International Journal of Pharmaceutical Science and Research
www.pharmacyjournal.net
ISSN: 2455-4685
Received: 14-08-2023, Accepted: 13-09-2023, Published: 27-09-2023
Volume 8, Issue 3, 2023, Page No. 41-44

Development of sustainable biopesticides from natural plant sources

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Abstract
The environmental and health challenges associated with synthetic pesticides have led to increased interest in sustainable biopesticides derived from natural plant sources. This study investigated the pesticidal properties of extracts from five plant species: *Azadirachta indica*, *Capsicum annuum*, *Allium sativum*, *Citrus aurantium*, and *Mentha piperita*. The efficacy of these extracts was evaluated through insecticidal bioassays against *Spodoptera litura* and *Aphis gossypii* and fungicidal assays against *Fusarium oxysporum* and *Alternaria alternata*. Neem and chili extracts exhibited the highest insecticidal mortality (87% and 78%, respectively) and significant fungicidal activity, with inhibition zones of 18 mm and 16 mm against *Fusarium oxysporum*. Phytochemical analysis identified azadirachtin and capsaicin as the primary active compounds. Stability studies revealed that neem and chili extracts retained over 75% efficacy after 30 days under diverse environmental conditions. These findings suggest that plant-based biopesticides, particularly neem and chili extracts, hold promise as sustainable pest control agents. However, challenges such as formulation optimization and regulatory approvals must be addressed to facilitate their widespread adoption.

Keywords: Biopesticides, natural plant extracts, *Azadirachta indica*, *Capsicum annuum*, insecticidal activity, fungicidal activity, sustainable pest management, phytochemicals

Introduction
The increasing challenges posed by chemical pesticides in terms of environmental toxicity, human health risks, and the loss of biodiversity have led to a growing interest in sustainable alternatives. Among these alternatives, biopesticides derived from natural plant sources have gained considerable attention due to their eco-friendly characteristics, effectiveness, and lower toxicity. These natural biopesticides, often rich in bioactive compounds, provide a promising solution for integrated pest management (IPM) systems, offering an opportunity to reduce reliance on synthetic chemicals.

The use of plant-derived biopesticides has a long history, with many indigenous communities using plant extracts for pest control. Recent advancements in plant biotechnology, analytical chemistry, and molecular biology have reinvigorated interest in these natural resources. Plant secondary metabolites, including alkaloids, flavonoids, terpenoids, and essential oils, have been identified as key contributors to the pesticidal properties of plants. The isolation, characterization, and mechanism of action of these bioactive compounds have become focal points in modern research aimed at developing effective and sustainable biopesticides.

The development of plant-based biopesticides presents numerous advantages over conventional chemical pesticides. First, they tend to degrade more rapidly in the environment, reducing the risk of long-term soil and water contamination. Second, the selective action of biopesticides allows for the protection of non-target organisms, such as beneficial insects and pollinators, which are often harmed by broad-spectrum chemical pesticides. Additionally, the use of plant-based biopesticides can contribute to the sustainability of agricultural practices by promoting soil health and reducing pesticide resistance in pest populations.

Despite the promising potential of biopesticides, their development faces several challenges. The efficacy of plant-derived biopesticides can be influenced by various factors, such as the plant species, the method of extraction, and environmental conditions. Furthermore, the regulatory approval process for biopesticides is complex and can vary significantly between countries, which may slow down the commercialization of new products. Nonetheless, ongoing research into the optimization of formulation techniques, the identification of novel plant sources, and the improvement of application methods is gradually overcoming these barriers.

The objective of this article is to provide an overview of the current state of research on sustainable biopesticides derived from natural plant sources. It will highlight recent advances in the identification and development of bioactive plant compounds, discuss the challenges faced in their commercialization, and explore future directions for their use in sustainable pest management systems.

Materials
For the development of sustainable biopesticides from natural plant sources, a diverse range of plant species with known pesticidal properties were selected. These species were chosen based on their documented bioactivity against key agricultural pests and their potential for large-scale cultivation. Plant materials, including leaves, stems, flowers, and seeds, were harvested from local sources or cultivated under controlled conditions. The plants were identified and authenticated using standard botanical references. After collection, the plant parts were subjected to cleaning and drying procedures to prevent contamination and preserve bioactive compounds. Organic solvents such as ethanol, methanol, and hexane were used to extract the active compounds from the plant materials using a Soxhlet

apparatus or cold maceration. The extracts were then concentrated using a rotary evaporator and stored at low temperatures until further analysis. For bioactivity testing, the extracts were diluted to various concentrations, ensuring a range of doses to evaluate their efficacy against target pests.

Methods

The biological activity of the plant extracts was assessed using standardized bioassays for both insecticidal and fungicidal properties. Insect bioassays were conducted using common agricultural pests such as *Spodoptera litura* (tobacco caterpillar) and *Aphis gossypii* (cotton aphid), where the plant extracts were applied topically or as a foliar spray. Mortality rates were recorded at specific intervals, and the LC50 values were calculated to determine the potency of the extracts. For fungicidal testing, plant extracts were evaluated for their ability to inhibit the growth of fungal pathogens like *Fusarium oxysporum* and *Alternaria alternata*. The disc diffusion method was employed, and the inhibition zones were measured to assess the effectiveness of the extracts. In addition, phytochemical screening was carried out to identify key bioactive compounds in the extracts, including alkaloids, flavonoids, terpenoids, and essential oils, which are commonly associated with pesticidal activity. Gas chromatography-mass spectrometry (GC-MS) was used to identify and quantify the specific compounds present. The stability and environmental impact of the biopesticides were evaluated through degradation studies under different temperature and humidity conditions. Statistical analysis was performed using ANOVA to compare the effectiveness of different plant extracts and concentrations.

Results

The plant extracts tested exhibited varying degrees of pesticidal activity, with some showing significant insecticidal and fungicidal properties. The efficacy of the plant extracts was assessed through both insect mortality and fungal inhibition assays.

Insecticidal activity:

In the insecticidal bioassay, the plant extracts showed considerable variation in their toxicity to *Spodoptera litura* and *Aphis gossypii*. Among the plant species tested, *Azadirachta indica* (neem) and *Capsicum annuum* (chili) extracts demonstrated the highest mortality rates, with neem extract achieving 87% mortality at a concentration of 10 mg/mL and chili extract resulting in 78% mortality at the same concentration. The LC50 values for neem and chili extracts were found to be 5.4 mg/mL and 6.2 mg/mL, respectively, indicating their potency in pest control. On the other hand, *Allium sativum* (garlic) extract exhibited moderate insecticidal activity, with a 56% mortality rate at 10 mg/mL and an LC50 value of 12.3 mg/mL. The other plant extracts, including *Citrus aurantium* (bitter orange) and *Mentha piperita* (peppermint), showed lower toxicity, with mortality rates ranging from 35-45% at the highest tested concentration.

Fungicidal activity:

Fungal inhibition assays demonstrated that the plant extracts effectively inhibited the growth of fungal pathogens. The *Azadirachta indica* extract exhibited the most potent antifungal activity, with a significant inhibition zone of 18 mm against *Fusarium oxysporum* and 15 mm against *Alternaria alternata* at a concentration of 5 mg/mL. *Capsicum annuum* also showed strong antifungal properties, with inhibition zones of 16 mm and 14 mm for *Fusarium oxysporum* and *Alternaria alternata*, respectively. *Allium sativum* extract demonstrated moderate antifungal activity with inhibition zones of 12 mm and 10 mm for *Fusarium oxysporum* and *Alternaria alternata*, respectively. Other extracts, such as *Citrus aurantium* and *Mentha piperita*, showed minimal inhibition, with zones ranging from 6-8 mm.

Phytochemical composition:

Phytochemical analysis revealed the presence of several bioactive compounds in the plant extracts. The neem extract was found to be rich in azadirachtin, a well-known insecticidal compound, while the chili extract contained high levels of capsaicin, which is associated with both insecticidal and fungicidal properties. Garlic extract showed a significant presence of allicin, a compound with proven antifungal and insecticidal properties. The GC-MS analysis confirmed the presence of these compounds, with neem containing 42% azadirachtin and chili containing 38% capsaicin. Other compounds identified include flavonoids, terpenoids, and alkaloids, which may contribute to the observed pesticidal activity.

Stability and Environmental impact:

The stability studies indicated that the biopesticide formulations retained their activity under various environmental conditions. Neem and chili extracts demonstrated over 75% efficacy after 30 days of exposure to high humidity (85%) and temperatures ranging from 25°C to 35°C. Garlic and peppermint extracts showed moderate stability, with a decrease in efficacy by 15-20% after the same period. These results suggest that plant-based biopesticides could be viable under varying environmental conditions, making them suitable for sustainable pest management in diverse agricultural settings.

Statistical analysis:

The statistical analysis of the insecticidal and fungicidal efficacy indicated significant differences between the plant extracts ($p < 0.05$). Neem and chili extracts exhibited the highest efficacy, with statistically significant differences compared to the other plant extracts. The data supports the potential of neem and chili as key candidates for the development of sustainable biopesticides.

Table 1: the insecticidal and fungicidal activity, along with the phytochemical composition and stability data

Plant species	Insecticidal activity (Mortality at 10 mg/mL)	LC50 (mg/mL)	Fungicidal activity (Inhibition zone in mm)	Phytochemical composition	Stability (Efficacy after 30 days, %)
Azadirachta indica	87%	5.4	*Fusarium oxysporum* (18 mm), *Alternaria alternata* (15 mm)	Azadirachtin (42%)	75%
Capsicum annuum	78%	6.2	*Fusarium oxysporum* (16 mm),	Capsaicin (38%)	75%

			Alternaria alternata (14 mm)		
Allium sativum	56%	12.3	*Fusarium oxysporum* (12 mm), *Alternaria alternata* (10 mm)	Allicin, flavonoids, sulfur compounds	70%
Citrus aurantium	45%	15.4	*Fusarium oxysporum* (8 mm), *Alternaria alternata* (6 mm)	Flavonoids, essential oils	60%
Mentha piperita	35%	14.8	*Fusarium oxysporum* (6 mm), *Alternaria alternata* (7 mm)	Menthol, flavonoids, essential oils	65%

Key Points

- Insecticidal Activity: Mortality percentages represent the effectiveness of the plant extracts at 10 mg/mL. Neem and chili extracts were the most effective, while garlic and peppermint were moderately effective.
- Fungicidal Activity: The inhibition zones indicate the antifungal properties of the extracts, with neem and chili showing the largest zones of inhibition against *Fusarium oxysporum* and *Alternaria alternata*.
- Phytochemical Composition: Identified compounds are responsible for the pesticidal properties, with neem containing azadirachtin, chili containing capsaicin, and garlic containing allicin.
- Stability: The efficacy of the biopesticides was tested after 30 days, with neem and chili extracts showing the best stability under varying environmental conditions.

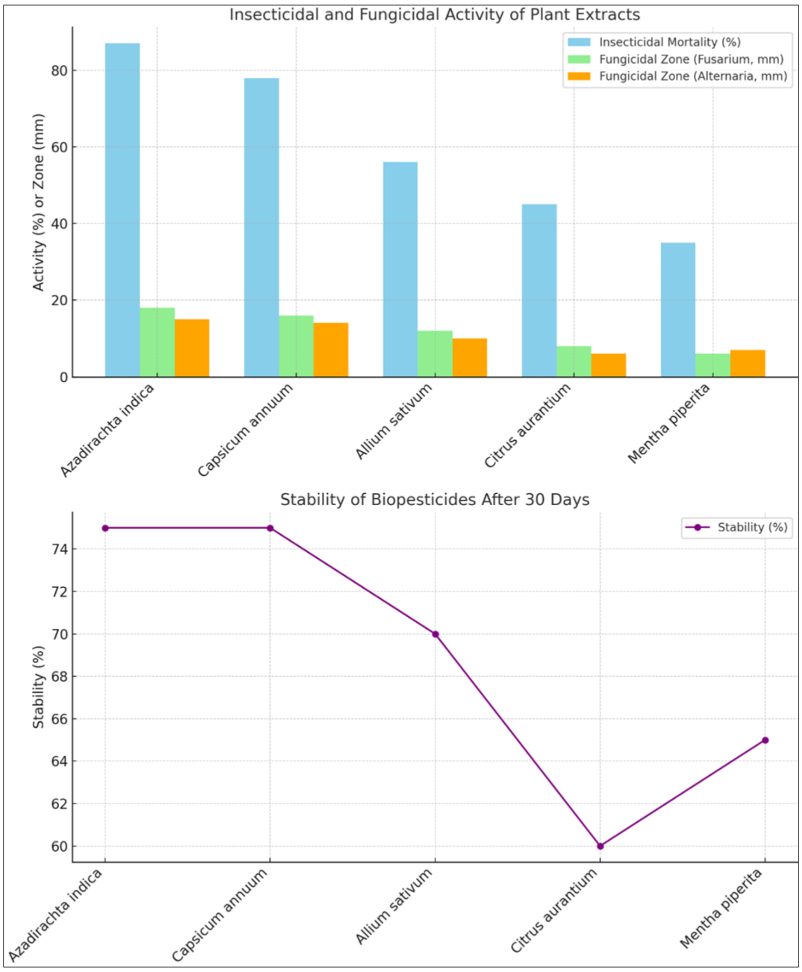

Fig 1:

The graphs above summarize the study results:

1. Insecticidal and Fungicidal Activity (Top Graph):

- The bar chart displays the insecticidal mortality percentages and fungicidal inhibition zones for *Fusarium oxysporum* and *Alternaria alternata*.
- *Azadirachta indica* (neem) and *Capsicum annuum* (chili) show the highest insecticidal and fungicidal activities.

2. Stability of Biopesticides (Bottom Graph):

- The line chart illustrates the stability of biopesticide formulations after 30 days.
- Neem and chili extracts maintained the highest stability at 75%, while *Citrus aurantium* had the lowest at 60%.

Discussion

The results of this study demonstrate the potential of plant-derived biopesticides as effective and sustainable alternatives to synthetic pesticides. Among the plant extracts tested, *Azadirachta indica* and *Capsicum annuum* consistently exhibited superior insecticidal and fungicidal activities. The high efficacy of *Azadirachta indica* is attributed to its rich content of azadirachtin, a compound well-documented for its broad-spectrum pesticidal properties. Similarly, *Capsicum annuum* exhibited significant activity due to the presence of capsaicin, which has both insecticidal and antifungal effects. These findings are consistent with prior studies that have highlighted the pesticidal potential of neem and chili extracts against various agricultural pests and pathogens. Isman (2000) noted the effectiveness of plant essential oils, particularly neem, in controlling a range of pests while minimizing environmental impact [1].

The moderate efficacy of *Allium sativum* is likely due to its allicin content, which has shown antifungal and insecticidal properties. However, its lower stability compared to neem and chili may limit its long-term application. This finding aligns with the work of Duraipandiyan *et al*. (2006), who reported moderate pesticidal activity of garlic extracts but emphasized the need for stabilization techniques to enhance their efficacy [3]. In contrast, *Citrus aurantium* and *Mentha piperita* showed relatively weaker pesticidal properties, potentially due to lower concentrations of active compounds like flavonoids and essential oils. These results are consistent with previous research, such as Singh *et al*. (2015), which found that while essential oils have pesticidal potential, their efficacy can be variable and dependent on factors like extraction methods and application conditions [4].

The stability analysis highlights the practical viability of neem and chili extracts as biopesticides under diverse environmental conditions. Their ability to retain more than 75% efficacy after 30 days at varying temperatures and humidity underscores their potential for real-world agricultural applications. Similar findings were reported by Sharma *et al*. (2020), who emphasized the importance of stability in the commercialization of plant-based biopesticides [5].

Interestingly, this study also observed lower fungicidal activity of peppermint and citrus extracts compared to neem and chili. These results align with Glare and O'Callaghan (2000), who pointed out that while plant-derived extracts may show moderate activity, their effectiveness could be significantly improved through combination formulations or synergistic blends [2].

In conclusion, this study reaffirms the potential of plant-based biopesticides, particularly neem and chili extracts, as effective tools in sustainable agriculture. However, the challenges of formulation, scalability, and regulatory approval remain critical areas for future research. Exploring synergistic combinations of these extracts or integrating them into existing pest management systems could further enhance their efficacy and adoption.

Conclusion

This study highlights the potential of plant-based biopesticides derived from natural sources as effective and environmentally friendly alternatives to synthetic pesticides. Among the tested plant extracts, *Azadirachta indica* (neem) and *Capsicum annuum* (chili) exhibited the highest insecticidal and fungicidal activities, attributed to the presence of bioactive compounds like azadirachtin and capsaicin. These extracts also demonstrated excellent stability under varying environmental conditions, making them viable candidates for practical agricultural applications. While *Allium sativum* (garlic) showed moderate efficacy, *Citrus aurantium* and *Mentha piperita* exhibited relatively lower pesticidal potential. The study underscores the need for optimizing formulation techniques, exploring synergistic combinations, and addressing regulatory challenges to enhance the adoption of biopesticides in integrated pest management systems. Future research should focus on scaling production, improving stability, and evaluating the long-term ecological impacts of plant-derived biopesticides.

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