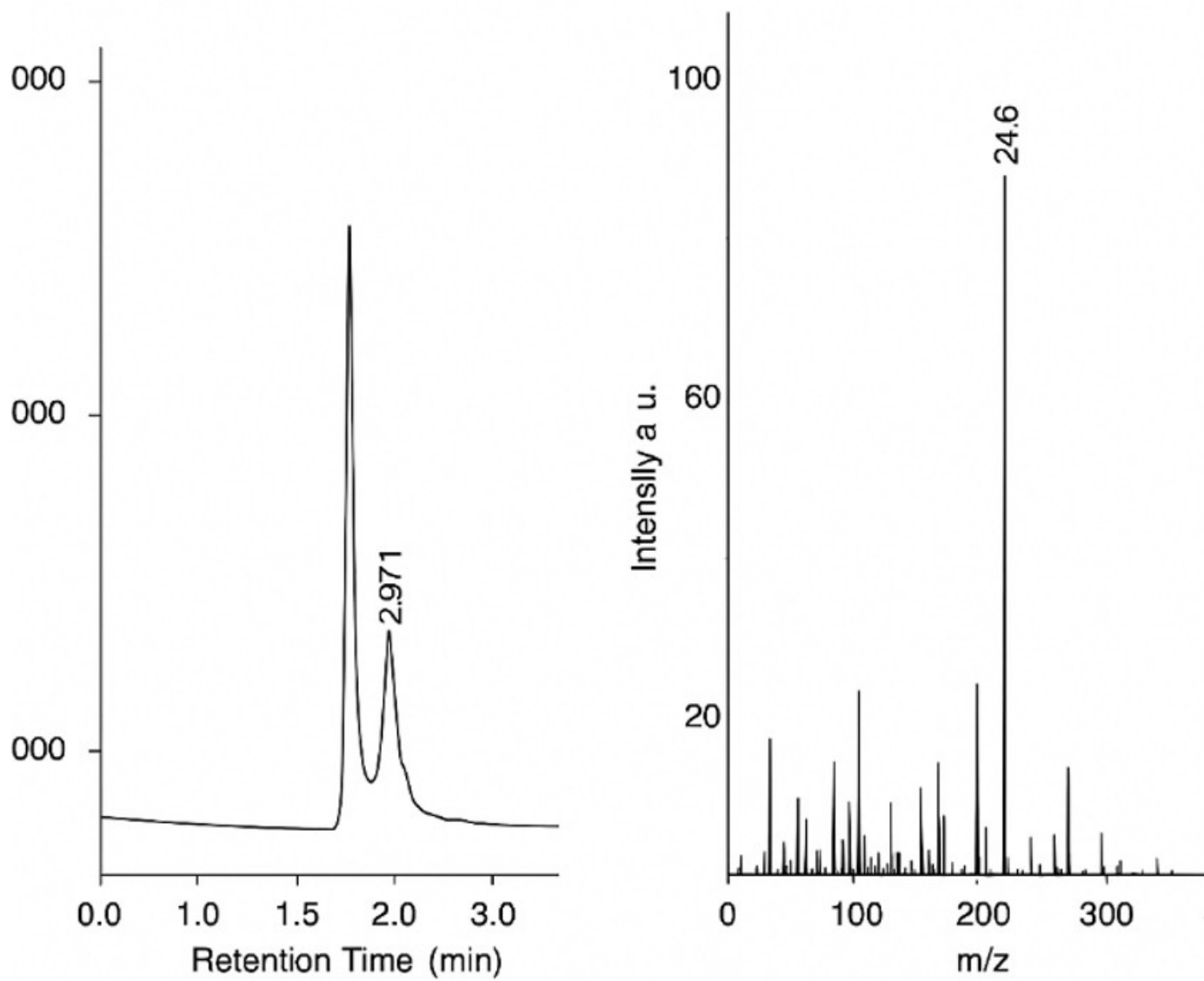


Figure 4

HPLC Chromatogram of Neem Extract and GC-MS Chromatogram of Neem Extract

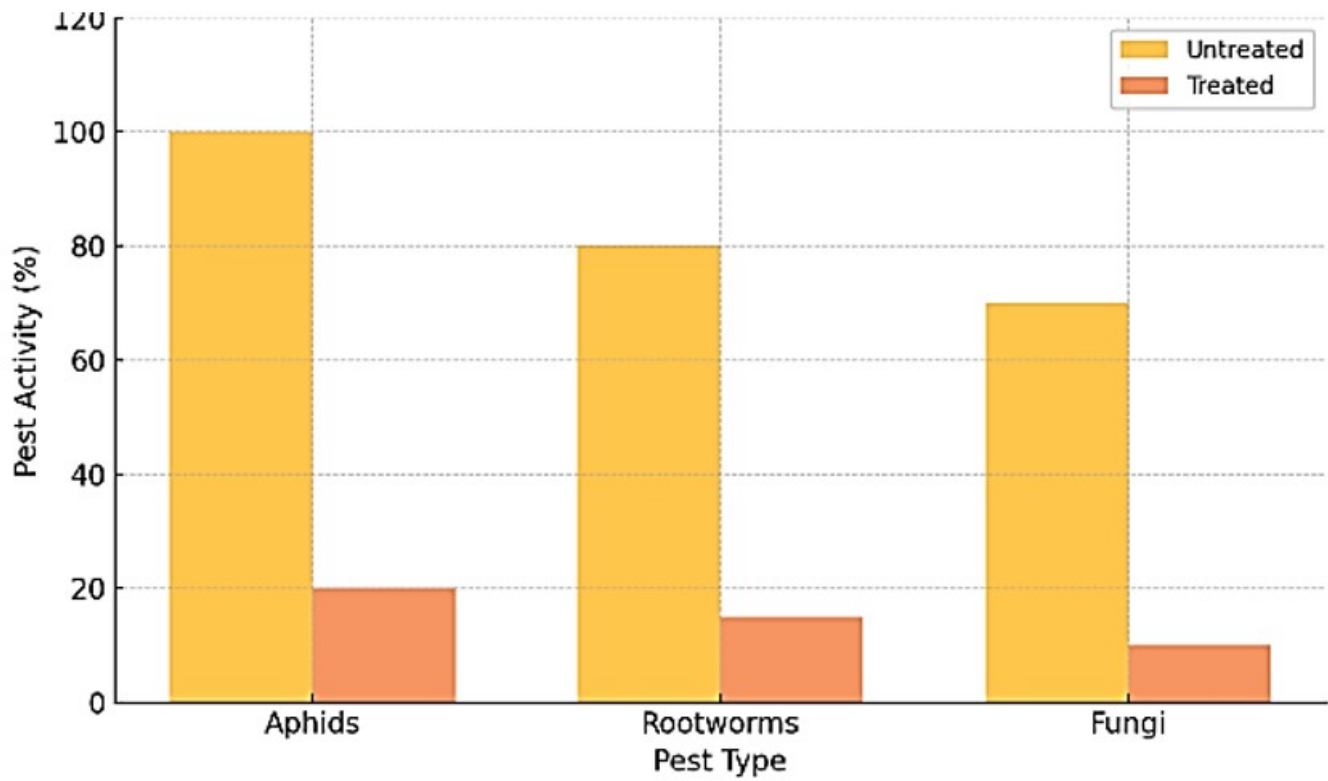


Figure 5

Insect Activity on Treated vs. Untreated Plants (Bar Chart)

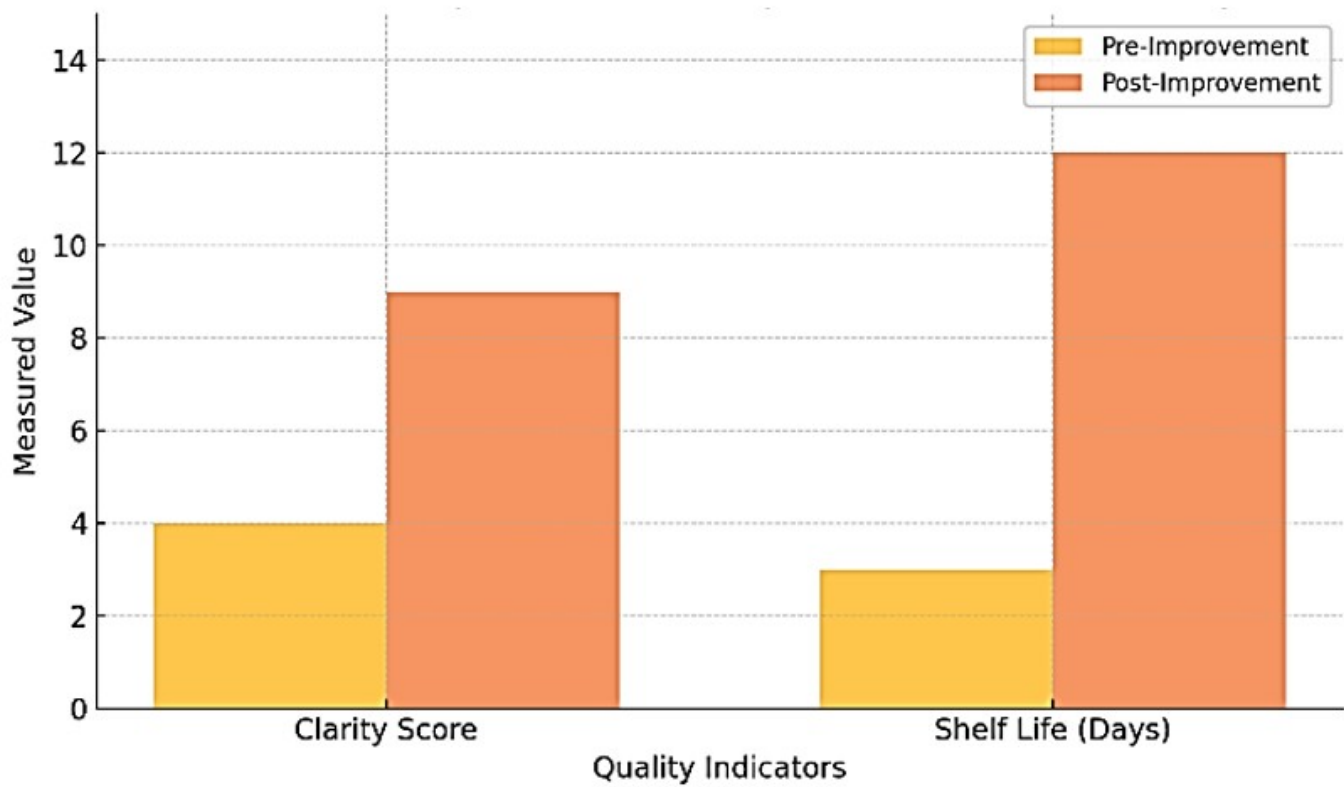


Figure 6

Pre- and Post-Improvement Comparison of Extract Clarity and Shelf Life

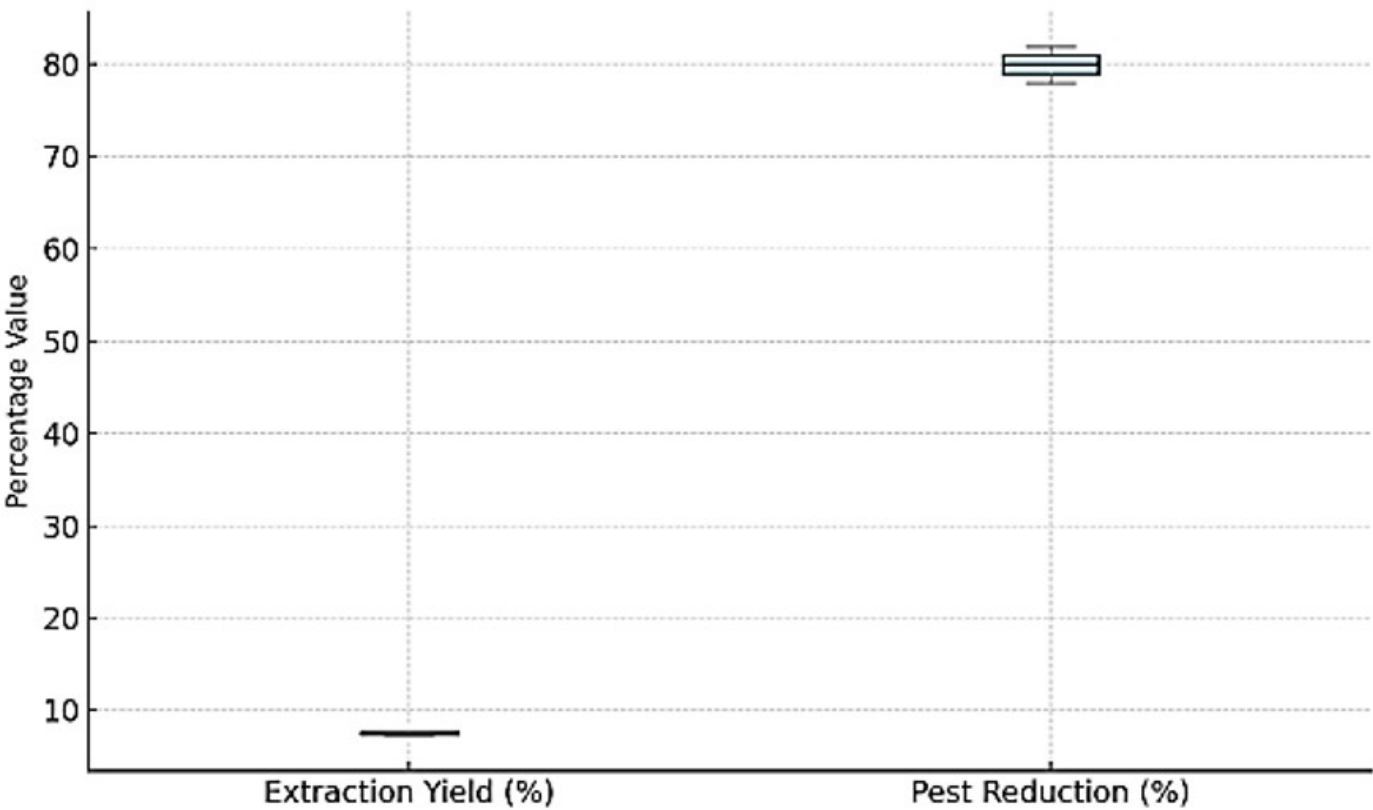


Figure 7

Yield and Efficacy Variance (Box Plot)