International Journal of Environmental Chemistry and Ecotoxicology Research,7(1)1-13,2025
ISSN: Print: 2399-3464,
ISSN: Online: 2399-3472
Website: https://www.eajournals.org/
Published by European Centre for Research Training and Development UK

Determination of The Termicidal Efficacy of Chitosan Nanoparticles Coated with Synergistic Blends of Selected Plants Extracts

C.O. Ikese*, S.T.Ubwa, S.H. Targba, Y.N. Akaasah, E.A. Omudu, C.N. Abah, O.V. Egbeneje, G.A. Odeh, R.A. Igoche, A.E. Yongo, D.T. Yoo
Department of Chemistry |Rev. Fr. Moses Orshio Adasu University, Makurdi - Nigeria[1]
*Correspondence: ichris@bsum.edu.ng , +234 8032497326

doi: https://doi.org/10.37745/10.37745/ijecer.16/vol7n113 Published August 11, 2025

Citation: Ikese C.O., Ubwa, S.T., Targba, S. H., Akaasah, Y.N., Omudu E.A., Abah, C. N., Egbeneje, O.V., Odeh, G.A., Igoche, R.A., Yongo, A.E., Yoo, D.T. (2025) Determination of The Termicidal Efficacy of Chitosan Nanoparticles Coated with Synergistic Blends of Selected Plants Extracts, *International Journal of Environmental Chemistry and Ecotoxicology Research*,7(1)1-13

Abstract: *The comparative termicidal efficacies of 4 synergistic plant extract blends; $A_{1:1:1}$, $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3}$ prepared from Olax subscorpioidea, Azadirachta indica and Cymbopogon citratus coated on chitosan nano particles, were assessed. The comparative mortalities and LD_{50} of the synergistic blends were determined at varied concentrations, using WHO susceptibility bioassays and probit analysis respectively. The results obtained show that all synergistic extract blends in the study possessed some level of termiticidal activity seeing as they achieved termite mortalities ranging between 25-100%. When graded with respect to mortality, the potency of the coated nanomaterials was in the order; $A_{1:3:1} > A_{3:1:1} > A_{1:1:1} > A_{1:1:3}$. The most potent synergistic blends were $A_{1:3:1}$ and $A_{3:1:1}$ as these produced termite mortalities as high as 96% and 100% respectively. Also, the termicidal efficacy of the synergistic blends appeared to be mostly attributable to the bioactive compounds in olax and neem rather than those from lemongrass. From the determined mortalities and LD_{50} of the coated nanomaterials, $A_{1:3:1}$, was found to be the most potent of the plant based termiticide.*

Keywords: termites, termicidal efficacy, Chitosan nanoparticles, *Olax subscorpiodea, Azadirachta indica, Cymbopogon citratus*.

INTRODUCTION

Termites (*Macrotermes natalensis)* are an important economic pest worldwide as they have been reported to cause substantial economic losses in agriculture, forestry and building construction [1,2]. Although an exact figure is not readily available in literature for every country, a 2019 report by the Food and Agricultural Organization of the United Nations, estimates the annual global economic losses associated with termite pest activity to be over $40 billion annually [3]. This figure varies depending on the source and the methods used to arrive at the estimate, but one thing is certain, the economic impact of termite pests is significant and of major concern [4].

Although a number of termite control methods such as the biological, physical and chemical methods are currently in use, they are plagued by special limitations that have hindered their effectiveness in termite control [5,6]. Whereas the biological and physical methods are more eco-friendly and easier to integrate with other termite control methods [7,8], they usually require highly specialized knowledge [9, 10] and are not yet as readily available and effective as the chemical methods [11, 12]. As a result, most termite control methods at the moment, rely heavily on synthetic chemical agents, which pose environmental and health risks [13]. Some of these include organochlorines, organophosphates, pyrethroids and fipronil, many of which are notorious for neurological damage, birth defects and environmental pollution among others. The development of eco-friendly termite control alternatives is essential to mitigating these risks. This has fueled the current surge to explore plant-based termiticides by many researchers [14]. Various Plant extracts have shown promise in termite control, including essential oils from plants like tea tree, lemongrass and eucalyptus [15, 16, 17].

However, the potential of plant-based termiticides is yet to be fully harnessed, as there is still a dearth of research needed to bridge the gap between the database of plants having termicidal effect, their individual modes of termicidal action and their synergistic termicidal power when compounded, with a view to enhancing their termicidal efficacy [18, 19, 20]. Consequently, this study seeks to develop eco-friendly termiticides from plant extracts by determining the synergistic termicidal efficacy of *Olax subscorpioidea, Azadirachta indica* and *Cymbopogon citratus* leaves extracts coated on chitosan nanoparticles with the chitosan nanoparticles acting as the termiticide matrix. The development of eco-friendly termiticides from plant extracts is crucial for reducing the environmental impact of termite control [21, 22, 23].

Olax subscorpioidea, Azadirachta indica and *Cymbopogon citratus* are native to Africa and widely distributed across the continent, particularly in tropical and subtropical regions [24, 25, 26]. They are known to thrive in diverse habitats ranging from rainforests to savannahs [27, 28, 29] and their extracts have been reported to show some level of activity against termites, thus hinting at their potential as viable termite control agents if their individual termicidal activities can be harnessed in a single synergistic termiticide.

MATERIALS AND METHODS

Sample Collection

Fresh leaves of *Olax subscorpiodea* (Olax), *Azadirachta indica* (neem), and *Cymbopogon citratus* (lemongrass) were harvested from Oju Local Government Area of Benue State, Nigeria and botanical identification carried out at the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja-Nigeria before conveying in polyethylene bags to the laboratory for extraction. Three thousand one hundred and twenty-five adult termites (*Macrotermes natalensis*) of approximately the same size were collected in batches of 125 termites from a termite mound using an aspirator into a plastic holding container, fitted with a mesh top 30 minutes prior to each mortality trial and kept in a shaded area, maintained at temperatures between 25-30 ^{o}C.

Preparation of plant extracts

Harvested leaves of olax, neem, and lemongrass were separately washed under running tap water to remove dirt, debris, and the decaying parts. The leaves were air-dried to brittle point for 96 hours at room temperature, after which they were separately ground into fine powder using a mistrial grinder and the

weight of the powder determined using a weighing balance [30]. The leave extract of each plant was separately prepared by acidified ethanolic extraction method [31]. In this method, 5.08 kg of leave samples, were separately washed, air-dried to brittle point, ground into leaf powder and macerated in 7.4 L absolute ethanol acidified with 100 mL concentrated acetic acid. The mixture was vigorously agitated once every 24 hours for 72 hours. This was then filtered using a muslin cloth and the residue re-macerated for extraction 4 more times. The resulting filtrates were then combined and concentrated using a rotary evaporator and thereafter evaporated to dryness before grinding to fine powder to obtain the extract powder.

Preparation of Synergistic blends of plant extracts

Four synergistic blends; $A_{1:1:1}$, $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3\ of}$ *Olax subscorpiodea* (Olax), *Azadirachta indica* (Neem), and *Cymbopogon citratus* (Lemongrass) were prepared by homogenizing accurately weighed-out portions of their extract powders in the ratios; 1:1:1, 3:1:1, 1:3:1, and 1:1:3 respectively, using a mistral grinder. These were then wet-mixed separately with 230 mL of distilled water in a 1000 mL beaker before evenly spreading it out on an aluminium foil paper to air-dry at room temperature for 72 hours. The dried mixtures were then ground into the fine powders of each synergistic blend.

Preparation of Chitosan Nanoparticles by Ionic Gelation

One kilogram of snail shell (Achatina achatina) was cleaned, washed and dried to remove impurities as reported by Ezeh et al. [32] before grinding into a fine powder with a mistral grinder. The resulting powder was then sieved using a 500 nanometres mesh to enhance its solubility. Demineralization was performed by adding 100 g of the sieved snail shell powder into 1000 mL of a 2M HCl solution, followed by continuous stirring for 2 hours to prevent effervescence while removing its carbonate and phosphate contents. The resulting insoluble fraction was filtered using Whatman filter paper and rinsed with deionized water until neutral pH was achieved. The insoluble, demineralized chitin fraction was oven-dried to a constant weight and set aside until deproteinization [33, 34, 35].

In the deproteinization phase, 50g of the demineralized chitin was placed in a 5000 mL beaker, and 870 mL of 10.3 M sodium hydroxide solution was added. The mixture was stirred on a hot plate for 5 hours, followed by 1 hour of cold-shaking at 150 rpm. The residue was filtered using Whatman filter paper and rinsed with deionized water until neutral pH was reached, this was then dried at 65 ^{o}C to a constant weight [33,36].

The deacetylation process, that converts chitin into chitosan was performed by treating the chitin obtained following demineralization and deproteinization with concentrated sodium hydroxide solution. The chitin was placed in a 1000 mL beaker and 750 mL of 12.3 M NaOH added and heated for 2 hours and 30 minutes in a water bath, this was followed by cooling for 30 minutes at room temperature. The mixture was then stirred using a magnetic stirrer for 4 hours at 200 rpm. The resulting chitosan was then washed with deionized water until the pH of the filtrate became neutral upon testing using litmus paper. The resulting chitosan was then filtered using Whatman filter paper and oven-dried to a constant weight at 40 ºC as described in literature [35,36, 37]. The resulting chitosan was then converted to nanoparticles using the protocol described by Zang et al. [38] in which the Sodium tripolyphosphate solution (TPP) was added drop wise to the chitosan solution under continuous magnetic stirring at room temperature. Care was taken throughout mixing to ensure uniform nanoparticle formation [39]. For complete complexation between chitosan and TPP to form, consistent stirring was maintained for approximately 30 minutes [38].

To isolate the chitosan nanoparticles, the nanoparticle suspension was centrifuged at 10,000 rpm for 15 minutes [39]. With utmost care, the supernatant containing unreacted TPP and acetic acid was decanted and discarded [40] thus recovering the chitosan nanoparticles. Residual TPP and acetic acid were removed by further resuspending in distilled water the precipitated chitosan nanoparticles and re-centrifuging repeatedly until the washings reached neutrality, thus indicating complete removal of excess TPP and acetic acid [41]. Lastly, the purified chitosan nanoparticles were collected by centrifugation and freeze-dried to obtain a dry powder [38].

Coating of synergistic extract blends on chitosan nanoparticles

The prepared chitosan nanoparticles were separately coated with the following $A_{1:1:1}$, $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3}$ by separately homogenizing weighed out amounts of each in the proportions shown in Table 1 to achieve the following concentrations of the synergistic extract in the particle mixture; 0.0, 20, 40, 60, 80 and 100 %. Untreated chitosan nanoparticles with concentration at 0.0 % contained no synergistic extract and served as the control.

Table 1: Blend proportion for compounding extract synergistic blends with chitosan nanoparticles

SN	Desired Concentration of synergistic Extract-in Particle Mixture (%)	Mass of synergistic blend (g)	Mass of chitosan nano particles (g)
1.	0	0.0	10.0
2.	20	2.0	8.0
3.	40	4.0	6.0
4.	60	6.0	4.0
5.	80	8.0	2.0
6.	100	10.0	0.0

Mortality trial and determination of temicidal efficacy of chitosan nanoparticles coated with synergistic plant extract blends.

Starting with $A_{1:1:1}$, 5 sheets of clean white filter paper were separately fitted into 5 replicate petri dishes before dousing the exposed upper surface with chitosan nanoparticles pre-coated with 20 % of a synergistic blend and another set of replicate petri dishes with uncoated chitosan nanoparticles (0% -control). Twenty adult termites were then placed on the doused paper surface in each petri dish to expose the termites to the coated and uncoated chitosan nanoparticles on the papers for 1 hour. After the exposure, the termites were returned to a holding bay and monitored for 24 hours for percent mortality. The procedure was repeated for the remaining concentrations of $A_{1:1:1}$ (i,e 40, 60, 80 and 100%) and the entire process was then repeated for the remaining synergistic extract blends; $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3}$.

A termite was classified as dead if it showed complete immobility, was unable to stand, crawl, or respond to gentle prodding. Termites exhibiting knockdown and unable to maintain coordinated flight or standing were categorized as moribund. Any termite capable of sustained flight, even with missing legs, was considered to be alive [42]. For each concentration of the extract-particle mixture, the number of dead and moribund termites was counted and expressed as a percentage of the initial 100-termite cohort using equation (1).

$$Observed\ mortality = \frac{\text{Total number of dead termites}}{100} \times 100 \qquad (1)$$

The observed mortality for the control group was also calculated in a similar manner and served as a critical baseline for comparison. where the control mortality exceeded 20%, the entire experiment was deemed unreliable and repeated. For control mortality between 5% and 20%, Abbott's formula [43] in equation 2 was applied to correct the observed mortality in the exposure groups. This statistical approach corrects for minor background mortality, and ensures accuracy of the results obtained. From a comparative assessment of the corrected mortalities obtained for each synergistic blend and the controls using One-way ANOVA, the termicidal efficacy or otherwise of each synergistic blend was ascertained.

$$corrected\ mortality = \frac{\%\ Observed\ mortality - \%\ Control\ \text{mortality}}{100 - \%\ \text{Control Mortality}} \times 100 \qquad (2)$$

Determination of LD_{50} by Probit Analysis

The LD_{50} for each synergistic extract blend was determined using probit analysis by converting their observed percent mortalities into probits using the Finney's Table [44] which was then plotted against the $\log_{10}$ of the concentrations of the test substance. A horizontal line was drawn at probit 5 on the y-axis, to intersect the fitted regression line representing the experimental data [45]. The x-axis coordinate of this intersection point corresponds to the log concentration, from which the LD50 was obtained by calculating the antilogarithm [45]. The LD_{50} for all synergistic blends were compared with a view to rank all synergistic blend in order of their efficacy.

Statistical Analysis

The results obtained were reported as Mean ± standard deviations of replicate determinations. The mortalities conferred by nanoparticles coated with synergistic extract blends and uncoated chitosan nanoparticles were compared by One-way Analysis of variance for any significant difference.

RESULTS AND DISCUSSION

The results obtained from the study are presented in Table 1 and in Figures 1-5. Table 1 shows the comparative mortalities of termites associated with different synergistic blends of the leave extracts of olax, neem and lemongrass at varied concentrations. Figures 1-5 shows the probit versus log concentration plots and hence the LD_{50} of $A_{1:1:1}$, $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3}$ synergistic blends coated on chitosan nanoparticles.

International Journal of Environmental Chemistry and Ecotoxicology Research,7(1)1-13,2025

ISSN: Print: 2399-3464,

ISSN: Online: 2399-3472

Website: https://www.eajournals.org/

Published by European Centre for Research Training and Development UK

Table 1: Comparative mortalities of termites with different synergistic blends of the leave extracts of Olax, Neem and Lemongrass at varied concentrations

S/N	Concentration (% w/w)	Plant Extract Synergistic Blend	Observed Mortality (%)	Control Mortality (%)	Corrected Mortality (%)	probit	Log conc.
1	20	$A_{1:1:1}$	34 ± 1	6 ± 1	29.78	4.45	1.301
		$A_{3:1:1}$	63 ± 1	10 ± 2	58.89	5.20	
		$A_{1:3:1}$	74 ± 1	14 ± 1	69.77	5.51	
		$A_{1:1:3}$	36 ±1	14 ± 1	25.58	4.64	
2	40	$A_{1:1:1}$	44 ± 3	6 ± 1	40.43	4.75	1.602
		$A_{3:1:1}$	70 ± 3	10 ± 2	66.67	5.41	
		$A_{1:3:1}$	78 ±4	14 ± 1	74.42	5.65	
		$A_{1:1:3}$	56 ±1	14 ± 1	48.84	5.15	
3	60	$A_{1:1:1}$	66 ± 1	6 ± 1	63.83	5.31	1.778
		$A_{3:1:1}$	79 ± 2	10 ± 2	76.67	5.71	
		$A_{1:3:1}$	96 ± 5	14 ± 1	95.35	6.65	
		$A_{1:1:3}$	74 ± 2	14 ± 1	69.77	5.64	
4	80	$A_{1:1:1}$	82 ± 4	6 ± 1	80.85	5.84	1.903
		$A_{3:1:1}$	90 ± 3	10 ± 2	80.00	5.84	
		$A_{1:3:1}$	100 ± 0	14 ± 1	100.00	8.09	
		$A_{1:1:3}$	84 ± 1	14 ± 1	81.40	5.99	
5	100	$A_{1:1:1}$	100 ± 0	6 ± 1	100.00	8.09	2.000
		$A_{3:1:1}$	97 ± 0	10 ± 2	96.67	6.75	
		$A_{1:3:1}$	100 ± 0	14 ± 1	100.00	8.09	
		$A_{1:1:3}$	100 ± 0	14 ± 1	100.00	8.09	

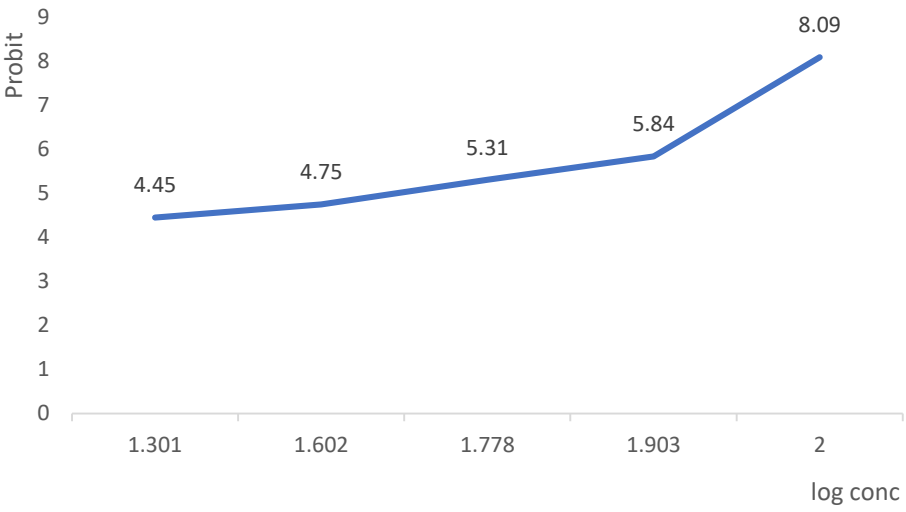

Figure 1: LD_{50} for $A_{1:1:1}$ Synergistic blend

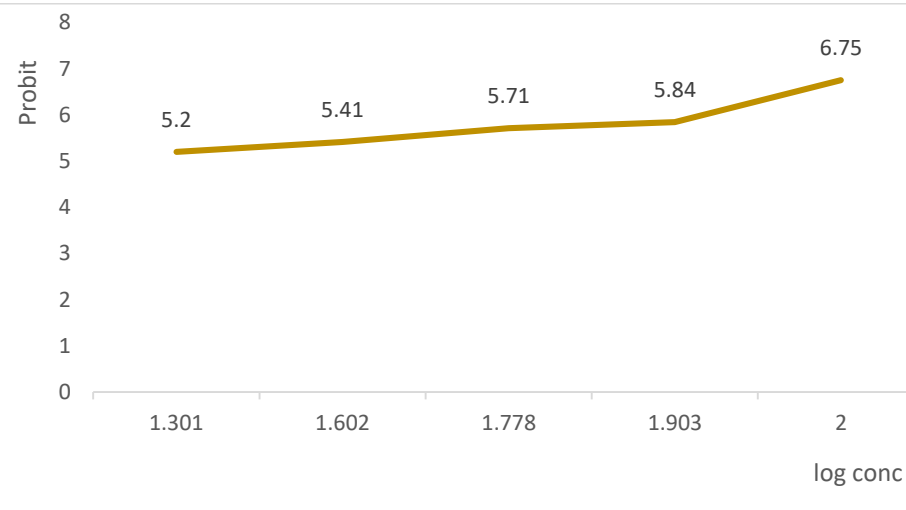

Figure 2: LD_{50} for $A_{3:1:1}$ Synergistic blend

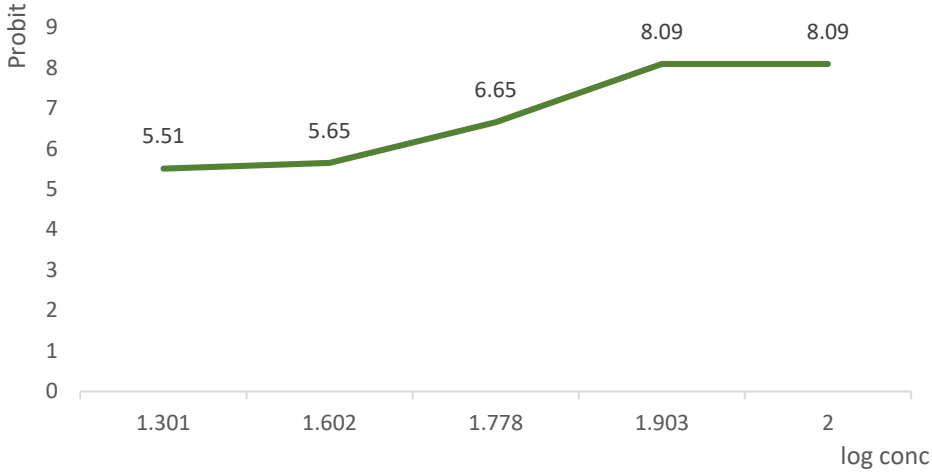

Figure 3: LD_{50} for $A_{1:3:1}$ Synergistic blend

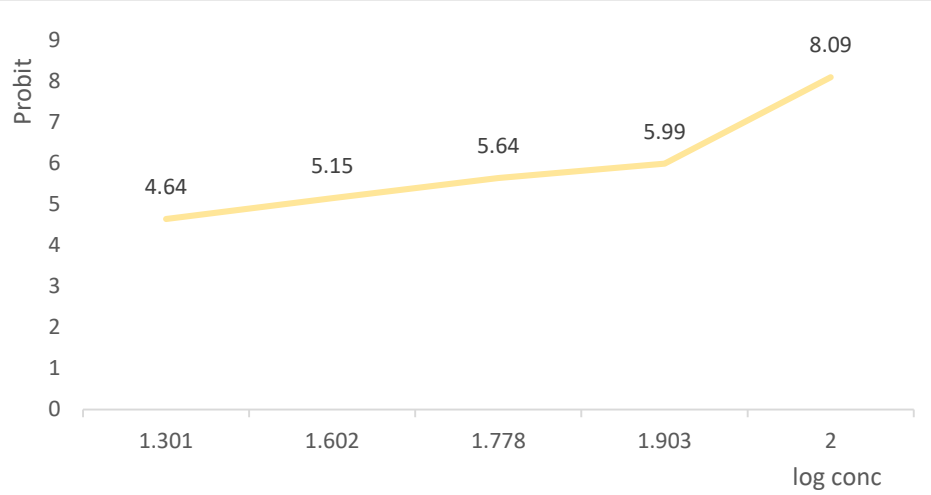

Figure 4: LD_{50} for $A_{1:1:3}$ Synergistic blend

The result in Table 1 shows that at all concentrations of the synergistic extracts coated on the nanomaterial, both the observed and corrected mortalities were significantly greater than their corresponding control mortalities obtained using the non-coated nano materials. This is a confirmation of the termicidal efficacy of all chitosan nanomaterials coated with synergistic extract blends.

From the results in Table 1, it can be seen that at synergistic blend concentrations < 100 %, there was a steady increase in mortality (both observed and control mortalities) provided that the proportion of olax and neem in the synergistic blend was greater or equal to the proportion of lemongrass in it as seen in mortalities conferred by $A_{1:1:1}$, $A_{3:1:1}$ and $A_{1:3:1}$. This is suggestive that the termicidal potency of the synergistic blends may be mainly attributable to bioactive compounds in olax and neem rather than those from lemongrass. However, when the termites were exposed to 100% synergistic blend, 100% mortality was recorded, thus suggesting that all synergistic blends were the most effective when brought in direct contact with termites as against when coated on nanoparticles.

Also, it can be seen From Table 1 that regardless of the variations in concentration of synergistic blends coated on the nanomaterials, $A_{1:3:1}$ consistently conferred the highest mortalities in all cases. This suggests that Azadirachta indica is the most important component of the synergistic blend. Also, a comparative assessment of the conferred mortalities in Table 1 shows that the potency of the coated nanomaterials is in the order; $A_{1:3:1} > A_{3:1:1} > A_{1:1:1} > A_{1:1:3}$.

The results in Figures 1, 2,3,4 and 5 allows for a comparison of the relative toxicities of $A_{1:1:1}$, $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3}$ to termites. This is because it permits the conversion into a straight line, of the otherwise sigmoid dose-response curve that would result from a direct plot of concentration against mortality [46-48]. The implication is that, each synergistic blend coated on nanomaterial affects termites differently at different concentrations, and it will be difficult to compare these differences using the ensuing sigmoid curve [49-51]. The antilogs of the log concentrations where the line intersects probit 5 in Figures 1, 2, 3, 4 and 5 correspond to LD_{50} values and these shows that the LD_{50} of $A_{1:3:1}$, $A_{3:1:1}$, $A_{1:3:1}$ and $A_{1:1:3}$ are 44%, 20%, 19% and 35% respectively. These are the concentrations at which 50% of the termite pests will be killed by each synergistic blend. The implication is that at these concentrations one encounters the same quantum of response hence the synergistic blends can be graded on the basis of their potency from the most potent to the least potent thus; $A_{1:3:1} > A_{3:1:1} > A_{1:1:3} > A_{1:1:1}$. While this reveals the concentration required to create the same magnitude of termite mortality with each synergistic blend, it also shows which among the treatments is the most effective pesticide for the control of termites.

CONCLUSION

The need for more research efforts to be tailored towards bridging the information gap that exist between the database of plants with termicidal effect, and their synergistic termicidal power when blended together, with a view to enhancing their termicidal efficacy cannot be overemphasized.

This study showed that at blend ratios of 1:1:1, 3:1:1, 1:3:1 and 1:1:3, leaf extracts of *Olax subscorpioidea, Azadirachta indica* and *Cymbopogon citratus* yield synergistic extract blends which when coated on chitosan nanoparticles, act as potent plant-based termiticide yielding mortalities between 25-100%. Whereas all synergistic blends coated on chitosan nanoparticles produced some termicidal effect, the potency of each blend was dependent on the concentration of the extract used. By mortality, the potency of the coated nanomaterials is in the order; $A_{1:3:1} > A_{3:1:1} > A_{1:1:1} > A_{1:1:3}$. The most potent synergistic blends

were $A_{1:3:1}$ and $A_{3:1:1}$ as these showed mortalities as high as 96% and 100% respectively. Also, the termicidal efficacy of the synergistic blends appear to be mainly attributable to the bioactive compounds in olax and neem rather than those from lemongrass. The synergistic blends were most effective when brought in direct contact with termites compared with when coated on chitosan nanoparticles. From both the determined mortalities and LD_{50} of the coated nanomaterials the synergistic blend; $A_{1:3:1}$, was found to be the most potent of the plant based termiticide.

Acknowledgements

The authors are grateful to Rev. Fr. Moses Orshio Adasu University, Makurdi for providing the laboratory space for this study and to the Tertiary Education Trust Fund (**TETFund**) for Funding this research via its institution-based research (IBR) grant.

Conflict of Interest

The authors declare no conflict of interest.

REFERENCES

[1]. Kambhampati, S., & Eggleton, P. (2018). Economic and ecological importance of termites: A global review. *Ecological Entomology,* 43(9), 1-10.

[2]. Rust, M. K., & Su, N.-Y. (2012). Global Economic Impact of Termites. *Annual Review of Entomology*, 57, 359-379.

[3]. Food and Agriculture Organization (FAO) (2019). The State of the World's Biodiversity for Food and Agriculture. FAO Commission on Genetic Resources for Food and Agriculture, Rome. Culled from http://www.fao.org/3/CA3129EN/ca3129en.pdf

[4]. Veerakone, S., & Bumpus, J. (2024). Termites: An Economic Drain–Analyzing Global Losses. *Pest Management Science*, 80(2), 579-587.

[5]. Hwang, Y. T., & Kwon, Y. J. (2021). A Review of the Evolution of Termite Control. *Journal of Pest Science,* 54(3), 655-668.

[6]. Sah, P., & Tiwari, S. (2023). Exploring Eco-friendly Alternatives to Termite Pest Management: Progress, Limitations, and Future Directions. *Pest Management Science*, 79(12), 3411-3422.

[7]. Hwang, Y. T., & Kwon, Y. J. (2023). A Review of the Evolution of Termite Control: Eco-Friendly Strategies and Integration with Other Methods. *Journal of Pest Science*, 54(5), 1061-1075.

[8]. Zhou, X., & Vasile, M. (2023). Eco-Friendly Innovations in Termite Control: The Role of Biological and Physical Methods. *International Journal of Pest Management*, 69(3), 234-241.

[9]. Sah, P., & Tiwari, S. (2023). Biological and Physical Control Methods for Sustainable Termite Management: Benefits and Challenges. *Pest Management Science*, 79(12), 3455-3465.

[10]. Alavi, S. R. A., Kafari, A. B. R., & Jamali, M. H. (2024). Termite Management by Entomopathogenic Fungi: Recent Advances and Sustainability Considerations. *Journal of Applied Microbiology*, 136(1), 45-59.

[11]. Khamis, R. M., Al-Rashidi, N., & Al-Malki, A. (2023). A Review of the Evolution of Termite Control. *Insects*, 14 (1), 25.

[12]. Callan, D., & Tschinkel, W. R. (2024). Biological Alternatives for Termite Control: A Comprehensive Review. *Journal of Entomological Science*, 59(2), 245-258.

[13]. Environmental Protection Agency. (2022). Human Health Issues Related to Pesticides. Retrieved October 2024, culled from [US EPA] (https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/human-health-issues-related-pesticides).

[14]. Mohammed, A. A., Abed, N. S., & Ibrahim, M. A. (2024). Termiticidal, biochemical, and morpho-histological effects of Eucalyptus and nutmeg essential oils against subterranean termites (Reticulitermes spp.). *Frontiers in Plant Science,* 14, Article 1292272.

[15]. Taderera, T., Nhamo, N., & Mavhodia, T. (2023). Efficacy of essential oils from aromatic plants against the subterranean termite, Reticulitermes spp. *Tropical Plant Research*, 10(1), 112-125.

[16]. Mansour, S. A., & El-Mougy, N. S. (2023). A Review on Natural Plant Extracts: A Potential Source for Eco-Friendly Termiticides. *Journal of Pesticide Science*, 48(4), 156-162.

[17]. Yusuf, S. K., Ekhator, I. E., & Ijeoma, A. (2024). Exploration of the Effectiveness of Plant-Based Insecticides against Termites: A Case Study of Eucalyptus and Lemongrass Extracts. *International Journal of Pest Management*, 70(1), 25-34.

[18]. Khalid, S. A., & Raza, A. (2023). Harnessing the Potentials of Plant-Derived Compounds as Eco-Friendly Termiticides: A Review. *International Journal of Pest Management*, 69(2), 147-158.

[19]. Junaid, M., Nisar, M., & Shaheen, I. (2023). Exploring the Underutilized Potential of Plant Extracts for Termite Management: Gaps and Opportunities. *Journal of Entomological Science*, 58(3), 301-312.

[20]. Ojo, A. A., & Akinbile, C. O. (2023). Integrating Plant-Based Termiticides into Pest Management: Challenges and Future Directions. *Journal of Applied Entomology,* 147(5), 208-220.

[21]. Kumar, S., & Tiwari, A. (2023). Green Alternatives for Termite Control: Efficacy of Plant-Derived Biopesticides Against Reticulitermes flavipes. *Environmental Science and Pollution Research,* 30(14), 13456-13467.

[22]. Ali, A., & Awan, U. (2023). Plant-Based Insecticides: Promising Solutions for Sustainable Termite Management. *Journal of Pesticide Science*, 48(2), 102-110.

[23]. El-Wakeil, N. E., & Hegazi, A. (2024). Evaluating the Environmental Impact of Plant Extracts-Based Termiticides: A Step Towards Ecological Sustainability. Sustainable Agriculture Research, 13(1), 45-56.

[24]. Akindele, S. O., & Adeyemo, O. K. (2023). Ethnobotanical Survey of Medicinal Plants in Osun State, Nigeria: A Study on Olax subscorpioidea. *Journal of Ethnopharmacology*, 305, Article 116065.

[25]. Ibrahim, T. M., & Ahmadu, A. A. (2023). The Role of Azadirachta indica in Traditional Medicine and Pest Control in Africa. *African Journal of Traditional, Complementary and Alternative Medicines,* 20(1), 12-25.

[26]. Adewale, M. I., & Adebayo, A. H. (2024). Cymbopogon citratus: Its Ethnobotanical Uses and Distribution in Africa. *Journal of Medicinal Plants Studies*, 12(2), 101-110.

[27]. Adewale, M. I., & Adebayo, A. H. (2023). Habitat Preferences and Ecological Adaptations of Olax subscorpioidea in Nigeria: Implications for Conservation. Journal of Ecology and Environmental Sciences, 58(2), 134-145.
[28]. Khan, A., & Fayaz, S. (2023). Ecological Distribution and Habitat Versatility of Azadirachta indica in Sub-Saharan Africa. African Journal of Ecology, 61(3), 345-356.
[29]. Nwankwo, D. I., & Akintola, J. O. (2024). Cymbopogon citratus: Adaptability and Habitat Variability in Africa's Ecosystems. Journal of Plant Studies, 12(1), 23-34
[30]. Ogbeide, O. A., Ighodaro, O. M., Okwuosa, C. N., & Isehunwa, O. O. (2020). Phytochemical and Antioxidant Properties of Selected Medicinal Plants in Nigeria. Journal of Medicinal Plants Research, 14(4), 54-63.
[31]. Segovia-Gómez, A., & Ferrer-Sueta, M. (2014). Termite control with Metarhizium anisopliae: field tests in Colombia. Journal of Insect Science, 14(4), 937-946.
[32]. Ezeh, O. C., Ugwuanyi, J. O., & Ogbu, M. (2023). Extraction and Characterization of Chitosan from Snail Shells Achatina fulica. Journal of Applied Sciences and Environmental Sustainability, 11(2024) 186-196.
[33]. Moosa, A. M., Ali, A., & Smith, J. (2016). Production and characterization of chitosan from the shells of snail specimens. International Journal of Biological Macromolecules, 93, 491-497.
[34]. Alaa, A. M., Amer, A. M., & Alhussainy, T. (2023). Characterization of Chitosan Obtained from Snail Shell (Achatina fulica) and its Excipient Potentials in Metronidazole Tablet Formulation. Journal of Food Quality and Hazards Control, 10(1), 37-43.
[35]. Duarte, M. A., Gomes, D. L., & Lira, L. F. (2000). Chitosan production from freshwater snail shells (Pomacea flagellata). Water Research, 34(9), 2153-2158.
[36]. Anusha, P., & Fleming, P. (2016). Preparation and characterization of chitosan from snail shell waste. International Journal of Advanced Research in Chemical Science, 3(8), 1-9.
[37]. Adekanmi, O. A., Kayode, O. J., & Mustapha, O. K. (2021). New process for synthesizing chitosan from snail shells. Journal of Physics: Conference Series, 1299(1), 012089.
[38]. Zang, X., Liu, Y., Zhang, S., & Zhao, H. (2015). Preparation and characterization of chitosan nanoparticles for the controlled delivery of curcumin. Journal of Nanomaterials, 2015, Article ID 562834. https://doi.org/10.1155/2015/562834
[39]. Liu, Y., Li, C., Zhang, Y., & Wu, X. (2020). Preparation of chitosan nanoparticles for the delivery of therapeutic agents to human cancer cells. Journal of Nanobiotechnology, 18(1), 123. https://doi.org/10.1186/s12951-020-00679-4
[40]. National Research Council. (2011). Chitosan: A Biopolymer with Special Properties and Applications [Report]. National Academies Press. https://doi.org/10.17226/13266
[41]. Jiang, Y., Wang, Y., Zhang, S., Li, L., & Wu, W. (2019). Preparation and characterization of chitosan nanoparticles for the delivery of doxorubicin. *Journal of Nanobiotechnology*, 17(1), 4. https://doi.org/10.1186/s12951-018-0432-1
[42]. Osman, K., Alhaj, N. A., & Crawford, J. W. (2021). The impact of environmental factors on the mortality of termites (Isoptera). *Insects,* 12(3), 250. https://doi.org/10.3390/insects12030250
[43]. Chi, J., Yang, Q., & Chen, H. (2017). A new method to determine the mortality of insects in bioassays using Abbott's formula. *Environmental Entomology*, 46(4), 789-797. https://doi.org/10.1093/ee/nvx080
[44]. Finney, D. J. (1971). Probit Analysis. Cambridge University Press.

[45]. Russell, R. L., Lacey, L. A., & Karam, M. L. (2020). Probit analysis in determining the LC50 and LD50 of insecticides against Drosophila suzukii (Matsumura). *Journal of Economic Entomology*, 113(3), 1385-1392. https://doi.org/10.1093/jee/toaa043

[46]. Morrison, H. G., & Barlow, J. G. (2021). Probit analysis for dose-response studies: A reliable methodology for determining lethal concentrations in environmental toxicology. *Environmental Toxicology and Chemistry,* 40(5),1422-1430. https://doi.org/10.1002/etc.4809

[47]. González, M. A., & García, M. (2022). Application of probit and logit models to estimate lethal doses in toxicological studies. *Journal of Hazardous Materials*, 423, 127115. https://doi.org/10.1016/j.jhazmat.2021.127115

[48]. Singh, A., & Kumar, V. (2020). Comparison of various statistical methods for analyzing dose-response data: A case study using probit analysis. Statistics in Medicine, 39(12), 1671-1683. https://doi.org/10.1002/sim.8553

[49]. Yardimci, B., & Ceylan, K. (2021). Comparison of the effectiveness of different termiticides against subterranean termites using dose-response analysis. *Journal of Economic Entomology,* 114(3), 1313-1321. https://doi.org/10.1093/jee/toab034

[50]. Baker, R. L., & Karp, K. K. (2020). Evaluation of different active ingredients for controlling termite populations: A quantitative approach. *Pest Management Science*, 76(8), 2665-2673. https://doi.org/10.1002/ps.5861

[51]. Roe, R. M., & Campbell, J. F. (2022). Effects of concentration on the efficacy of diverse termiticides against Coptotermes spp. in laboratory bioassays: Implications for field applications. *Insects,* 13(1), 34. https://doi.org/10.3390/insects13010034