

# Plant-Based Biopesticide from Madagascar *Melia azedarach*

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## Abstract

The growing utilization of natural pesticides from plants increases the need to develop new alternative sources. The Madagascar *Melia azedarach* presents the advantage of producing Azadirachtin D, one of the bioactive molecules that functions as an antifeedant, repellent, insecticide, and growth regulator. The plant, which originated from Madagascar does not biosynthesize the toxic molecule Meliatoxin A and its derivatives, making it an interesting alternative for plant-based biopesticide. This work presents the origin, chemical analyses, and an example of the application of a plant-based biopesticide from the leaves of *Melia azedarach*.

## Keywords

*Melia azedarach*, Biopesticides, Azadirachtin, Meliatoxin, Antifeedant

## 1. Introduction

Synthetic pesticides have caused ecological damage and induced serious health hazards among workers during manufacture, formulation, and field applications. To surpass the problems of synthetic chemical hazards, one of the best control measures is the use of plant-origin products [1]. More than 6000 plant species are known to have insecticidal properties, and many of them are used by farmers in developing countries [2]. Exploring the scientific bases of those insecticidal properties and transforming local knowledge into agricultural applications are the major long-term objectives of this present study. Among natural products, one of the

most promising natural compounds is Azadirachtin A, an active compound extracted from the *Azadirachta indica*, whose antiviral, antifungal, antibacterial, and insecticidal properties have been known for several years [3]. Azadirachtin A is active in nearly 550 insect species [4].

*Melia azedarach* is reported to have a low or lack of Azadirachtin A content, which limits its ability to function as a potent biopesticide compared to its *Azadirachta indica* counterpart [5]. Furthermore, *Melia azedarach* produces the toxic compound meliatoxin, which can be absorbed by the cultivated vegetation and will have a negative effect on animals and humans if *Melia azedarach* is used as a biopesticide source. The only exception to date is the *Melia azedarach* from Argentina, also called “paraíso,” which has been consistently reported to be free of meliatoxins A and B [5]. In this present work, we report the potential use as a biopesticide of Madagascar *Melia azedarach* that can biosynthesize Azadirachtin D but not the toxic compounds meliatoxins.

## 2. Literature Review

### 2.1. Biopesticides

The global biopesticide market was valued at around USD 5 billion in 2023 and is projected to reach nearly USD 15 billion by 2029, reflecting sustained double-digit annual growth [6]. In 2025, biopesticides are experiencing significant global market growth, projected to reach USD 8 billion to USD 9 billion, driven by increasing environmental awareness, demand for sustainable agriculture, and supportive government regulations. North America is a leader in the market, and key trends include technological advancements such as nano-biopesticides [7], their integration with precision agriculture and Artificial Intelligence for targeted application, and the ongoing development of new bio-based products by innovative companies [8].

Biopesticides are composed of natural elements such as microorganisms and their metabolites. Currently, these substances have gained popularity and are used in sustainable production models, including organic agriculture and integrated pest management, which are characterized by partially or completely avoiding inputs of synthetic origin. There is a general belief that biopesticides are less harmful than chemical pesticides due to their natural origin, low persistence, and lower toxicity [9].

Plant-based biopesticides are composed of bioactive compounds present in plants and are known to have pesticidal effects. The biopesticides derived from plants are eco-friendly, safe, and beneficial for the environment. They do not harm the soil, water, or air quality of the environment. Hence, plant-derived pesticides should be used for pest control. One study highlights the importance and traditional uses of local plants as biopesticides. It provides baseline data for the formulation of herbal biopesticides which could be safe for the environment and human health care [10].

## 2.2. Azadirachtins

Azadirachtin A is the most active ingredient in many plant-based biopesticides [11]. Azadirachtin, a complex tetraterpenoid limonin with potent insecticidal properties, is the most widely used biological pesticide worldwide. Its versatile pharmacological applications include the inhibition of tumor growth and anti-malarial, anti-bacterial, and anti-inflammatory properties. Azadirachtin plays a pivotal role in pest control and novel drug development. The primary source of azadirachtin is the neem tree (*Azadirachta indica* A. Juss), with an azadirachtin content ranging from 0.3% to 0.5%. Despite the market demand for botanical pesticides reaching approximately 100,000 tons per year, the annual neem production in China is only 1.14 tons. Although azadirachtin can be obtained through plant extraction or chemical synthesis, the quantity obtained does not meet the market demand in China. The sluggish pace of azadirachtin biosynthesis results from the limited availability of genetic information and the complexity of the synthetic pathway. Recent advancements in azadirachtin biosynthesis hold promise as an efficient collection method. The study explored the physicochemical properties, biological activities, mechanisms of action, and acquisition methods of azadirachtin. The study also established a theoretical foundation for the scientific application and efficient synthesis of azadirachtin, offering valuable reference information to the industry [12].

Azadirachtin D is a derivative of Azadirachtin A with the same mode of action as a biopesticide. Both molecules act as insecticides primarily by disrupting insect hormones (ecdysones and juvenile hormone) and neuroendocrine pathways, leading to impaired molting and development, ultimately breaking the insect's life cycle. They also function as antifeedants, reducing food intake, and can cause mid-gut cell necrosis, cell division inhibition, and muscle flaccid paralysis. Some evidence also suggests they can bind to actin, disrupting the insect cytoskeleton [13]. **Figure 1** displays the structure of Azadirachtins A and D.

## 2.3. *Melia azedarach*

Synonyms: *Melia australasica*, *Melia bukayun*, *Melia composita*, *Melia japonica*, *Melia sempervirens*, *Melia toosendan* [14].

Common names: Chinaberry tree, Pride of India, bead-tree, Cape-lilac, syringaberry tree, Persian lilac, Indian lilac, white cedar [14].

Chinaberry tree (*Melia azedarach*) is a perennial tree (family *Meliaceae*) that is generally found in disturbed areas, road edges, and openings in forests or thickets. It is native to China, Japan, the Indian sub-continent, southeastern Asia, and large parts of northern and eastern Australia. Chinaberry trees have historically been used as ornamental plants, as shade trees, and for firewood. It can grow as a single-stemmed tree up to 50 feet tall. The deciduous leaves can grow up to 2 feet long, are arranged alternately along the stem, and are bipinnately compound and toothed. Flowers are pink to purple (often lilac colored) and are arranged in numerous small clusters that bloom in spring. Chinaberry produces numerous

yellow to yellow-green wrinkly drupe fruits that are about 0.4 - 0.5 inches in diameter. It thrives under a large variety of environmental climates and soils and releases compounds in its leaves and roots that inhibit the growth of other plants, resulting in large bare patches around trees. Chinaberry fruits are dispersed through wildlife and via waterways. This plant is poisonous to humans, livestock, and mammals if ingested [14].

*Melia azedarach* is one of the important medicinal plants from the *Meliaceae* family; medicinal properties cited by traditional practitioners are quoted for each and every plant part (leaves, fruits, seeds, and roots) [15]. Pharmacological investigations verify different health maintenance activities of *Melia azedarach*: antinephrolithiasis, hepatoprotective, antibacterial, anti-parasitic, antiulcer, anthelmintic, antioxidant, and antipyretic actions. Extensive literature on its chemistry is also available [16]-[18].

*Melia azedarach*, an invasive yet abundant plant species in Africa, presents a promising solution for plant-based biopesticides. Known for its insecticidal properties, its biodegradable and eco-friendly extracts could replace harmful pesticides, offering a sustainable approach to managing Fall Army Worm (FAW) infestations and ensuring agricultural productivity. The report focuses on developing a bio-pesticide from *Melia azedarach* to mitigate FAW impacts while protecting ecosystems [19].

Compounds derived from plant extracts, such as those from the fruits and leaves of *Melia azedarach* Linn (chinaberry tree), which contain azadirachtin, have been utilized for crop pest control. However, these natural compounds are susceptible to degradation during extraction and under field conditions. To address this, extraction processes involving supercritical fluid and microencapsulation via spray drying were used to obtain and preserve biopesticidal molecules. This study focuses on producing a biopesticide from *Melia azedarach* fruit extract using supercritical CO<sub>2</sub>, and microencapsulation via spray drying with a blend of cheese whey and gum Arabic. The extract was obtained from chinaberry fruits rich in azadirachtin (736.92 µg/L) using supercritical CO<sub>2</sub> at 250 bar and 50°C for 120 min, with a yield of 3.44%. The extract was then encapsulated using cheese whey and gum Arabic (70:30) as encapsulating agents at extract: encapsulating agents' ratios of 1:10 and 1:15. Mortality rates of *Tetranychus urticae* Koch adult females and their eggs, in contact and residual assays with both non-encapsulated and encapsulated extract, did not significantly differ ( $p > 0.05$ ) from the commercial acaricide abamectin. This study introduces a potential new biopesticide that utilizes chinaberry extract obtained through supercritical CO<sub>2</sub> extraction and encapsulation for industrial scale-up studies, and this approach holds promise for the production of an effective biopesticide for crops affected by two-spotted spider mites [20].

*Melia azedarach* L. is reported to be poisonous to farm and domestic animals and humans. It was shown that a series of limonoids, meliatoxins A1, A2, B1, and B2, are the poisonous principles [21]. Another study reported a way to avoid the

formation of meliatoxin by grafting *Azadirachta indica* onto *Melia azedarach* as rootstock. The results showed that *Azadirachta indica* grafted onto *Melia azedarach* rootstock produces azadirachtin, and also that its fruits are free of meliatoxins from rootstocks [22]. This last literature review confirms how important it is to find *Melia azedarach* species that can biosynthesize azadirachtin but not meliatoxins, which is the subject of this present article. **Figure 2** shows the chemical structure of meliatoxins A1, A2, B1, and B2.

### 3. Methodology

The methodology adopted is as follows:

To locate the exact origin of the *Melia azedarach* tree (region, altitude, and longitude)

To verify scientifically if the tree is truly the *Melia azedarach* species (leaf verification and picture comparison to an identified sample of *Melia azedarach*)

To collect leaves of *Melia azedarach* for a biopesticide assay

To analyze the leaf extract by LC/MS for Azadirachtin and Meliatoxins content.

To formulate a biopesticide sample for real-scale test application on rice field culture

#### 3.1. LC-MS Analysis of *Melia azedarach* Leaves

3.2 g of leaf material was submerged in 1 liter of 80:20 MeOH/H<sub>2</sub>O for 48 hours while stirring. After 48 hours, the solvent was filtered off via Buchner filtration.

The sample was then partitioned with 1 liter of hexane twice. The methanol extract was then dried down via rotary evaporation, which yielded 1.60 g of an oily solid sample.

This was resuspended and diluted to a concentration of 0.5 mg/mL for LC-MS analysis.

3  $\mu$ L of sample was injected onto a Waters BEH C18 reverse-phase column (150  $\times$  4.6 mm, 1.7  $\mu$ m) UPLC coupled with a mass spectrometer and TUV, and run with a gradient Elution of 5% - 100% MeCN/H<sub>2</sub>O + 0.1% formic acid over 7 minutes. Wavelength Detection was set to 220 nm and 254 nm, and ESI positive mode was used for mass spectrometry detection with a mass range of 50 - 1800 amu.

#### 3.2. Real-Scale Application Materials and Methods

##### 3.2.1. Objective

The main goal was to test the effect of aqueous extracts of *Melia azedarach* leaves on pests responsible for the disease resembling rice blast observed in some plots of our experimental station.

##### 3.2.2. Plant Materials

The plant used as a biopesticide was *Melia azedarach*.

- Parts were mainly leaves and seeds, crushed and macerated for 24 hours, then filtered.

- Collection site: Tsimbazaza Botanical Park.
- Rice varieties: certified FOFIFA seeds, X265, and SEBOTA 70/251 (originally from Brazil).

### 3.2.3. Other Materials

- Pruning shears and plastic bags: for collecting leaves and seeds.
- Scale: for weighing before maceration.
- Grinder or mortar: for coarse crushing.
- Containers: for maceration with well or rainwater.
- Fine mesh cloth: for filtering.
- 20-liter backpack sprayer.
- Locally made weeders and grinders.

### 3.2.4. Methods

#### 1) Steps of the Biological Treatment

The frequency and timing of biological treatments were the same as those used in chemical treatments.

#### 2) Soil Treatment:

Apply 2 - 5 kg of *Melia azedarach* leaves per are, 3 - 4 weeks before transplanting, to improve soil fertility and health.

#### 3) Seed Treatment:

Rinse seeds in a macerated leaf extract (5 g/L).

#### 4) Nursery Treatment:

Spray *Melia azedarach* leaf extract on 3 - 5-day-old seedlings.

#### 5) Post-Transplanting Treatment:

Apply the first spray 15 days after transplanting, and another before panicle initiation.

#### 6) Crop Rotation:

Use the same biopesticide treatments on legumes and vegetable crops.

#### 7) Compost:

Prepared on site from plant residues and manure, with two turnings before soil preparation.

#### 8) Rice-Growing Practices

Seed preparation (selection, sorting, rinsing, and sowing).

Nursery preparation for SRI, using only biological inputs (compost and biopesticide).

Field preparation and seed treatment.

Line transplanting, one seedling per hill, in plots pretreated with leaf extract.

First spray: 15 days after transplantation.

Second spray: before panicle initiation.

#### 9) Preparation of *Melia azedarach* Extract

Procedure:

Wash the leaves and shade-dry for 2 days.

Weigh 2 kg of fresh leaves.

Coarsely grind.

Macerate in 5 L of well water for 24 hours.

Filter.

Dilute 1 L of the filtrate with 5 L of water.

Spray preventively or curatively.

#### 10) Preliminary Trials

Before rice field applications, similar *Melia azedarach* leaf extracts were tested:

In the laboratory, against rice weevils that infest cereals and starches.

In the field, against giant ants in a hibiscus (Bissap) plantation.

Both applications gave successful results, motivating the use of the extract against rice diseases such as blast fungus.

## 4. Results

### 4.1. Origin and Location of *Melia azedarach* Tree

The *Melia azedarach* tree is located in Tsimbazaza, Antananarivo.

Tsimbazaza, located within Antananarivo, has an approximate longitude of 18.90°E and an altitude of around 1280 meters (4199 ft) above sea level. The city itself sits at this elevation, with a longitude on the central plateau of Madagascar.

Longitude: Approximately 18.90°E.

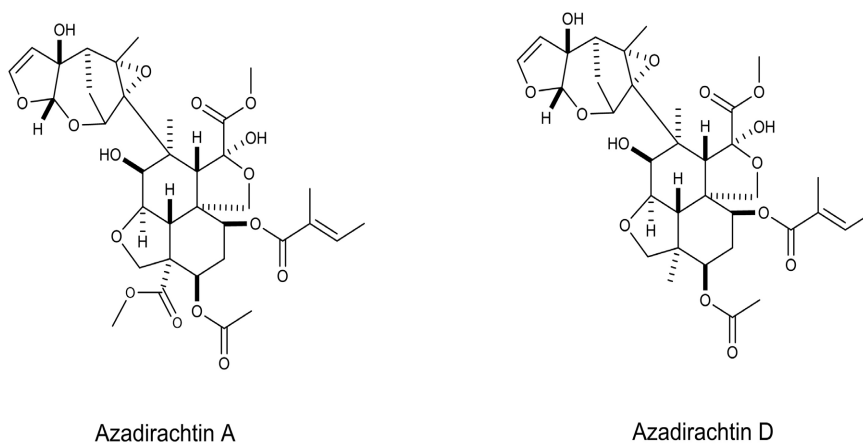
Altitude: Around 1.280 m (4199 ft).

**Figure 3** shows the *Melia azedarach* tree.

### 4.2. Scientific Verification of the Leaves of *Melia azedarach*

The tree and leaf verification of *Melia azedarach* collected is consistent with the literature description [14], which is described as alternate, bi-pinnately compound, and up to 45 cm long, with 3 - 5 pairs of leaflets, each containing 25 - 75 pinnules. The leaflets are typically ovate to elliptic, 2.5 - 7 cm long, with serrate or toothed margins and an acuminate (tapering to a point) apex. The leaflets are dark green on top and lighter below, with a base that is usually asymmetric. The Malagasy vernacular name is “Voandelaka.”

**Figure 4** displays the picture of *Melia azedarach* leaves.



**Figure 1.** Chemical structure of Azadirachtins A and D.





Figure 4. *Melia azedarach* leaves.

### 4.3. Liquid Chromatography/Mass Spectrometry (LC/MS) Analyses

Liquid chromatography coupled with mass spectrometry was used to analyze *Melia azedarach* extract for its Azadirachtins and Meliatoxins content. Reverse phase column C18 was used to separate the molecules from the extract of *Melia azedarach*. Time of flight (TOF) technique analyzer was used to detect the ions produced by Electrospray ionization (ESI) with minimal fragmentation from a liquid sample. The results indicated that Azadirachtin D is the pesticidal content of the Madagascar *Melia azedarach* with  $m/z$  at  $677 = [M + H]^+$ . The toxic compounds Meliatoxins A1, A2, B1, and B2 were not detected with  $m/z$  at  $659 = [M + H]^+$  for A1 and B1,  $m/z$  at  $645 = [M + H]^+$  for A2 and B2. Figure 5 displays the presence of Azadirachtin D from Madagascar *Melia azedarach*. Figure 6 displays the results of all samples analyzed by LC/MS including the sample from *Azadirachta indica* and the presence of Azadirachtin D but not meliatoxins.

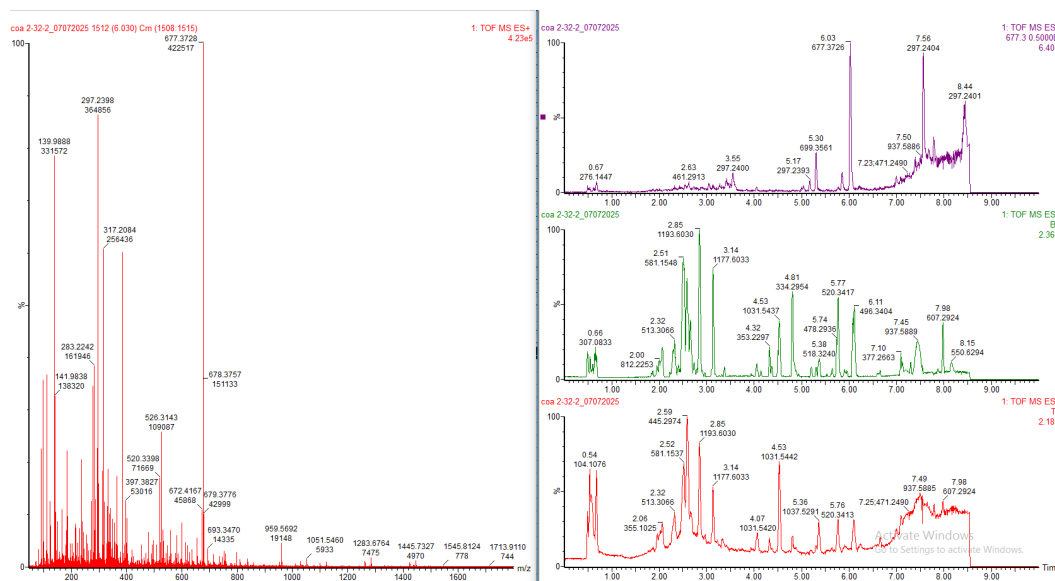
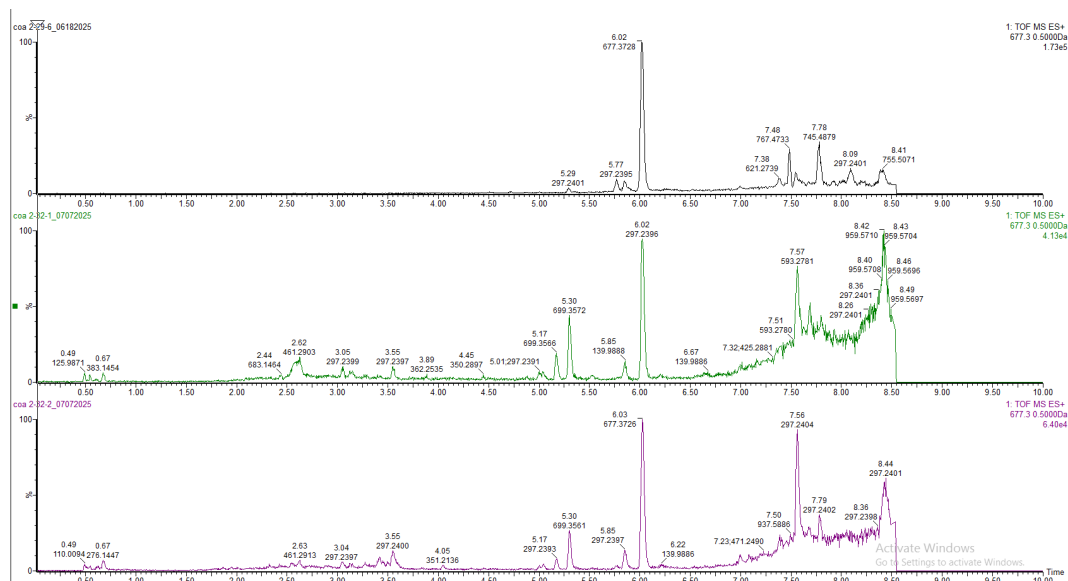


Figure 5. Presence of Azadirachtin D from Madagascar *Melia azedarach* leaves.



**Figure 6.** Presence of Azadirachtin D in all samples analyzed, Madagascar *Melia azedarach* leaves and *Azadirachta indica* leaves. Absence of Meliatoxins.

#### 4.4. Formulation of *Melia azedarach* Biopesticide and Test in Rice Field

The main objective of this work is to transform local knowledge into agricultural applications. With respect to this objective, the development of cost-effective and straightforward procedures to formulate plant-based biopesticides from *Melia azedarach* constitutes a primary long-term goal of the present study. An example of a plant-based biopesticide from *Melia azedarach* without extraction is the use of crushed fruits or leaves of the chinaberry tree to deter or kill insects. By preparing an aqueous suspension or powder from these plant parts, local communities have traditionally applied them to crops to protect against various pests by leveraging the plant's natural insecticidal, antifeedant, and growth-regulating compounds. While formal extraction is a standard process, a simple biopesticide can be created from *Melia azedarach* (chinaberry) by preparing a water-based solution from its leaves.

##### 1) Simple example of aqueous pesticide from *Melia azedarach*.

###### Ingredients

- *Melia azedarach* leaves: A generous amount (e.g., 2000 grams).
- Water: 5 liters.
- Mild soap or detergent: Approximately 20 grams, or a few drops of liquid soap. The soap acts as a surfactant, helping the mixture to stick to plant leaves.

##### 2) Instructions

**Collect and prepare:** Gather fresh *Melia azedarach* leaves. You can either chop them coarsely or pound them to help release their compounds.

**Soak:** Add the prepared leaves to the water and let them soak overnight, for about 12 to 24 hours.

**Strain:** After soaking, strain the liquid through a cloth or sieve to remove the

plant matter.

Add soap: Dissolve the soap in a small amount of water before mixing it into the strained liquid. Stir thoroughly.

Apply: Pour the solution into a spray bottle and apply it directly to the affected plants, targeting the undersides of the leaves where many pests hide.

### 3) Best practices and safety

Application timing: For best results, spray in the evening. The active compounds are sensitive to sunlight, which can reduce their effectiveness.

Test first: Always test the solution on a small section of the plant first to ensure it does not cause damage before spraying the entire plant.

Use it fresh: The pesticide is most potent when freshly prepared. While it can be stored for a limited time in a cool, dark place, its effectiveness diminishes over time.

Repel, not kill: This type of biopesticide primarily acts as a repellent and feeding deterrent, rather than as an instant killer, disrupting the life cycle of pests.

Caution: Though natural, handle all pesticides with care. Wear protective gloves and clothing during preparation and application. Do not use any utensils or containers for food preparation after using them for this pesticide.

#### 4.4.1. How It Works

1) This method creates a simple “aqueous extract.” By soaking the plant material in water, you dissolve some of the pesticidal compounds naturally present in the leaves. When sprayed on plants, these compounds:

Act as an anti-feedant: Discouraging pests from feeding on the treated plants.

Repel insects: The strong scent can drive away pests.

Disrupt insect growth: The solution can interfere with the pest’s growth and development, similar to the action of azadirachtin from the related neem tree.

2) To use *Melia azedarach* leaf powder as a pesticide, grind the dry leaves into a fine powder and then apply the powder directly to affected plants when there is no rain, ensuring thorough coverage, especially under the leaves.

### 3) Preparation and Application

Gather and dry leaves:

Collect *Melia azedarach* (Chinaberry) leaves, clean them, and dry them in the shade for approximately 15 days.

Powder the Leaves:

Grind the dried leaves into a fine powder using a mortar and pestle or an electric grinder.

Dusting Method:

Dust the powdered leaves directly onto the plants. Ensure that there is no moisture on the plant surfaces, as this will reduce the powder’s effectiveness.

#### 4.4.2. Real-Scale Application

### 1) Context

Between 2016 and 2018, the experimental area used the Improved Rice-

Growing System (IRGS), combining compost and ammonium phosphate. Yields did not exceed 1.5 tons/ha.

Around 2022, the appearance of a disease resembling rice blast (pyriculariose) motivated us to adopt the System of Rice Intensification (IRGS), with the exclusive use of biological inputs.

The IRGS is characterized by:

- Early transplanting (8 - 15 days after sowing instead of 20 - 45),
- Single seedlings transplanted one by one (instead of 3 - 5 per hill),
- Low water level (<1 cm instead of 15 cm in conventional systems),
- 2 to 3 mechanical weedings,
- Crop rotation practices between rice cycles.

Trials using biopesticides based on *Melia azedarach* leaves, pulp, and/or seeds, formulated at the CNRIT, produced promising results and encouraged the transition to the SRI system.

## 2) Site Description

The study took place in Anjomakely, on rice plots belonging to one of the researchers involved in this publication. The site is located 15 km from the administrative headquarters of CNRIT, our workplace in Antananarivo, the capital of Madagascar.

Geographic coordinates: Latitude 20°8'60"S, Longitude 47°30'0"E, Altitude 1539 m (5052 ft).

The total experimental area is 25 are (including canals and dikes), divided into several plots.

In 2022, two plots (2.4 are total) were infected by disease, measuring 1.1 are and 1.3 are, respectively (**Figure 7, Figure 8**).

- The first plot was treated twice at 10-day intervals before harvest.
- The second plot served as the untreated control.

Yields obtained:

- 6 tons/ha for the treated plot
- 5 tons/ha for the control plot

A yield increase of approximately 17% was observed in favor of the plot treated with *Melia azedarach* leaf extract.

The yield calculation method consisted of threshing, winnowing, and sun-drying the harvested rice before weighing. The production was then stored in plastic bags.

## 3) Explicit yield calculation

Plot A (treated) has an area of 1.3 are and produced 78 kg of dry paddy, corresponding to a yield of 6 tons·ha<sup>-1</sup>.

Plot B (untreated control) has an area of 1.1 are and produced 55 kg of dry paddy, corresponding to a yield of 5 tons·ha<sup>-1</sup>.

The relative difference between the treated and untreated plots was, therefore:

$$(6 - 5)/6 = 0.17$$

indicating a 17% higher yield in the treated plot. Both plots are adjacent and

exhibit a visually similar infestation rate estimated at 35% - 45% of plants.

#### 4) Climatic Overview

At the experimental site, climatic observations were divided into two periods:

- 2015-2020: Annual rainfall between 900 and 1100 mm during the early family-scale operations.
- Since 2022: Due to climate change and increasing irregularities, rainfall has dropped to below 900 mm/year.

#### 5) Climate Analysis (2022-Present)

- Average annual precipitation: <900 mm
- Average temperature: ~25°C (hottest months: July–October)
- Average humidity: 50% - 90% from transplanting until March

### 4.4.3. Results

Successful outcomes without chemical additives.

Effective use of biological inputs and gradual yield increases.

Almost total eradication of the disease in 2022 — only 1 infected plant out of 2000 the following year.

#### 1) Advantages of *Melia azedarach*

Easy to prepare.

Green leaves are available for much of the year.

Leaves can be stored in dried form (a finding from this study).

Very low-cost preparation, especially when trees grow near the fields.

#### 2) Conclusion on the real-scale application results

When the disease visibly affected about 30% of plants, a biological treatment based on *Melia azedarach* (seeds, pulp, leaves, and/or extracts) was applied to infected plots, leaving a control plot untreated.

A significant yield increase of about 17% was observed in the treated plots.

The disease was eradicated with a single treatment.

From 2023 to 2025, systematic biological treatments were applied from sowing to harvest, including during off-season crops. Currently, only 1 infected plant per 2,000 is observed.

Results have far exceeded expectations: one treated plot yielded 12 tons/ha, a record for this production zone.

However, climate change now disrupts traditional agricultural calendars.

#### 3) Perspectives

Conduct microbiological analyses to confirm the disease's exact nature — essential for continuing this research.

Expand this innovative biological protocol to tomatoes, potatoes, and legumes, especially black-eyed peas, which are a major export crop.

Plan for large-scale applications in partnership with rice-growing operators.

Establish local *Melia azedarach* plantations for large-scale projects.

Create a specialized production unit managed by a cooperative to ensure a large-scale biopesticide supply.



**Figure 7.** Rice infected with disease and not treated with biopesticide.



**Figure 8.** Rice treated with *Melia azedarach* biopesticide.

## 5. Conclusions

This work demonstrates that the Madagascar *Melia azedarach* biosynthesizes Azadirachtin D, one of the bioactive molecules that act as pesticidal agents from plants. The plant also does not produce the toxic compounds Meliatoxins. The presence (or absence) of Meliatoxins in *Melia azedarach* depends strongly on geographic origin, plant part, and even season. *Melia azedarach* shows strong chemotype diversity depending on geography and genetics. Madagascar populations may represent a non-meliatoxin chemotype. Environmental factors such as climate, soil, and seasonal patterns can also influence secondary metabolite biosynthesis.

The practical impacts of this finding are as follows:

For toxicology: Madagascar *Melia azedarach* leaves are considered much less

toxic to cattle and other livestock than other variants because they lack meliatoxins.

For commercial/biopesticide use: This makes Madagascar *Melia azedarach* more attractive as a raw material for isolating other limonoids (e.g., azadirachtins), since the problematic mammalian toxins (meliatoxins) are absent.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

## References

- [1] Mondal Debashri, M. and Tamal, M. (2012) A Review on Efficacy of *Azadirachta indica* A. Juss Based Biopesticides: An Indian Perspective. *Research Journal of Recent Sciences*, **1**, 94-99.  
[https://www.isca.me/rjrs/archive/v1/i3/15.ISCA-RJRS-2012-067\\_Done.pdf](https://www.isca.me/rjrs/archive/v1/i3/15.ISCA-RJRS-2012-067_Done.pdf)
- [2] Walia, S. and Koul, O. (2008) Exploring Plant Biodiversity for Botanical Insecticides. Sustainable Crop Protection, Biopesticide Strategies. Kalyani Publishers, 191-206.  
[https://www.researchgate.net/publication/278683826\\_Sustainable\\_Crop\\_Protection\\_Biopesticide\\_Strategies](https://www.researchgate.net/publication/278683826_Sustainable_Crop_Protection_Biopesticide_Strategies)
- [3] Harikrishnan, R., Nisha Rani, M. and Balasundaram, C. (2003) Hematological and Biochemical Parameters in Common Carp, *Cyprinus Carpio*, Following Herbal Treatment for *Aeromonas Hydrophila* Infection. *Aquaculture*, **221**, 41-50.  
[https://doi.org/10.1016/s0044-8486\(03\)00023-1](https://doi.org/10.1016/s0044-8486(03)00023-1)
- [4] Anuradha, A. and Annadurai, R.S. (2008) Biochemical and Molecular Evidence of Azadirachtin Binding to Insect Actins. *Current Science*, **95**, 1588-1593.  
[https://www.researchgate.net/publication/255648423\\_Biochemical\\_and\\_molecular\\_evidence\\_of\\_azadirachtin\\_binding\\_to\\_insect\\_actins](https://www.researchgate.net/publication/255648423_Biochemical_and_molecular_evidence_of_azadirachtin_binding_to_insect_actins)
- [5] Murray, B.I. (2005) Chapter Six—Tropical Forests as Sources of Natural Insecticides. *Recent Advances in Phytochemistry*, **39**, 145-161.
- [6] Marrone, P.G. (2024) Status of the Biopesticide Market and Prospects for New Bioherbicides. *Pest Management Science*, **80**, 81-86. <https://doi.org/10.1002/ps.7403>
- [7] Nuruzzaman, M., Rahman, M.M., Liu, Y. and Naidu, R. (2016) Nanoencapsulation, Nano-Guard for Pesticides: A New Window for Safe Application. *Journal of Agricultural and Food Chemistry*, **64**, 1447-1483. <https://doi.org/10.1021/acs.jafc.5b05214>
- [8] Vaschetto, L. (2025) Biopesticides in 2025: The Science Behind Sustainable Pest Control.  
<https://www.azolifesciences.com/article/Biopesticides-in-2025-The-Science-Behind-Sustainable-Pest-Control.aspx>
- [9] Soto-Barajas, M.C., Archundia, D., Martínez, O.G.R., López, E., Almazan, J. and Prado, B. (2025) Current and Future Perspectives on Biopesticides Analysis in Soil. *Journal of Natural Pesticide Research*, **12**, Article 100120.  
<https://doi.org/10.1016/j.napere.2025.100120>
- [10] Bhat, S.S., Soni, P.K., Menasinakay, V.S., Biswal, S.K. and Kumar, S. (2021) Plants

- Used as a Traditional Biopesticide. In: Kumar, S., Ed., *Medico Biowealth of India*, Volume III, Ambika Prasad Research Foundation, 1-7.  
<https://www.researchgate.net/publication/35477273>
- [11] Raizada, R.B., Srivastava, M.K., Kaushal, R.A. and Singh, R.P. (2001) Azadirachtin, a Neem Biopesticide: Subchronic Toxicity Assessment in Rats. *Food and Chemical Toxicology*, **39**, 477-483. [https://doi.org/10.1016/s0278-6915\(00\)00153-8](https://doi.org/10.1016/s0278-6915(00)00153-8)
- [12] Su, X., Liang, Z., Xue, Q., Liu, J., Hao, X. and Wang, C. (2023) A Comprehensive Review of Azadirachtin: Physicochemical Properties, Bioactivities, Production, and Biosynthesis. *Acupuncture and Herbal Medicine*, **3**, 256-270.  
<https://doi.org/10.1097/hm9.0000000000000086>
- [13] Subrahmanyam, B. (1990) Azadirachtin—A Naturally Occurring Insect Growth Regulator. *Proceedings: Animal Sciences*, **99**, 277-288.  
<https://doi.org/10.1007/bf03186397>  
<https://www.ias.ac.in/article/fulltext/anml/099/03/0277-0288>
- [14] <https://www.cal-ipc.org/plants/profile/melia-azedarach-profile/>
- [15] Huma, Q., Muhammad, A., Abida, A., Naveed, I. R., Sammer, F. and Muhammad, S. (2016) Ethnopharmacological and Phytochemical Account of Paradise Tree (*Melia azedarach* L.: Meliaceae). *Pure and Applied Biology*, **5**, 5-14.  
<https://doi.org/10.19045/bspab.2016.50002>
- [16] Hieu, T.T., Chung, N.T., Dung, V.C. and Duc, D.X. (2022) Chemical Composition and Bioactivities of *Melia azedarach* (Meliaceae): A Comprehensive Review. *Current Organic Chemistry*, **26**, 2160-2187.  
<https://doi.org/10.2174/1385272827666230130140839>
- [17] Cao, F., Chen, J., Lin, Z., Lin, H., Liu, B., Chen, Z., *et al.* (2024) Chemical Constituents from the Fruit of *Melia Azedarach* and Their Anti-Inflammatory Activity. *Antioxidants*, **13**, Article 1338. <https://doi.org/10.3390/antiox13111338>
- [18] Zeng, J., Ma, R., Wang, L., Zhang, S., Song, H., Yang, Y., *et al.* (2018) Chemical Constituents from the Leaves of *Melia azedarach*. *Natural Product Research*, **33**, 2860-2863.  
<https://doi.org/10.1080/14786419.2018.1501690>
- [19] Laskar, R. and Ivantsova, M.N. (2025) Formulating a Bio-Pesticide Using *Melia Azedarach* Plant Extracts. 127-131.  
[https://elar.ufru.ru/bitstream/10995/141983/1/978-5-6051237-3-6\\_2025\\_037.pdf](https://elar.ufru.ru/bitstream/10995/141983/1/978-5-6051237-3-6_2025_037.pdf)
- [20] Vicinieski, R.P., Nolibos Almeida, R., Fonseca Duarte, P., Guizolfi, T., Johann, L., da Silva, G.L., *et al.* (2024) Production of Biopesticide from *Melia Azedarach* Linn Extract Obtained by Supercritical Fluid Extraction for the Control of *Tetranychus urticae*. *Biocatalysis and Agricultural Biotechnology*, **56**, Article 103018.  
<https://doi.org/10.1016/j.bcab.2024.103018>
- [21] Oelrichs, P.B., Hill, M.W., Vallely, P.J., MacLeod, J.K. and Molinski, T.F. (1983) Toxic Tetranortriterpenes of the Fruit of *Melia Azedarach*. *Phytochemistry*, **22**, 531-534.  
[https://doi.org/10.1016/0031-9422\(83\)83039-8](https://doi.org/10.1016/0031-9422(83)83039-8)
- [22] Forim, M.R., Cornélio, V.E., das G. F. da Silva, M.F., Rodrigues-Filho, E., Fernandes, J.B., Vieira, P.C., *et al.* (2010) Chemical Characterization of *Azadirachta indica* Grafted on *Melia azedarach* and Analyses of Azadirachtin by HPLC-MS-MS (SRM) and Melia-toxins by MALDI-MS. *Phytochemical Analysis*, **21**, 363-373.  
<https://doi.org/10.1002/pca.1208>