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Efficacy of *Azadirachta indica* seed extract against leaf miner on lemon seedlings under Namibian nursery conditions

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Abstract
This study compared the effectiveness of two concentrations of *Azadirachta indica* (neem) seed extract in controlling citrus leaf miner (*Phyllocnistis citrella*) infestation on lemon seedlings. The experiment was conducted at the University of Namibia, Ogongo Campus Tree Nursery, using a Completely Randomized Design with three treatments replicated four times. The treatments comprised of neem Seed Extract Concentration 1 (1.5 kg/10 L water), neem Seed Extract Concentration 2 (2.5 kg/10 L water), and a control (tap water), applied to twenty lemon seedlings per treatment over five months. A two-way ANOVA indicated that treatment, time, and their interaction significantly influenced infestation percentage, leaf desiccation, and estimated leaf curling extent ($p< 0.001$). Treatments accounted for the greatest variation in infestation percentage (38.13%) and leaf curling extent (24.05%), while time contributed most to variation in leaf desiccation (36.29%). The control group recorded the highest infestation (51.1%), followed by neem seed extract concentration 1 (23.3%), and neem seed extract concentration 2 (23.2%). No significant difference was observed between Concentration 1 and Concentration 2, indicating that both neem extract concentrations significantly reduced infestation compared to the control. Leaf desiccation differed significantly among treatments, with the control group showing the highest desiccation (28.43%), followed by neem seed extract concentration 1(13.27%), and neem Seed extract concentration 2 (10.54%). Similarly, the highest leaf curling extent was recorded in the control group (3.238), followed by neem seed extract concentration 1 (2.342) and neem Seed extract concentration 2 (1.975). Across the study period, infestation and associated symptoms increased, with December exhibiting the highest infestation rates and leaf damage. These findings demonstrate that neem seed extracts are effective in mitigating citrus leaf miner damage in lemon seedlings, supporting their potential use as sustainable alternatives to synthetic pesticides.

Keywords: Biopesticide, seed extract, pest control, integrated pest management, lemon seedlings

1. Introduction
The citrus leaf miner, scientifically known as *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae: Phyllocnistinae), is a major pest affecting citrus production worldwide, particularly in nurseries and young orchards where seedlings are more prone to infestation [1, 2]. The larvae of this pest feed on young leaves, creating serpentine mines that reduce photosynthetic efficiency, distort leaf development, and increase plant susceptibility to secondary infections such as citrus canker (*Xanthomonas citri*), ultimately leading to stunted growth and yield losses [3, 4]. Effective management of *P. citrella* is critical for maintaining the health and productivity of citrus seedlings, particularly in the early developmental stages when plants are most vulnerable. According to [5] conventional pest management strategies rely heavily on synthetic insecticides, which, despite their effectiveness, pose significant environmental and economic concerns. The repeated application of chemical pesticides has been linked to the development of insecticide resistance, contamination of soil and water resources, and adverse effects on non-target organisms, including pollinators and natural enemies of pests [6]. Furthermore, growing consumer demand for residue-free agricultural products has driven research into eco-friendly alternatives, particularly botanical pesticides derived from plants with insecticidal properties [7, 8]. Reported that among botanical insecticides Neem (*Azadirachta indica*, A. Juss), also known as nim or margosa, is a fast

mahogany-growing tree that belongs to the *Meliaceae* family, and it has gained considerable attention due to its bioactive compounds, primarily azadirachtin, which act as an insect growth regulator, feeding deterrent, and oviposition. *A. indica-based* formulations have demonstrated efficacy against various agricultural pests, including aphids, whiteflies, caterpillars, and leaf miners [9]. However, the effectiveness of neem extracts varies depending on factors such as extraction method, solvent type, concentration, and target pest species [10]. Despite its well-documented pesticidal properties, limited studies have specifically evaluated the efficacy of different neem seed extract concentrations in controlling *P. citrella* infestations in citrus seedlings [11]. This study aims to compare the effectiveness of different *A. Indica* seed extract concentrations in controlling leaf miner infestations in lemon seedlings. By determining the most effective concentration for pest suppression, this research will contribute to the development of an environmentally sustainable pest management strategy for citrus nurseries. Additionally, the findings will provide insights into the potential of neem-based formulations as a viable alternative to chemical pesticides, aligning with the global movement toward integrated pest management (IPM) and sustainable agriculture.

2. Materials and Methods

2.1 Description of the Study Area

The study was conducted at the Forestry Nursery of the University of Namibia, Ogongo Campus, located at 17°40'38" S latitude and 15°18'02" E longitude in the Omusati region, Namibia. The area is characterized by a semi-arid climate, with an annual rainfall ranging from 400 to 600 mm and temperatures varying between 22°C and 36°C. The soil in the study area is predominantly sandy loam, supporting a savannah woodland vegetation type (12). During the experiment, the lemon seedlings were grown under an 85% shade net to simulate nursery conditions.

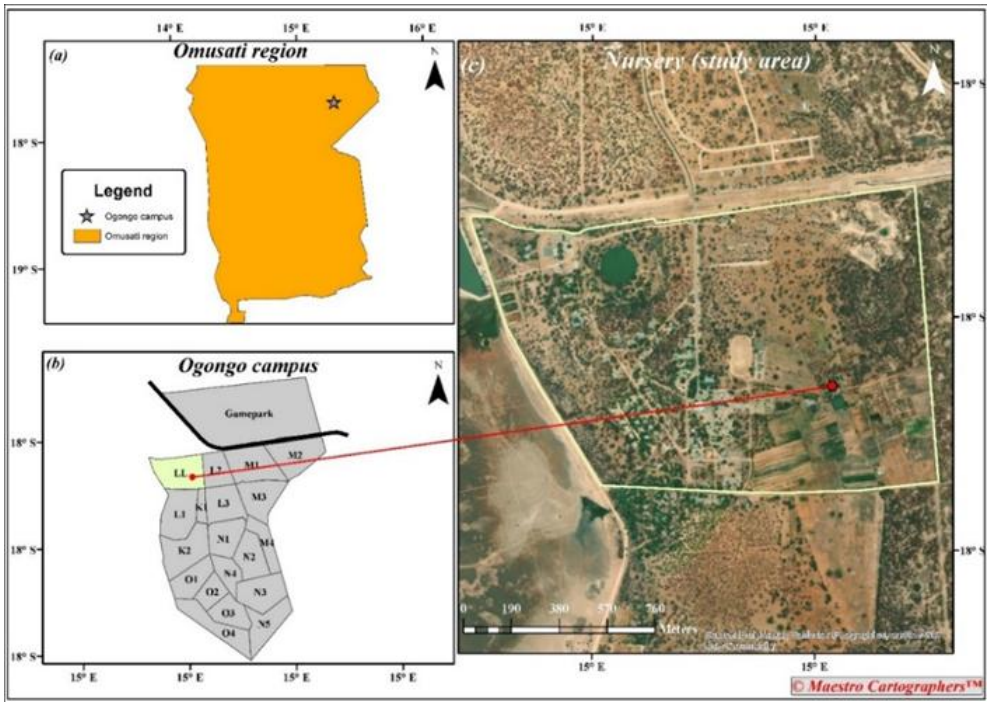

Fig 1: Location of Ogongo Campus in the Omusati region.

2.2 Experimental Design

The experiment was laid out in a Randomized Complete Design (RCD) with three treatments, each replicated four times. The treatments included two concentrations of *Azadirachta indica* (neem) seed extract, concentration 1 and concentration 2, and a control - tap water (C-W). Four-month-old lemon (*Citrus limon*) seedlings grown from seeds were used for the experiment. Each treatment group consisted of 20 seedlings per replicate, resulting in 80 seedlings per treatment and a total of 240 seedlings for the entire study.

TREATMENTS	R1	R2	R3	R4
Neem Seed Extract Concentration1 (NSEC1: 1.5kg)	NSEC2	NSEC1	C-W	NSEC2
Neem Seed Extract Concentration 2(NSEC2: 2.5kg)	C-W	NSEC2	NSEC1	C-W
Control C (WATER)	NSEC1	C-W	NSEC2	NSEC1

Fig 2: Experimental design layout.

2.3 Preparation of Botanical Extracts

Dry neem seeds were collected from trees in the vicinity of the University of Namibia, Ogongo Campus. The seeds were cracked open to extract the kernels, which were then shade-dried and ground using an electric grinder. Two batches of seeds were prepared. The first neem seed extract concentration (NSEC1) comprised of 1.5 kg seed powder to 10 liters of water. The second neem seed extract concentration (NSEC2) comprised of 2.5 kg of seed powder to 10 liters of water. The mixtures were soaked overnight to allow for maximum extraction of active compounds and then filtered through cheesecloth to remove solid residues. The resulting neem extracts were used for the application.

2.4 Application of Treatments

Treatments were applied as a foliar spray, seedlings were sprayed using hand sprayer bottles weekly from 4 months after emergence, with 1L/20 seedlings for seed extract concentrations; tap water of the same ratio was used for the control group. The spraying process was carried out over a

period of five months.

2.5 Data Collection

Field observations were carried out from July to December 2024, during which both quantitative and qualitative data were collected. Three types of data were recorded: infestation percentages, leaf desiccation, and estimated leaf curling extent. Baseline data had been collected before spraying, followed by monthly data collection after treatment application.

Infestation percentages were assessed by counting number of mined leaves per seedling. To calculate the mean infestation percent, formula 1 below was used.

$$Mean\ Infestation\ percentages\ (\%) = \frac{Total\ number\ of\ infested\ \ leaves/seedling}{Total\ Number\ of\ leaves/\ seedling} \times 100 \quad 1.$$

Leaf desiccation was assessed by counting the number of drying leaves per seedling. To calculate mean leaf desiccation, formula 2 below was used.

$$Mean\ Leaf\ desiccation\ (\%) = \frac{Total\ number\ of\ drying\ leaves/seedling}{Total\ Number\ \ of\ leaves/\ seedlings} \times 100 \quad 2.$$

The estimated leaf curling extent was determined by classifying the curled portion of leaves per seedling using a numerical scale from 0 to 100. To determine the extent of leaf curling, the curled portion of leaves per seedling was assessed, and the extent of all the leaves curling was assigned a class (Table 1).

Table 1: Scale, description and visual reference for estimated leaf curling extent in lemon seedlings

Scale	Description	Visual (Picture)
0-25	No visible curling or Slight curling on the edges	
26-50	Moderate curling affecting less than 50% of the leaf	
51-75	Extreme curling with significant distortion	
76-100	Leaf is almost entirely curled or distorted	

2.6 Data Analysis

All statistical analyses were performed using GenStat 18.1 for Windows. Normality of the data was assessed using a Q-Q plot. The plotted points appeared to lie approximately along the diagonal reference line, indicating that the data did not deviate substantially from normality. Since data met the normality assumption, a parametric two-way analysis of variance (ANOVA) was conducted to determine significant differences among treatments. Fisher's protected least significant difference test was used to show treatments that differ significantly. A significance level of $\alpha = 0.05$ was used as the threshold for statistical significance.

3. Results

3.1. Effect of Neem seed extract concentrations in controlling Leaf miner on lemon seedlings

A two-way ANOVA was conducted to evaluate the effects of two different concentrations of Neem extracts (NSEC1, NSEC2, and Control) on infestation percentage, leaf desiccation percentage, and estimated leaf curling in the control of leaf miners on lemon seedlings. The analysis revealed a significant main effect of treatment on the measured parameters ($p < .001$), indicating that Neem extract spray had a statistically significant impact on pest infestation and associated damage symptoms.

Table 2: ANOVA for treatment effects on infestation percentage, leaf desiccation percentage, and estimated leaf curling extent in controlling leaf miner on lemon seedlings

Source of variation	d.f.	Infestation%		Leaf desiccation %		Leaf curling extent(scale)	
		P value	Var%	P value	Var%	P value	Var%
Tr	2	<0.001	38.13	<0.001	25.34	<0.001	24.05
Tim	5	<0.001	25.13	<0.001	36.29	<0.001	19.41
(Tr×Tim)	10	<0.001	17.81	<0.001	12.75	<0.001	2.48
Total	17		81.07		74.38		45.94

Tr represents the Treatment, Tim denotes Time (in months), and Tr × Tim refers to the interaction between Treatment and Time. The model contributed 81.07% of the total variation in infestation percentage, 74.38% in leaf desiccation percentage, and 45.94% in the extent of leaf curling (on a scale). Among the factors, Treatment contributed the most to the variation in infestation percentage and leaf curling extent, whereas Time had the greatest influence on leaf desiccation percentage

The mean infestation percentage, leaf desiccation percentage, and estimated leaf curling extent, along with their standard errors for each treatment, are summarized in Table 2. The Control group recorded the highest infestation percentage (M = 51.1), followed by NSEC1 (M = 23.3), while NSEC2 had the lowest (M = 23.2), suggesting a slight difference in the effectiveness of the different concentrations of the Neem extract. For the leaf desiccation variable, the Control group exhibited the highest mean desiccation (M = 28.4), followed by NSEC1 (M = 13.3), and NSEC2 recorded the lowest (M = 10.5), indicating a notable reduction in leaf damage under both neem extract concentrations.

A similar pattern was observed in the estimated leaf curling extent: The Control group (tap water (C-W)) recorded the highest mean curling score (M = 3.238), followed by NSEC1 (M = 2.342), while NSEC2 showed the lowest mean value (M = 1.975), further supporting the difference in treatment effectiveness.

Pairwise comparisons using Fisher's protected least significant difference (LSD) test revealed no significant difference in infestation percentages between NSEC1 and NSEC2 concentrations. However, both NSEC concentrations were significantly different from the Control (C-W). A significant difference was also observed between both NSEC concentrations and the Control group in terms of leaf desiccation and estimated leaf curling extent.

Table 3: Descriptive statistics for Infestation% %, leaf desiccation%, and estimated leaf curling extent across all treatments in controlling leaf miner on Lemon seedlings.

Treatment	Infestation %	Leaf desiccation%	Estimated leaf curling extent (scale)
NSEC1	23.3±0.67 b	13.27± 0.61 b	2.342± 0.07 b
NSEC2	23.2±0.66 b	10.54±0.61 c	1.975±0.05 c
C-W	51.1±1.62 a	28.43±1.24 a	3.238±0.06 a

Note: Means with different letters within the column are significantly different at $p< 0.05$

3.2. Effect of time on treatments in controlling leaf miner on Lemon seedlings

To assess the effects of time (from July to December) on infestation percentage, leaf desiccation percentage, and estimated leaf curling extent, a two-way ANOVA was performed. The analysis indicated a significant main effect of time on all three variables ($p< .001$), as summarized in Table 1 above. Among the factors, Time contributed the most to the variation in leaf desiccation percentage (36.29%), followed by infestation percentage (25.13%), then leaf curling extent (19.41%).

The mean infestation percentage, leaf desiccation percentage, and estimated leaf curling extent, along with their corresponding standard errors for each month, are presented in Table 3. Among the months, December recorded the highest mean infestation percentage, while August showed the lowest. A similar pattern was observed for the leaf desiccation and estimated leaf curling extent variables. Pairwise comparisons using Fisher's Protected Least Significant Difference (LSD) test revealed no significant differences in infestation percentages between most months, except between September and October, and between October and November. For leaf desiccation, significant differences were found between most months, except between October and November, and between November and December. A related pattern was observed for estimated leaf curling extent; however, significant differences were found between September and October, and between November and December Table 4.

Table 4: Descriptive statistics for Infestation%, leaf desiccation%, and estimated leaf curling extent across Time (months)

Time	Infestation %	Leaf desiccation%	Estimated leaf curling extent (scale)
JULY	11.5±1.14 d	0.00±0.00 e	1.758±0.088 d
AUGUST	26.0±1.14 c	12.23 ±1.00 d	2.125±0.091 c
SEPTEMBER	37.7±1.37 b	15.72±0.85 c	2.533±0.096 b
OCTOBER	38.1±1.57 b	24.66±1.32 b	2.542±0.097 b
NOVEMBER	39.9± 2.14 ab	24.95±1.38 ab	3.008± 0.080 a
DECEMBER	41.8± 2.34 a	26.90±1.57 a	3.142±0.078 a

Note: means with different letters within the column are significantly different at p< 0.05

4. Discussion

The results of this study highlight the remarkable effectiveness of neem seed extract concentrations (NSEC1 and NSEC2), which significantly outperformed the untreated control in reducing leaf miner infestation, leaf desiccation, and leaf curling ($p\leq 0.001$). This aligns with previous studies by [13, 14], who reported the effectiveness of neem-based biopesticide against various insect pests due to the presence of azadirachtin, a bioactive compound that disrupts insect growth, feeding, and reproduction. However, a closer analysis revealed that while both neem concentrations were effective, there was no statistically significant difference between NSEC1 and NSEC2 in reducing infestation percentage. Despite this, NSEC2 consistently recorded the lowest mean values across all three parameters, suggesting a potential advantage of higher concentration though not statistically significant for infestation control, similar to findings by [15, 16], who observed a dose-dependent insecticidal effect of neem extracts on agricultural pests.

Interestingly, physical observations during the experiment revealed that NSEC2, although numerically more effective, appeared to cause mild phytotoxic effects such as leaf burn on some seedlings, especially when compared to NSEC1. This suggests that while higher concentrations of neem extract may enhance pest suppression, they may also increase the risk of plant stress or damage, especially in young or sensitive crops. This trade-off warrants further investigation to determine the most appropriate and safe application rates. The performance of NSEC1 was slightly lower than NSEC2, which may be attributed to variations in the dosage applied, as reported by [17].

In addition to infestation percentages, the study also assessed the percentage of leaf desiccation and the estimated extent of leaf curling. The results indicated that NSEC2 significantly reduced leaf desiccation percentages compared to both NSEC1 and the control. Supporting the view of [18] findings that neem-based biopesticides can improve plant health by reducing leaf desiccation in insect-infested plants. NSEC1 also reduced leaf desiccation percentages, though to a lesser extent than NSEC2, supporting the observations of [17] who observed that the effectiveness of neem extracts can vary depending on the extraction method and concentrations.

The control group exhibited the highest leaf desiccation percentages, consistent with [19], discoveries claiming that untreated plants tend to suffer greater leaf desiccation due to prolonged pest damage. Surprisingly, the current investigation found that the extent of leaf curling followed a similar pattern to the leaf desiccation parameter, with NSEC2 demonstrating the lowest mean percentages compared to NSEC1 and the control. This result reflects those of [15], who also found that neem-based treatments at high concentrations significantly reduce leaf curling caused by sap-sucking pests, which contradicts to that of [20], who observed limited efficacy of neem extracts in controlling leaf curling in certain pest species, possibly due to environmental degradation of azadirachtin.

While this study confirms neem's ability to reduce visible pest damage, it also supports the need for balanced application, particularly since the slight seedling burn associated with NSEC2 could offset its benefits if not carefully managed. This aligns with conflicting results from [20], who reported reduced efficacy of neem under certain environmental or crop conditions, potentially due to phytotoxicity or compound degradation.

Over the six months, infestation percentages, leaf desiccation, and leaf curling were generally higher during the initial months (July to September) and gradually declined in the later months (October to December). This trend aligns with the seasonal patterns of pest activity observed by [21], who reported that leaf miner populations tend to decrease with changing environmental conditions. The consistent performance of NSEC2 treatment supports its potential for long-term pest control, reinforcing the claims made by. However, the efficacy of neem-based products may vary depending on environmental conditions, as highlighted by [20], who noted that extreme temperatures and rainfall could reduce the effectiveness of botanical pesticides. In contrast, the control group exhibited the highest infestation percentages, leaf drying percentages, and leaf curling percentages throughout the study, reinforcing the vulnerability of untreated lemon seedlings to leaf miner infestation. Despite some conflicting reports on the efficacy of neem-based products, the superior performance of NSEC2 in this study suggests the potential of neem seed extract as an environmentally friendly alternative to chemical pesticides. The control group consistently recorded the highest infestation levels, leaf desiccation, and leaf curling extent, emphasizing the susceptibility of untreated lemon seedlings. While NSEC1 and NSEC2 both reduced damage effectively, the minor phytotoxic symptoms observed under NSEC2 treatment suggest that NSEC1 may offer a more balanced option, especially under certain environmental conditions or for more sensitive crops. Nevertheless, the overall results support neem-based products as viable alternatives to synthetic pesticides.

This study's findings support the concepts of Integrated Pest Management (IPM), which promotes the use of botanical pesticides to reduce the adverse effects of synthetic chemicals on both the environment and human health. The reliable effectiveness of neem seed extract treatments highlights its role in sustainable agriculture, especially in areas like Namibia, where farming systems are particularly susceptible to climate change. Future research should investigate optimal application rates, any possible phytotoxic effects from high concentrations, and interactions with other biopesticides or environmental factors to improve the safety and efficacy of these treatments.

5. Conclusion and Recommendations

This study aimed to determine the effects of different concentrations of *Azadirachta indica* seed extracts concentrations in controlling *Phyllocnistis citrella* infestation on lemon seedlings under nursery conditions. The findings revealed that NSEC2 (2.5 kg/10L water) was the most effective, achieving the lowest infestation percentage, leaf drying percentage, and estimated leaf curling extent; however, because of the slight seedling burn associated with NSEC2 could offset its benefits if not carefully managed. Therefore, future research should explore the use of low neem concentrations NSEC1 (1.5kg/10L water) to optimize pest control. Additionally, conducting experiments across different seasons would provide insights into the seasonal effectiveness of neem extract. Further studies on improved formulations or application methods, such as combining neem extract with other organic treatments, could enhance its efficacy and longevity.

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7. Competing interests: The authors declare that they have no competing interests. The research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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