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Susceptibility of *Macrotermes subhyalinus* (Ramburi) (Isoptera: Terminidae) to Oil of *Acalypha godseffiana* (Muell. Arg.

Oni, M.O.[1*], Kofoworola, T.[1], Ogungbite, O.C.[2], Ofuya, T.I.[1] and Adunola, P.[1]

[1] Department of Crop, Soil and Pest Management, Federal University of Technology, PMB 704, Akure, Nigeria.
[2] Department of Plant Science, University of Ado-Ekiti, Ado- Ekiti. Nigeria
*Corresponding author: mercyyinka2007@yahoo.co.uk

ABSTRACT

Oil from the leaf of Acalypha godseffiana plant was screened for bioactivity against Macrotermes subhyalinus in-vivo and in-vitro. Toxicity of the oil to termite was tested at 0 (control), 1, 2, 3 and 4% concentrations and each treatment were replicated four times. Repellent activity of the oil was tested using filter paper cut into equal halves labelled G and C. The G was treated with 0.5ml of the Solvent and C with 1, 2, 3 and 4% concentration of the oil and number of termites on each sides of the paper were counted after 0-1, 1-2, 2-4 and 4-6h post- treatment. Result obtained showed that only 4% concentration of the oil achieved 100% insect mortality and repellence within 2-4h and 24h respectively and its effect was significantly different ($p < 0.05$) from other concentration levels. However, LC50 and LC95 5.94 (5.58-6.99) and 7.53(6.82-7.98) reflected that low concentration of the oil are required to achieve 50 and 95% termite mortality within 24hr. Results obtained from field experiment showed that Terminalia superba wood-block treated with same concentration of the oil had similar effect on termite survival and no traces of infestation except in the control. The results of this study confirm that some components of A. godseffiana have insecticidal potential. Phytochemical analysis of A. godseffiana oil revealed flavonoid as the major component of the oil. This signaled flavonoid possibly have played significant role in the observed bioactivity. Further studies are highly imperative to confirm toxicity of flavonoids in A. godseffiana to M. subhyalinus.

Key words: *Macrotermes subhyalinus, Acalypha godseffiana*, flavonoid, mortality, repellent activity.

INTRODUCTION

Termite is one of the most important insect pests, which plays a vital and integral role in the destruction of many woods and wood products. It is a rural and urban insect pest because it can be found in almost every terrestrial habitat (Adeyemo *et al.,* 2015). *Macrotermes subhyalinus* is a popular species of termite known for its destructive activity among individual. It is capable of causing more than 90% or total destruction of forest wood as well as finished wood if left unchecked (Yamada *et al.,* 2003). Ekpo and Onigbinde (2007) reported *M. subhyalinus* as a destructive insect pest that attacks not only the forest products but as well attacks agricultural produce and products such as maize, sugarcane, cocoa, groundnut, beans and vegetables.

Over the years, the control and management of this insect pest has overwhelmingly depended on the use of synthetic chemical insecticides until recently when the adverse effects of these chemical insecticides on human and environmental health was discovered (Ahmed *et al.,* 2005: Adeyemo *et al.,* 2015). Because of the public awareness of the danger associated with the use of synthetic chemical insecticides, government of many countries banned their use (Isman 2000). Therefore, researches have shifted toward the use of plant materials as they were believed to contain myriads of allelochemicals that could be insecticidal in nature while many of them have proven to be highly medicinal and ecofriendly (Ashamo *et.al* 2006: Oni, 2014: Ogungbite *et.al* 2014). Akinkurolere *et al.,* (2006) however reported that, tropical regions of the world including Nigeria are well endowed with botanicals of different species that have insecticidal properties.

Acalypha godseffiana (Muell. Arg.) is a medicinal plant proven to have anti-malarial and anti-fungi efficacy. Its effectiveness in the treatment of gastrointestinal disorder and fungal skin infection has also been reported (Ogundaini, 2005). However, we are not aware of any report on insecticidal activity of this plant on any urban insect. Likewise, its field application has not been well established. This present study investigated toxicity of *A. godseffiana* leave oil on termite, *M. subhyalinus.*

MATERIALS AND METHODS

Insect collection

Subterranean termites *M. subhyalinus* were collected from a termitarium inside the campus of Federal University of Technology, Akure, Nigeria. The termites were placed in petri-dishes and taken to the research laboratory immediately to acclimatize at ambient temperature of 28± 2 °C and relative humidity of 75± 5% for 24 h.

Collection of plant material

The leaves of *A. godseffiana* were sourced from teaching and research farm of same institution. Sands and other dirts were washed off from leaves, cut into smaller pieces,air dried in an open laboratory, grinded into fine powder using an electric blender (Panasonic Blender MX134). The powder were further seived through 1mm aperture sieve and kept in plastic containers with tight lids and stored in Refridgerator at 4°C prior to use.

Phytochemical analysis of the plant oil and the wood used

The alkaloid and Saponin content of the oil extract and the wood was determined using the method of Obadoni and Okchuko (2001) while the tannin, flavonoid and phytate were determined using the method of (Ovais Ullah Shirazi *et.al*. 2014). The wood *T. superba* was obtained from major sawmill in Akure. Nigeria.

Extraction of oil

The oil was extracted from the powders of *A. godseffiana* using ethanol as solvent. About 150g of the leaf powder was soaked in 500ml of ethanol for three days and vigorously stirred occasionally with a glass rod. The supernatant was decanted using a double-layered Whatman No.1 filter paper. Solvent was evaporated from the resulting extract solution by using a-rotary evaporator at 30 to 40°C with rotary speed 3 to 6rpm for 8h (Udo, 2011). The resulting extract slurry was air-dried to remove traces of the solvents. From this main stock,oil concentrations of 1, 2, 3 and 4% were made diluting 0.1, 0.2, 0.3 and 0.4ml of plant extract in 9.9, 9.8, 9.7 and 9.6ml solvent respectively.(Ashamo and Akinawonu, 2012;Ileke *et.al* 2014).

Toxicity

The effect of oil extract of A. *godseffiana* on the mortality of the insect was tested by treating a filter paper with 1, 2, 3 and 4 % of plant oil. The control set-up was treated with 0.5ml ethanol each, in separate petri-dish. Water was sprayed on the sides of the container to maintain relative humidity of 75±5%. The set-ups were replicated three times. Number of dead termites was recorded in percentages after 1,4 and 24 h of treatment.

Repellent assay

The procedure was as described above with slight modifications. Filter paper was cut into equal halves placed in petri-dishes which was labeled G and C. The G was treated with 0.5ml of ethanol and C with 1, 2, 3 and 4% of the plant oil. A full disk was carefully remade by attaching the tested half to the negative control half with tape. The set-up was placed in each petri-dish with the seam oriented in one of four different randomly selected directions to avoid any insecticidal stimuli affecting the distribution of termites. Ten insects were released at the center of the filter paper disk, and covered immediately. The experiment was replicated four times. Number of termites found on each strip were recorded at 0-1, 1-2, 2-4 and 4-6 h after treatment. Percentage repellency (PR) was calculated using the formula;

$$\%R = \frac{N_C - N_T}{N_C + N_T} \times 100$$

where Nc is the number of insect present in the negative control half and Nt is the number of insects present in the treated half.

Treatment of *Terminalia superba*

The wood sample was cut into 2 x 3 cm in triplicates and were oven dry for 24hr at the temperature of 50°C. Initial weight of the wood was taken after oven dry. The *Terminalia superba* wood were treated with 1, 2, 3 and 4% of *A. godseffiana* leaf oil. The treated wood-blocks were taken to a site where there is termite and spread with control randomly and covered up to prevent water from entering and to provide dark environment for the insects. Insect infestation was observed four weeks after treatment. Oil-treated set-up served as control. The experiment was replicated four times. Weight of treated woods were taken and percentage weight loss in wood was noted.

Statistical analysis

Data obtained were subjected to One-way analysis of variance at 0.05 significant levels while means were separated with New Duncan's Multiple Range Tests using SPSS version 17. Also, data obtained on insect mortality were subjected to Probit analysis to calculate the lethal dosage required to achieve 50 and 95% mortality of the insect (Finney, 1971).

RESULTS

Percentage mortality of *M. subhyalinus* exposed to A. *godseffiana* plant leaf oils.

The effect of *A. godseffiana* oil on insect survival was presented in Table 1. Except at 4h of exposure {$F_{4,\ 10}$= 6.847, p > 0.06 (4h)}, statistically significant differences existed between the treatments at $F_{4\ ,10}$= 4.250, p < 0.029 (1h); $F_{4,\ 10}$=25.273, p < 0.0001(24hr). Termite mortality increased with increase in concentration of oils and period of exposure.

Table 1: Mortality of *M. subhyalinus* exposed to *A. godseffiana* leaf oil

Concentration (%)	Mortality (%) in hour		
	1	4	24
1	0.00± 0.00[a]	36.66± 6.66[a]	60.00 ±10.00[b]
2	10.00± 10.00[a]	43.33 ± 6.66[b]	80.00± 0.00[bc]
3	20.00 ± 10.00[a]	53.33 ± 14.52[b]	86.66 ± 6.66[c]
4	30.00 ± 0.00[b]	60.00 ± 10.00[b]	100.00 ± 0.00[c]
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	10.00± 10.00[a]

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at p< 0.05 using New Duncan's Multiple Range Test.

Table 2: Lethal concentration (LC50) and (LC95) of *A. godseffiana* oil to *M. subhyalinus* at 4 h of exposure.

Slope ± S.E	Intercept ± S.E	X^2	LC_{50}(50%FL)	LC_{95}(95%FL)	Sig.
0.985 ± 0.163	-3.83 ± 0.067	99.95	5.94 (5.58-6.99)	7.53(6.82-7.98)	0

Table 3: Percentage of termites, *Macrotermes subhyalinus* repelled during exposure to *Acalypha godseffiana* leaf oil

Oil Concentration (%)	% Repellency in hour			
	0-1	1-2	2-4	4-6
1	0.00 ± 0.00[a]	33.33± 6.66[b]	60.00 ±11.54[b]	80.00 ±11.54[b]
2	20.00 ± 0.00[b]	46.66 ± 6.66[b]	66.66± 6.66[b]	100.00±0.00[c]
3	33.33 ± 6.66[c]	53.33 ± 6.66 [b]	93.33 ± 6.66[c]	100.00±0.00[c]
4	66.66± 6.66[d]	86.66 ± 13.33[c]	100.00± 0.00[c]	100.00±0.00[c]
Control	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00± 0.00[a]	0.00 ±0.00[a]

Each value is a mean ± standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different at p< 0.05 using New Duncan's Multiple Range Test.

Within 24 hours of application 2, 3 and 4% concentration of oils achieved 80 - 100% and their effect was significantly ($p < 0.05$) different from other concentrations except 1% concentration that recorded 60% termite mortality. Regardless of the concentration used the effect of *A. godseffiana* oils on the termites was significantly different from that of the control.

Lethal concentration LC_{50} and LC_{95} of *A. godseffiana* oils tested against *M. subhyalinus* at 4 h of exposure.

The amount of *A. godseffiana* oil required to achieve 50 and 95% mortality of *M. subhyalinus* is presented in Table 2. *A. godseffiana* oil appeared effective against *M subhyalinus* as it recorded the lowest concentration required to achieve 50 and 95% mortality of the termites within 4hr of application when compared with the control. It was observed that only 5.94 (5.58-6.69) was required to achieve 50% insect mortality while, 7.53 (6.82-7.98) was required to achieve 95% mortality of the termites.

Repellent activity of *A. godseffiana* oil extract against *M. subhyalinus*

Table 3 shows the data on test of ability of *A. godseffiana.* Statistically, significant differences existed between the treatments at $F_{4, 10}$= 43.25, p < 0.00 (0-1h); $F_{4, 10}$= 15.92, p < 0.00 (1-2h); $F_{4, 10}$=15.92, p <0.00(2-4hr)); $F_{4, 10}$=70.50, p <0.00(4-6hr). Mortality of termites increased with increase in concentration of the oil extract and period of application. Within 24 hours of application 2, 3 and 4% concentration of the oil achieved highest mortality of 80 - 100% and their effect was significantly ($p < 0.05$) different from other concentrations except 1% concentration that recorded 60% mortality of the termite. Regardless of the concentration used the effect of *Acalypha godseffiana* oil extract against *M. subhyalinus* was significantly different from that of the control.

Phytochemical composition of *A. godseffiana* and *T. superba*

Figure I and II showed the phytochemical composition in *A. godseffiana* and *T. superba* respectively. The phytochemical constituents found in *A. godseffiana* include

Alkaloid, Saponin, Tannins, Flavonoids and Phenols. Alkaloid was found in 0.5mg/g, Saponin 0.1mg/g, Tannins 1 mg/g, Flavonoids 3.1 mg/g and Phenols 1.1 mg/g. Furthermore, the phytochemical constituent of *T. superba* are Alkaloid 32 mg/g, Saponin 5mg/g, Tannin 19mg/g, Flavonoids 11mg/g. Alkaloid as the major constituent recorded, connodes it as an index of high *T. superba* susceptibility to *M. subhyalinus* feeding activity.

Table 4: Phytochemical composition of wood material

Phytochemicals	Cconstituents
Alkaloid	+++
Anthraquinonoid	–
Flavonoid	+
Steroid	–
Tannin	++
Saponin	+
Cardiac glycosides	+

Note: strongly present (+++) ; present (++) ; slightly present (+)

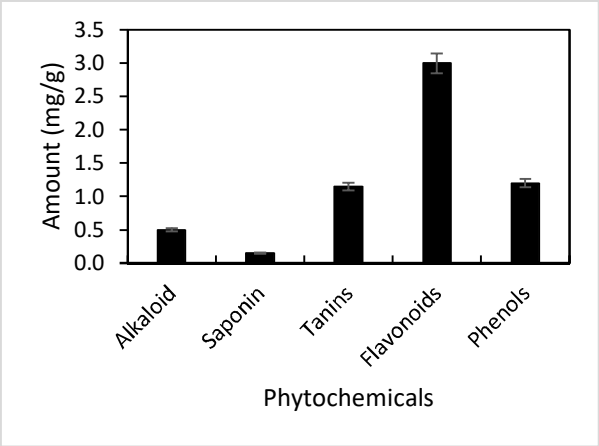

Figure 1: Phytochemical composition of *A. godseffiana* oil extract

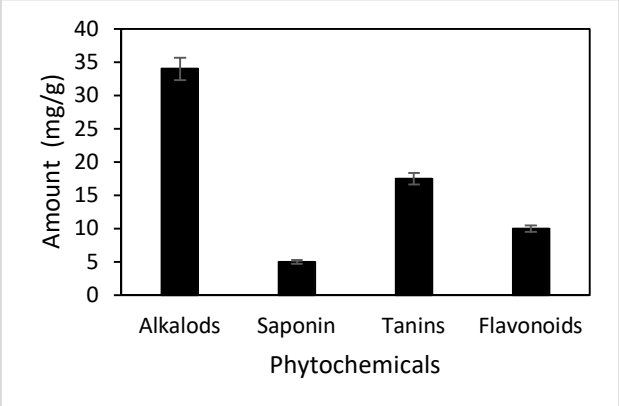

Figure 2: Phytochemical composition of *Terminalia superba* infested by *M. subhalinus*

DISCUSSION

Botanicals of different species have been reported of having medicinal values. In fact, before the discovery of many nowadays drugs, botanicals have been the major backbone of human medicine. Also, Isman (2006) opined that even before the discovery of many synthetic chemical insecticides, botanical extracts and powders have been the major weapons used by farmers to combat insect pests both on the field and in storage.

In this research, the result obtained showed that higher concentration of *A. godseffiana* leaf oil significantly affected high mortality of *M. subhyalinus*. High mortality induced by oils could be due to their ability to disrupt the normal respiratory ability of the insects. Also, the oils may have blocked the spiracle of termites Ajayi, (2012) and thereby lead to suffocation or death. The result of this research showed that, low concentration of the oil was required to achieve 50 and 95% mortality of the insects. The ability oils to effect high mortality of *M. subhyalinus* could also be due to the presence of phytochemicals in the leaf oil. The phytochemical analysis result of the oil extract showed the presence of alkaloid, saponin, tannin, flavonoids and phenols. Many of these phytochemicals have been reported to have significant effects on the survival of insects. Yang *et al*. (2006) opined that saponin, tannin, flavonoid, and many other phytochemicals have a level of high contact toxicity against wide range of insect pests. The chemicals have been noted to disrupt normal respiratory activity of insect and thereby cause their death, in corroboration with Ajayi *et al*. (2012). The result obtained agreed with the work of Adeyemo *et al.,* (2015) in which different plant materials were found to reduce termite infestation on wood.

Furthermore, the result obtained on repellent activity of oil showed that, the oil significantly repelled the termites. The high repellent activity of *A. godseffiana* oil against the insect could be due to inability of the insect to resist the toxic substance present in the oil. The result obtained in this research agreed with the work of Yang *et al.,* (2014) in which plant crude oil was used to protect stored product. The field application of this oil showed that, the termites were unable to feed on the wood of *T. superba* treated with the oil *A. godseffiana* at different concentrations tested. From the observations, it was noted that only the control treatments were infested by the insect while no traces of infestation were noticed on the oil treated woods. The result obtained confirmed the result obtained on repellent activity of the oil. The oil must have repelled the termites and thereby prevented the infestation of the treated wood. The results of this research revealed that oil of *A. godseffiana* is a promising botanical in the control of *M. subhyalinus*. Probit analysis showed that low oil concentration of the oil is required to achieve high mortality of the termites. Since

Plate I: Feeding effect of *M. subhyalinus* on *Terminalia superba*

botanical oil is one of the alternatives upon which insect pest control is based, there is a need for more research work on botanicals which could effectively compete with and replace many popular synthetic chemical insecticides. To increase the chance of winning the battle against termites as well as other insects, more research to be done on *A. godseffiana* oil to obtain biocontrol agent for control of termite both on the field and in storage.

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