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# Research Article
# Repellent and Insecticidal Activities of the Root Extracts of *Chromolaena odorata* and *Mimosa diplotricha* Against *Macrotermes* Species

[1,2]Osariyekemwen Uyi, [1]Adeyemi Daniel Adetimehin and [1]Ogechi Perpetual Ogu

[1]Department of Animal and Environmental Biology, University of Benin, P. M. B. 1154, Benin, Nigeria
[2]Department of Zoology and Entomology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa

## Abstract

**Background and Objective:** Although a number of studies have empirically evaluated the use of extracts from several indigenous plants against insect pests, but little is known about the use of extracts from invasive alien plants to control these noxious species. This study investigated the repellent and insecticidal activities of the root extracts of two invasive alien plants, *Chromolaena odorata* and *Mimosa diplotricha* against *Macrotermes* species. **Materials and Methods:** Four concentrations of the aqueous root extracts of the two plants were tested on the worker caste of *Macrotermes* species using the filter paper impregnation technique after which percentage repellency was monitored for 30 min. In a similar experimental setup mortality was monitored for 12, 24 and 36 h. **Results:** The highest concentration [10% (w/v)] of the root extracts of *C. odorata* and *M. diplotricha* significantly repelled 98 and 100% of *Macrotermes* species, respectively, following a 30 min exposure period. Mortality of *Macrotermes* species caused by the root extracts of *C. odorata* and *M. diplotricha* was high and observed to be concentration and exposure time dependent. The highest concentration [10% (w/v)] of *C. odorata* and *M. diplotricha* root extracts accounted for 100% mortality against *Macrotermes* species after a 36 h exposure period. Following a 36 h exposure period, the median lethal concentrations ($LC_{50}$) of *C. odorata* and *M. diplotricha* against the termites were 1.72 and 4.12% (w/v), respectively. **Conclusion:** This study elucidates the repellent and insecticidal activities of the root extracts of *C. odorata* and *M. diplotricha* for the first time and suggests that the root extracts of both plants can be used for the control of *Macrotermes* species.

Key words: *Chromolaena odorata*, *Mimosa diplotricha*, termites, aqueous extracts, toxicity

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Corresponding Author: Osariyekemwen Uyi, Department of Animal and Environmental Biology, University of Benin, P.M.B. 1154, Benin, Nigeria
Tel: +234 80 380 13012 12 Fax: +234 052 602370



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Despite the fact that termites play beneficial roles in ecosystem functioning, they are also very destructive and are a major threat to crops and household properties[1,2]. Crops such as yam, cassava, sugarcane, groundnuts, sorghum and maize are prone to infestation and damage by termites[3]. In addition, termites also attack stored grains such as maize and rice[3]. Termites are commonly responsible for the mortality of tree seedlings in forestry, plantations and also cause a considerable damage to buildings and other wooden structures such as fence poles and utility poles[4].

*Macrotermes* species (Blattodea: Termitinae) are members of the fungus growing termites belonging to the subfamily Macrotermitinae. In Africa, *Macrotermes* is and remains a serious pest of some agricultural crops such as cassava, citrus plants, coconut, coffee, groundnut, sorghum, sugarcane, sweet potato and tree plantations[3,5]. They are thought to be responsible for the majority of crop damages and high levels of tree mortality in forestry[4]. Furthermore, damage to stored products by pests including termites provide entry for secondary infestation by pathogens especially *Aspergillus* spp., consequently causing indirect yield loss and contamination of products[6].

The fascinating results produced by synthetic insecticides against pest such as termites, have been overshadowed by recent debates surrounding their hazards to human health and effects on non-target organisms amongst other issues[7]. Furthermore, their frequent usage sometimes result in the development of insecticide resistance in target species. The challenge of finding sustainable alternatives to these synthetic insecticides has led to the bio-prospecting of plants with repellent and toxicological properties[6]. Natural products of plant origin have been reported to be useful and desirable tools in pest management because they are effective[8,9]. Several experiments using plant extracts and powders in human and animal health protection, agriculture and in household pest management have been particularly promising. For example, the potential toxicological (pesticidal) activities of some plants such as *Azadirachta indica* (L.) (Meliaceae), *Chrysanthemum indicum* (L.) (Asteraceae), *Cineraria folium* (L.) (Asteraceae), *Anchomanes diffuronis* (Schott) (Araceae), *Aframomum melegueta* (K. Schum) (Zingiberaceae), *Jatropha curcas* (L.) (Euphoriaceae) and *Annona muricata* (L.) (Annonaceae) against pests of medical, agricultural, economic, storage and veterinary importance have been adequately documented[10-13].

Although a number of studies have empirically evaluated the use of extracts or powders from several indigenous plant species against insect pests including termites[14-16], but little is known about the use of extracts from invasive alien plants to control these noxious species[9,16]. *Chromolaena odorata* is an invasive weedy shrub native to the Americas[17]. Its natural habitat range stretches from southern Florida, USA to Northern Argentina including the Caribbean Islands. The weed is known to be invasive in some countries in South-East Asia, West Africa, East, Central and Southern Africa and parts of Australia[17-19]. Following the weed's introduction into Nigeria in the late 1930s, it is now known to be widespread in the southern parts of Nigeria where it is recognized as an agricultural weed as well as a beneficial plant[20]. *Mimosa diplotricha* is a fast growing, annual (short-lived) or perennial shrubby leguminous vine native to the Americas[21]. Although, the nativity of the weed has been traced to Brazil, its natural habitat range in the Americas stretches from southern Mexico to northern Argentina including the Caribbean Islands[21]. Following the introduction of the weed into Nigeria in the late 1980s, it is now known to be present in most states in southern Nigeria where it threatens agriculture and biodiversity conservation and livelihoods[21]. The repellent and insecticidal activities of the root extracts of the invasive alien plants, *C. odorata* and *Mimosa diplotricha* C. Wright ex Sauvalle (Mimosaceae) against termites are unknown. Therefore, the objective of this study was to investigate the repellent and insecticidal activities of the aqueous root extracts of *C. odorata* and *M. diplotricha* against the worker caste of *Macrotermes* species.

## MATERIALS AND METHODS

**Collection and preparation of plant powders:** Fresh roots of *C. odorata* and *M. diplotricha* plants were collected from an open farmland at Dentistry quarters, within the vicinity of University of Benin Teaching Hospital (UBTH), Benin city (6°39'N, 5°56'E), Nigeria. Following collection, the roots were chopped separately into pieces, washed with running water and shade-dried for about 7 days and thereafter oven-dried at 60°C for 72 h. The dried roots were blended into a fine powder using an electric blender (Braum Multiquick Immersion Hand Blender, B White Mixer MR 5550CA, Germany) and then preserved at room temperature in an air-tight and water-proof container for further use.

**Extract preparation:** Different concentrations of the root extracts of each plant were prepared based on weight per volume (w/v), as 2.5, 5.0, 7.5 and 10.0 g of the grinded root powder of each plant material was mixed with 100 mL of water in separate flasks to obtain a crude extracts of four different concentration levels of 2.5, 5.0, 7.5, 10.0% (w/v), respectively. Each of the flask containing different concentrations (2.5, 5.0, 7.5, 10.0% (w/v) of the root extracts was shaken thoroughly for 30 min to ensure quick and even distribution of the solutes (= root powders) in the solvent (= water) .

**Insect collection:** *Macrotermes* species worker caste were collected from termite mounds in an open farmland at the Faculty of Agriculture, University of Benin, Benin city, Nigeria. At the site of collection, termite mounds were dug up using a spade and mounds containing termites were put in a plastic box. The termites were dusted off the mound using camel brush and placed in a polythene plastic box measuring $22\times17\times7cm^3$. Plant materials were added to the plastic box as feeds for the termites. Then the top part of the box was covered with a Muslin cloth to allow adequate air circulation (in and out of the box) and also to prevent the escape of the termites. Moistened wad of cotton was placed into the plastic box to maintain the required moisture level (more than 60%) for termite survival. The box carrying the termites was then transported to the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin city, Nigeria and placed in a cool dark area until needed for the experiment. Termites were used 24 h after collection. Both the repellency and mortality tests were conducted in the Laboratory of the Department of Animal and Environmental Biology, University of Benin, Benin city, Nigeria, between October 2016 and March 2017.

**Repellency bioassay:** The filter paper impregnation technique[15] was used to perform the repellency bioassay. Whatman No. 1 filter paper cut into two equal parts (half part treated and the other half left untreated) were placed inside a Petri dish of 8.5 cm diameter. The distance between the two halves was 1 cm. The treated half had 1 mL of one of four treatments (2.5, 5, 7.5 and 10% (w/v)) of the botanical extracts of either plants while the untreated half was treated 1 mL of water. Both treatments were administered using a 2 mL syringe. Five replicates were used for all four concentrations. Ten worker termites were introduced into the center of the Petri dish, specifically at the point of demarcation between the treated and untreated filter papers and the Petri dish was placed in darkness to minimize the effect of light on the termites. The numbers of termites on both the treated and untreated filter papers were recorded from each Petri dish 30 min after treatment application. Repellency was determined based on the number of termites which stayed on the extract-treated filter paper. Percentage repellency was calculated using the Eq.:

$$\text{Repellency (\%)} = \frac{(\text{U -T})}{\text{U}}\times 100$$

Where:
U = Number of termites on the untreated filter paper
T = Number of termites on the treated filter paper

**Mortality bioassay:** To perform the mortality bioassay, the filter paper impregnation technique was employed[15]. Whatman No. 1 filter paper was placed inside a Petri dish of 8.5 cm diameter. The filter paper was treated with 2 mL of one of four concentrations [2.5, 5.0, 7.5 and 10.0% (w/v)] of the aqueous root extracts of *C. odorata* and *M. diplotricha*. Ten termites were placed in a Petri dish treated with one of the four treatments of the aqueous root extracts. Each treatment was replicated five times. The experiment was laid out using completely randomized design (CRD). A control treatment where tap water was used to treat the filter paper was also set-up for comparison. All experimental units were placed in darkness at a temperature of $26\pm3°C$ and relative humidity of 60-70% for 2 days. Termites' mortality was recorded at 12 h interval until 100% mortality was recorded. Mortality (%) was calculated using the following Eq.:

$$\text{Mortality (\%)} = \frac{\text{Number of dead termites}}{\text{Total number of termites}}\times 100$$

**Statistical analysis:** Control treatments, where the termites were not exposed to the aqueous root extracts of *C. odorata* and *M. diplotricha* plants caused 0.0% repellency and mortality; hence the controls were not included in the statistical analyses. The percentage repellency of the different concentrations of *C. odorata* and *M. diplotricha* root extracts [2.5, 5.0, 7.5 and 10.0% (w/v)] was analyzed with a Generalized Linear Model (GLZ) assuming a normal distribution with an identity link function. When the overall results were significant in the GLZ analysis, the difference between the treatments means was compared using the Tukey's Honest Significant Difference (HSD) test. The relationships between percentage mortalities of *Macrotermes* species and concentrations of *M. diplotricha* root extract across all three exposure times was analyzed with linear regression analyses, as was the relationships between mortalities (%) of *Macrotermes* species and concentrations of *C. odorata* root extract across all three exposure times. The relationships between mortalities (%) of termites and exposure durations for the two different plant species across the all treatment concentrations were analyzed using linear regression analyses. The effects of the root extracts of the two plants and the concentration levels the extracts on termite mortality was analyzed using General Linear Model Analysis of Variance (GLM ANOVA). Probit regression was used to calculate the concentration of the root extract estimated to cause 50% mortality ($LC_{50}$), the temperature causing 50% of tested individuals to die in a given period. With the exception of the GLZ, GLM ANOVA and probit regression that were performed using SPSS statistical software, version 16.0 (SPSS, Chicago, IL, USA), all other analyses were performed using Genstat 12.0 (VSN International, Hemel Hempstead, UK) and Microsoft Excel.

## RESULTS

**Repellency test:** Following a 30 min exposure period of *Macrotermes* species to different concentrations of the root extract of *M. diplotricha*, percentage repellency against the termites significantly differed (GLZ: Wald $\chi^2 = 9.242$, df = 3, p = 0.026) with the 2.5% w/v concentration exhibiting the least percentage repellent activity (78%) (Fig. 1a). There were no significant differences in percentage repellent activities between the 5.0, 7.5 and 10.0% (w/v) treatments, although the 10.0% (w/v) appears to have exhibited the highest percentage repellency (100%) (Fig. 1a). Following a 30 min exposure of *Macrotermes* species to different concentrations of the root extracts of *C. odorata* plants, percentage repellency (against the termites) did not differed ($\chi^2 = 0.490$, df = 3, p = 0.921) (Fig. 1b). Although percentage repellency did not differed between the treatments, the 10.0% (w/v) concentration exhibited the highest percentage repellency (98%), while the 2.5% w/v treatment exhibited the least repellency (86%) (Fig. 1b).

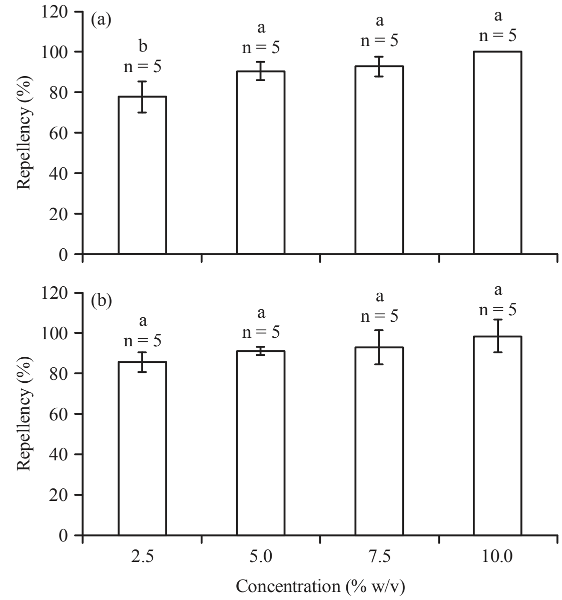


Fig. 1(a-b): Percentage (mean±SE) repellency of different treatments of (a) *Mimosa diplotricha* and (b) *C. odorata* root extracts against *Macrotermes* species following a 30 min exposure period. Means capped (following GLZ) with different letters are significantly different [after Tukey's Honest Significant Difference (HSD) test: p<0.05]. Sample sizes are given in parenthesis

**Mortality test:** Termites' mortalities were very high irrespective of exposure times and concentrations of *M. diplotricha* root extract with mortality ranging between 76 and 100% (Fig. 2a-c). Irrespective of exposure time (12, 24 or 36 h), linear regression analyses showed significant positive relationships between percentage mortalities of *Macrotermes* species and concentrations of *M. diplotricha* root extract as mortality increased with increased concentrations of the root extracts (Fig. 2a-c). The root extract of *C. odorata* plants caused considerable mortalities in *Macrotermes* species irrespective of exposure times and concentrations with mortality ranging between 10 and 94% (Fig. 3a-c). There were significant positive correlations between percentage mortality levels of *Macrotermes* species and concentrations of the root extract of *C. odorata* plants across all three exposure trials (Fig. 3a-c). When termites were exposed to the root extracts of *C. odorata* and *M. diplotricha* for 36 h, the $LC_{50}$ values were 1.72 and 4.12% (w/v), respectively. There were linear relationships between percentage mortalities of termites and exposure durations of the insects at the different concentrations of *M. diplotricha* root extract (2.5% w/v: $R^2 = 0.989$; 5.0% w/v: $R^2 = 0.964$, 7.5% w/v: $R^2 = 0.987$, 10.0% w/v: $R^2 = 0.899$) (Fig. 4a). Similarly, there were linear relationships between percentage mortalities of termites and exposure durations of the insects at the different concentrations of *C. odorata* root extract (2.5% w/v: $R^2 = 0.946$, 5.0% w/v: $R^2 = 0.907$, 7.5% w/v: $R^2 = 0.995$, 10.0% w/v: $R^2 = 0.987$) (Fig. 4b). Overall, termites mortality differed according to plant species

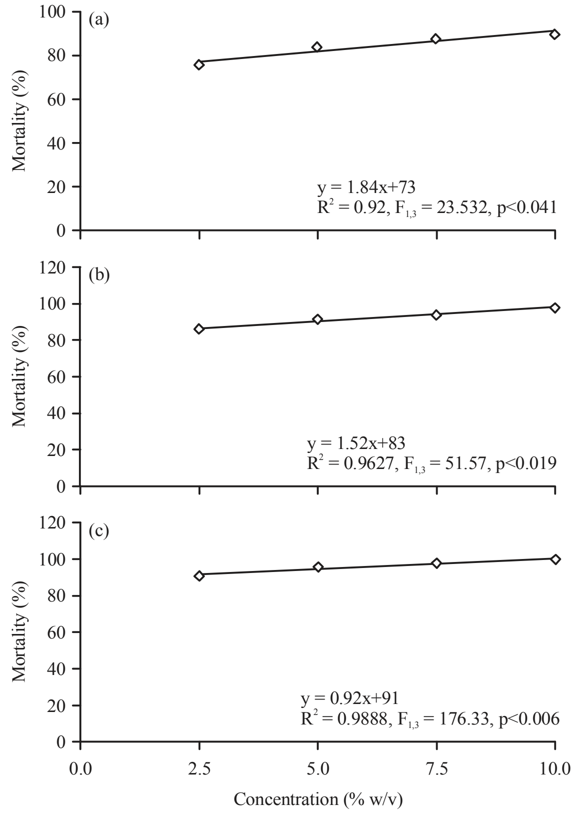


Fig. 2(a-c): Relationships between mortality (%) of *Macrotermes* species and concentrations of *Mimosa diplotricha* root extract following (a) 12 h, (b) 24 h and (c) 36 h exposure period

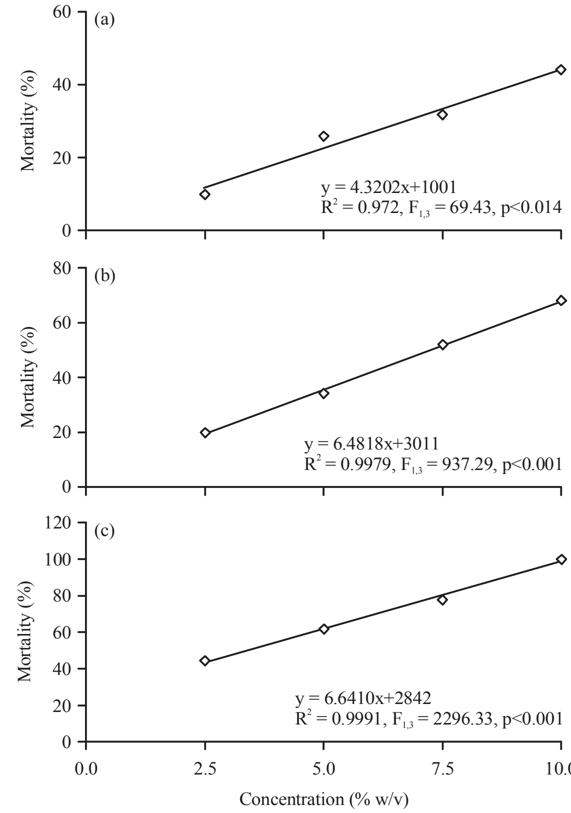


Fig. 3(a-c): Relationships between mortality (%) of *Macrotermes* species and concentrations of *Chromolaena odorata* root extract following a (a) 12 h, (b) 24 h and (c) 36 h exposure period

(*M. diplotricha* versus *C. odorata*, GLM ANOVA: $F_{1,39} = 32.18$; $p<0.001$) and concentrations ($F_{3,39} = 6.14$, $p = 0.002$) (Fig. 5). With the exception of the equal mortality levels between *M. diplotricha* and *C. odorata* at the 10.0% (w/v) concentration, *M. diplotricha* exhibited significantly higher mortalities across concentrations compared to *C. odorata* (Fig. 5).

## DISCUSSION

This study investigated the repellent and insecticidal activities of *M. diplotricha* and *C. odorata* aqueous root extracts on the worker caste of *Macrotermes* species. The study was undertaken as part of the global initiative to introduce and practice more sustainable environmentally friendly methods of controlling termites, a serious economic pest of agricultural crops, household furniture, forest trees, plantations and building structures. The results from this study revealed that the root extracts of *M. diplotricha* and *C. odorata* displayed some repellent and insecticidal activities against *Macrotermes* species.

This study demonstrated that the aqueous root extracts of *C. odorata* significantly repelled the worker caste of *Macrotermes* species, although repellency was a function of concentration of the root extract used. Studies focusing on the repellent activities of the leaf powders or extracts of *C. odorata* against insect pests are not uncommon[16,22]. For example, cowpea grains treated with the leaf powder of *C. odorata* exhibited significant repellent activity against the adults of *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae)[9]. The high repellent activities (98%) demonstrated by the highest concentration (10% w/v) of the root extract of *C. odorata* is consistent with the findings of other authors[15,16], who reported high repellency with increasing concentrations of the plant powders or extracts against insect pests including termites. The lack of a significant difference in termite repellency between all four concentrations of *C. odorata* root extract (2.5. 5.0, 7.5 and 10.0% w/v) not only suggests that low concentrations of the *C. odorata* can significantly repel *Macrotermes* species, but also demonstrates the excellent repellent properties of *C. odorata* root extract.

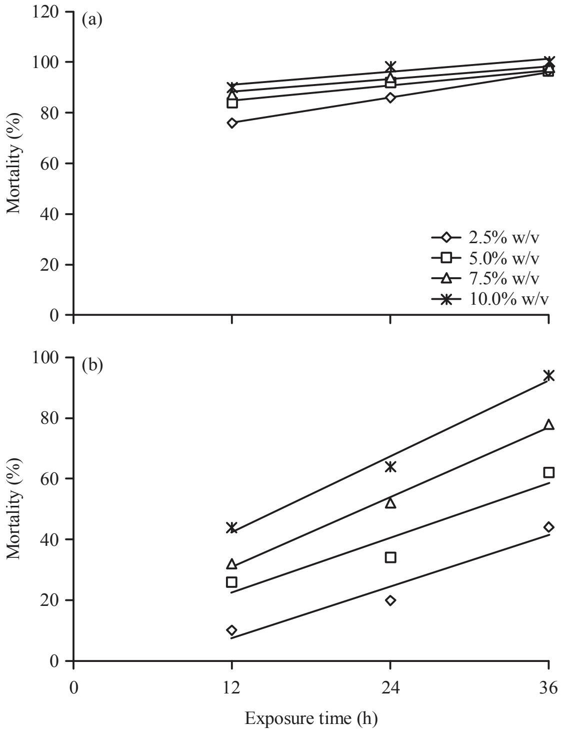


Fig. 4(a-b): Relationships between percentage mortalities of *Macrotermes* species and exposure durations of termites to different concentrations of (a) *Mimosa diplotricha* and (b) *Chromolaena odorata* root extracts

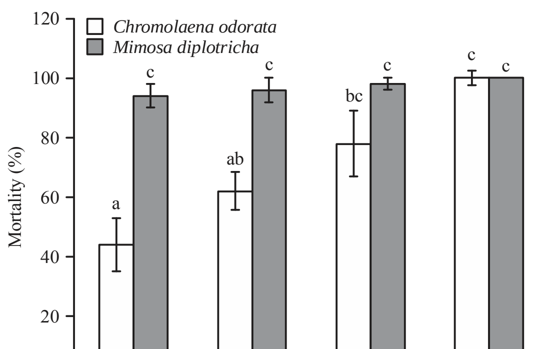


Fig 5: Percentage mortality of *Macrotermes* species treated with different concentrations of the root extracts of *Chromolaena odorata* and *Mimosa diplotricha* over a 36 h period. Means capped with different letters are significantly different (after Tukey's HSD test: $p<0.05$)

The results from this study revealed that the root extract of *M. diplotricha* significantly repelled *Macrotermes* species and as with *C. odorata* root extract, the repellent activities were dependent on the concentrations of root extract used. Following a 30 min exposure period, the highest concentration of *M. diplotricha* root extract (10% w/v) significantly repelled 100% of *Macrotermes* species. The lack of a significant difference in repellency between the 5.0, 7.5 and 10.0% (w/v) treatments suggested that low concentrations of the *M. diplotricha* can also cause significant mortality against the investigated termite species. To date no studies exist on the repellent activities of *M. diplotricha* root extract against insect pests, consequently, this study is the first to document the repellent activities of *M. diplotricha* root extract.

Undoubtedly, the insecticidal activity of *C. odorata* root extract against *Macrotermes* species reported in this study has not been previously documented, but studies reporting the insecticidal activities of the leaf or root powders and leaf extracts of *C. odorata* against insect pests are not uncommon[9,16,23,24]. For example, Uyi and Igbinoba[16] reported that the root powder of *C. odorata* caused significant mortality (100%) against *C. maculatus* following a 72 h exposure period. In this study, the highest concentration (10% w/v) of the root extract of *C. odorata* caused 100% mortality against *Macrotermes* species, following a 36 h exposure period. The high mortality demonstrated by the highest concentration of *C. odorata* root extract is in agreement with the findings of other authors[15,24,25]. who reported high mortalities with increasing concentrations of either plant extracts, oils or powders against insect pests including pest of agricultural and economic importance.

Admittedly, mortality of *Macrotermes* species caused by the root extract of *M. diplotricha* was high and observed to be both concentration and exposure time dependent. Following a 36 h exposure period, the root extracts of *M. diplotricha* at the highest concentration (10% w/v) accounted for 100% mortality against *Macrotermes* species. The results from this study is consistent with the findings of other authors[15,24,25], who reported high mortalities with increasing concentrations of plant extracts, oils or powders against insect pests. Although many studies often report that insect (including termites) mortality always increase with an increase in exposure period to plant extracts or powders[15,16], but only a few performed regression analysis to empirically demonstrate significant correlations in insect mortality between concentrations of plant extracts (or powders) and exposure time. This study used linear regression analyses to show significant positive relationships between percentage mortalities of *Macrotermes* species and concentrations of *C. odorata* and *M. diplotricha* root extracts and empirically demonstrated that mortality increased with increased concentrations of the root extracts.

The high repellent and mortality exhibited by the root extract of *C. odorata* against *Macrotermes* species could be attributed to the presence of one or more bio-active compounds present in the roots of the plant. Studies on the phytochemical composition of the roots of *C. odorata* revealed that the roots of the plant contain secondary metabolites (= phytochemicals) such as alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins[26,27] and this might plausibly explain the reason for the high repellency and mortality of *C. odorata* root extract against *Macrotermes* species reported in this study. Although, the root extract of *M. diplotricha* demonstrated significant repellent and insecticidal activities against *Macrotermes* species, little or nothing is known about its phytochemistry. The high repellent and insecticidal activities of *M. diplotricha* root extracts might be attributed to the presence of phytochemicals as has been documented for other invasive alien plants[27,28]. Studies focusing on the insecticidal activities of the leaf, stem, flower and root extracts of a congener of *M. diplotricha*, *Mimosa pudica* (L.) (Mimosaceae) revealed that the various plant parts demonstrated significant insecticidal activity against the larvae of *Aedes aegyptii* (F.) (Diptera; Culicidae)[29]. The reason behind the insecticidal activity of *Mimosa* species could be attributed to the presence of alkaloids, flavonoids, tannins and sterols, terpenoids. Nevertheless, further studies on the phytochemical composition of the roots of *M. diplotricha* are necessary to either validate or rebut this conjecture.

## CONCLUSION

The excellent repellent and insecticidal activities of *C. odorata* and *M. diplotricha* in this study suggests that the root extracts of both plants can be used as sustainable alternatives to conventional insecticides and extracts of indigenous plants, in the control of *Macrotermes* species in Nigeria and other countries where the pest is a menace. Furthermore, both *C. odorata* and *M. diplotricha* are agricultural nuisance and their use as bio-pesticides could help curb the invasiveness of these alien plants, thus making it a win-win situation for both agriculturists and farmers.

## SIGNIFICANCE STATEMENT

This study discovers the repellent and insecticidal activities of the root extracts of two invasive alien plants, *C. odorata* and *M. diplotricha* that can be beneficial for the control and management of *Macrotermes* species. This study uncovers the positive attributes of *C. odorata* and *M. diplotricha* that many researchers were unable to explore. Thus a new environment friendly insecticide can developed from the findings of this study.

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