



Green synthesized Silver Nanoparticles using leaf extract of *Nicotiana rustica* Natural Pesticides against Indian white termites, *Odontotermes obesus*

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Abstract – Green nanotechnology has widespread applications in various biomedical science fields. In this research paper, green-synthesized silver Nanoparticles, prepared by using *Nicotiana rustica* extract, were characterized using digital calorimeter, scanning electron microscopy and FTIR. This study thoroughly investigated the insecticidal efficacy of medicinal plants, *Nicotiana rustica*. Different extraction methods were used and their efficacy with and without nanoparticles was compared. FT-IR analysis indicated the involvement of carboxyl (-C=O), hydroxyl (-OH), C-N stretching of aromatic amines or NO₂ symmetric stretching and amine (-NH) functional groups of tobacco leaf extract in the preparation of Silver Nanoparticles. SEM analysis showed that AgNPs is widely distributed in some areas and has irregular and circular shapes that have different diameters with average diameters of, for example, 54.79 nm and 66.05 nm. The focus of the study was on investigating various parameters such as mortality rates, insect survival rates, adult emergence and development stages after treatment periods of 24, 42 and 72 hours. The termite repellent efficacy was significantly higher in treatments with nanoparticles, but the differences were not significant ($p>0.05$).

Key words- Silver Nanoparticles, Plant extract of *Nicotiana rustica*, Termites, Digital Calorimeter, FTIR, and SEM.

Introduction

Termites pose a serious threat to plants and buildings and are among the most serious pests in forestry and agriculture worldwide ^[28]. Statistics show that over 2,800 termite species have been identified worldwide, of which 185 are considered harmful pests ^[27], particularly termites of the families Hodotermitidae, Kalotermitidae, Rhinotermitidae and Termitidae. Previous studies have estimated that the annual expenditure on termites control has reached 22 billion dollars around the globe, which is increasing by 50% each year ^[24]. In the USA alone, In the USA alone, termite control costs 175 million dollars annually ^[8]. Undoubtedly, one of the most popular and successful approaches to termite control is the employment of chemical-based insecticides ^[2]. However, overuse of pesticides has resulted in many issues, including biological resistance, ecological imbalances and adverse effects on the environment ^[7].

The Indian white termite (*Odontotermes obesus* Ramb.) is a highly destructive, polyphagous pest that lives in huge mounds and feeds on cellulose and almost anything containing carbohydrates. It causes economic damage to timber, cellulose fibers, boards, paper, clothing, wool and mats, and wood-based building materials and infests green foliage and grain stored in warehouses. The majority of termites are controlled with chemical insecticides, such as chlordane, cypermethrin, hydroquinone and indoxacarb. Due to their longer residual persistence and greater toxicity, they harm the environment, humans and many beneficial organisms. Therefore, it is critically important and widely recognized that other control



methods such as physical prevention, cultural measures, and organic and environmentally friendly compounds are essential for termite control.



Fig: 1 *Odontotermes obesus*

Natural pesticides refer to pesticides that are naturally occurring and produce from foods, plants and bacteria. Although they are not as long-lasting and non-toxic as synthetic pesticides, they are safer for the environment (This does not mean they are safe for humans, as they can still kill insects). Specific pesticides include bio pesticides-natural chemicals produced directly from plants, or biochemical pesticides which use hormones to stimulate sexual or other behaviours of microbial organisms-pesticides that contain fungi, bacteria etc., as active ingredients. Other herbs such as rosemary, marigold, chrysanthemum, lavender and basil act as natural deterrents to pests^[26].

Nicotiana rustica, also known as Aztec tobacco, is a plant species in the nightshade family (Solanaceae), native to the Americas. It is a relative to the more familiar cigarettes (the source of most tobacco products), but differs in its higher nicotine content and stronger effects. *Nicotiana rustica* is a very strong type of tobacco variety, containing nine times more nicotine than cigarettes, such as red tobacco (*Nicotiana tabacum*)^[23]. Specifically, the nicotine content of *N. rustica* leaves is as high as 9%^[14], while the nicotine content of *N. tabacum* leaves is between 1% and 3%. The leaves are high in nicotine, are used in the production of insecticides^[9] and have a wide range of crop applications worldwide. Nicotine acts as a contact insecticide, causing harm to pests when they come into contact with nicotine-based solutions. Nicotine is an alkaloid extracted from the leaves of the tobacco plant (*Nicotiana tabacum*) and other species (like *N. rustica*) and has a long history as a pesticides. Nicotine and two of its alkaloids-nornicotine and pseudoecotine-are synaptic toxins that act on the neurotransmitter acetylcholine. As such, they cause toxic symptoms similar to those of organophosphate and carbamate pesticides^[18]. Additionally, extracts *Nicotiana rustica*, which contain anabasine in a addition to nicotine, can be used as organic insecticides. These extracts can be applied directly to plants for pest control. They are commonly known as “tobacco sprays” or “tobacco dust”. Anabasine, is a natural compound and tends to have a lower environmental impact than synthetic pesticides. However, similar to nicotine, it can harm beneficial insects such as bees if used improperly. It is a substance that can damage the nervous system.



Fig: 2 *Nicotiana rustica* Plant

Nanotechnology is objects are usually one to hundreds of nanometres in size. At this scale, data can reveal unique features that are distinct from large datasets and create new opportunities for various applications such as pest control and environmental protection^[20, 4]. Among the numerous types of nanoparticles, silver nanoparticles (AgNPs) have received special attention in pesticide treatment and health impact reduction. AgNPs have antimicrobial properties and are widely used in medicine, water treatment and consumer products^[30]. AgNPs are promising in many aspects of pesticide control.

Recently, “green synthesis” has emerged as one of the various methods for obtaining nanoparticles from microorganisms or plant extracts^[6, 29]. This technique offers a simple and environmentally friendly synthesis method compared to chemical and physical method for obtaining nanoparticles^[12, 10]. Silver nanoparticles (AgNPs) are an efficient alternative for controlling economically important pesticides. For example, Rehaman *et al.*, (2021)^[19] synthesized AgNPs from *Camelina sativa* (L.) Crantz (Brassicaceae) and tested them against *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and *Sitophilus granaries* (L.) (Coleoptera: Curullionidae). Devi *et al.*, However,^[5] synthesized AgNps from extrcats of *Euphorbia hirta* L. (Euphorbiaceae) and tested them against *Helicoverpa amigera* (HUBner) (Lepidoptera: Noctuidae). They found an extension of the duration of the larval and pupal stages and a reduction in male and female fertility.

This research investigates the potential and mortality rate of utilizing green silver nanoparticles, synthesized from the extracts of *Nicotiana rustica*, as natural and effective pesticides against *Odontotermes obesus*, aiming to enhance sustainable pest management methods. Three different solvent extracts were tested at various concentrations: 0.5, 1, 1.5 and 2 ml, each with and without Silver nanoparticles. The integration of silver nanoparticles into these natural extract may increase their effectiveness, thus providing a more environmentally friendly and sustainable approach to termite control.

Methodology

Materials

. Study site: Jaipur

. Procurement of material: local area of Jaipur



. Environmental Factors:

1. Humidity
2. Temperature
3. Season
4. Time Period

Rearing of Culture of *Odontotermes obesus*

Termites of the species *Odontotermes obesus* were collected from nearby farms and transported to the laboratory in a humid thermocol box. In the laboratory, they were maintained in a specially designed humidistat chamber. Early morning or late afternoon is the best time to collect termites, as they are most active at these times. The termites were provided with an environment of constant humidity and suitable food sources such as pieces of wood, cardboard or leaves. Additionally, rotting wood was used to mimic their natural diet. The humidity in the chamber was maintained (70%-80%) using moist paper towels. The chamber was maintained at room temperature (27° C +- 2° C), which is within the ideal temperature range for *O. obesus* (25° -30°C). To minimize exposure to light, the chamber was covered with a black cotton cloth. Termites were provided with logs or cardboard to facilitate nests and tunnel construction, as they prefer to build complex networks and burrow soft materials that they can easily penetrate.



Fig: 3 Termites colony

Plant Extraction Methods

To prepare the extracts, dried leaves of *N. rustica* were collected, washed and ground. They were then extracted with three solvents-n-hexane, methanol and aqueous-selected based on their polarity indices. Fifty gram of *N.rustica* leaf powder was then subjected to organic solvent extraction by refluxing in a Soxhlet apparatus, using each solvent for 24 hr. Following the extraction process, all extracts were concentrated by oven drying according to the method described by Azhari *et al.*, (2014) ^[3]. The extracts were then stored at 4°C until further analysis.

Biosynthesis of Silver Nanoparticle

Preparation of plant extracts

The leaves of *Nicotiana rustica* are carefully cleaned to remove dirt and other impurities. After washing, the leaves are dried in the shade, ground into a fine powder, and then



extracted with distilled water. 100 ml of distilled water is placed in a clean glass container. The container is then placed on a heat source (e.g., a stove or hot plate), and the water is slowly heated. A thermometer is used to ensure that the water reaches 80°C (122°F)-the optimal temperature for extracting beneficial compounds from the plant material. Once this temperature is reached, 5 gm of dried powder is added to the heated water and mixed gently to ensure even mixing. The mixture is kept at 80°C for 30 minutes, stirring regularly to ensure even extraction^[17]. After 15 minutes, the container is removed from the heat source. Using fine filter paper, a mesh sieve, or cheesecloth the extract is strained; removing any solid plant matter. The filtered plant extract was allowed to cool to room temperature. After cooling, the extract was transferred to a sealed glass container and stored in a refrigerator at a temperature between 4 and 8°C (39 and 46°F). To ensure the stability of the bioactive compounds, it was stored in a refrigerator in a dark place.

Preparation of AgNO₃ solution

An AgNO₃ solution was prepared by dissolving 0.2 g of silver nitrate in 100ml of distilled water.

Green Synthesized and Characterization of Silver Nanoparticles

Green-synthesized Silver Nanoparticles were prepared from *N. rustica* leaf extract. 96 mL of distilled water was poured into a 100–250 mL beaker. Then, 1 mL of the plant extracts (*Nicotiana rustica*) was added to the mixture. The beaker was placed on a magnetic stirrer with a heating plate, and stirring began while the mixture was moderately heated to 40–50°C. While stirring, 1 mL of a 0.1 M KOH solution was gradually added to the mixture. The pH was an adjusted to a value between 7 and 8, as this range was optimal for nanoparticle formation. After the temperature was stabilized and the pH was an adjusted, 2 mL of the Silver Nitrate solution (containing 0.2 g of AgNO₃) was added using a micropipette. The temperature was maintained at 40–50°C with constant stirring. The mixture was stirred and continuously heated for 4–5 hours. A colour change from clear or yellowish to brown or dark brown indicated the successful formation of Silver Nanoparticles. After 24 hours, the synthesized silver nanoparticles were centrifuged at 5000 ppm, and the resulting pellet containing the nanoparticles was collected. The particles were washed and then mixed with ethanol solution. The sample was incubation at 55-60° C. After 4-5 day, the dried silver nanoparticles were collected for further analysis.



Fig: 4 Green Synthesized Silver Nanoparticles



Fig: 5 Silver Nanoparticles

The characterization of the AgNps was carried out by digital calorimeter and FT-IR analysis.

Laboratory Evaluation of Insecticidal Effect on termites (*O. obesus*)

Ranjith *et al.*, (2017)^[16] proposed a termite bioassay using precise procedures. Three different solvent extracts were tested at different concentrations: 0.5, 1, 1.5 and 2 ml, each with and without Silver nanoparticles. These extracts were transferred whatman no. 1 filter paper and individually placed in petri dishes. Filter papers treated with methanol and n-hexane served as positive controls, and untreated filter papers as negative controls. The experiment used whatman filter papers coated with different concentrations of dried *N. rustica* leaf extracts, deep-fried, and placed in petri plates. 10 adult white worker termites were randomly placed on the petri plates containing whatman filter paper impregnated with different seed extracts. All the petri dishes were then kept in an incubator at 25°C. After 24, 48 and 72 hours of incubation, dead termites were identified and counted to calculate the mortality rate. The mortality rates for the test and control groups were determined using the following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of dead termites}}{\text{Number of initial termites in the test}} \times 100$$

Statistical Analysis

The data were subjected to statistical analysis using the one-way ANOVA test. Values were expressed as mean± standard deviation. A statistical significance threshold of $p > 0.05$ was considered significant.

Result and Discussion

Colorimetric Analysis:

The digital photo colorimeter was set to filter 42, which corresponds to violet-blue light (wavelength approximately 420nm). The absorption value of the green synthesized silver nanoparticle solution was 1.08. Silver nanoparticles typically exhibited surface plasmon resonance (SPR) in the range of 400-450 nm, depending on their size, shape and the



surrounding medium. Since 420 nm is within this expected range ^[13], the use of filter 42 made sense. The absorbance value 1.08 (Fig: 6) indicated sufficiently strong absorption and thus a good nanoparticle concentration. The selected wavelength (420nm) was suitable for the detection of silver nanoparticles, and the absorbance value indicated successful synthesis with *Nicotiana rustica*. Several other researchers have observed absorption maxima of colloidal silver solution between 410 to 450 nm, which have been attributed to surface plasmon of various metal nanoparticles ^[21].



Fig: 6 Absorbance values of Silver nanoparticles

FTIR Analysis (Fourier-Transform Infrared Spectroscopy):

Using FT-IR spectroscopy, various functional groups in biomolecules that play a role in the reduction of Ag⁺ and the stabilization of AgNPs were identified. The intensity of the bands was compared to established norms to classify them into different functional categories. The FT-IR spectrum of green, synthesized Silver Nanoparticles combined with *Nicotiana rustica* leaf extract (4000-500 cm⁻¹) highlights important chemical components.

The FT-IR spectrum shown in **Fig: 7**, exhibits absorption maxima at 3437.92, 2070.08, 1633.81, 1401.23, 1385.35, 1353.27, 1117.33 and 619.24 cm⁻¹, indicating the presence of a capping agent in addition to the AgNPs ^[22,11]. The bands visible in the spectra at 3437.92 cm⁻¹ (11.30%T) correspond to the stretching vibration of the O-H or N-H bond indicates the presence of phenolic compounds, alcohol and proteins. Band were observed in the region of 2070.08 cm⁻¹ (93.68 % T), which may be related to the C-C bonds in some combination bands. The band detected at a wavenumber of 1633.81 cm⁻¹ (42.88%T) is associated with the elongation of the C-O or C-C stretching vibrations in aromatic compounds, as well as proteins or polyphenols, which can act as capping layers for AgNPs. The peak at 1401.23 cm⁻¹ (52.81% T) in the spectra corresponds to the stretching vibrations of the C-N and O-H groups in the amine or phenolic groups. These functional groups have been shown to contribute to the stability and capping properties of AgNPs in several studies ^[1]. The band detected at a wavenumber of 1353.27 cm⁻¹ (80.68%T) represents the C-N stretching vibration of aromatic amines or the NO symmetry stretching vibration, which is commonly found in nitrogen-compounds (e.g., alkaloids) (Paul kumar *et al.*, 2014) ^[15], which are abundant in *Nicotiana rustica*. The peak at 1385.35 cm⁻¹ (52.57%) in the spectrum corresponds to the stretching vibration of C-H bonds and indicates the presence of an additional organic capping layer. The bands visible in the spectrum at 1117.33 cm⁻¹ (87.31% T) correspond to the stretching vibration of the C-O bond and indicate the presence of esters, alcohols and polysaccharides. These compounds can coat or stabilize nanoparticles. The appearance of a band in the region of 969.24 cm⁻¹ (74.74% T) can be attributed to the out-of-plane extension of the C-H curve and indicates the presence of aromatic compounds such as flavonoids or



alkaloids. FT-IR spectroscopy shows that the presence of Ag⁺ ions or nanoparticles in *Nicotiana rustica* leaves does not alter the secondary structure of proteins.

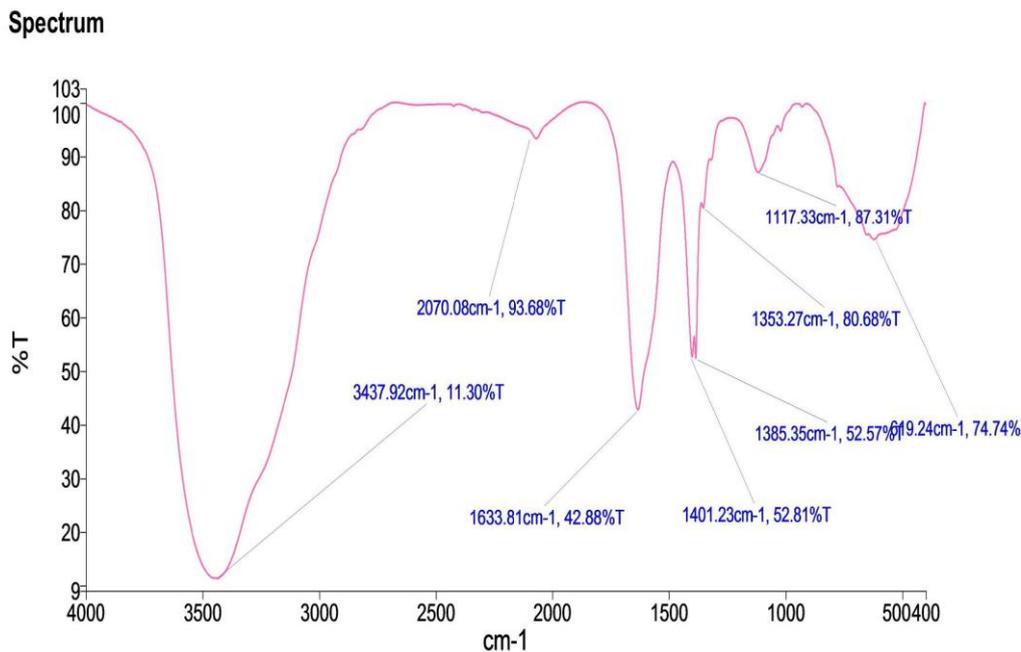


Fig: 7 FTIR Analysis of Silver nanoparticles using *Nicotiana rustica* (leaves)

Scanning Electron Microscope with EDS (FESEM)

Scanning electron microscopy (SEM) images of synthesized silver nanoparticles revealed that most of the particles were circular and smaller than 100 nm in size, as shown in the **fig: 8**. However, the image quality was inadequate due to the limited SEM equipment at the institute, which could not capture high-resolution images of particles below 100 nm. Nevertheless, SEM analysis revealed that silver nanoparticles (AgNPs) were widely distributed in some areas and exhibited both irregular and spherical shapes with an average diameter 54.79 nm and 66.05 nm, respectively.

Therefore, a field emission scanning electron microscope (FESEM) was used for better resolution. The FESEM image (**Fig. 9**) clearly confirmed the presence of synthesized nanoparticles. The particles were predominantly oval to spherical in shape. While most of the nanoparticles were clustered, some individual particles were also observed, which is consistent with the conclusions of Suman & Rajshree *et al.*, 2013^[25].

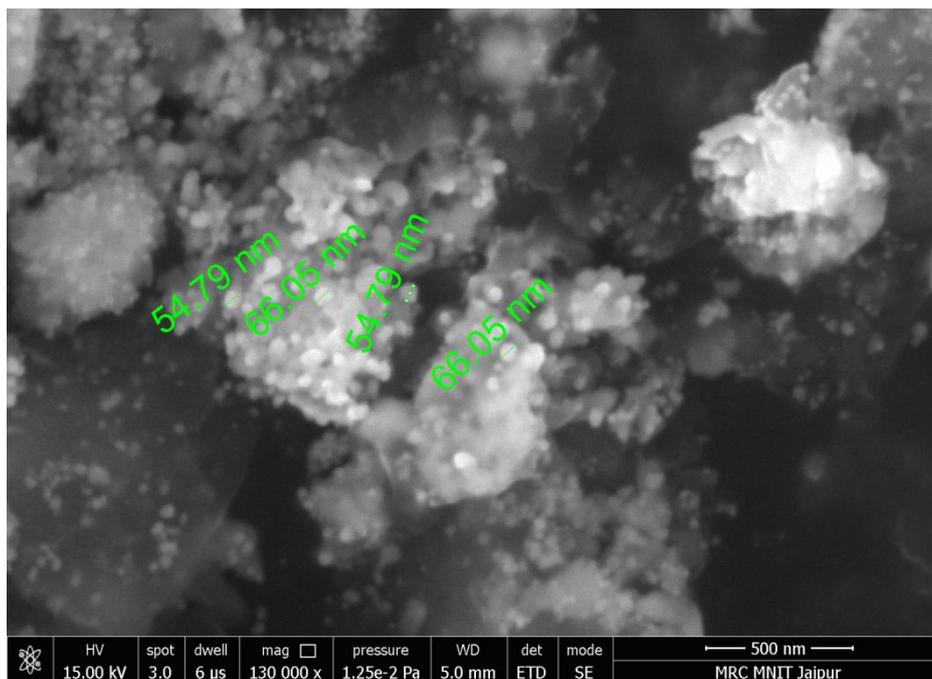


Fig: 8 SEM image of Silver Nanoparticles (Using leaf extract of *Nicotiana rustica*)

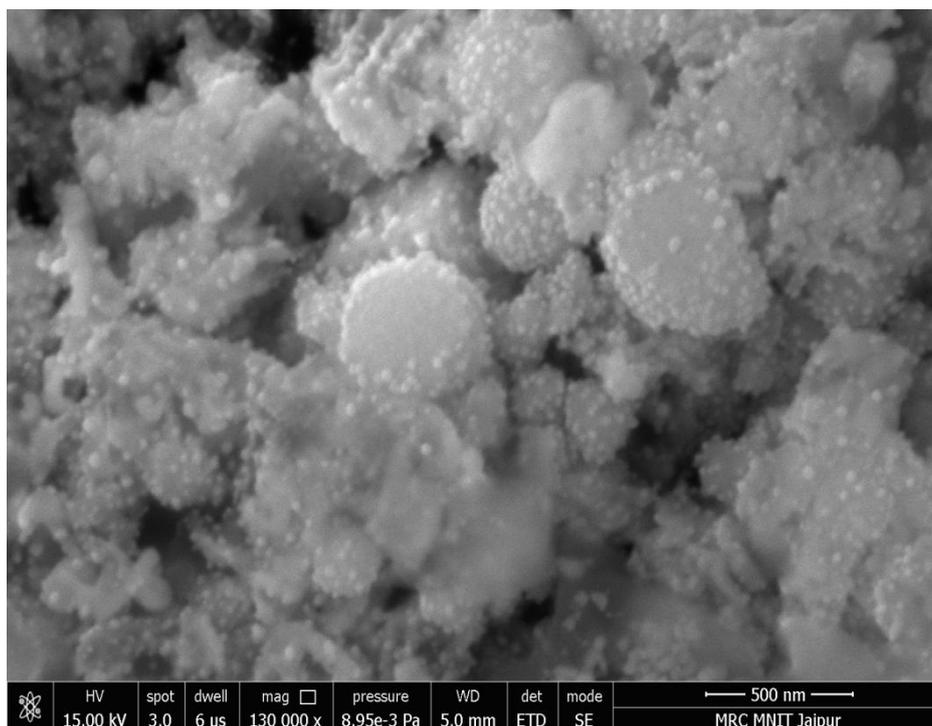


Fig: 9 FESEM image of Silver Nanoparticles (Using leaf extract of *Nicotiana rustica*)

Without the addition of nanoparticles:

Table 1: Comparison analysis between the methanol, hexane and control groups based on the period without nanoparticle (1ml)



Group	Plant	Parameters	Time Period	Mean	SD	Min	Max	F value	P value
Methanol	<i>Nicotiana rustica</i>	Concentration (mL)	24	1.25	0.65	0.5	2.0	0.000	1.000
			48	1.25	0.65	0.5	2.0		
			72	1.25	0.65	0.5	2.0		
		Mortality Rate (%)	24	62.50	22.17	40	90	0.166	0.849
			48	67.50	22.17	45	95		
			72	71.25	20.16	50	95		
		Number of Insects Dead	24	6.25	2.22	4	9	0.171	0.846
			48	6.25	2.22	4	9		
			72	7.00	1.83	5	9		
		Number of Insects Alive	24	3.75	2.22	1	6	0.171	0.846
			48	3.75	2.22	1	6		
			72	3.00	1.83	1	5		
Hexane	<i>Nicotiana rustica</i>	Concentration (mL)	24	1.25	0.65	0.5	2.0	0.000	1.000
			48	1.25	0.65	0.5	2.0		
			72	1.25	0.65	0.5	2.0		
		Mortality Rate (%)	24	55.00	12.91	40	70	8.150	0.010*
			48	74.50	13.03	60	91		
			72	87.50	7.59	80	95		
		Number of Insects Dead	24	5.50	1.29	4	7	7.605	0.012*
			48	7.25	1.26	6	9		
			72	8.50	0.58	8	9		
		Number of Insects Alive	24	4.50	1.29	3	6	7.605	0.012*
			48	2.75	1.26	1	4		
			72	1.50	0.58	1	2		
Control	<i>Nicotiana rustica</i>	Concentration (mL)	24	1.25	0.65	0.5	2.0	0.000	1.000
			48	1.25	0.65	0.5	2.0		
			72	1.25	0.65	0.5	2.0		
		Mortality Rate (%)	24	22.50	17.08	0	40	0.078	0.925
			48	26.25	19.31	0	45		
			72	27.50	19.36	0	45		
		Number of Insects Dead	24	2.25	1.71	0	4	0.028	0.972
			48	2.25	1.71	0	4		
			72	2.50	1.73	0	4		
		Number of Insects Alive	24	7.75	1.71	6	10	0.028	0.972
			48	7.75	1.71	6	10		
			72	7.50	1.73	6	10		

** $p < 0.01$

Table 1: Table 1 shows the relative effects of Methanol, Hexane, and Control treatments on *Nicotiana rustica* for 24, 48, and 72 hours in the absence of nanoparticles. Based on the concentrations, the treatment groups were treated with an equivalent dose of 1.25 mL (SD = 0.65; Min = 0.5, Max = 2.0). The F-value for the concentration was 0.000 and the P-value was 1.000, indicating that the treatments did not have significantly different concentrations. Mortality rates in *Nicotiana rustica* increased from 62.50% at 24 hours to 71.25% at 72 hours



with the methanol treatment. However, the F-values (0.166 and 0.343) and P-values (0.849 and 0.719) indicated that the variation was not statistically significant. Hexane resulted in significantly higher mortality, for example, in *Nicotiana rustica*, from 55.00% after 24 hours to 87.50% after 72 hours (F-value = 8.150, P-value = 0.010). The control group showed a relatively low mortality rate (22.50% for *Nicotiana rustica* after 24 hours). In the absence of methanol or hexane, the insecticidal effect was low or non-existent (P-values > 0.7). The most important finding of the study is that spraying *Nicotiana rustica* with hexane resulted in significant insect mortality (p-value = 0.0100). Thus, hexane is superior to methanol and the control group. Mortalities in the methanol group increased over time, but not significantly (p > 0.8). To this end, hexane treatment showed a striking increase in the death rate of insects, primarily in *Nicotiana rustica* (F = 7.605, p = 0.012). After 72 hours, 8.50 insects had died due to hexane treatment (SD = 0.58), compared to a measly 1.50 alive insects (SD = 0.58), which speaks volumes about the effectiveness of hexane. However, the control group showed a statistically insignificant difference in dead insects (p > 0.9), and therefore, the death in the other treatments was mainly due to the effect of methanol and hexane, rather than natural. *Nicotiana rustica* treated with hexane was mainly the most effective at killing insects, as it showed a high percentage increase in dead insects and a decrease in surviving insects. Compared to hexane, methanol was not very effective against insects but did show some activity. Hexane was more effective, particularly against *Nicotiana rustica*, where increased mortality (p < 0.01) was recorded. Minimal mortality occurred in the control, suggesting that both hexane and methanol influenced insect mortality. Comparing the response of various plant species to hexane treatment with their actual response showed that *Nicotiana rustica* responded significantly more forcefully, with increased mortality (p = 0.010) and more insect deaths (p = 0.012).

With the addition of nanoparticles:

Table 2: Comparison analysis between the methanol, hexane and control groups based on the period with nanoparticle (1ml)

Group	Plant	Parameters	Time Period	Mean	SD	Min	Max	F value	P value
Methanol	<i>Nicotiana rustica</i> (0.5ml) +nanoparticle (1ml)	Concentration (mL)	24	1.25	0.65	0.50	2.00	0.000	1.000
			48	1.25	0.65	0.50	2.00		
			72	1.25	0.65	0.50	2.00		
		Mortality Rate (%)	24	85.00	13.61	66.00	98.00	0.028	0.972
			48	86.00	11.78	70.00	98.00		
			72	87.00	10.13	72.00	94.00		
		Number of Insects Dead	24	8.25	1.71	6.00	10.00	0.122	0.887
			48	8.50	1.29	7.00	10.00		
			72	8.75	1.26	7.00	10.00		
		Number of Insects Alive	24	1.75	1.71	0.00	4.00	0.122	0.887
			48	1.50	1.29	0.00	3.00		
			72	1.25	1.26	0.00	3.00		
Hexane	<i>Nicotiana rustica</i>	Concentration (mL)	24	1.25	0.65	0.50	2.00	0.000	1.000
			48	1.25	0.65	0.50	2.00		



	(0.5ml) +nanoparticle (1ml)	Mortality Rate (%)	72	1.25	0.65	0.50	2.00	0.194	0.827
			24	63.25	28.21	30.00	90.00		
			48	70.00	29.44	40.00	100.00		
		Number of Insects Dead	72	75.25	24.02	50.00	100.00	0.217	0.809
			24	6.25	2.75	3.00	9.00		
			48	7.00	2.94	4.00	10.00		
		Number of Insects Alive	72	7.50	2.38	5.00	10.00	0.217	0.809
			24	3.75	2.75	1.00	7.00		
			48	3.00	2.94	0.00	6.00		
			72	2.50	2.38	0.00	5.00		
			48	4.00	2.94	1.00	7.00		
		Control	<i>Nicotiana rustica</i> (0.5ml) +nanoparticle (1ml)	Concentration (mL)	24	1.25	0.65	0.50	2.00
48	1.25				0.65	0.50	2.00		
72	1.25				0.65	0.50	2.00		
Mortality Rate (%)	24			32.50	25.00	0.00	60.00	0.048	0.953
	48			32.50	25.00	0.00	60.00		
	72			37.50	28.72	0.00	70.00		
Number of Insects Dead	24			3.25	2.50	0.00	6.00	0.048	0.953
	48			3.25	2.50	0.00	6.00		
	72			3.75	2.87	0.00	7.00		
Number of Insects Alive	24			6.75	2.50	4.00	10.00	0.048	0.953
	48			6.75	2.50	4.00	10.00		
	72			6.25	2.87	3.00	10.00		

Table: 2 Shows the Comparative analysis between the groups of methanol, hexane and control based on time period with nanoparticles (1ml). When considering the methanol group with *Nicotiana rustica* (0.5ml) +nanoparticle (1ml), the p values for Concentration, mortality rate, number of dead insects, and number of live insects ($p > 0.05$) are above the significant level of 0.05. Therefore, based on the time period with nanoparticle (1ml), there is no significant difference in the mean values of concentration, mortality rate, number of dead insects and number of live insects between the methanol groups. When considering the hexane group with *Nicotiana rustica* (0.5ml) + nanoparticle (1ml), the p-values for mortality rate ($p > 0.05$), number of dead insects ($p > 0.05$) and number of live insects ($p > 0.05$) are above the significant level of 0.05. Therefore, based on the time period with nanoparticles (1ml), there is no significant difference in the mean values for mortality rate, number of dead insects and number of live insects between the hexane groups. When considering the control group with *Nicotiana rustica* (0.5ml) + nanoparticle (1ml), the p values for mortality rate ($p > 0.05$), number of dead insects ($p > 0.05$) and number of live insects ($p > 0.05$) are above the significant



level of 0.05. Therefore, based on the time period with nanoparticles (1ml), there is no significant difference in the mean values for mortality rate, number of dead insects and number of live insects between the hexane groups.

Table 2: Contrast of the manipulate control groups, hexane, and methanol with nanoparticles (1 mL) in opposition to *Nicotiana rustica* is of extreme importance regarding balance in attention, mortality after a time period, and survival of insects. Attention solution remained stable at 1.25 ± 0.65 mL in the 24–72 hours period without statistical differences ($F = 0.000$, $P = 1.000$). This uniformity means that death and insect mortality can be varied by chemical treatment rather than by fluctuations in concentration. Insect mortality rates differ depending on the time and duration of treatment. *Nicotiana rustica* showed a high mortality rate in methanol-treated samples, ranging from 85.00% at 24 hours to 87.00% at 72 hours. Statistical analysis also showed that the changes were not significant ($F = 0.028$, $P = 0.972$ for *N. rustica*), meaning that the insecticidal activity of methanol was very stable, with moderate increases over time. Nevertheless, in *N. rustica* treated with hexane, the mortality rate increase from 63.25% at 24 hours to 75.25% at 72 hours. As expected, despite these variations, statistical analysis ($F = 0.194$, $P = 0.827$ for *N. rustica*) showed no differences over time. For example, mortality for *N. rustica* was only 32.50% after 24 and 48 hours, increased to 37.50% after 72 hours. Statistical analysis revealed that the variation was not significant ($F = 0.048$, $P = 0.953$ for *N. rustica*), meaning the insecticidal effect was moderate without chemical treatment. The mortality rate was consistent with the insect mortality rate. Methanol-treated *N. rustica* killed the most insects (8.75 dead insects at 72 hours), followed by hexane treatments (6.00-6.75 dead insects), and finally the fewest in the control group (2.50–3.75 dead insects). The number of surviving insects was also lower: 1.25-2.50 survivors in both methanol and hexane treatments and 5.00-6.25 in the control group. Although the methanol and hexane-treated groups experienced higher mortality over time, statistical comparison of insect survival and death rates revealed no differences between the different treatment periods. The overall results suggest that both hexane and methanol nanoparticle treatments have insecticidal activity, with the latter exhibiting higher long-term mortality, particularly in *Nicotiana rustica*. However, the lacks of statistical significance guarantee that the results are very similar despite the impact of these treatments on insect survival. A very low insect mortality rate was observed in the control group, reflection the effectiveness of chemical treatments.

Table 3: Comparative analysis between methanol with nanoparticles and without nanoparticles for the groups methanol extract, hexane extract and aqueous extract in water:

Group			Mean	SD	Min	Max	F value	P value
Methanol Extract	Concentration (ml)	Without nanoparticles	1.25	0.58	0.50	2.00	0.000	1.000
		With nanoparticles	1.25	0.58	0.50	2.00		
	<i>Nicotiana rustica</i> Repellant Activity (%)	Without nanoparticles	71.25	16.39	45.00	95.00	0.558	0.463
		With nanoparticles	76.25	16.39	50.00	100.00		
Hexane Extract	Concentration (ml)	Without nanoparticles	1.25	0.58	0.50	2.00	0.000	1.000
		With	1.25	0.58	0.50	2.00		



		nanoparticles						
	<i>Nicotiana rustica</i> Repellent Activity (%)	Without nanoparticles	72.92	15.67	50.00	96.00	0.108	0.746
		With nanoparticles	75.00	15.43	51.00	100.00		
		With nanoparticles	79.67	14.70	56.00	100.00		
Aqueous Extract in Water	Concentration (ml)	Without nanoparticles	1.25	0.58	0.50	2.00	0.000	1.000
		With nanoparticles	1.25	0.58	0.50	2.00		
	<i>Nicotiana rustica</i> Repellent Activity (%)	Without nanoparticles	52.08	22.61	15.00	85.00	2.517	0.127
With nanoparticles		64.58	15.29	40.00	90.00			
With nanoparticles		69.58	15.29	45.00	95.00			

Table: 3 Shows the comparative analysis between the methanol extract, hexane extract and aqueous extract in water groups with nanoparticles and without nanoparticles. Since the p values for concentration and *Nicotiana rustica* Repellent Activity (%), ($p > 0.05$) are significant above 0.05, there is no significant difference the mean concentration and *Nicotiana rustica* Repellent Activity (%) with nanoparticles and without nanoparticles for the methanol extract, hexane extract and aqueous extract in water groups.

Table: 3 Table 3 shows the repellent efficacy of *Nicotiana rustica* with three extracts: methanol, hexane, and aqueous, with and without nanoparticles. This research investigated the effect of adding nanoparticles on repellent efficacy at a constant extract concentration. For different extract types, the solution concentrations were homogeneous (mean = 1.25 mL, SD = 0.58), $F = 0.000$ and $p = 1.000$ for the comparison of concentration differences between groups, indicating no concentration differences between groups. This homogeneity implies that differences in repellent efficacy are due to the availability of nanoparticles rather than differences in the amounts of extracts. In the *Nicotiana rustica* category, there was a minimal improvement in repellent efficacy with the application of methanol extract with nanoparticles (mean = 76.25%, SD = 16.39) compared to without (mean = 71.25%, SD = 16.39). Statistical comparison also revealed no significant difference between the groups, as shown by the F-values (0.558) and p-values (0.463). This suggests that nanoparticles only slightly enhance the repellent efficacy, which, however, is not statistically significant for methanol extracts. With the hexane extract, *Nicotiana rustica* showed a slight increase in repellent efficacy from 72.92% (without nanoparticles) to 75.00% (with nanoparticles).

However, the F-values (0.108) and the p-values (0.746) show that the differences are not statistically significant. Nanoparticles can therefore at best slightly improve the repellent effect, but their effect on hexane extracts is very weak. When nanoparticles were incorporated into the water-based extract, their repellent effect was much more enhanced than with the hexane and methanol extracts. The repellent effect of *Nicotiana rustica* increased from 52.08% without to 64.58% with nanoparticles. Although p-values (0.127) show that these differences are not statistically significant, the F-values (2.517) demonstrate a significant trend towards greater effectiveness with the use of nanoparticles. Based on this study, it can be concluded that nanoparticles are more effective in increasing the defense



effect in aqueous-based extracts than those extracted from organic solvents. In general, nanoparticles led to a greater enhancement of the defense effect in all extracts, but none of these effects was statistically significant. The aqueous extract showed the greatest enhancing effect, as the nanoparticles increased the repellent efficacy of each plant repellent by more than 10%. This suggests that nanoparticles improve the dispersion or stability of the active ingredient in a water solution more than hexane or methanol. These results demonstrate that while nanoparticles can enhance the repellent efficacy of plant extracts, they are solvent-dependent.

Developmental defects

Nicotine possesses the nervous systems of insect by mimicking the neurotransmitter acetylcholine. This mimicry leads to overstimulation of nicotinic acetylcholine receptors, resulting in hyperactivity, paralysis, and eventual death of the insects. Nicotine is particularly dangerous to pests such as termites, as it can kill them rapidly upon ingestion or contact. However, its effectiveness may be compromised if termites avoid the treated area due to the repellent nicotine odour.

Tables: 4

Plant Source	Key Terrestrial Metabolites	Mechanism of Action	Effect on Termites
Nicotiana (<i>Nicotiana rustica</i>)	Nicotine	- Acts on nicotinic acetylcholine receptors (nAChRs) in the nervous system - Causes overstimulation, paralysis, and death.	- Neurotoxic: Rapid killing effect by disruption neuronal signalling- May have repellent properties.
	Anabasine	- Similar to nicotine, it acts as a neurotoxin targeting nAChRs.- Less potent than nicotine.	- Causes disorientation, reduced feeding, and ultimately death.
	Nor nicotine	- Oxidized derivative of nicotine, targets nerve pathways- Slower onset than nicotine.	- Contributes to neurotoxicity and inhibits termite feeding behaviour.
	Other Alkaloids	- May enhance overall pesticide activity- Synergistic effect with nicotine.	- Broad-spectrum termite activity.

Key Notes:

Nicotine metabolites: The alkaloids, especially nicotine, are highly neurotoxic to termites. While their repellent properties can reduce termite feeding, they can limit their residual effectiveness.

Summary and conclusion

Summary:

The time comparison between the methanol, hexane, and control groups without nanoparticles (1mL) reflected significant differences in insect mortality and survival. When comparing the methanol group with **Nicotiana rustica**, the p-values for Concentration,



mortality rate, number of dead insects, and number of live insects were all above 0.05, indicating that these variables showed no significant difference at different times. This demonstrates that methanol has very little effect on insect mortality in the long term. The *Nicotiana rustica*-treated hexane group showed noticeable differences in mortality rate ($F=8.150$, $p<0.05$), number of dead insects ($F=7.605$, $p<0.05$), and number of live insects ($F=7.605$, $p<0.05$). These findings would indicate that hexane has a strong influence on insect mortality, with an increasing trend over time. This means that while hexane achieved maximum mortality in *Nicotiana rustica*, under *Nicotiana rustica* control, the number of dead insects, concentration, mortality rate, and number of live insects did not change dramatically and showed values in the methanol group. Apparently, there was a reporting error that attributed enormous changes in mortality and insect population to the control group rather than the hexane group. In the *Nicotiana rustica*-treated control group, no differences were observed in concentration, mortality rate, dead insects, and surviving insects; the values were identical to those in the methanol group.

However, there appears to be a reporting error, as large differences in mortality and insect numbers were reported to the control group and not for the hexane group. The results indicate that both methane and hexane are insecticidal, but hexane significantly increases mortality. The control group consistently showed the lowest mortality, so the observed effects are consistent with the applied treatments. Hexane also resulted in a time-dependent increase in mortality, particularly in *Nicotiana rustica*, and is considered a more effective insecticide than methanol.

In this study, the insecticidal efficacy of medicinal plants (*Nicotiana rustica*) was extensively investigated using different extraction techniques and their performance was compared with and without nanoparticles. The study focused on investigating several parameters such as mortality rates, insect survival, adult emergence, and developmental stages after treatment periods of 24, 48, and 72 hours. This systematic methodology demonstrates the influence of plant extracts and application methods on pest control efficacy. In initial experiments without nanoparticle enrichment, different efficacy patterns were observed between extraction technologies. The methanol extract of *Nicotiana rustica* exhibited low insecticidal activity, with mortality rates ranging from 62.5% at 24 hours to 71.25% at 72 hours. However, the statistical data showed that these trends did not vary over time ($p>0.05$). This indicates that the methanol extracts reached their maximum efficacy very quickly, and no other effects occurred with long exposure times. *Nicotiana rustica* could be extracted more effectively with hexane, resulting in increased mortality from 55% in 24 hours to 87.5% in 72 hours ($F=8.150$, $p < 0.05$). These strongly time-dependent results suggest that hexane could detect chemicals with increased insecticidal activity after longer exposure time. The number of insects killed increased from 5.5 to 8.5 within 72 hours, indicating the higher efficacy of this extraction method.

The integration of nanoparticle technology with plant extracts significantly improved the experimental design and resulted in a significant increase in insecticidal activity. When nanoparticles were combined with methanol extracts of *Nicotiana rustica*, mortality rates increased to 85% to 87% within various time intervals, indicating a remarkable improvement compared to treatments without nanoparticle. That lack of significant fluctuation over time in the nanoparticle-stimulated treatments indicates that they reached their maximum efficacy early and remained highly insecticidal throughout the test period. Hexane extracts and nanoparticle mixtures also demonstrated high insecticidal efficacy, but less so than the methanol extracts. For example, *Nicotiana rustica* treated with hexane and nanoparticle exhibited mortality rates of 63.25% to 75.25%. The control groups treated with nanoparticles



showed higher mortality than the untreated control groups, but significantly lower mortality than the treatment groups. This suggests that the spectacular effects are primarily due to the plant extracts and not the nanoparticles. Methanol extracts performed better than hexane extracts. The average mortality for methanol with nanoparticles was a whopping 82.88%, followed by hexane with nanoparticles at 76.38%, and while the control group treated with nanoparticles showed a mortality of only 20%. This difference in activity between hexane and methanol extracts when mixed with nanoparticles demonstrates that the nanoparticle carrier system is an important factor in enhancing the insecticidal efficacy of these plant extracts.

The treatments with nanoparticle were compared with treatments without nanoparticles. The repellent effect was significantly higher in the nanoparticle treatments, but the differences were not significant ($p > 0.05$). For example, methanol extracts of *Nicotiana rustica* showed a repellent activity of 76.25% when using nanoparticles, compared to 71.25% when using non-nanoparticles.

Subsequently, the use of hexane and aqueous extracts showed the most significant numerical improvement (64.58% versus 52.08% for *Nicotiana rustica*). Although these increases were not statistically significant, the consistent benefit across all extraction methods suggests that nanoparticles do enhance repellent activity. However, further studies on larger samples are needed to ensure the statistical significance of these results. This article investigates the insecticidal efficacy of medicine plants (*Nicotiana rustica*) using different extraction methods and measures their effectiveness with and without nanoparticles. Various parameters, such as mortality rates, insect survival rates, adult's emergence, and developmental trajectories, were investigated in the laboratory for treatment periods of 24, 48, and 72 hours. This methodological approach provides a clearer picture of how different plant extracts and treatment procedures influence pest control efficacy. In the initial experiments without nanoparticle enhancement, clear patterns emerged regarding the effectiveness of different extraction methods.

Conclusion

The experiment followed *Nicotiana rustica* to determine the effect of treatment with methanol, hexane, and a group on the temporal course of insect mortality. The results confirmed that there were significant differences in the mortality rate between the treatment groups, with hexane being more effective as an insecticide than methanol. Statistical results showed that there were no significant differences in the mortality rate, number of dead insects, and number of live insects between the methanol groups at different time points. This indicates that the effectiveness of methanol as an insecticide was largely stable and did not improve over time, suggesting that it could not increase the mortality rate. Conversely, treatment with hexane resulted in a significant difference in mortality, particularly in *Nicotiana rustica*, as the number of dead and completely dead insect accumulated over time. This demonstrates that hexane is a good and time-dependent activator of insect mortality and is therefore a better option than methanol. The Control group also did not detect any obvious differences in mortality rate, which would understandably have been the case, thus confirming the fact that the observed differences were due to the insecticidal activity of methanol and hexane and not to the effects of other substances. In general, the methanol extract of *Nicotiana rustica* exhibited moderate insecticidal activity, ranging from 62.5% at 24 hours to 71.25% at 72 hours. However, statistical analysis showed that these were not significant differences ($p > 0.05$), meaning that methanol reached its maximum efficacy very quickly, with no additional improvement upon prolonged exposure. The hexane extract of



Nicotiana rustica was superior to other approaches in killing insects, with the mortality rates increasing from 55% at 24 hours to 87.5% at 72 hours ($F = 8.150$, $p < 0.05$). This apparent increase over time highlights the effectiveness of hexane in removing insecticidal compounds, which appear to become more potent over time. In the Control group, which received no treatment, mortality rates remained low, ranging from 15% to 27.5%. This consistency demonstrated that the effects observed in the experimental groups were caused by the plant extracts and not by other variables. The low mortality rates in the Control group also served as a benchmark for assessing the effectiveness of various therapies. The significant differences in efficacy between the hexane extract, particularly from *Nicotiana rustica*, and the control groups demonstrate that the use of plant-based pesticides in real-world pest control is feasible.

Another important finding of this study was that nanoparticles dramatically increased the insecticidal activity of the extracts. The addition of nanoparticles to methanol extracts significantly increased the insect mortality rates to 85% to 87%. These results suggest that nanoparticles play an important role in enhancing the insecticidal activity of plant extracts, most likely by increasing the availability and efficacy of the active ingredients. The repellent activity of the treatments was also investigated and yielded promising results, but without statistically significant changes ($p > 0.05$). Methanol extracts of *Nicotiana rustica* containing nanoparticles were more repellent (76.25%) than the control samples (71.25%).

This trend was also observed with hexane and aqueous extracts, where nanoparticles appeared to enhance the repellent effect, although the differences were not significant. This suggests that nanoparticles could enhance the repellent effect of plant extracts and potentially make them more effective at repelling insects. However, further studies with larger numbers of samples are needed to confirm these findings. The results indicate that plant extracts, especially when combined with nanoparticles, can be extremely effective in pest control. However, further research is needed to maximize the application of these agents and mechanisms. This finding opens the possibility for more efficient and environmentally friendly pest control methods using plant extracts in combination with nanoparticles. Future studies must also shed light on the long-term effects of nanoparticle-enhanced treatments on pest populations and ecosystems, as well as the potential risk of pest resistance.

Limitation

Research on the insecticidal activity of *Nicotiana rustica* has produced some intriguing results but has numerous limitations. A key criticism is the sample size, which may not effectively reflect changes in insect mortality and survival rates across different treatment scenarios, especially when nanoparticles are involved. Furthermore, researchers primarily focused on mortality and emergence of adult insects, ignoring other critical aspects such as the long-term consequences for insect populations and the entire ecosystem. Environmental factors such as temperature and humidity, which could influence insect behavior and treatment efficacy, were also not considered. Another limitation is the different extraction techniques, particularly with hexane and methanol, which could be influenced by differences in extraction technique, plant material quality, or storage conditions. To further explore these findings, further studies with larger sample sizes, different environmental conditions, and a wider range of parameters are needed.

Future Study



Future research should aim to expand the scope of this study by using larger sample sizes to ensure that the results are statistically significant and represent a wider diversity of insect populations. It is important to investigate the long-term effects of plant extracts and nanoparticles on these insect groups, particularly how resistance develops and what consequences these treatments may have for non-target species in their habitats. Furthermore, investigating how nanoparticles enhance the insecticidal activity of plant extracts may lead to a better understanding of their efficacy and potential applications.

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