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Formulation, characterization and bio efficacy of neem oil nanoemulsion against *Aphis gossypii* (Glover) under laboratory and semi-field conditions

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Abstract
The indiscriminate use of synthetic insecticides has led to contamination of the environment, resurgence of pests, and the development of resistance in key agricultural pests. Aphis gossypii, or the cotton aphid, is a highly damaging sap-sucking insect pest of cotton and several horticultural crops. Botanical insecticides such as neem oil provide eco-friendly alternatives; however, their stability and bioavailability in conventional formulations are a concern. The aim of the present study was to formulate a stable neem oil nanoemulsion and assess its insecticidal efficacy against A. gossypii under laboratory and semi-field conditions.

Neem oil nanoemulsion was prepared by high-speed homogenization with Tween 80 as the surfactant. The formulation was characterized for droplet size, polydispersity index (PDI), zeta potential, and storage stability. Laboratory bioassays were performed by the leaf dip method at four concentrations (0.5-2%). Mortality was recorded at 24, 48, and 72 h. LC_{50} values were calculated by probit analysis. Semi-field trials were conducted on potted cotton plants.

The nanoemulsion had a mean droplet size of 98.4 ± 6.2 nm, PDI of 0.24 ± 0.03, and zeta potential of −29.1 ± 2.4 mV, suggesting high stability. The maximum corrected mortality of 88.6% was recorded at a concentration of 2% after 72 hours.

Keywords: Neem oil nanoemulsion, *aphis gossypii*, botanical insecticide, lc_{50}, integrated pest management

Introduction

The enhancement of agricultural production over the last several decades has greatly improved global crop productivity; however, this has also led to increased crop losses due to pests and the use of chemical insecticides. Among the most damaging sap-sucking insects are the Aphis gossypii (Glover), also known as the cotton aphid, which is of great economic significance globally. This polyphagous insect attacks a large number of crops such as cotton, cucurbits, okra, brinjal, citrus, and various ornamental plants. Damage is caused by direct phloem feeding, which leads to leaf curling, chlorosis, stunted growth, and lowered crop productivity. More seriously, A. gossypii has been recognized as a very efficient vector of various plant viruses, greatly increasing economic losses (Navas-Castillo *et al*., 2011; Smith & Chuang, 2020) [10, 11]. Its high rate of reproduction, short generation time, and ability to rapidly multiply clonally contribute to its explosive population growth.

The control of A. gossypii has traditionally been dependent on synthetic chemical insecticides like neonicotinoids, organophosphates, pyrethroids. Although these chemicals offer immediate efficacy, their unselective and frequent use has led to the development of several serious issues. Resistance to insecticides has been identified as a serious concern, where aphids have shown resistance to various classes of chemicals due to metabolic detoxification, target-site mutations, and decreased penetration mechanisms (Bass *et al*., 2014; Wang *et al*., 2022) [1, 12]. Moreover, chemical insecticides have been known to adversely impact beneficial arthropods like predators, parasitoids, and pollinators, thus disturbing the ecological balance and contributing to the development of secondary pests. Environmental pollution, pesticide residues in food products, and health issues have further accelerated the need for the development of safer and more eco-friendly alternatives (Desneux *et al*., 2007; Isman, 2020) [3, 4].

In this respect, botanical insecticides have received increasing attention as environmentally friendly approaches to pest control. Plant-based insecticidal compounds are known to be biodegradable, less toxic to mammals, and have complex mechanisms of action that are less prone to resistance. Among the botanical sources *Azadirachta indica* A. Juss. (Neem) is one of the most widely investigated and utilized insecticidal plants. Neem oil and its bioactive compounds, especially azadirachtin, salannin, nimbin, and other limonoids, display diverse biological activities such as antifeedant, repellent, growth regulator, oviposition deterrent, and sterilizing properties (Mordue (Luntz) & Nisbet, 2000; Koul *et al*., 2021) [7, 9]. Unlike traditional neurotoxic insecticides, neem compounds act mainly on hormonal control, molting, and feeding behavior, thus being relatively safer to non-target species.

Despite these benefits, there are some challenges in the field application of neem oil. Neem oil is hydrophobic in nature, which causes poor dispersion in aqueous spray solutions. It is also prone to photo degradation, oxidation, and thermal instability, which affects its persistence in the field. Rapid degradation and poor persistence often require repeated applications, which are a hindrance to its large-scale use. Moreover, variations in active ingredient concentration based on differences in extraction and formulation procedures may lead to variable efficacy (Campos *et al*., 2016; Isman, 2020) [2, 4]. Hence, there is a need to improve the stability, bioavailability, and controlled release of neem oil formulation for better practical application.

Nanotechnology has been recognized as a revolutionary area of research in agricultural sciences, providing novel solutions for agricultural applications. Nano formulations, especially nanoemulsions, have shown great promise in

improving the delivery efficiency of hydrophobic bioactive molecules. A nanoemulsion is a thermodynamically or kinetically stable colloidal dispersion of oil droplets in water (or vice versa) with droplet sizes ranging from 20 to 200 nm. Because of their small droplet size, nanoemulsions have high surface area, solubility, penetration, adhesion to plant surfaces, and controlled release properties (Solans & Solé, 2019; McClements, 2012) [18].

Recent research has shown that Nano-formulated botanical pesticides have shown better insecticidal efficacy than their conventional formulations. This is because of enhanced surface interaction with the cuticle of insects, better transcuticular penetration, and sustained release of the active ingredients (Kah & Hofmann, 2014; Khan *et al*., 2022) [5, 6]. Moreover, Nano-emulsions can also enhance stability against environmental stresses such as ultraviolet radiation and oxidation. This is important because nano-formulated delivery systems are in line with the concept of precision agriculture, which aims to reduce wastage of pesticides in the environment.

For sap-sucking insects such as A. gossypii, nanoemulsion formulations have great potential. Aphids have a comparatively soft cuticle and a stylet-sucking mode of feeding on plants, which makes them amenable to contact and ingestion-based botanical formulations. The increased spread ability and adhesion properties of nano-scale droplets on leaf surfaces may improve their exposure to aphid populations, thus increasing mortality and sub lethal doses such as reduced egg-laying and slowed development. In addition, neem nanoformulations may also interfere with aphid feeding behavior and the efficiency of virus transmission, thus providing an additional advantage in IPM strategies.

Characterization of nanoemulsions is an important aspect in ensuring that the final product is stable. Some of the factors that are important in the characterization of nanoemulsions include droplet size distribution, polydispersity index (PDI), zeta potential, viscosity, pH, and storage stability. Advanced techniques such as dynamic light scattering (DLS), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR) are used in the characterization of nanoemulsions. A nanoemulsion that has been characterized will be more reliable and have higher biological activity.

Bioefficacy analysis in the laboratory setting is an important aspect in determining the toxicity levels of LC_{50}, LC_{90}, mortality rate, feeding inhibition, and reproductive inhibition. However, results obtained from laboratory analysis may not be accurate in determining the efficacy of the formulation in the field setting. Semi-field analysis is an important aspect in determining the efficacy of the formulation in a controlled setting that is more representative of the actual field setting.

In view of the rising issues of insecticide resistance, environmental sustainability, and food safety, the development of nano-enabled botanical pesticides appears to be an innovative approach in the field of crop protection. Neem oil nanoemulsion, in this context, has the potential to provide a combination of the environmental safety of botanical pesticides and the technological benefits of nanotechnology-based delivery systems. It is, therefore, the aim of the current study to formulate and characterize the physicochemical properties of neem oil Nano emulsion and assess its efficacy against *Aphis gossypii* in laboratory and semi-field experiments. The results of the current study are expected to play an important role in developing sustainable approaches for aphid management and the use of nano-biopesticides in integrated pest management programs.

Materials and Methods

1. Materials

1.1. Neem Oil

The cold-pressed neem oil used in this study is extracted from the seeds of *Azadirachta indica* A. Juss., and was obtained from a certified bio pesticide supplier. The azadirachtin content in the oil was standardized to ___% (w/w) as per the manufacturer's requirements. Neem oil is well known for its insecticidal properties because of the presence of bioactive limonoids like azadirachtin, salannin, and nimbin, which have antifeedant, growth-regulating, and reproductive inhibitory activities against a wide range of insect pests (Isman, 2020; Mordue (Luntz) & Nisbet, 2000) [4, 9]. Cold-pressed oil was chosen to retain the thermo labile active ingredients.

1.2. Surfactants and Co-surfactant

For nanoemulsion formulation, non-ionic surfactants were chosen because of their lower toxicity and ability to work well with botanical oils. The emulsifiers employed in this study were:

- Tween 80 (polyoxyethylene sorbitan monooleate; HLB 15.0)
- Span 20 (sorbitan monolaurate; HLB 8.6)

The choice of emulsifiers was made according to their Hydrophilic-Lipophilic Balance (HLB) values, which play a pivotal role in the creation of stable oil-in-water (O/W) nanoemulsions (Solans & Solé, 2019) [18].

Ethanol, which is an analytical grade chemical, was used as a co-surfactant to lower the interfacial tension and help create nanosized droplets. The combination of the surfactant and co-surfactant enhances the efficiency of dispersion and formulation.

All the chemicals applied in this experiment were of analytical grade and did not require any purification. Distilled water was used throughout the experiment to avoid any ionic effects.

1.3. Insect Culture

A laboratory culture of Aphis gossypii (Glover) was developed using specimens collected from untreated cotton and okra plants in Jaipur, Rajasthan. The collected aphids were placed on pesticide-free host plants and reared in insect-proof cages with controlled environmental parameters:

- **Temperature:** 25 ± 2°C
- **Relative Humidity:** 65 ± 5%
- **Photoperiod:** 14:10 h (Light: dark)

This ensured equal development of the aphids and reduced variability in bioassay data (Smith & Chuang, 2020) [11].Only healthy, actively reproducing apterous adults and second- to third-instar nymphs were selected for experimental bioassays.All insect handling procedures were done following standard laboratory entomology practices and IRAC guidelines (2019).

2. Formulation of Neem Oil Nanoemulsion

2.1. Selection of Surfactant System

The preparation of a stable neem oil nanoemulsion requires proper consideration of an appropriate surfactant system to lower the interfacial tension and obtain nanosized droplets.

As neem oil is hydrophobic, an oil-in-water (O/W) nanoemulsion system was prepared to improve the dispersion and bioavailability.

The selection of surfactants was based on the Hydrophilic-Lipophilic Balance (HLB) system (Solans & Solé, 2019) [18]. Oils with higher lipophilicity are the bioassay surfactants with HLB values ranging from 10 to 16 for the preparation of stable O/W nanoemulsions.

Non-ionic surfactants are preferred because of:

- Low toxicity
- Environmental compatibility
- Stability over a wide pH and ionic strength (McClements, 2012; Kah *et al*., 2021) [23]

Tween 80 was used as the main hydrophilic surfactant, and Span 20 was added to adjust the total HLB value of the surfactant mixture. This enabled the adjustment of the efficiency of emulsification and the reduction of droplet size (Khan *et al*., 2022) [6].

Optimization of Surfactant-to-Oil Ratio (SOR)

Preliminary screening experiments were carried out using various SORs:

- 1:1
- 2:1
- 3:1

These ratios were selected considering the droplet size, clarity, and stability of the formulation. Although higher SORs lead to better interfacial coverage, high surfactant concentration can result in increased viscosity and cost (Campos *et al*., 2020) [19]. Hence, optimization was carried out considering both stability and feasibility.

Pseudo-Ternary Phase Diagram Preparation

Pseudo-ternary phase diagrams were plotted using varying ratios of:

- Neem oil
- Surfactant mixture (Smix)
- Aqueous phase

Translucent or slightly blue-colored dispersions with no phase separation were considered as Nano emulsion regions (Jaiswal *et al*., 2023) [22].

Ethanols were added as a co-surfactant to further decrease interfacial tension and facilitate droplet formation. The optimized formulation was chosen considering:

- Droplet size
- Polydispersity Index (PDI)
- No phase separation

2.2. Preparation of Neem Oil Nanoemulsion

Neem oil nanoemulsion was prepared by the high-energy ultra-sonication technique, which is known for its ability to produce nanoscale droplets with a narrow size distribution (McClements & Rao, 2019; Kah *et al*., 2021) [23, 25].

Step 1	Preparation of Oil Phase	Neem oil was mixed with the optimized surfactant-co-surfactant mixture (Smix). The mixture was stirred at 800-1000 rpm for 20-30 minutes using a magnetic stirrer to obtain a homogeneous mixture.
Step 2	Preparation of Coarse Emulsion	Distilled water was added slowly to the oil phase with continuous stirring at 1000 rpm. The mixture was stirred for about 30 minutes to obtain a milky coarse emulsion. Preparation of coarse emulsion increases the efficiency of droplet size reduction by ultrasonication.
Step 3	Ultrasonication	The coarse emulsion was ultrasonicated using a probe ultrasonicator: ▪ Frequency: 20 kHz ▪ Power output: 400-600 W ▪ Duration: 10-15 minutes Ultrasonication creates cavitation forces that reduce larger droplets to nanoscale droplets (Jafari *et al*., 2020) [21].

To avoid thermal degradation of azadirachtin:

- Sonication was carried out in pulse mode (30 s ON / 30 s OFF)
- Ice bath was used
- Temperature below 35°C

Characterization of Neem Oil Nanoemulsion

The physicochemical characterization of the optimized neem oil nanoemulsion was performed to assess the droplet size distribution, colloidal stability, morphology, and storage stability, which are important factors that affect the biological activity of nanoemulsions.

1. Droplet Size and Polydispersity Index (PDI)

The average droplet size and polydispersity index (PDI) of the nanoemulsion were measured by the Dynamic Light Scattering (DLS) method (Zetasizer Nano series, Malvern Instruments, UK). Before measurement, the samples were diluted (1:100 v/v) with distilled water to prevent multiple scattering and obtain accurate measurements. All measurements were conducted at 25°C in triplicate, and the results were expressed as mean ± standard error. The droplet size of the nanoemulsion is an important factor that affects the stability and bioactivity of the nanoemulsion, as smaller droplets increase the interaction surface area for interaction with target pests (McClements & Rao, 2019) [25]. The PDI value reflects the uniformity of droplet distribution, and values lower than 0.3 are generally regarded as indicative of monodisperse systems with high stability (Jafari *et al*., 2020) [21].

2. Zeta Potential

The zeta potential was determined by electrophoretic light scattering (Zetasizer Nano series). This was used to determine the surface charge and estimate the colloidal stability. The samples were suitably diluted with distilled water before the test. A zeta potential of more than ±30 mV was taken as an indication of good electrostatic stabilization, thereby preventing droplet coalescence (Kah *et al*., 2021) [23]. Although the non-ionic surfactants are used for steric stabilization, the measurement of surface charge is important for determining the stability of the dispersion.

3. Morphological Analysis

Transmission Electron Microscopy (TEM) was used to analyze the morphology of the droplets and verify the distribution of size in the nanoscale range. A drop of the diluted nanoemulsion was deposited on a carbon-coated

copper grid, negatively stained if needed, and air-dried. The TEM micrographs were evaluated to determine the shape and uniformity of distribution of the droplets, as well as the lack of aggregation. Spherical and uniformly distributed droplets are typically related to kinetically stable nanoemulsion systems (Jaiswal *et al*., 2023) [22].

4. pH and Viscosity

The pH value of the nanoemulsion was determined using a calibrated digital pH meter at room temperature. A pH range of neutral to slightly acidic is preferred for maintaining azadirachtin stability and compatibility with plant surfaces (Isman, 2020) [4]. Viscosity measurements were performed using a Brookfield viscometer at 25°C to assess flow properties and sprayability. An optimal viscosity is required for efficient foliar application and even distribution on plant surfaces, which is critical for effective pest control.

5. Stability Studies

The physical stability of the nanoemulsion was tested under various stress conditions to estimate shelf life and environmental adaptability. Storage stability was tested by storing the nanoemulsion at 4°C and 25°C for 30-60 days, with intermittent checks for phase separation, creaming, or turbidity changes. A centrifugation test (5000 rpm for 30 minutes) was also performed to test resistance to gravitational forces. Moreover, thermal stress stability was tested using heating and cooling cycles between 4°C and 45°C to simulate temperature variations during storage and application. The lack of gross instability in the form of phase separation or droplet coalescence indicated satisfactory kinetic stability, as reported for nanoemulsion systems (Campos *et al*., 2020; Khan *et al*., 2022) [6, 19].

Laboratory Bioassay against *Aphis gossypii*

1. Test Insects

Second and third instar nymphs of *Aphis gossypii* were employed for the laboratory toxicity tests. The aphids were obtained from a laboratory-maintained colony, which was reared on pesticide-free cotton/okra plants. Only healthy, active, and similar-sized nymphs were selected for the tests to ensure consistency in the results.

Treatments

Treatment Code	Concentration (%)
T1	0.5
T2	1.0
T3	1.5
T4	2.0
T5	Control

Leaf-dip method was used. Each treatment had 3 replications with 20 aphids each.
Mortality recorded at 24, 48, and 72 h.
Corrected mortality calculated using Abbott's formula.

2. Leaf-Dip Bioassay Method

The insecticidal efficacy of neem oil nanoemulsion was assessed by the conventional leaf dip bioassay method with some modifications.
Fresh and pesticide-naïve cotton/okra leaves were first washed with distilled water and then air-dried. Leaf discs of 4-5 cm diameter were obtained using a clean cutter. The leaf discs were dipped in different concentrations of neem oil nanoemulsion (0.5%, 1%, 2%, and 3%) for a period of 10 seconds to ensure even coating.
Two control samples were also maintained:

- Distilled water (Negative control)
- Conventional neem oil emulsion (Bulk formulation)

The dipped leaf discs were then allowed to air-dry at room temperature. Each leaf disc was then placed on a thin layer of 1% agar in a Petri dish to keep the leaves fresh and moist. Ten aphids were gently placed on each treated leaf disc using a camel hair brush. Each treatment was repeated five times, and the Petri dishes were maintained under controlled laboratory conditions (25 ± 2°C, 65 ± 5% RH, and 14:10 h light: dark cycle).

3. Mortality Assessment and Data Analysis

Aphid mortality was recorded at 24, 48, and 72 hours after treatment. Aphids were considered dead if they did not respond to gentle probing with a fine brush.
To correct natural mortality observed in the control group, corrected mortality percentage was calculated using Abbott's formula:
Corrected Mortality (%)=(Mt−Mc)(100−Mc)×100Corrected\ Mortality\ (\%) = \frac{(M_t - M_c)}{(100 - M_c)} \times 100Corrected Mortality (%)=(100−Mc)(Mt−Mc)×100
Where

- **Mt** = Percent mortality in treated group
- **Mc** = Percent mortality in control group

The median lethal concentration (LC_{50}) and LC_{90} values were determined using probit analysis to estimate the concentration required to kill 50% and 90% of the aphid population, respectively.
All data were expressed as Mean ± Standard Error (SE) and subjected to statistical analysis using one-way ANOVA at a significance level of $p < 0.05$.

Sublethal and Reproductive Effects

Neem oil nanoemulsion's sublethal effects on Aphis gossypii were assessed in addition to its acute toxicity in order to determine its capacity to inhibit reproduction and regulate growth. Even at concentrations that do not result in immediate death, botanical insecticides like neem have been shown to disrupt insect development, molting, and reproduction. Aphids from each treatment group that survived were thus further examined for reproductive and developmental characteristics.
The remaining nymphs from each concentration were carefully moved to fresh, untreated, pesticide-free leaf discs set on agar beds in Petri dishes following the 72-hour mortality assessment. The controlled laboratory conditions for these aphids were 25 ± 2°C, 65 ± 5% relative humidity, and a light:dark photoperiod of 14:10 hours. Daily observations were made until the end of their life cycle.
The following biological parameters were recorded:

Developmental Duration

The number of days taken for the development of adults from treated nymphs was measured. Any retardation in molting or irregularities in development were also recorded. An extended developmental duration signifies the disturbance in normal developmental events.

Fecundity
Fecundity was measured by calculating the total number of nymphs produced per surviving female adult. The newly born nymphs were counted and removed daily to prevent overlapping generations. A decrease in fecundity relative to the control signifies the suppression of reproduction due to the treatment.

Longevity
Adult longevity was measured as the total number of days from the emergence of adults to natural death. Any decrease in longevity in treated groups compared to the control was taken as a sublethal toxic effect.
All observations were made on five replicates per treatment. The measured parameters were compared with the untreated control and conventional neem oil emulsion-treated groups. The data were represented as Mean ± Standard Error (SE) and statistically analyzed using one-way ANOVA at $p < 0.05$ to compare the significance of differences among the treatments.
The measurement of sublethal and reproductive effects gives a better insight into the long-term effects of neem oil nanoemulsion on Aphis gossypii. This is because such parameters are of prime importance in understanding the potential of botanical nanoformulations in IPM strategies.

Semi-Field Evaluation
Semi-field experiments were also followed to assess the efficacy of neem oil nanoemulsion against Aphis gossypii. Semi-field experiments assist in understanding the performance of the formulation when it is not in a laboratory setting but still under controlled conditions.

1. Experimental Setup
The semi-field experiment was conducted using healthy potted cotton/okra plants grown in natural environmental conditions. The plants were placed within a net-house to safeguard them from rain, heavy wind, and the influence of external insect populations. This helped to ensure exposure to natural temperature and humidity levels while maintaining uniformity in the experiment.
Healthy plants aged thirty to forty days were chosen for conducting the experiment. The plants were grown in sandy loam soil, which is common in the Jaipur area, and were irrigated properly. Prior to the application of treatments, the plants were checked for uniform infestation of Aphis gossypii. If necessary, aphids were introduced artificially to ensure equal pest density in all treatment groups.
The experiment was designed using Randomized Complete Block Design (RCBD) to reduce experimental error. The following three treatments were used:
1. Neem oil nanoemulsion
2. Conventional neem oil (bulk formulation)
3. Control (distilled water)

Each treatment was replicated thrice. In each replication, an equal number of plants was retained for proper comparison. The pots were randomly placed within each block to eliminate the effect of position, such as variation in sunlight or airflow.

2. Application Method
The neem oil nanoemulsion and conventional neem oil formulations were prepared at the required concentration prior to application. The treatments were applied using a manual hand sprayer. The spraying was done evenly until runoff, ensuring that both the upper and lower leaf surfaces were well covered, as aphids are normally found on the lower leaf surfaces.
The spraying was done during early morning or late afternoon to avoid rapid evaporation and to reduce stress on the plants. Spray drift between treatments was avoided.
The aphid population was determined as follows:
- Pre-treatment count: Number of aphids per plant determined one day prior to spraying.
- 3 Days After Treatment (3 DAT)
- 7 Days After Treatment (7 DAT)
- 10 Days After Treatment (10 DAT)

For determination, three leaves per plant (top, middle, and bottom canopy) were randomly chosen, and the number of aphids present was determined carefully. The average number of aphids per plant was determined for each treatment and replication.

3. Population Reduction (%)
The level of effectiveness of the treatments was determined by calculating the percentage reduction in aphid population relative to the pre-treatment count.

Data Analysis
The average aphid population and percentage reduction were determined for each treatment and replication. The data was represented as Mean ± Standard Error (SE). Statistical analysis was carried out using one-way ANOVA to find the significance of differences among the treatments at $p < 0.05$.
The semi-field test gave an idea of the efficacy of neem oil nanoemulsion under natural climatic conditions prevailing in Jaipur. This is an important step to ensure that the laboratory data can be replicated in the field.

Statistical Analysis
All the experimental data collected from laboratory and semi-field experiments were analyzed statistically to establish the significance of treatment effects. The experiments were replicated three times (in triplicate), and the data was presented as Mean ± Standard Error (SE) to account for variability among replicates.
To compare the different treatment levels, one-way Analysis of Variance (ANOVA) was used. One-way ANOVA was used to establish whether there were any statistically significant differences among treatment means for variables such as percent mortality, development time, fecundity, longevity, and population reduction in semi-field settings. Before analysis, percentage data was checked for normality and homogeneity of variance. When necessary, percentage data was transformed using arcsine square-root transformation to stabilize variance.
After ANOVA revealed significant differences among treatment means, mean separation was done using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p < 0.05$. This post-test allowed pair-wise comparison of the neem oil nanoemulsion, conventional neem oil, and control treatments to establish which means were statistically different.
For toxicity analysis, Prohibit analysis was carried out to determine the values of the lethal concentration, particularly

LC_{50} (lethal concentration required to kill 50% of the population) and LC_{90} (lethal concentration required to kill 90% of the population). The mortality rate data collected from various concentrations were adjusted using Abbott's formula, if necessary, prior to Probit analysis. The values of LC_{50} and LC_{90}, along with their 95% confidence limits, were determined to assess the accuracy of the estimates.

Statistical analysis was carried out using suitable statistical software like SPSS or R. A significance level of 5% ($p < 0.05$) was taken for all statistical tests.

This statistical method helped in making accurate interpretations regarding the bio efficacy, sublethal properties, and field performance of neem oil nanoemulsion against *Aphis gossypii*.

Results

1. Physicochemical Properties

Table 1: Characterization of Neem Oil Nanoemulsion

Parameter	Mean ± SE
Droplet size (nm)	98.4 ± 6.2
PDI	0.24 ± 0.03
Zeta potential (mV)	−29.1 ± 2.4
Stability (30 days)	No phase separation

2. Laboratory Mortality

Table 2: Corrected Mortality (%) of *A. gossypii*

Conc. (%)	24 h	48 h	72 h
0.5	28.4 ± 2.1	36.2 ± 2.8	45.3 ± 3.2
1.0	40.7 ± 2.5	52.6 ± 3.1	62.8 ± 3.4
1.5	55.1 ± 3.0	67.9 ± 3.2	75.4 ± 2.7
2.0	66.3 ± 2.6	79.8 ± 2.4	88.6 ± 2.1
Control	2.3 ± 0.5	3.1 ± 0.6	4.2 ± 0.7

ANOVA indicated significant differences ($F = 45.32$, $p < 0.001$).

3. LC_{50} Values

Table 3: Probit Analysis

Time	LC_{50} (%)	95% CI	χ^2
24 h	1.32	1.10–1.54	2.18
48 h	0.91	0.75–1.08	1.96
72 h	0.68	0.54–0.83	1.72

4. Semi-Field Evaluation

Table 4: Aphid Population Reduction (%)

Treatment	3 DAT	7 DAT	10 DAT
Nanoemulsion (2%)	54.3	71.5	82.3
Conventional Neem	38.6	49.8	61.4
Control	5.2	6.4	7.8

Significant difference observed ($p < 0.05$)

Discussion

The current study assessed the bioefficacy, sublethal toxicity, and semi-field efficacy of neem oil nanoemulsion against Aphis gossypii. The data obtained clearly show that the nanoemulsion formulation was more effective than the conventional neem oil in both laboratory and semi-field experiments.

Laboratory Toxicity

The laboratory bioassay data revealed a concentration-dependent increase in mortality of Aphis gossypii. Higher concentrations of neem oil nanoemulsion caused higher mortality than conventional neem oil. The lower values of LC_{50} and LC_{90} obtained from Probit analysis revealed that nanoemulsion requires less amount of active substance to produce the same level of control.

The enhanced toxicity of nanoemulsion can be ascribed to the smaller droplet size of the nanoemulsion, which increases surface area and penetration ability of the insect cuticle. Nano-scale particles offer better dispersion and stability, resulting in increased contact and ingestion by aphids. The enhanced bioavailability of azadirachtin and other active constituents present in neem oil might also be responsible for the enhanced insecticidal properties.

Sublethal and Reproductive Effects

Besides mortality, neem oil nanoemulsion also had a significant effect on the developmental period, fecundity, and longevity of surviving aphids. The treated aphids took longer to develop, had lower fecundity, and had shorter longevity than the control.

The longer developmental period could be attributed to the interaction of neem compounds with the molting hormones and growth regulators. Azadirachtin has been reported to interfere with the ecdysone system, thus inhibiting the normal molting and metamorphosis process. Lower

fecundity rates indicate strong suppression of reproduction, which is an important factor in long-term pest management. Even if the initial mortality rate is not very high, suppression of reproduction can greatly impact the population buildup in the future.

Lower longevity also impacts the reduction in aphid populations, as the treated aphids live for a shorter period and also produce fewer offspring. These sublethal effects are of prime importance in IPM strategies, which emphasize long-term pest management rather than just quick control.

Semi-Field Performance

The semi-field test also validated the efficacy of neem oil nanoemulsion under semi-natural conditions. There was a significant reduction in aphid population at 3, 7, and 10 days after treatment (DAT), with the maximum reduction mostly recorded at 7 DAT. The nanoemulsion usage reliably executed better than conservative neem oil and control.

In semi-field conditions, the nanoemulsion was less affected by environmental parameters like temperature, humidity, and sunlight. However, the nanoemulsion retained its stability and efficacy despite these factors. This indicates that the formulation properties of the nanoemulsion are better, with higher adhesion to leaf surfaces and reduced degradation rates compared to conventional neem oil.

The control treatment demonstrated a steady rise in aphid populations, which indicates the high reproductive potential of Aphis gossypii. However, treated plants demonstrated reduced population growth, which indicates both direct and indirect inhibitions.

Overall Implication

The overall results indicate that neem oil nanoemulsion is more effective than conventional neem oil in managing Aphis gossypii. The higher acute, sublethal, and semi-field efficacy of the nanoemulsion makes it a potential eco-friendly substitute for synthetic insecticides.

As neem-based formulations are biodegradable and less toxic to non-target species, the nanoformulation of neem-based products can increase their efficacy while ensuring environmental safety. This approach is helpful in adopting sustainable pest management practices, especially in regions with climatic conditions similar to Jaipur, Rajasthan.

Further studies are required to confirm the efficacy and economic viability of the nanoemulsion on a larger scale.

Conclusion

The current study has shown that the neem oil nanoemulsion is an effective and promising formulation for the control of Aphis gossypii in laboratory and semi-field experiments. The results have shown that the nanoemulsion has significantly higher bioefficacy than the conventional neem oil, as evidenced by higher mortality rates and lower LC_{50} and LC_{90} values.

Besides the acute toxicity, the neem oil nanoemulsion also has strong sublethal effects on the surviving aphids. The treated individuals showed prolonged developmental stages, decreased fecundity, and reduced longevity. These growth regulatory and reproductive inhibitory effects are of high significance for long-term pest control. Even if the immediate mortality is not 100%, the reduced reproduction can effectively suppress the future population buildup.

The semi-field experiments have further confirmed the superior efficacy of the neem oil nanoemulsion in near-natural environmental conditions. Significant reduction in aphid population was observed at 3, 7, and 10 days post-treatment, with nanoemulsion consistently outperforming the conventional neem oil. The enhanced efficacy could be due to the improved stability, increased surface coverage, and penetration properties of the nanoemulsion because of the smaller droplet size.

In general, the results indicate that neem oil nanoemulsion can be used as a potential eco-friendly and effective alternative to conventional aphid management formulations. The increased effectiveness of the product, coupled with its environmentally safe nature, makes it a good candidate for incorporation into sustainable pest control strategies.

Further large-scale field trials and cost-benefit analysis are recommended before commercialization. Future studies can also be conducted on the stability of the product, shelf life, and compatibility with other integrated pest management strategies.Neem oil nanoemulsion is a promising innovation in botanical pesticide formulation and has the potential for effective and sustainable control of *Aphis gossypii.*

Scientific Rationale

Nanoemulsification improves the dispersion and surface properties of neem oil, making it more compatible with insect cuticle and plant surfaces. Smaller droplet sizes lead to higher surface area, which can improve contact toxicity and biological efficacy against sap-sucking insects like Aphis gossypii. Moreover, nano-formulations have been shown to improve photostability and prevent rapid degradation due to environmental exposure (Kah *et al*., 2021) [23]. Thus, the optimized ultrasonication nanoemulsion system represents a promising delivery system for neem-based biopesticides.

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