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Synergism of three botanical termiticides as wood protectants against subterranean termites, *Macrotermes subhyalinus* (Rambur, 1842)

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Abstract

Background: The increased interest in the harmful effects of most chemical pesticides on the ecosystem has continually served as an impetus to search for safer and eco-friendly pesticides from plant origin. In this study, the termiticidal potentials of extract mixtures of *Azadirachta indica* (A. Juss.), *Nicotiana tabacum* (L.), and *Jatropha curcas* (L.) against *Macrotermes subhyalinus* (Rambur, 1842) infesting *Triplochiton scleroxylon* (K. Schum) wood blocks were investigated in the field (open and under shade) and laboratory conditions. Weight loss in wood blocks, level of wood damage, and termite mortality were used as indices of wood protection potential of the botanical mixtures. The level of repellent ability of the extracts mixture was also determined. For the laboratory bioassays, ten termites (worker/soldier) were used per treatment and each treatment was replicated thrice. Profile of components of the three mixtures was also obtained using head space–solid-phase micro-extraction, gas chromatography–mass spectrophotometry (HS-SPME, GC-MS) analysis.

Results: Extracts of *A. indica* plus *N. tabacum* achieved 100% mortality of worker within 4 h while those of *N. tabacum* plus *J. curcas* and *A. indica* plus *N. tabacum* plus *J. curcas* achieved 100% mortality of termites at 6 h post-treatment. Also, extract of *A. indica* plus *N. tabacum* and *A. indica* plus *N. tabacum* plus *J. curcas* evoked 100% mortality of soldier termites at 6 h. Termites exposed to *N. tabacum* plus *J. curcas* for 1, 2, 3, and 4 h were the most repelled at 73, 87, 73, and 73%, respectively. The extract of *J. curcas* plus *A. indica* plus *N. tabacum* offered the highest protection against termite damage in the open field (6.17%). The botanicals were ineffective under shade. Insecticidal compounds like (S)-3-(1-Methyl-2-pyrrolidinyl) pyridine; Methyl ester, Hexadecanoic acid; (Z, Z)-9, 12-Octadecadienoic acid; Anthracene; 2-Hydroxy-Cyclopentadecanone; and n-Hexadecanoic acid were found in the extracts.

Conclusion: These results suggest that the botanical mixtures could confer some protection against termites. Also, the knowledge about the components and varied level of potency under different conditions may be vital in developing biorationals against *M. subhyalinus*.

Keywords: Antifeedant, *Azadirachta indica*, *Jatropha curcas*, *Nicotiana tabacum*, Termiticidal

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Background

Termites (Order: Isoptera) are cosmopolitan groups of social insects mostly found in the tropics, sub-tropics, and temperate regions of the world (Su & Scheffrahn, 2000). Presently, there are close to 3000 named termite species in 282 genera worldwide, with about 1000 species in Africa (Kambhampati & Eggleton, 2000; van Huis, 2017). The types commonly found in Western Africa include *Ancistrotermes*, *Cubitermes* (Termitidae), *Odontotermes*, *Microtermes*, and *Macrotermes* (Abdurahaman, 1990; Mitchell, 2002). In spite of their beneficial role in increasing the soil fertility through the mixing of soil organic matter, termites are best described as pests of most plant products due to their deleterious activities on most timbers, agricultural crops, and wooden materials in buildings (Culliney & Grace, 2000; Ekhuemelo, Abu, & Anyam, 2017; Lax & Osbrink, 2003). In fact, the infamous activities of termites on plant products, especially wooden structures, have been attributed to their strong affinity for ligno-cellulolytic materials in wood (Ajayi, 2012). In cases of severe infestations, their activities could result in the loss of structural strength of buildings. The damage caused by termites alone is reported to be more than the combined annual destruction caused by fires, tornadoes, and earthquakes in monetary terms (Ahmed & French, 2005; Isman, 2006). Consequently, termites are known to cause losses worth billions of dollars annually in many countries of the world (Olaniyan, Ibikunle, Olayanju, Olagoke, & Olawoore, 2015).

In sub-Sahara Africa, *Macrotermes subhyalinus* (Rambur) is one of the commonest and most damaging termite species in agro-forestry areas (Pearce, 1997). This species of African subterranean termite has been reported in Nigeria by Ajayi (2012) as well as Ajayi, Adedara, and Oyeniyi (2018). *M. subhyalinus* was reported to be capable of causing total destruction of crops and wooden structures within few months under favorable climatic conditions due to their voracious feeding behavior (Malaka, 1996; Nyeko & Olubayo, 2005). In an effort aimed at tackling the various menace associated with termite infestation on wooden structures, pest managers have relied heavily on the application of synthetic termiticides to the structural peripherals, especially in large-scale control. However, due to the increased awareness on the various harmful effects of most synthetic termiticides on human and environmental health, termite control in most developing nations is currently being focussed on the use of non-chemical methods, including the use of plant-based termiticides (Kasseney, Nyamador, Mondedji, Ketoh, & Glitho, 2016; Rust, 2014).

In the last few decades, botanicals and its products have played an integral role as suitable substitute to most synthetic insecticide in the management of various insect pests. Several plants species have therefore been investigated for their anti-termite potentials under laboratory and field conditions. Most of the plants were observed to exhibit toxic, repellent, and antifeedant properties against several species of termite (Acda, 2014; Ajayi et al., 2018; Osipitan, Jegede, Adekanmbi, & Ogunbanwo, 2013; Raina, Bedoukian, Florane, & Lax, 2012). For instance, *Azadirachta indica* (A. Juss.), *Nicotiana tabacum* (L.), and *Jatropha curcas* (L.) have been established to possess some termiticidal properties (Ekhuemelo et al., 2017; Shiberu, Ashagre, & Negeri, 2013; Singh & Sushilkumar, 2008). But, a detailed study that involved the combination of extracts from *A. indica*, *N. tabacum*, and *J. curcas* for the control of *M. subhyalinus* remained to be explored. The insecticidal potency of any plant usually depends on the quantity and nature of bioactive compounds in such plant. There is possibility of having a synergistic or antagonistic effect on the efficacy of these botanicals against insect pests if mixed together due to possible chemical reactions between the components of different plants in the mixture. Consequently, mixing of these three plants as termiticides could either increase or reduce their potency against termites. Thus, this study seeks to screen mixtures of *N. tabacum*, *A. indica*, and *J. curcas* for their bioactivity against *M. subhyalinus* infesting wooden structures in Nigeria. This is aimed at achieving a botanical termiticide with higher efficacy against *M. subhyalinus* infesting wooden structures.

Materials and methods

Study location

This study was carried out within the premises of the Federal University of Technology, Akure (FUTA). It is located at latitude 7.2972° N and longitude 5.1461° E of the equator, and situated at elevation of 353 meters above sea level (Owoeye & Ibitoye, 2016).

Termite culture

Termites were harvested majorly from a large open mound equipped with a funnel-like large ventilation shafts near the study area. Some were however harvested from surface termite tunnels at night when they forage for food as well as infested logs of wood. The harvested termites in the study area had earlier been identified as *Macrotermes subhyalinus* (Rambur) in a study conducted by Ajayi et al. (2018). Dark chambers were constructed using the method of Ali, Ahmed, Sheridan, and French (2016) with some modifications. The chambers were constructed with wood in the dimension of 0.5 m × 0.5 m × 1 m (length × width × height). Black polythene materials was used to line the inner parts of the chambers, while perforated black polythene was used to cover the upper part to ensure efficient gaseous

exchange. The chambers contained layers of moist soil up to 5 cm to mimic the natural environment of the termites. Termites were kept in dark chambers in the laboratory throughout the duration of the experiments.

Sourcing and preparation of wood sample

Triplochiton scleroxylon (K. Schum) was used as substrate wood for this experiment. It was obtained from Ibitoye Sawmill that situates at Road-block area, Akure, Ondo State. *Triplochiton scleroxylon* was used as substrate due to previous records of its high susceptibility to insect pests (Louppe, 2008; Owoyemi, Adiji, & Aladejana, 2017). The wood was cut into a dimension of 4 cm × 4 cm × 6 cm (width × height × length) at the Department of Forestry and Wood Technology, Federal University of Technology, Akure (FUTA). The wood blocks were then oven-dried to a constant weight at 104 °C for 24 h (ASTM, 2003).

Collection of plant materials

Leaves of tobacco—*Nicotiana tabacum*, Neem—*Azadirachta indica* and seeds of Physic nut—*Jatropha curcas* were sourced locally from different locations in Akure, Ondo State, Nigeria. Each plant part was separately air-dried under ambient (26 ± 2 °C, 70 ± 5% rh) conditions in Laboratory III of Biology Department, FUTA till their moisture contents was reduced to approximately 12%. Moisture content was determined using oven dried method. The percentage moisture content was estimated using the formula below:

$$MC = \frac{W_1 - W_2}{W_2} \times \frac{100}{1}$$

Where
W_1 = Initial weight
W_2 = Oven-dry weight (ASTM, 2003)

The plant parts were then separately pulverized using electric blender (Marlex grinder, Model Excella) before being stored in air-tight plastic containers and kept inside refrigerator (4 °C) for further studies. Each plant material was labeled separately to avoid mix-up.

Preparation of plant extracts

Aqueous extracts of each plant material used in this study were prepared according to the method of Ajayi et al. (2018) with little modifications. Powders of each of the three plant parts weighing 10 g were properly admixed together in dual (ratio 1:1) and tri-combinations (ratio 1:1:1). Each of the mixture was later soaked in 100 mL of distilled water and left to infuse for 24 h. The aqueous extracts obtained from each of the mixture were well-labeled and kept inside the refrigerator (4 °C) for further studies.

Bioassay procedures

Laboratory experiment using impregnated filter paper techniques was used to evaluate acute toxicity and repellence properties of the botanical mixtures against *M. subhyalinus* while field experiments in open landscape and under shade conditions were used to assess the level of wood damage by termites.

Toxicity test on termites

Impregnated filter paper technique, described in FAO method 15 (Anonymous, 1974) was modified for termite toxicity test in this study. A whole 9 mm Whatman No. 1 filter paper was impregnated with aqueous extracts of plant materials prepared and placed in Petri dishes with an underlay of thin layer of moist soil. Impregnation of filter paper was done by dipping the paper into the extracts for 10 s and then quickly placed in labeled Petri dishes. Ten termites (worker/soldier) were then placed on the treated wet filter papers before the dishes were covered. Number of dead termites was recorded at 1-h intervals for 6 h. Filter paper dipped in distilled water served as control. Each treatment was replicated three times.

Repellent test on termites

One half of filter paper was immersed in extracts of mixtures of the plants and the other half was immersed in distilled water. The two treated half filter papers were placed in Petri dishes that were 2 cm apart on underlay of moist soil. The filter papers were separated with plastic inhibitory bars to prevent termites from moving to either side. Ten worker termites were introduced into the 2 cm space between the two halves of treated filter paper and left for 5 min before removing the plastic inhibitory bars. The setup was checked at 1-h intervals for 4 h. All experiments were replicated three times.

The formula: [PR = 2(C − 50)] (Talukder & Howse, 1993) was used to determine repellency, where PR = percentage repellency and C = percentage of termite on control half of the filter paper. Same procedure was repeated for soldier termites.

Field trial on termites

The wood protection ability of the aqueous extracts was assessed in the open field landscape and under the shade. Termite infestation on treated wood blocks was assessed using weight loss and visual rating. The treatment of the wood blocks was done based on the methods described by Syofuna, Banana, and Nakabonge (2006) with little modifications. The wood blocks were treated with extracts of mixtures of *A. indica*, *N. tabacum*, *J. curcas*, as well as ordinary distilled water (control) and each treatment was replicated four times. The wood blocks were soaked for 24 h in the extracts of

mixture of the three plants and were positioned on termite-infested ground. A distance of 30 cm was maintained between treatments while 10 cm was maintained between replicates of the same treatment (ASTM, 2003). The wood blocks were left in the field for 12 weeks. Each wood block was observed weekly for signs of termite infestation. Visual rating was done according to the method described by Pearce (1997) and modified by Syofuna et al. (2006). After the lapse of the 12 weeks, remnants of the wood blocks were removed, rinsed under a stream of water, and oven-dried at 104 °C to approximately 12% moisture contents. Final weight of each wood block was recorded. The damage done to each wood block was determined by recording weight loss.

Head space–solid-phase micro-extraction, gas chromatography–mass spectrophotometry analysis of the botanical mixtures

The profile of compounds in the botanical mixture of the plant parts was evaluated using the method of Ajayi et al. (2018) with little modifications. The phytochemical constituents of the dual (*A. indica* + *N. tabacum* and *N. tabacum* + *J. curcas*) and tri-combinations (*A. indica* + *N. tabacum* + *J. curcas*) of powders of the three plant parts was done using head space–solid-phase micro-extraction, gas chromatography-mass spectrometry (HS-SPME, GC-MS) analysis. All the analyses were carried out in the Laboratory of Organic Chemistry, Faculty of Pharmaceutical Science of University of Sao Paulo, Black Ribeirao, Brazil. Powders of each botanical mixture was further ground with an electric blender (model number: QBL-18L40; 350 W) until it passed through a sieve with mesh size of 50 μm before being utilized for the HS-SPME step. Powder weighing 60 mg from each botanical mixture was sealed in 2 mL vials and stored at – 18 °C for 2 h before HS-SPME sampling. The SPME device was inserted into the sealed vial containing the plant sample and the fiber was exposed to the headspace (equilibration time: 15 min; temperature: 50 °C; sampling time: 120 min). After sampling, the SPME device was immediately inserted into the GC injector and the fiber was thermally desorbed for 15 min at 230 °C. Analyses were carried out on an Agilent GC-6890 Plus system coupled to a 5973 MSD (Little Falls, DE, and USA). For HS-SPME-GC-MS analysis, the injector was operated in the splitless mode at 230 °C for 5 min and thereafter split in ratio 1:10. The chromatographic conditions were column, HP—5MS (25 m × 0.25 mm i.d., 0.3 mm film thickness; Agilent); oven temperature programme from 40 °C (held for 3 min) to 125 °C (0 min) at 4 °C min^{-1}, then at 1.5 °C min^{-1} to 140 °C (held for 4 min) and finally at 5 °C min^{-1} to 250 °C (held for 5 min); carrier gas was Helium at a flow rate of 1.0 mL min^{-1} in constant flow mode. Interface temperature was 280 °C

and the MS were obtained in the electron impact mode at 70 eV, scan mode, range *m/z* 35–350 at a rate of 2.36 scans s^{-1}. Data were acquired in a PC system using software ChemStation G1701CA (Agilent). Tentative identification of the compounds in each botanical mixture was done by comparing their spectra with mass spectral databases of National Institute of Standards and Technology (NIST, 2011).

Data analysis

Mortality data as well as data on repellency of botanicals were converted to percentages. The results were later represented on charts using Microsoft Excel (Microsoft Office 2013). Student *T* Test was used to compare damage to treated wood under shade and in open field. The data on weight loss was subjected to one-way analysis of variance (ANOVA) (Akinneye & Oyeniyi, 2016). Tukey's HSD test was used to separate significant means of the data obtained from non-significant means at α = 0.05. All analyses were done using the Statistical Package for Social Sciences (SPSS) Version 19.0.

Results

Toxicity of botanical mixture to *M. subhyalinus*

All the extracts of botanical mixtures evoked varied level of termite mortality which increased with increase in exposure time. In the test on worker caste of *M. subhyalinus*, extract of *Azadirachta indica* plus *Nicotiana tabacum* achieved complete mortality (100%) within 4 h exposure (Fig. 1). The mixture of *N. tabacum* plus
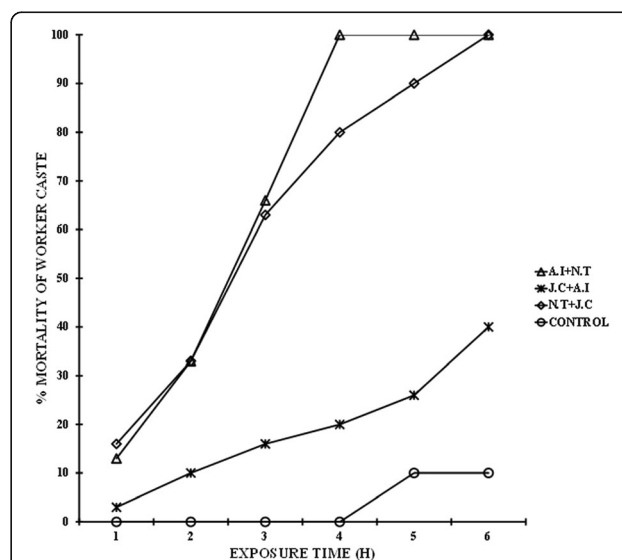

Fig. 1 Percentage mortality of worker caste of *Macrotermes subhyalinus* on filter papers treated with bi-mixed aqueous extracts. A.I+N.T = *Azadirachta indica* plus *Nicotiana tabacum*. J.C+A.I = *Jatropha curcas* plus *Azadirachta indica*. N.T+J.C = *Nicotiana tabacum* plus *Jatropha curcas*

Jatropha curcas (Fig. 1) as well as *A. indica* plus *N. tabacum* plus *J. curcas* (Fig. 2) achieved 80 and 83% mortality at 4 h, respectively; but at 6-h exposure, both mixtures achieved 100% mortality. In contrast, extract of *J. curcas* plus *A. indica* was the least effective against workers caste due to the lowest mortality of 20% at 4 h and 40% at 6 h (Fig. 1). Similarly, with soldier caste, the combination of *J. curcas* plus *A. indica* was the least effective of the extract mixture with the lowest mortality of 6% at 2 h and 46% at 6 h (Fig. 3). Extract of *N. tabacum* plus *J. curcas* achieved 52 and 92% mortality at 2 and 6 h respectively while the extracts of *A. indica* plus *N. tabacum* and *A. indica* plus *N. tabacum* plus *J. curcas* achieved 100% mortality of soldier caste of *M. subhyalinus* at 6 h (Fig. 4).

Repellency of botanical mixtures to *M. subhyalinus*

There were more termites on the half of the filter papers treated with combination of *J. curcas* plus *A. indica* extract and this resulted in repellency of – 47, – 20, – 47, and – 47% of termites at 1, 2, 3, and 4 exposure hours, respectively, compared with the corresponding half that was treated with distilled water only. In contrast, 33, 33, 47, and 47% of termites were repelled by extract of *A. indica* plus *N. tabacum* at 1, 2, 3, and 4 h, respectively. The mixture of *J. curcas* plus *A. indica* plus *N. tabacum* repelled 60, 20, 47, and 47% of termites at 1, 2, 3, and 4 h respectively. However, the mixture of *N. tabacum* plus *J. curcas* achieved the highest repellency with 73, 87, 73, and 73% termites repelled at 1, 2, 3, and 4 h, respectively (Fig. 5).
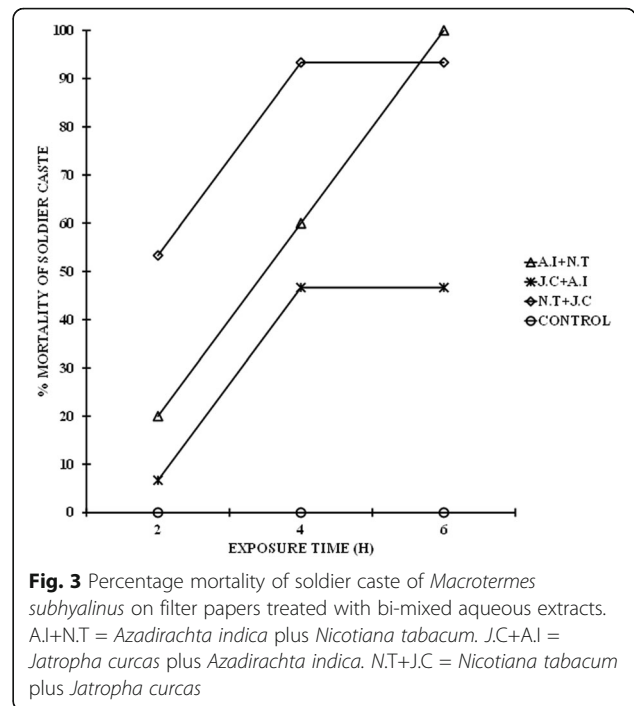

Fig. 3 Percentage mortality of soldier caste of *Macrotermes subhyalinus* on filter papers treated with bi-mixed aqueous extracts. A.I+N.T = *Azadirachta indica* plus *Nicotiana tabacum*. J.C+A.I = *Jatropha curcas* plus *Azadirachta indica*. N.T+J.C = *Nicotiana tabacum* plus *Jatropha curcas*

Visual rating of extract-treated wood blocks

After 12 weeks, no visible damage was observed on the control wood blocks treated with distilled water under the shaded environment (Fig. 6). Among the extract treated wood blocks, the mixture of *N. tabacum* plus *J. curcas* offered the highest protection due to the least visible damage by termites (33.3%). The highest damage was however observed in wood blocks treated with the
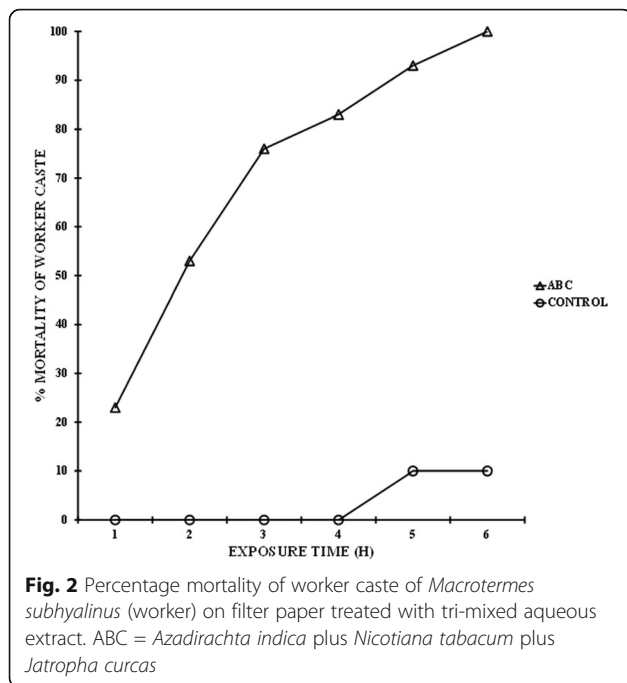

Fig. 2 Percentage mortality of worker caste of *Macrotermes subhyalinus* (worker) on filter paper treated with tri-mixed aqueous extract. ABC = *Azadirachta indica* plus *Nicotiana tabacum* plus *Jatropha curcas*

Fig. 4 Percentage mortality of soldier caste *Macrotermes subhyalinus* (worker) on filter paper treated with tri-mixed aqueous extract. ABC = *Azadirachta indica* plus *Nicotiana tabacum* plus *Jatropha curcas*
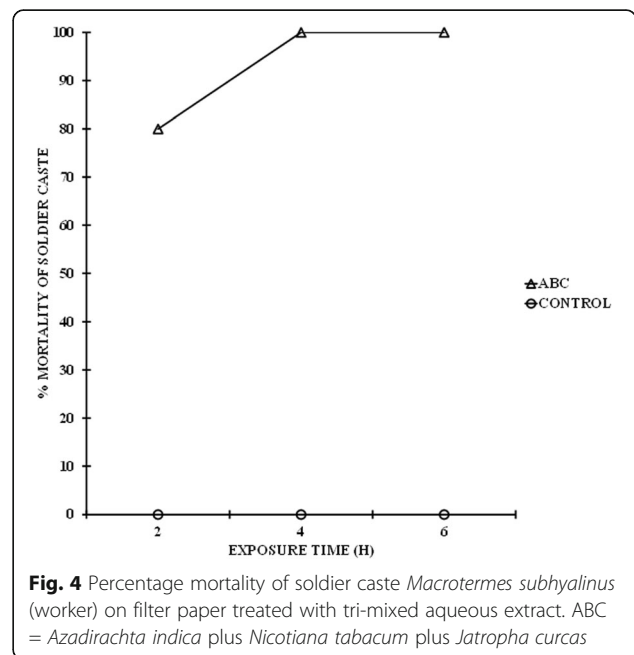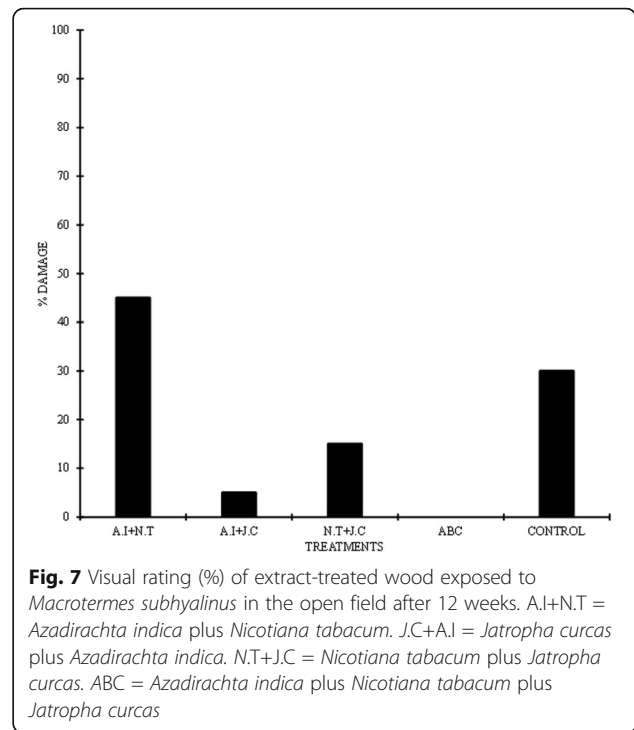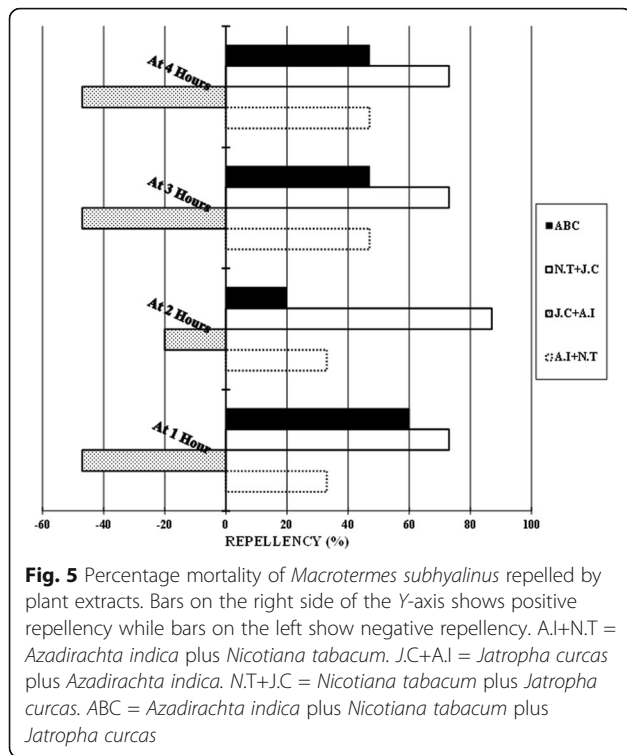
Fig. 5 Percentage mortality of *Macrotermes subhyalinus* repelled by plant extracts. Bars on the right side of the *Y*-axis shows positive repellency while bars on the left show negative repellency. A.I+N.T = *Azadirachta indica* plus *Nicotiana tabacum*. J.C+A.I = *Jatropha curcas* plus *Azadirachta indica*. N.T+J.C = *Nicotiana tabacum* plus *Jatropha curcas*. ABC = *Azadirachta indica* plus *Nicotiana tabacum* plus *Jatropha curcas*


Fig. 7 Visual rating (%) of extract-treated wood exposed to *Macrotermes subhyalinus* in the open field after 12 weeks. A.I+N.T = *Azadirachta indica* plus *Nicotiana tabacum*. J.C+A.I = *Jatropha curcas* plus *Azadirachta indica*. N.T+J.C = *Nicotiana tabacum* plus *Jatropha curcas*. ABC = *Azadirachta indica* plus *Nicotiana tabacum* plus *Jatropha curcas*

mixture of *A. indica* plus *J. curcas* (Fig. 6). On the contrary, wood blocks treated with the mixture of the three extracts in the open field offered complete protection because no visible damage was reported on them (Fig. 7). The mixture of *A. indica* plus *J. curcas* also protected

the wood blocks better (5.0%) when compared to wood blocks treated with distilled water (30%), *N. tabacum* plus *J. curcas* (15.0%) and *A. indica* plus *N. tabacum* (45.0%).
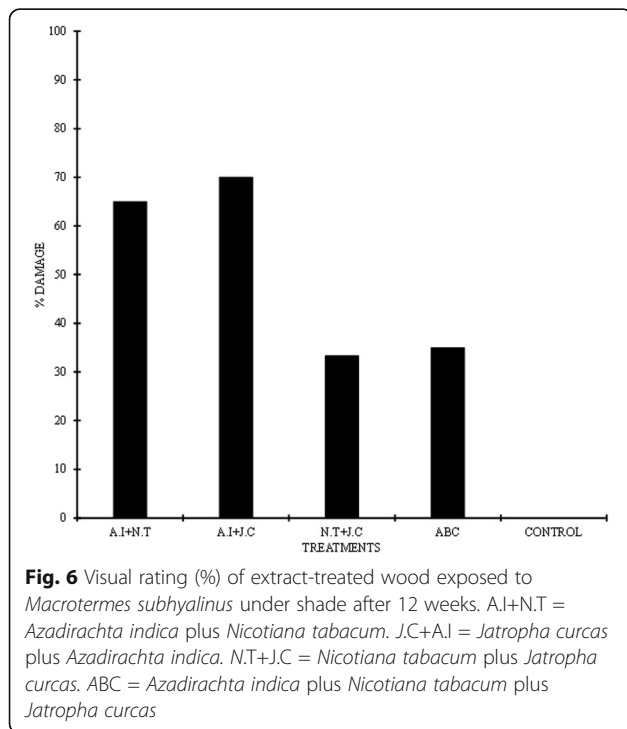
Weight loss in extract-treated wood blocks

The weight loss in extract-treated wood blocks under shade and in the open field is presented in Table 1. Wood blocks under the shade treated with extract of *N. tabacum* plus *J. curcas* had the highest weight loss (50.35%) that was significantly different ($F_{4, 15} = 57.950$; $p < 0.001$) from the weight loss recorded in other treated wood blocks. The weight loss in wood blocks treated with extract of *A. indica* plus *N. tabacum* (43.05%) and *A. indica* plus *J. curcas* (43.80%) were not significantly different ($F_{4, 15} = 57.950$; $p = 0.996$). The lowest weight

Fig. 6 Visual rating (%) of extract-treated wood exposed to *Macrotermes subhyalinus* under shade after 12 weeks. A.I+N.T = *Azadirachta indica* plus *Nicotiana tabacum*. J.C+A.I = *Jatropha curcas* plus *Azadirachta indica*. N.T+J.C = *Nicotiana tabacum* plus *Jatropha curcas*. ABC = *Azadirachta indica* plus *Nicotiana tabacum* plus *Jatropha curcas*

Table 1 Percentage weight loss (Mean ± S.E) in extract-treated wood exposed to subterranean termites under shade and in the open field

Treatment	Shaded environment	Open environment
	Mean weight loss (%)	Mean weight loss (%)
A.I + N.T	43.05 ± 1.29c	43.81 ± 1.32d
A.I + J.C	43.80 ± 1.29c	13.10 ± 1.89ab
N.T + J.C	50.35 ± 1.75d	17.92 ± 1.71bc
ABC	36.42 ± 0.65b	6.17 ± 1.65a
Control	21.29 ± 1.93a	22.71 ± 1.58c

Mean values followed by the same lower-case letter(s) down the column are not significantly different at α = 0.05 using ANOVA and Tukey's test

Table 2 Student *t* test comparing weight loss in extract-treated wood exposed to subterranean termite under shade and in the open field

Treatment	Pair	Mean ± S.E	N	SD	df	t.cal	Sig.	Remarks
A.I + N.T	Shade	43.05 ± 1.29	4	2.58	6	− 0.41	0.70	NS
	Open	43.81 ± 1.32	4	2.65	6			
A.I + J.C	Shade	43.80 ± 1.29	4	2.58	6	13.43	0.00	S
	Open	13.10 ± 1.89	4	3.77	6			
N.T + J.C	Shade	50.35 ± 1.75	4	3.50	6	13.26	0.00	S
	Open	17.92 ± 1.71	4	3.42	6			
ABC	Shade	36.42 ± 0.65	4	1.29	6	17.06	0.00	S
	Open	6.17 ± 1.65	4	3.30	6			
Control	Shade	21.29 ± 1.93	4	3.86	6	− 0.57	0.60	NS
	Open	22.71 ± 1.58	4	3.16	6			

A.I+N.T = *Azadirachta indica* plus *Nicotiana tabacum*
J.C+A.I = *Jatropha curcas* plus *Azadirachta indica*
N.T+J.C = *Nicotiana tabacum* plus *Jatropha curcas*
ABC = *Azadirachta indica* plus *Nicotiana tabacum* plus *Jatropha curcas*

loss of 36.42% was recorded in wood blocks treated with the mixture of the three botanicals (*N. tabacum* plus *A. indica* plus *J. curcas*). Interestingly, the control setup had the least weight loss (21.29%) which was significantly different ($F_{4, 15} = 57.950$; $p < 0.001$) from the weight loss of other treatments in shaded environment.

In the open field experiment, the wood blocks treated with extract of *A. indica* plus *N. tabacum* had the highest weight loss (43.81%) which was significantly different ($F_{4, 15} = 75.658$; $p < 0.001$) from the weight loss of other treatments including control. However, the weight loss in woods treated with extract of *A. indica* plus *J. curcas* (13.10%) was not significantly different ($F_{4, 15} = 75.658$; $p = 0.279$) from the weight loss in woods treated with extract of *N. tabacum* plus *J. curcas* (17.92%). Woods treated with extracts mixtures of the three botanicals had the lowest weight loss (6.17%).

Student *T* test revealed no significant difference between the weight loss in woods treated with extract of

A. indica plus *N. tabacum* (t (4) = − 0.41, $p = 0.70$) and the control (t (4) = − 0.57, $p = 0.60$) in both open field and under shade experiment (Table 2). However, the weight loss in woods treated with extract of *J. curcas* plus *A. indica* (t (4) = 13.43), *N. tabacum* plus *J. curcas* (t (4) = 13.26), and *A. indica* plus *N. tabacum* plus *J. curcas* (t (4) = 17.06) in both environments were significantly different from each other ($p < 0.001$).

Profile of compounds in the mixtures of *A. indica* + *N. tabacum*, *N. tabacum* + *J. curcas*, and *N. tabacum* + *J. curcas* + *A. indica*

Ten compounds were identified in the profile of the mixture of *A. indica* plus *N. tabacum* as shown in Table 3. Hexadecanoic acid, Methyl ester had the highest abundance of 46.39% and this was followed by (S)-3-(1-Methyl-2-pyrrolidinyl) pyridine with 16.02%. Twelve compounds were revealed in profile of the mixture of *N. tabacum* plus *J. curcas* as shown in Table 4. The most abundant compound was (Z,Z)-9,12-Octadecadienoic acid (37.249%), followed by cis-13-Octadecenoic acid with 23.672%. Table 5 showed that nine compounds were present in the mixture of *N. tabacum* plus *J. curcas* plus *A. indica*. The compound with the most abundance was Fluoranthene (49.20%) and this was followed by Pyrene with 18.70%. The chromatogram of chemical profile of various compounds in different mixtures of botanical extract is shown in Figs. 8, 9, and 10.

Discussion

The ability of various mixtures of the botanical extracts to elicit termite mortality showed that all the plant extracts possesses termiticidal properties. This is in agreement with earlier studies by several scholars where the termiticidal efficacies of all the botanical extracts used in this study were reported (Ekhuemelo et al., 2017; Epilla & Ruyooka, 1988; Gold, Whiteman, & Pimbert, 1991; Shiberu et al., 2013; Singh & Sushilkumar, 2008). However, the rate of termite mortality depends on the nature

Table 3 Compounds identified in mixture of *Azadirachta indica* plus *Nicotiana tabacum*

RT (min)	Name of compounds	CAS No.[a]	% of total peak area
9.736	(S)-3-(1-Methyl-2-pyrrolidinyl) pyridine	000054-11-5	16.021
9.759	2-(1-methyl-2-pyrrolidinyl)-Pyridine	023950-04-1	4.993
9.782	(S)-3-(1-Methyl-2-pyrrolidinyl) pyridine	000054-11-5	6.500
16.642	12-Methyl-E, E-2, 13-octadecadien-1-ol	1000130-90-4	2.701
35.677	Hexadecanoic acid, Methyl ester	000112-39-0	46.390
20.024	trans-13-Octadecenoic acid	000693-71-0	7.780
20.041	Oleic Acid	000112-80-1	3.534
20.276	13-methyl-, Oxacyclotetradecane-2, 11-dione	074685-36-2	8.181
20.322	cis-Vaccenic acid	000506-17-2	3.899

[a]Chemical abstracts service registry number

Table 4 Compounds identified in mixture of *Nicotiana tabacum* plus *Jatropha curcas*

RT (min)	Name of compounds	CAS No.[a]	% of total peak area
9.536	(S)-3-(1-Methyl-2-pyrrolidinyl) pyridine	000054-11-5	18.866
16.642	Neophytadiene	000504-96-1	2.726
17.850	Methyl ester, Hexadecanoic acid	000112-39-0	2.253
18.874	n-Hexadecanoic acid	000057-10-3	1.730
18.920	n-Hexadecanoic acid	000057-10-3	0.907
18.948	n-Hexadecanoic acid	000057-10-3	0.659
18.994	n-Hexadecanoic acid	000057-10-3	0.470
20.007	2-Methyl-Z,Z-3,13-octadecadienol	1000130-90-5	0.661
20.030	Methyl ester, trans-13-Octadecenoic acid	1000333-61-3	0.403
20.110	2-Hydroxy-Cyclopentadecanone	004727-18-8	0.018
20.259	Oleic Acid	000112-80-1	0.267
20.276	2,6-dihexadecanoate l-(+)-Ascorbic acid	028474-90-0	0.084
20.310	n-Hexadecanoic acid	000057-10-3	0.076
21.328	(Z,Z)-9,12-Octadecadienoic acid	000060-33-3	37.249
21.374	cis-13-Octadecenoic acid	013126-39-1	23.672
21.546	(Z)-9,17-Octadecadienal	056554-35-9	9.957

[a]Chemical abstracts service registry number

of plant in the botanical mixture, exposure time, and caste of the termite.

The admixture of *N. tabacum* with either *A. indica* or *J. curcas* have a synergistic impact on the efficacy of *A. indica* or *J. curcas* as a termiticide, especially at the highest exposure time. For instance, complete mortality was evoked in worker and soldier castes of *M. subhyalinus* exposed to all botanical mixtures containing *N. tabacum* within 6-h exposure when compared to those exposed to mixture without *N. tabacum*. The only exception was observed in soldier caste of *M. subhyalinus* exposed to mixture of *N. tabacum* and *J. curcas*, where 92% mortality was recorded within 6-h exposure. The soldier termites are usually less mobile than the worker termite since they are less involved in the collection of cellulose for the colony unlike the worker termites (Barbosa-Silva
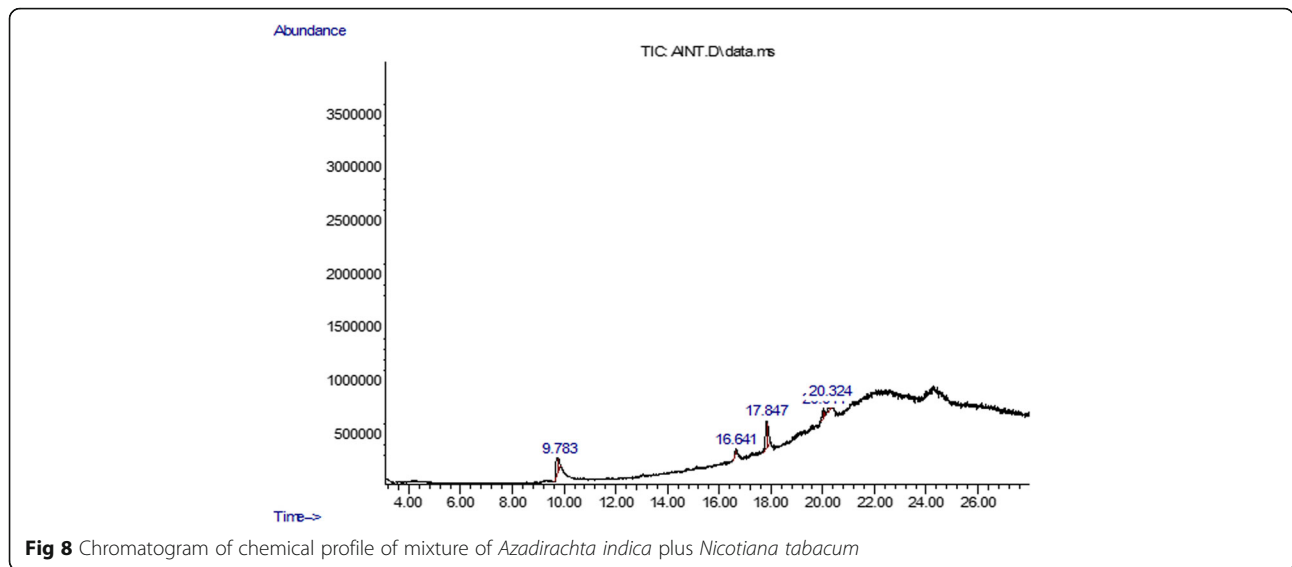
& Vasconcellos, 2019; Watanabe, Gotoh, Miura, & Maekawa, 2014). The characteristic lower mobility of the soldier termite on wet treated filter paper could have reduced their ability to pick up more plant extracts unlike worker termites and this could be responsible for the observed lower mortality in soldier termite. Highly mobile arthropods in a poisoned environment are known to pick up higher dosage of toxins and also expend greater amount of conserved energy on mobility, thus left with little or no energy to withstand the poison (Oyeniyi, Gbaye, & Holloway, 2015a, 2015b). This could be responsible for the observed higher mortality of the worker termite in this study.

Also, the higher potency of botanical mixture containing *N. tabacum*, to worker and soldier castes of *M. subhyalinus* may be attributed to the presence of various

Table 5 Compounds identified in mixture of *Nicotiana tabacum* plus *Jatropha curcas* plus *Azadirachta indica*

RT (min)	Name of compounds	CAS No.[a]	% of total peak area
9.621	(S)-3-(1-Methyl-2-pyrrolidinyl) pyridine	000054-11-5	2.554
13.609	Fluorene	000086-73-7	2.383
16.585	Phenanthrene	000085-01-8	6.198
16.699	Anthracene	000120-12-7	14.031
17.844	Methyl ester, Hexadecanoic acid	000112-39-0	5.350
18.931	n-Hexadecanoic acid	000057-10-3	-0.910
20.018	Methyl ester, 11-Octadecenoic acid	052380-33-3	2.499
20.356	Pyrene	000129-00-0	18.698
21.099	Fluoranthene	000206-44-0	49.197

[a]Chemical abstracts service registry number

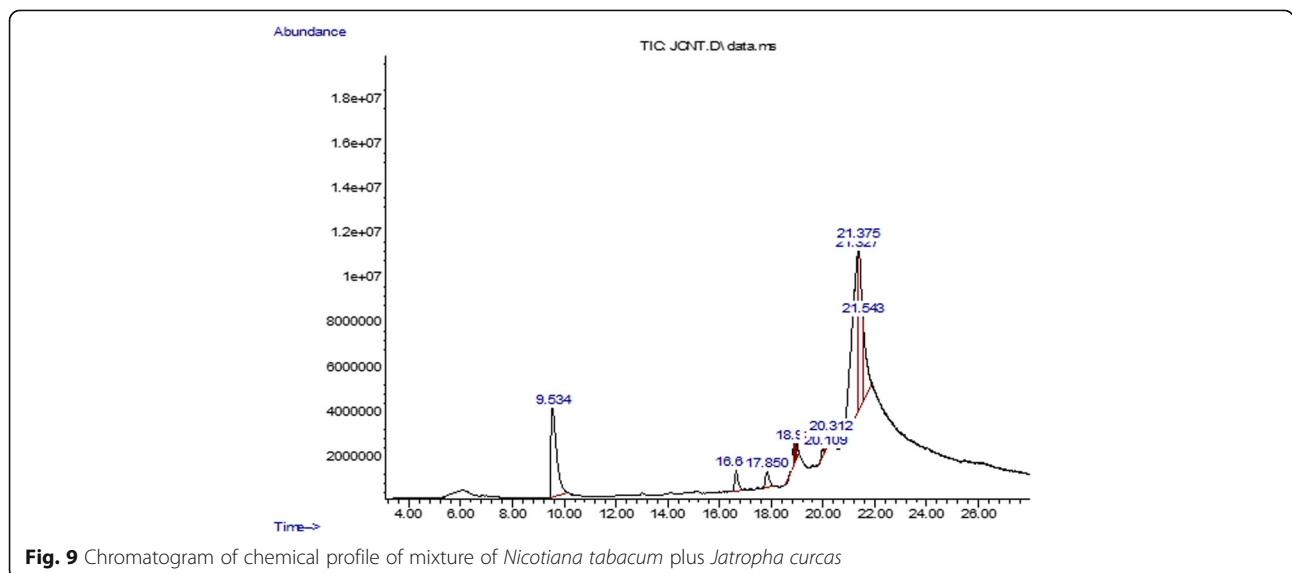**Fig 8** Chromatogram of chemical profile of mixture of *Azadirachta indica* plus *Nicotiana tabacum*
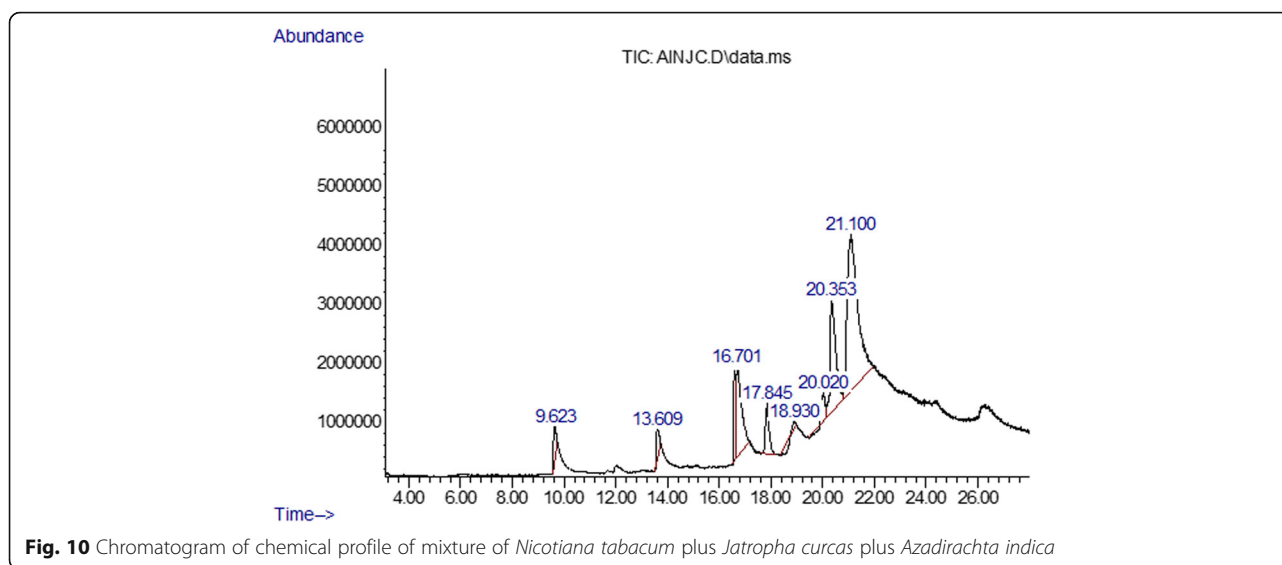
toxic and insecticidal compounds identified in the botanical mixture containing *N. tabacum.* Some of these compounds include (S)-3-(1-Methyl-2-pyrrolidinyl) pyridine (also called Nicotine), oleic acid, (Z,Z)-9,12-Octadecadecanoic acid, hexadecanoic acid, and Anthracene. Nicotine, for example, has been established as a fast-acting nerve toxin and a very potent anti-termite agent (Shiberu et al., 2013). Oleic acid had also been established as the main insecticidal compound responsible for the insecticidal efficacy of *Nigella sativa* (Ranunculaceae) against *Callosobruchus chinensis* (L.). Likewise, hexadecanoic and octadecanoic acid, as well as their alkyl esters identified in the ethanol and hexane crude extracts of *Cassia fistula* (L.) were reported to be insecticidal (Barakat et al., 2004). Consequently, termites exposed to nicotiana-containing botanical extracts, especially at the

highest exposure, might have suffered continuous nervous disruption due to various insecticidal compounds in the mixture, resulting in the termination of vital functions and ultimately death (Muhammad, 2015). Although, previous studies had established that *J. curcas* and *A. indica* were highly toxic to termites when applied singly and their high efficacy has been linked to the presence of insecticidal compounds like jatrophin in *J. curcas* and azadirachtin in *A. indica* (Gold et al., 1991; Jembere, Getahun, Negash, & Sevoum, 2005; Schmutterer & Singh, 1995; Solsoloy, 1993), admixture of both botanical extracts in this study resulted in an antagonistic reaction due to the lowest mortality observed in both worker and soldier castes of *M. subhyalinus.*

Extracts of all the mixtures of the plants but for *J. curcas* plus *A. indica* repelled the termites. This indicates

Fig. 9 Chromatogram of chemical profile of mixture of *Nicotiana tabacum* plus *Jatropha curcas*

Fig. 10 Chromatogram of chemical profile of mixture of *Nicotiana tabacum* plus *Jatropha curcas* plus *Azadirachta indica*

that the botanical mixtures containing *N. tabacum* were able to repelled *M. subhyalinus* regardless of the exposure time. However, of all the botanical mixture containing *N. tabacum*, highest repellence (≥ 73%) was exhibited by *N. tabacum* plus *J. curcas* extract against *M. subhyalinus*. The high repellence observed in termites exposed to extract of *N. tabacum* plus *J. curcas* may be linked to the presence of antifeedant and toxic compounds like oleic acid, (Z,Z)-9,12-Octadecadienoic acid, and (S)-3-(1-Methyl-2-pyrrolidinyl) pyridine in the GC-MS analysis of the mixture (Barakat et al., 2004; Deshpande, Adhikary, & Tipris, 1974; Mathur, 1998; Tare & Sharma, 1991). These compounds might have been responsible for the ability of the mixtures to repel and prevent termites from feeding on wood. Even though the repellency of neem plant against termites is well documented (Deka & Singh, 2001; Epilla & Ruyooka, 1988; Gold et al., 1991), admixture of *A. indica* with *J. curcas* in this study reduced the repellence ability of *A. indica* against termite, thus signaling an antagonistic effect. It could therefore be inferred that extract combination of *J. curcas* with *A. indica* could not serve as termite repellent because several termites were observed on filter paper treated with the extract of the plant mixture.

The field test shows that wood blocks treated with *N. tabacum* plus *J. curcas*, *A. indica* plus *J. curcas*, and *N. tabacum* plus *J. curcas* plus *A. indica* under shade had significantly higher weight loss and shows higher signs of infestation than their counterparts in the open field. This suggests that treated wood suffered more damage under the shade than in the open field. This could be attributed to variation in the environmental conditions, between the two experimental sites. Generally, shaded environment are characterized by lower temperature and higher humidity when compared to open environment due to reduced penetration of sunlight. Termites are known to be very active under humid and moderately warm conditions and usually rely on moisture from soil, wood, and mud tubes for their protection from desiccation (McManamy, Koehler, Branscome, & Pereira, 2008).

The favorable condition under the shade could have therefore attracted more termites to degrade the wood blocks than in the open field. The higher damage of the wood blocks under the shade also suggested that the botanical mixtures were less effective on the wood blocks under high humid and low temperature conditions. Also, the pleasant scent of the extracts of the plant mixtures under the shade might have also attracted more termites to the wood blocks treated with extracts than those treated with distilled water. Since the extracts were observed to be less effective under the shade, higher infestations by termites were therefore observed on wood blocks treated with extracts that those treated with distilled water. This study also showed that the botanical mixture exhibited largely antagonistic reaction under the shade. It is however important to note that the aforementioned reasons are still speculative. Further studies are therefore suggested to better elucidate on the mechanisms underlying the reduced efficacy of the botanical mixtures under the shade when compared to control. The trend was, nevertheless, reversed in the open field condition with higher weight loss and infestation level observed on wood blocks treated with distilled water than extract-treated wood blocks.

Extracts from the tri-mixture of the botanicals better protected the wood from termite damage than those from bi-mixture. The ability of the extract from the tri-mixed plants to better protect the wood against termite in the open field could indicate a higher synergistic

potential of various compounds in the three botanicals. This also suggests that the three extract mixture could offer good protection to wooden structures against termite destructive activity in the open field. The greatest termite damage was however observed in wood treated with extract combination of *A. indica* plus *N. tabacum* in the open field. This may probably indicate that the bioactive components in the mixture of *A. indica* plus *N. tabacum* might be photosensitive.

In modern research, there is a shift toward the knowledge of molecular bioactivity of sample treatments and their effects on target organisms. This has necessitated the profiling of the compounds formed from the combinations of botanicals used in this study. This was important to evince the particular compounds responsible for bioactivity on termites. Abdul-Rahuman and Venkatesan (2008) found that oleic acid and linoleic acid (Z,Z)-9,12-Octadecadienoic acid)—found in the mixture of *N. tabacum* and *J. curcas*—were quite potent toxics against the 4th larvae of *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus*. E, Z-3, 13-Octadecadien-1-ol identified in *N. tabacum* and *J. curcas* are kairomones used for controlling the Western Poplar clearwing moth. Palmitic acid (Methyl ester, Hexadecanoic acid)—a fatty acid ester found in all the mixtures profiled—can be used as a pesticide (Muhammad, 2015). Anthracene—found in the mixture of the three botanicals—is a solid polycyclic aromatic hydrocarbon used as wood preservatives, insecticides, and coating materials (Barnes, 2019).

Conclusion
The current study clearly showed that the admixture of extracts of *N. tabacum* with *J. curcas* or *A. indica* had a synergistic impact on the termiticidal properties of *J. curcas* and *A. indica*. On the contrary, the mixture of *J. curcas* with *A. indica* resulted in an antagonistic effect due to considerable reduction in the termiticidal properties of the resulting mixture. The mixture of the three botanical extracts also offered the best protection to the treated wood blocks in the open field condition. The mixture of the botanicals also influenced the quantity and nature of bioactive compounds in the botanical extracts. Anthracene, (S)-3-(1-Methyl-2-pyrrolidinyl) pyridine (also called Nicotine), oleic acid, (Z,Z)-9,12-Octadecadecanoic, and hexadecanoic acid identified in the botanical mixtures had been reported to be insecticidal and could therefore be used in formulating ecologically tolerable termiticides.

Abbreviations
ASTM: American society for testing and materials; MC: Moisture content; PR: Percentage repellency; C: Percentage concentration; HS-SPME: Head space–solid-phase micro-extraction; GC-MS: Gas chromatography-mass spectrometry; ANOVA: Analysis of variance; SPSS: Statistical package for social sciences; h: Hours

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