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EFFICACY OF GREEN SYNTHESIZED NANOPARTICLE PRESERVATIVES DEVELOP FROM *AZADIRACHTA INDICA* AND *EUPHORBIA TIRUCALLI* ON SOME SELECTED WOOD SPECIES AGAINST TERMITE ATTACK

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Abstract:

In this study, the effects of green-synthesized CuO nanoparticles on the wood species of Daniellia oliveri, Terminalia superba, and Albizia ferruginea were examined. Wood samples were cut into 35mmx35mmx350mm, oven dried and weighed. Plant extracts with recognized wood-preserving qualities; Azadirachta indica and Euphorbia tirucalli extracts were used alongside copper (II) sulfate pentahydrate (CuSO4.5H2O) to develop copper II oxide (CuO) nanoparticles. For a 12-week period, the copper oxide nanoparticle formulation's effectiveness against termites was evaluated on the selected wood species by exposing them to termites at the timber graveyard site located at the Federal University of Technology, Akure. Scanning electron microscopy (SEM) along with energy dispersive x-rays (EDX) was used to characterize the developed nanoparticles (NPs). The SEM images revealed the presence of spherical CuO nanoparticles. Copper and oxygen were confirmed to be present in significant fractions by the EDX profile. Results showed that the percentage weight loss of untreated wood samples and wood samples treated with 100% plant extract recorded weight losses values of 70.43% and 57.01%, respectively, whereas the weight losses of the nanoparticles from CuO derived from Azadirachta indica and Euphorbia tirucalli were 21.98% and 24.62%, respectively. The three formulations of CuO nanoparticles from the two plants extract showed similar effectiveness in protecting the treated wood samples from termites.

Key word: *nanoparticles; copper II oxide; termites; Azadirachta indica and Euphorbia tirucalli.*

INTRODUCTİON

Wood is a biological material with a wide range of characteristics, including the ability to be degraded when exposed to bio-deteriorating agents (Borges *et al.* 2018). It is also a choice material in diverse application when compared to other competing materials such as metals and concrete. However, wood of several species has low durability in terms of how long they last in several areas of building applications especially in their natural state even used in hazardous environments or areas where they are prone to attack by biological agents such as insects and fungi. In many tropical countries, wood and wood-based products are prone to insect attacks such as termites; also, their level of severity and prevalence may vary among and within individual countries. In different part of Nigeria, termites represent a major insect pest that ravages and causes severe economic losses to wood products. Since termites are major structural pests of wood in building (Su *et al.* 2000; Eko *et al.* 2015), both homeowners and builders are always considering how to control them (Owoyemi 2008). Even domestic items like books and clothing are not safe from termites. To minimize the extent of damage to wood materials and other wood products, numerous treatments have been used including treatment with copper chrome arsenate (CCA), spent engine oil and creosote-based oils (Olaniran *et al.* 2010). However, majority of conventional wood preservatives have been limited to specific areas of use, due to emission of harmful chemicals and excessive energy consumption during their production with negative effects on the environment (Gomper 2013; EU 2001). Researchers are now faced with the challenge of formulating preservatives that are both economically viable and have low toxicity. According to Weir *et al.* (2008), nanotechnology is the design, characterization, development, and use of materials, devices, and systems by regulating their shape and size at the nanoscale. It has numerous uses in industry, commercial products, and medicine. Since green synthesized nanoparticle can be used in low concentrations, with low energy requirements, and without producing toxic chemical by-products, nanotechnology has demonstrated that it can provide a solution to this problem (Jadoun *et al.* 2021; Christophe and Bilal 2022). According to Borges *et al.* (2018), a variety of metals including silver (Ag), boron (B), copper (Cu), zinc (Zn), and titanium (Ti) have been employed in the manufacture of nanoparticles that show promise for protecting wood. Additionally, plants are a wonderful source of compounds that preserve wood (Singh and Singh 2012; González-Laredo *et al.* 2015). Dhanalakshmi and Rajendran (2013), reported that humans have long used copper and copper complexes as water purifiers, antibacterial, antifouling agents, algaecide, fungicides, and nematocides. *Azadirachta indica and Euphorbia tirucalli* extract have been reported to be promising for protecting wood against termite attack but problem of fixation still persist (Tor *et al.* 2007; Agbidye *et al*. 2020 and Okanlawon *et al*. 2020). Therefore, this work aimed to assess the efficacy of green synthesized copper oxide nanoparticle derived from *Azadirachta indica* and *Euphorbia tirucalli* against termites attack with a view to develop copper oxide (CuO) nanoparticle against termites attack on wood.

MATERİAL AND METHOD

Synthesis of copper oxide nanoparticles

In order to develop CuO nanoparticles, leaves of *Azadirachta indica* and stem of *Euphorbia tirucalli* and also CuSO4.5H2O were employed. The leaves of *Azadirachta indica* and stem of *Euphorbia tirucalli* were properly cleaned in distilled water before being dried and ground into fine powder for the preparation of an aqueous extract. 150ml of sterile, twice-distilled water were combined with 25g of the leaves. Whatman filter paper No. 1 was used to filter the mixture, which was then heated at 60°C for 10 minutes. The mixture was then kept at 4°C for later use. The extracts and the precursor solution of CuSO4.5H2O (0,025M) were preheated separately before being combined in proportions of 1:2, 1:4, and 1:6 in a water bath at 60°C (Majumder 2012). The resultant solution was stirred at 1000 rpm for 10 minutes and kept at room temperature for 3h. Presence of copper oxide nanoparticles was indicated by change in colour from bluish green to dark green. The solution thus obtained containing copper oxide nanoparticles was used for the experiment.

Characterization of biologically synthesized CuO nanoparticles

Energy dispersive X-ray (EDX) and scanning electron microscopy (SEM) (Gemini Ultra 55 with ESB detector at 5,0kV) were used to analyze the produced nanoparticles. Drop casting method was used to apply a small amount of the nanoparticle formulation on a cleaned, small (5mm×5mm) silicon wafer piece. The wafer was completely dried in the air and maintained in a desiccator with a vacuum for 48 hours. Gold was applied to the specimens, and SEM images were captured.

Treatment of wood samples

Three indigenous wood species which are: *Daniellia oliveri, Terminalia superba* (Afara) and *Albizia ferruginea* (Ayinre) were used for the study. Wood samples of each wood species were cut into size 35mm×35mm×450mm respectively. A total of one hundred and thirty five wood sample were used for the study. The control samples for this research were in two groups. The first group were treated with the 100% of each of plant extract while the second group were not treated with any preservative. This is to serve as basis for comparing durability characteristics nanoparticle treated wood. Five (5) wood sample were obtain from each of the selected wood species, making it fifteen (15) wood sample for each formulation (1:2, 1:4, and 1:6) from each of the plant source, making it ninety (90) wood samples for the nanoparticle treatment while the control, fifteen (15) wood samples were treated with 100% plant extract for each of the plants source, totalling thirty (30) and the remaining fifteen (15) wood samples were without treatment. Pressure impregnating method were used for the treatment. Wood samples were introduced into the impregnation equipment and subjected to a vacuum of 2 mbar for 5 min. Thereafter, the impregnation solution containing nanoparticles was used for the treatment, and the wood samples were impregnated at a pressure of 4 Bar for 3 hour. On removal of the treated samples from the impregnation equipment, they were air-dried at room temperature for 48 h to evaporate the excess of water avoiding the appearance of cracks. The treated samples were weighed and percentage absorption of the preservative was calculated using the following equation 1.

$$\%\ absorption = \left(\frac{T_1 - T_2}{T_1}\right) \times 100 \quad (1)$$

where: T_1 is the dry weight of sample before treatment (in grams);
T_2 is weight of sample after treatment (in grams).

Field Test

The field work was carried out in the timber graveyard of Federal University of Technology Akure, Ondo state located on longitude 5.13^{0}E and latitude 7.31^{0}N. The sites were cleared, thoroughly weeded and then sprinkled with wood shavings to stimulate termite activities. Both treated and untreated wood samples (used as control) of each of the three wood species were buried at a depth of 225mm below the ground surface and at a spacing of 100mm by 100mm. The samples were inspected weekly for a period of twelve (12) weeks. This is to assess the condition of the samples during the exposure periods and grade the extent of termite attack through visual assessment according to ASTM D2017-05 on a scale of 0 to 10. At the end of the exposure for twelve (12), the samples were removed from the field, dried until constant weight was achieved (at a temperature of 103±2°C) and re-weighed to assess the extent of attack by termites. Weight loss due to termite attack was calculated using the following equation 2:

$$\%\,Weight\ Loss = \left(\frac{T_2 - T_3}{T_2}\right) \times 100 \quad \text{.......} \quad (2)$$

where: T_2 is weight after preservative treatment;
T_3 is weight after termite attack (in grams).

The experimental design was Randomized Complete Block Design (RCBD), analysis of variance (ANOVA) was used to test for significant differences between plant extract sources, formulation and wood species. A comparison of the means was conducted employing Duncan Multiple Range Test (DMRT) to identify which groups were significantly different at $P<0.05$ when the ANOVA indicated a significant difference among plant extract sources, formulation and wood species.

RESULTS

Characterization of nanoparticle preservatives develop from Azadirachta indica and Euphorbia tirucalli L

The result of Scanning electron microscope (SEM) with Electron Dispersive X-rays of nanoparticles synthesized from CuO nanoparticles and leaf extracts of *Azadirachta indica* and *Euphorbia tirucalli* and prepared at three mixing ratio (1:2, 1:4 and 1:6) is shown in (Figure 1A to1C and 2D to 2F). SEM image confirmed the presence of CuO nanoparticles that are spherical in nature. The particles were well separated without agglomeration and the average size of the major axis of the particle size is 45 µm in the case of nanoparticles synthesized from CuO and *Azadirachta indica* leaf extract mixing ratio 1:2. In the case of nanoparticles synthesized from CuO and *Azadirachta indica* leaf extracts at mixing ratio 1:4 and 1:6 the average; size of the major axis of the particle size were 120 µm and 89.3 µm respectively (Fig. 1a-c). The average size of the major axis of the particle size of nanoparticles synthesized from CuO and *Euphorbia tirucalli* stem extract ratio of 1:2 is 36.2µm. Also, In the case of nanoparticles synthesized from CuO and *Euphorbia tirucalli* leaf extract mixing ratio 1:4 and 1:6 and the average size of the major axis of the particle size were 45.1µm and 24.3µm respectively (Fig. 2d-f). The EDX profile also confirmed the presence of copper and oxygen in major fractions (Fig. 2d-f).

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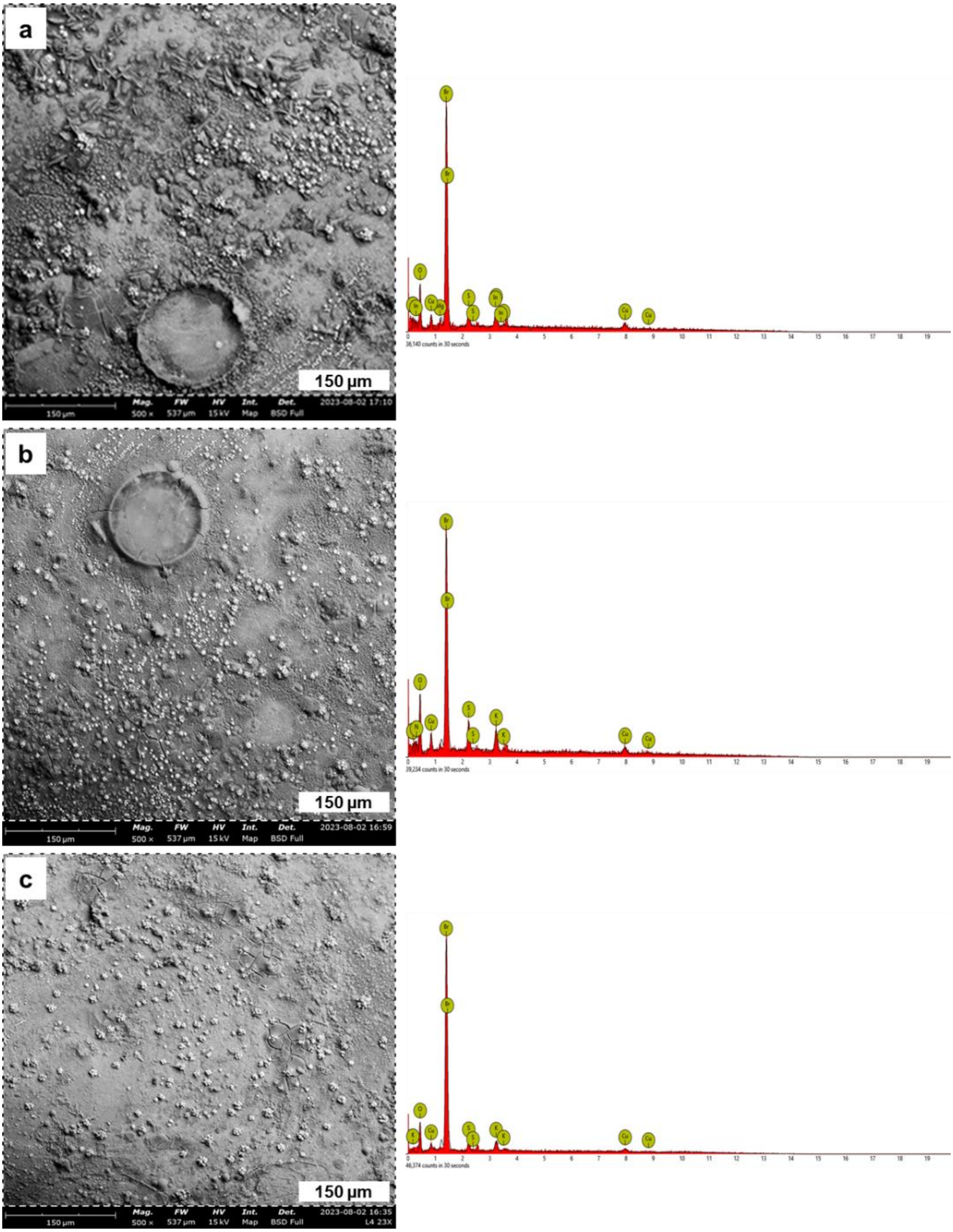

Fig. 1.
SEM and EDX images of nanoparticles synthesized from CuO and Azadirachta indica extracts at the three mixing ratios of 1:2 (a), 1:4 (b), and 1:6 (c).

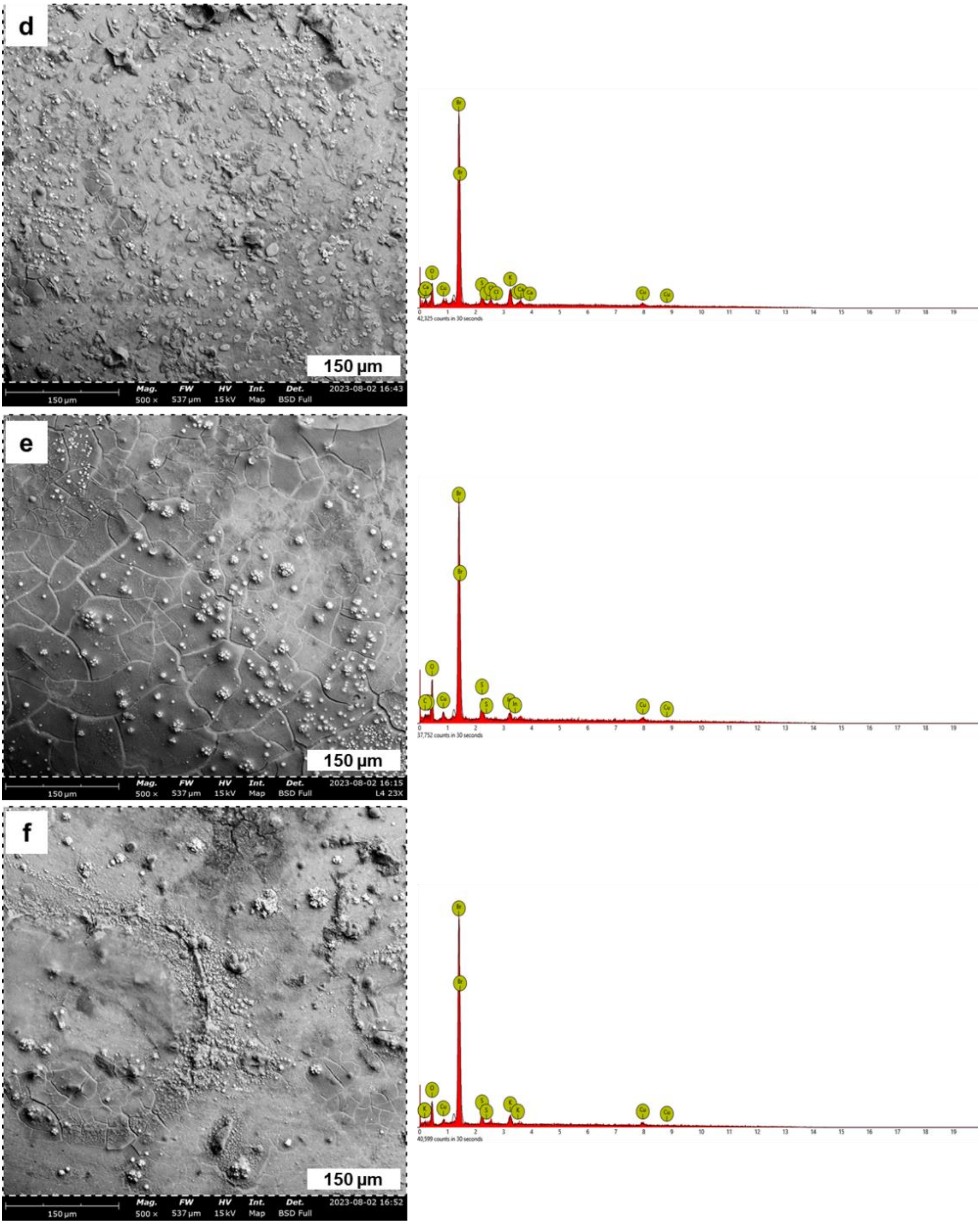

Fig. 2.
SEM and EDX images of nanoparticles synthesized from CuO and Euphorbia tirucalli extracts at the three mixing ratios 1:2 (d), 1:4 (e), and 1:6 (f).

Absorption of synthesis CuO Nanoparticle from E. tirucalli and A. indica

The result of absorption of nanoparticles synthesized from CuO, *E. tirucalli and A. indica* is presented in Table 1 and 2. These results showed that percentage of absorption of the nanoparticles from CuO, *E. tirucalli and A. indica* by the three wood species ranges from 7.29% to 36.23%. *D. oliveri* had the highest percentage of absorption of 36.23% while *A. ferruginea* had the least mean value of 7.29%. The result of the effect of mixing ratios of plant extract with precursor solution of $CuSO4.5H2O$ on percentage of absorption further revealed that mixing ratio 1:4 has the least absorption value of 16.27% while there was not significant difference among the two other mixing ratios with mean absorption of 20.19% to 22.14%.

Table 1

Mean separation of the percentage of absorption (%) of wood species

wood species	Percentage of absorption (%)
A. ferruginea	7.29 ± 3.21^{c}
T. superba	15.99 ± 6.59^{b}
D. oliveri	36.23 ± 12.85^{a}

Table 2

Mean separation of the percentage of absorption (%) of the mixing ratio

Mixing ratio	Percentage of absorption
mixing ratio 1:4	16.27^{b}
mixing ratio 1:6	20.19^{a}
100% plant	20.75^{a}
mixing ratio 1:2	22.14^{a}

Result of ASTM Visual Rating for the nanoparticle preservatives develop from Azadirachta indica and Euphorbia tirucalli

The ASTM visual assessment results from this study indicates that the nanoparticle preservatives develop from CuO, *Azadirachta indica* and *Euphorbia tirucalli* and the various formulations investigated were effective in preventing and/or limiting termite attack on the wood of the three selected wood species compared to the use of 100% plant extract and untreated counterparts as shown in Figu. 3a-c. The result of T. *superba treated* with CuO nanoparticle produce from *E. tirucalli* shows that that termite started to attack wood sample with the mixing ratio1:2, 1:4 and 1:6 at 11th, 8th and 10th week respectively and at the end of the twelve weeks of study had visual rating value of 9.8, 8.2 and 9.6 respectively. The result of *T. superba* treated with CuO nanoparticle produce from *A. indica* shows that that termite started to attack wood sample with the mixing ratio1:2, 1:4 and 1:6 at 6th, 4th and 10th week respectively and at the end of the twelve weeks of study had visual rating value of 8.4, 8.8 and 9 respectively. The result of the other batches of wood sample that serve as control revealed that termites started to attack wood sample treated with 100% *A. indica*, 100% *E. tirucalli* and untreated wood sample at the first week and the end of the twelve week had visual rating value of 0 respectively (Fig. 3a). The result of *D. oliveri* treated with CuO nanoparticle produce from *E. tirucalli* shows that that termite started to attack wood sample with the mixing ratio1:2, 1:4 and 1:6 at 4th, 6th and 3rd week respectively and at the end of the twelve weeks of study had visual rating value of 8.2, 9.2 and 8.8 respectively. The result of *D. oliveri* treated with CuO nanoparticle produce from A. indica shows that that termite started to attack wood sample with the mixing ratio1:2, 1:4 and 1:6 at 4th, 6th and 4th week respectively and at the end of the twelve weeks of study had visual rating value of 8.2, 8.8 and 8.2 respectively. The result of the other batches of wood sample which serve as control revealed that termites started to attack wood sample treated with 100% *A. indica,* 100% *E. tirucalli* and untreated wood sample at the 2nd , 1st and 1st week respectively and the end of the twelve week had visual rating value of 4.6, 1.8 and 0 respectively (Fig. 3b). The result of *A. ferruginea* treated with CuO nanoparticle produce from *E. tirucalli* shows that that termite did not attack all the wood sample with the mixing ratio1:2, 1:4 and 1:6 and at the end of the twelve weeks of study had visual rating value of 10 respectively. The result of *A. ferruginea* treated with CuO nanoparticle produce from *A. indica* shows that that termite started to attack wood sample with the mixing ratio 1:4 and 1:6 at 8th and 5th week respectively and mixing ratio1:2 had no attack for the twelve week. However, at the end of the twelve weeks of study mixing ratio1:4 and1:4 had visual rating value of 9.4 and 9.4 respectively. The result of the other batches of wood sample which serve as control revealed that termites started to attack wood sample treated with 100% *A. indica*, 100% *E. tirucalli* and untreated wood sample at the 2nd, 4th and 1st week respectively and the end of the twelve week had visual rating value of 8.8, 9 and 8 respectively (Fig. 3c). Furthermore, the study established that the extent of the protection varied among species and with the preservatives. With respect to the wood species, *A. ferruginea* performed showed the highest resistance to termites, while wood samples treated with formulation 1:4 of CuO and extracts from the two plant sources recorded the best performance of all the formulation investigated.

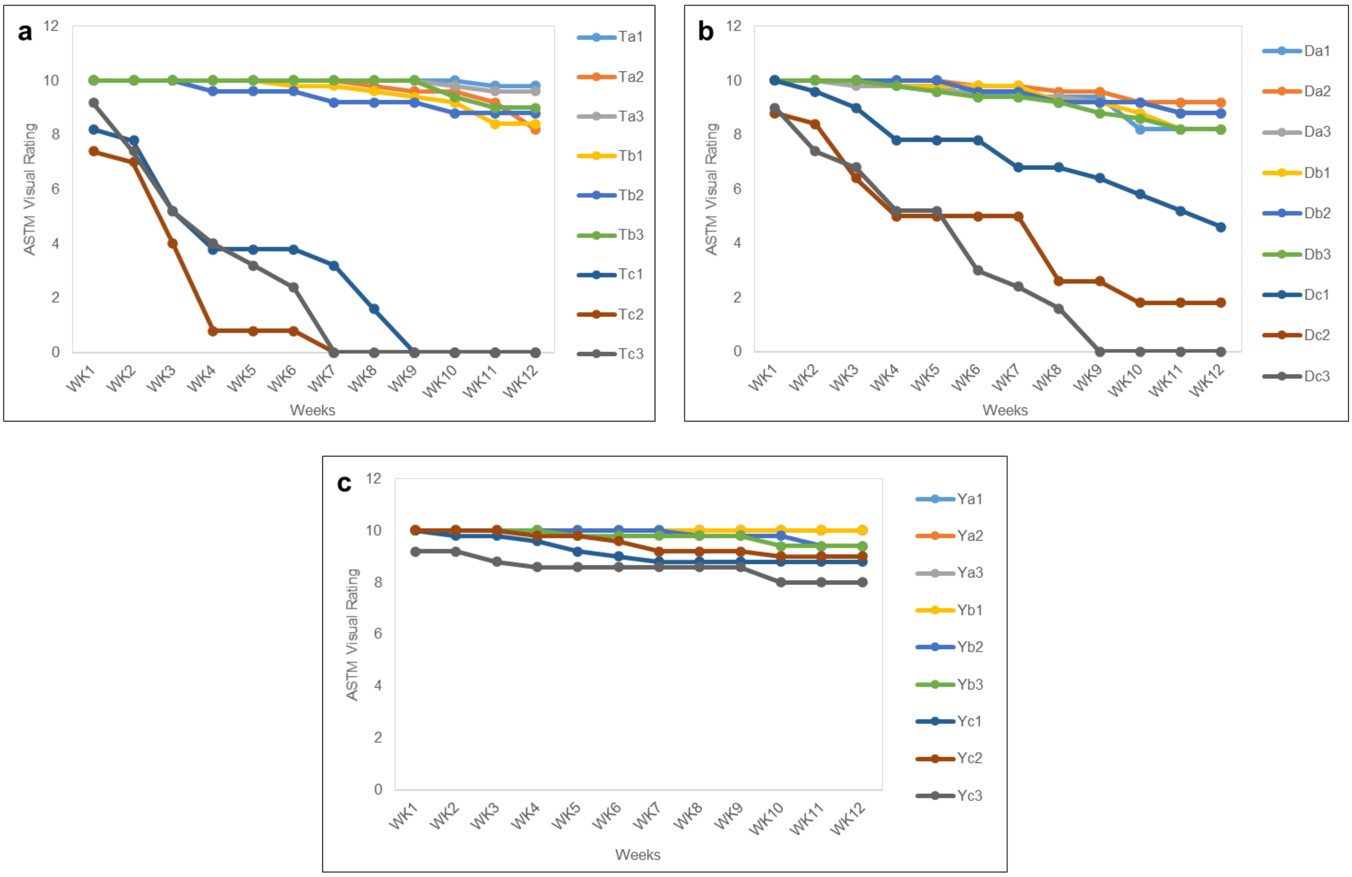

Fig. 3.
ASTM Visual rating of wood samples; T. superba (a), D. oliveri (b), and A. ferruginea (c) treated with different formulation ratios of nanoparticles from CuO and plant extracts. a1, a2 and a3 indicate mixing ratio 1:2, 1:4 and 1:6 and also c1, c2 and c3 indicate mixing ratio 100% A. indica, 100% E. tirucalli and untreated.

Percentage weight loss of synthesis CuO Nanoparticle from *E. tirucalli and A. indica*

The result of the percentage weight loss of wood samples treated with nanoparticles synthesized from CuO, *E. tirucalli and A. indica* is presented in Tables 3 to 5. These results showed that percentage weight loss of the wood species after treatment with nanoparticles from CuO, *E. tirucalli and A. indica* ranges from 6.79% to 39.42%. *D. oliveri* had the highest percentage weight loss of 39.42% while *A. ferruginea* had the least mean value of 6.79%. The outcome of the effect of the formulations of *E. tirucalli* and *A. indica* extract with precursor solution of CuSO4.5H2O on the wood species further revealed that the percentage weight loss for *T. superba,* formulation ratio 1:2 of CuO and *E. tirucalli* had the lowest mean weight loss of 4.85%, while 100% plant extract of *E. tirucalli* and untreated samples recorded the highest weight loss value of 100%. *D. oliveri* treated with formulation ratio 1:6 of CuO and *E. tirucalli* recorded the lowest mean weight loss value of 16.97%, while untreated samples had the highest mean weight loss value of 100%. *A. ferruginea* treated with formulation ratio 1:2 of *CuO and A. indica* had the lowest mean weight loss value of 1.72%, while those treated with 100% plant extract recorded the mean weight loss of 11.62%.

Table 3

Descriptive Statistics of Percentage Weight Loss of synthesised CuO Nanoparticle by E. tirucalli and A. indica

wood species	CuO Nanoparticle source	Formulation ratio	Percentage weight loss		
			species	CuO Nps source	Wood
T. superba	*E. tirucalli*	1:2	4.85±4.20	31.24±41.30	39.40±42.87
		1:4	10.72±11.59		
		1:6	9.39±6.98		
		100% plant	100±0.00		
	A. indica	1:2	13.38±10.62	32.40±38.23	
		1:4	10.87±10.61		
		1:6	10.75±10.75		
		100% plant	94.61±12.02		
	Untreated	Untreated	100±0.00	100±0.00	
D. oliveri	*E. tirucalli*	1:2	29.78±19.81	36.91±34.87	39.42±33.64
		1:4	18.35±7.28		
		1:6	16.97±14.61		
		100% plant	82.53±39.06		
	A. indica	1:2	24.06±18.58	26.79±16.27	
		1:4	15.73±12.68		
		1:6	23.29±12.07		
		100% plant	44.07±7.40		
	Untreated	Untreated	100±0.00	100±0.00	
A. ferruginea	*E. tirucalli*	1:2	5.17±3.07	5.71±5.75	6.79±7.25
		1:4	3.28±2.92		
		1:6	5.14±2.63		
		100% plant	9.25±10.40		
	A. indica	1:2	1.72±0.85	6.74±6.98	
		1:4	6.26±7.46		
		1:6	7.38±10.54		
		100% plant	11.62±1.19		
	Untreated	Untreated	11.29±12.61	11.29±12.61	

Mean ± std

Table 4

Mean separation of wood species and CuO Nanoparticle source

Species	Percentage Weight loss	CuO/extracts Nanoparticles	Percentage Weight loss
A. ferruginea	6.79^{b}	*A. indica*	21.98^{b}
T. superba	39.40^{a}	*E. tirucalli*	24.62^{b}
D. oliveri	39.42^{a}	Untreated	70.43^{a}

Means with the same superscript are not significantly different at ($p \leq 0.05$)

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Table 5

Mean separation of the percentage weight loss of the formulation ratio of plant extract/precursor solution of CuSO4, 5H2O

Formulation ratio	Percentage Weight Loss
mixing ratio 1:4	10.87^c
mixing ratio 1:6	12.15^c
mixing ratio 1:2	13.16^c
100% plant	57.01^b
Untreated	70.43^a

Means with the same superscript are not significantly different at ($p \le 0.05$)

DISCUSSION

Efficacy of nanoparticles from CuO, Azadirachta indica and Euphorbia tirucalli L as wood preservatives

According to this study's findings, the wood species chosen for this work showed the ability to absorb nanoparticles formulated from CuO and plant extracts (Table 1). T. superba and D. oliveri had the highest absorption proportion, whereas A. ferruginea absorbed relatively little of it. This could be as a result of the different densities of the wood species employed for the study. This variation in absorption by the wood species may be related to their porosities. According to Shiran and Liping (1993), wood density has a direct linkage to its porosity, and then it is logical to say that pore volumes available for absorption determines the level pf uptake. In low density species, absorption may be higher due to numerous pore spaces available, whereas in higher density wood species limited pore spaces may result to low absorption. Aside from the effect of density, the anatomical properties of the wood species may also influence their absorption. It has been found that even in low density wood species, absorption may be low if there are presence of tyloses and other occluding crystals in the vessels and also the ray parenchyma cells (Olaniran *et al*. 2022). Thus, it is possible for wood species of low density to experience low absorption depending on their anatomical properties. The use of plant extracts is highly advantageous in wood preservation since they readily available, renewable, efficient, quick, and easy to process (Iravani 2011; Vijayaraghavan and Ashokkumar 2017). The protection provided by nanoparticles formulated from CuO, E. tirucalli and A. indica in this study was demonstrated by the resulting weight loss of wood species. It was found that wood species treated with nanoparticles from CuO and plant extracts of E. tirucalli and A. indica recorded weight loss values of 21.98% and 24.62% respectively, compared to the untreated samples and those treated with only plant extracts which recorded weight loss values of 70.43% and 57.01% respectively. According to Shiny et al. (2019), the effectiveness of the synthesized plant extract and copper oxide nanoparticle formulations is showing promising results, and studies are ongoing to create a stable and environmentally friendly wood preservative formulation of metal nanoparticles and plant extracts. Furthermore, Akhtari and Nicholas (2013) found that all formulations of CuO and ZnO tested were successful in preventing termites from destroying wood, with copper marginally outperforming zinc and micronized copper outperforming amine copper. The outcome of our investigation also showed that termite resistance to attack on wood treated with the nanoparticles from CuO and two sources of plant extracts and their formulation ratios were the same. This is evident from the non-significance of the three formulation ratios on weight loss of selected wood species after treatment. This implies that any of these formulations can be used in the treatment of wood species for adequate protection from termites.

CONCLUSION

Nanoparticles formulated from CuO, A. indica leaf and E. tirucalli stem extracts were used to have proven to be efficient as a wood preservative. For a period of twelve weeks, it was discovered that every formulation of nanoparticles from plant extracts and CuO adequate protection against termites during the field tests. These formulations could offer great solutions in the production of environmentally friendly bio-preservatives compared to the presently used chemicals which are now under some restrictions due to human toxicity and negative consequences they have on the environment.

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