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Pesticidal efficacy of three tropical herbal plants' leaf extracts against *Macrotermes bellicosus,* an emerging pest of cocoa, *Theobroma cacao* L.

A. V. Oyedokun*, J. C. Anikwe, F. A. Okelana, I. U. Mokwunye and O. M. Azeez

ABSTRACT

Insecticidal activity of the *Phyllanthus amarus*, *Acassia albida* and *Tithonia diversifolia* leaf crude extracts were evaluated against the workers of *Macrotermes bellicosus in vitro*. Different concentrations of the aqueous and ethanolic extracts (12.5%, 25%, 50%, 66.7%, and 75%) were tested against *M. bellicosus* which differed significantly ($P < 0.05$) from the control and standard (Thiamethoxam–Actara 25 WG). The aqueous extracts of *P. amarus*, *A. albida* and *T. diversifolia* caused 40-56%, 24-60% and 42-88% mortality, respectively after 140 minutes of exposure (MOE) to the extracts. The control resulted in 0% mortality, while the standard caused 100% mortality during the same period of 140 MOE. Similarly, ethanolic extracts of *P. amarus*, *A. albida* and *T. diversifolia* resulted in a significantly ($P < 0.05$) higher percentage mean mortality of 64 – 91 %, 36.4 – 76 % and 36 – 68 %, respectively. The toxicity ratings of the aqueous extracts ranged between low in 50% concentration of *A. albida* and very high in 75% concentration of *T. diversifolia* extracts, whereas, toxicity ratings of ethanolic extracts ranged between moderately low in 50% concentration of *A. albida,* as well as 12.5 % concentration of *T. diversifolia* and very high in 75 % concentration of *P. amarus*. Ethanol enhanced/potentiated the bio-insecticidal activities of some of the plant extracts used in this study. Further studies are needed to be carried out to know the fractional constituents of each plant species that is insecticidal in all the plants used in this study because different active compounds may have different biocidal properties, with different mixtures of the active ingredients so as to maximize the plants' insecticidal properties.

Key words: *Phyllanthus amarus, Acassia albida*, *Tithonia diversifolia, Macrotermes bellicosus,* Thiamethoxam, mortality.

INTRODUCTION

It is widely known that some higher plants exhibit biocidal properties. Plant extracts have been used locally in herbal preparations to cure ailments even before the advent of orthodox medicine in many developing countries. For instance, garlic had a wide spectrum of actions; not only is it antibacterial, antiviral, antifungal and antiprotozoan, but it also has beneficial effects on the cardiovascular and immune systems, Harris *et al*. (2001). The knowledge and use of plants as well as their extracts as protectants against grains and other foodstuffs had been in existence since time immemorial (Dales, 1996; Isman, 2000). Plants contain bioactive organic chemicals in the form of metabolites and about 400,000 secondary metabolites from Neem (*Azadiracta indica*) were reported to have effective insecticidal properties against field and store pests. Nimonol, Tetracylic triterpenoids, and others have been isolated from Neem leaves (Govindachari *et al.*, 1999; Bina *et a*l., 2004). Extracts from tobacco, rotenone, and pyrethroids have been studied extensively for their pesticidal activities. Flavonoids, Alkaloids, Saponins, Sesquiterpenes, Limonoids, Phenols, Stilbenes and Coumarins of plant origin have been reported to possess toxic, growth regulating and anti-feedant effects against a host of insect pests (Sunita and Lalijee, 2008).

Macrotermes bellicosus (Isoptera: Termitidae) generally referred to as harvester termites or War-like termite or African termite are mainly found in Africa, the Middle East, and Asia. Termites attack cocoa plants on the field by attacking the trunks and pods of cocoa causing the plant and the pods to dry up after severe infestations, Anikwe *et al.*, (2009). *Macrotermes bellicosus* normally feed on dead vegetation, their tunnels may weaken plant stems, causing them to collapse or giving access to fungus and other diseases. Where bark is gnawed from trees, the phloem may be interrupted, causing the death of the tree. The mud runways with which some species cover the plants to reach dead wood may cause the plant, e.g. tea, to wither and collapse. Cocoa trees, sugar cane, young coconut trees, cotton and wheat plants are among crops that may be affected by termites. *M.*

bellicosus is not of very great importance in this respect but its nest-mounds get in the way of agricultural machinery and have to be blown up with explosives or leveled with bulldozers, thus increasing the cost of mechanized farming, road construction and building-site clearance. Perhaps the most familiar termite damage is to the wooden fabric of buildings and furniture. Various earth-dwelling species, though not *M. bellicosus*, tunnel underground from their nests and enter the building through its foundations or any wooden part in contact with the ground. To obtain the wood for their food they make extensive tunnels through the structures, weakening them and eventually causing their collapse (Mackean, 2006). Hence, *M. bellicosus* is fast attaining a new pest status (minor to major) and it is important in the economic entomology of cocoa in Nigeria.

Phyllanthus amarus Schum and Thonn is an annual plant that belongs to the family *Euphobieacea*. The plant is a common weed of cultivated fields and it is widespread in West Africa (Akobundu and Agyakwa, 1998). The active ingredients include phyllantine, phyllantinol, phylochrysine, phylltetralin and hypophyllantine (Thyagarajan *et al.,* 1998). Other bioactive compounds include bioflavanoids, quercertin, quercertol, quercitrin, rutin, alkaloids, glycosides saponins, and catechins. *Acassia albida* belongs to *Mimosaceae* family.It is commonly known as Ana Tree. It is native to the Transvaal and Southwest Africa. It has been reported to be useful in treating fevers, diarrhea, hemorrhage, colds and opthalmia, leprosy, pneumonia, etc. in East and West Africa, FAO (1980); Felker (1981). *Tithonia diversifolia* (Hemsl.) is considered to be a medicinal plant that is widely used in folk medicine to treat various illnesses. It is commonly known as Mexican Sunflower, tree marigold, shrub sunflower or Japanese sunflower (English), "sepeleba" in (Yoruba Language), pua renga (Cook Island). Ethno botanical surveys have shown that extracts from the plant exhibited antimalaria, antidiarrhetic, antiinflammatory, antibacterial, antiproliferation properties and its effectiveness in the treatment of haematomas and wounds had been demonstrated as well (Rungeler *et al.,* 1998; Tona *et al.,* 1998). The leaf is reported to contain sequiterpene lactones taginin C as an active component against *Plasmodium*, diversifolin, diversifolin methyl ether and tirotundin as active components against inflammatory activity (Rungeler *et al.,* 1998).

Prophylactic use of synthetic insecticides had been the readily available control measure against termites' infestation on the field but the insecticides are expensive and most times not readily available to peasant farmers. Due to the environmental limitations and increasing legal restrictions on the use of many synthetic pesticides, there is a need for the development of alternative control measures that will be cheap, environmental friendly and easily adaptable by local farmers through the abundant flora diversities that exist in the tropics.

MATERIALS AND METHODS

This study was carried out in the Entomology Laboratory of the Cocoa Research Institute of Nigeria, Ibadan at a temperature of 28 ± 3^0C and relative humidity of 75 ± 5%. The field survey for the termite infestation frequency was conducted on a forth night basis in all the 9 zones (longitude 3.85 ^{o} N and latitude 7.22 ^{o}E) and the adjoining offices of Cocoa Research Institute of Nigeria, Headquarters Ibadan in the dry seasons (January to March of 2010 and 2011). The total number of active castes and the newly emerging castes per zone was observed visually, counted and tagged with metal plate labels. The number of cocoa trees with signs of infestation per zone (mud tubes on the bark, tunneled bark and mounded trees) was observed, counted, and tagged with ribbon. The total number of active and newly emerging castes from each zone was summed up, as well as the total number of infested trees, into five replicates per zone for mean separation.

Collection of Materials

Fresh leaves, healthy leaves of *P. amarus*, *A. albida* and *T. diversifolia* were collected from the field at CRIN Headquarters, Ibadan. Likewise, the workers of *M. bellicosus* used for this bioassay were collected along with the caste's soil bulk particles from the zone 8 cocoa plantations of CRIN Headquarters, Ibadan with the aid of a spatula and very soft camel hair brush into a tray and taken to the laboratory for this study. The termites were kept in aerated plastic cages in the laboratory at 27 ± 3^oC and relative humidity of 75 ± 5%.

Extract preparation

The plant extracts were prepared as described by Adedire and Akinneye (2003) with some modifications. The collected leaves were thoroughly rinsed in distilled water twice to remove dirt and were allowed to drain on a wire mesh. 200g each of the fresh leaves of *P. amarus*, *A. albida* and *T. diversifolia* were weighed separately into sterile mortar and pestle. Aqueous extracts were prepared by pounding the weighed leaves of each plant material into 200 ml of distilled water with which the stock solution of each extract was prepared. These mixtures were allowed to stay for 24 hours, heated at 60^oC for 45 minutes in a water bath (Tecan-WB3, Techne Incorporation, New Jersey, USA), later shaken thoroughly and sifted with muslin cloth. The filtrates were the stock solution that were serially diluted (water: extract) into different concentrations 1-8v/v: 12.5%, 1-4v/v: 25%, 1-2v/v: 50%, 2-3v/v: 66.7%, and 3-4v/v: 75%. Ethanolic extract was prepared using 200 ml of ethanol (99.5 %) (Absolute AR grade (Thornton and Ross Laboratories, England) as the solvent for 200 g fresh, healthy leaves each of *P. amarus*, *A. albida* and *T. diversifolia* following the extraction and dilution methods of the aqueous extract described above. The ethanolic extract was serially-diluted with distilled water at the same rate as described above.

Bioassay

The topical application test was carried out on the workers of *M. bellicosus* using different concentrations (12.5, 25, 50, 66.7 and 75 %) of the aqueous and ethanolic extracts to evaluate the contact action and toxicity ratings of the plant extracts used in this study. The control was without any treatment while the standard was Thiamethoxam (Actara 25 WG) at the rate of 8g insecticide/litre of water. The sterile Petri dishes were lined with filter papers to fit and the lids were perforated to allow for aeration. Five worker caste of termites (workers are responsible for caste construction and high foraging ability) were picked randomly into each Petri dish (9 cm diameter) from the collection tray with soft camel brush. There were five treatments per extract (aqueous and ethanolic). One drop of each aqueous and ethanolic extract concentrations was applied on the dorsal surface of each termite in each Petri dish using sterilized Burkard, England Micro-syringe (1ml capacity). Each treatment was replicated five times. Mortality counts were recorded after every 20 minutes up to 140 minutes. A termite was considered dead when it was lying flat on its back and showing no sign of movement of its body after being touched with soft camel brush.

Statistical Analysis

The data obtained from all the bioassay treatment replicates were converted to percentage (using Percentage mortality = total number of dead termites after treatment/total number of termites before treatment X 100) and the percentage mortality per treatment was subsequently subjected to analysis of variance and the means were separated using Tukey's Studentized Range (HSD) Test of SPSS 17.0 Version. The mean values of field observations on the number of mounds per zone and the number of infested trees were separated using Tukey's Studentized Range (HSD) at $P < 0.05$.

RESULTS

From field observation and surveys, *M. bellicosus* is fast attaining a new pest status from minor to major insect pest of cocoa, based on the frequency of the signs of infestation and damage observed in cocoa plantations at CRIN Headquarter, Ibadan, Nigeria. A total of 1512 active Termitaria were counted in all the 9 Zones as well as the areas surrounding the offices at CRIN Headquarters, Ibadan, during the survey period while a total of 873 cocoa trees were found with *M. bellicosus*

Table 1. Caste numbers and infested trees by *M. bellicosus* in CRIN, Headquarters, Ibadan, Nigeria in the dry seasons of 2010 and 2011.

Zones	Caste Number Mean ± SE	Infested Trees Mean ± SE
1.	137.40 ± 15.42[abc]	78.00 ± 2.12[bc]
2.	161.00 ± 1.70[bc]	104.00 ± 1.790[d]
3.	124.40 ± 2.00[ab]	79.00 ± 3.13[bc]
4.	120.00 ± 1.30[ab]	87.00 ± 2.61[c]
5.	182.00 ± 2.76[cd]	108.00 ± 3.69[d]
6.	120.00 ± 6.78[ab]	83.00 ± 1.14[c]
7.	91.60 ± 2.23[a]	68.00 ± 0.89[b]
8.	265.20 ± 15.88[e]	126.00 ± 4.89[e]
9.	218.00 ± 7.07[de]	118.00 ± 4.47[de]
Office surroundings	94.00 ± 23.81[a]	22.00 ± 2.21[a]

Mean following the same letter (s) in a column are not significantly different ($P < 0.05$) following Tukey's Studentized Range (HSD).

infestation. Zone 8 accounted for the highest number castes/ termitaria and infested trees which were significantly different ($P < 0.05$) from other surveyed zones (Table I). Total number of active and emerging castes in Zone 7 was the lowest, while office surroundings had the lowest number of infested trees.

The percentage mean mortality effects of aqueous extract of *P. amarus* on the workers of *M. bellicosus* following tropical application tests are shown in Table II. There was no significant difference ($P < 0.05$) among the insecticidal activities (contact) of aqueous extracts of *P. amarus* at concentrations, *A. albida* at 12.5% and *T. diversifolia* at 12.5% resulting in 40.00%, 40.00%, 36.00% and 42.20% percentage mean mortality, while other concentrations resulted in percentage mean mortality that were significantly different ($p < 0.05$) from one another, the control and the standard (Table II). The standard insecticide significantly differed ($p < 0.05$) from all the treatments' percentage means mortality and the control, resulting in 100% mortality of *M. bellicosus* after the maximum period of exposure (140 minutes). Percentage mean mortality of *M. bellicosus* that resulted from the aqueous extract of *P. amarus* at 25% concentration treatment was not significantly different from 50% concentration of aqueous extract of *T. diversifolia* with mean mortality percentages of 56.00% and 58.00%, respectively. Likewise, percentage mean mortality rate of 52.00% and 52.00% were recorded for the aqueous extracts of *A. albida* at 25% and *A. albida* at 66.7% concentrations

Table 2. Percentage means mortality effects (mean ± SE) of *M. bellicosus* after 140 minutes of exposure to crude aqueous and ethanolic extracts of *P. amarus*, *A. albida* and *T. diversifolia* at different concentrations.

Plant extract	**Concentration % (v/v)**	**Aqueous extract (% mean mortality)**	**Ethanol extract (% mean mortality)**
P. amarus	12.5	44.00 ± 1.79^{de}	80.00 ± 1.70^{i}
A. albida	12.5	36.00 ± 2.28^{c}	76.00 ± 1.10
T. diversifolia	12.5	42.20 ± 1.24^{cde}	36.00 ± 1.10^{b}
P. amarus	25.0	56.00 ± 2.00^{gh}	76.00 ± 2.28^{hi}
A. albida	25.0	52.00 ± 0.63^{fg}	48.00 ± 1.41^{c}
T. diversifolia	25.0	64.00 ± 0.89^{ij}	60.00 ± 2.00^{de}
P. amarus	50.0	40.00 ± 0.89^{cd}	64.00 ± 1.41^{ef}
A. albida	50.0	24.00 ± 1.79^{b}	36.40 ± 1.72^{b}
T. diversifolia	50.0	58.00 ± 1.67^{ghi}	46.00 ± 2.19^{c}
P. amarus	66.7	48.00 ± 1.89^{ef}	72.00 ± 0.89^{gh}
A. albida	66.7	52.00 ± 0.89^{fg}	60.00 ± 2.098^{de}
T. diversifolia	66.7	68.00 ± 2.76^{k}	55.20 ± 1.02^{d}
P. amarus	75.0	40.00 ± 1.09^{cd}	91.60 ± 0.748^{j}
A. albida	75.0	60.00 ± 2.83^{hi}	63.60 ± 0.748^{ef}
T. diversifolia	75.0	88.00 ± 1.41^{l}	68.00 ± 1.67^{fg}
Control	0.00	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Standard	8g/litre of water	100.00 ± 0.00^{m}	100.00 ± 0.00^{k}

Mean values of different superscript letters are significantly different ($P < 0.05$) following Tukey's Studentized Range (HSD) Test.

respectively. *T. diversifolia* at 75% concentration recorded the highest percentage mean mortality of *M. bellicosus*, in all the aqueous extracts evaluated and *A. albida* at 50% concentration recorded the lowest percentage mean mortality (Table 2).

Table 2 shows the percentage mean mortality of *M. bellicosus* treated to different concentrations of *P. amarus*, *A. albida*, and *T. diversifolia* after the maximum period of contact with the extracts. Ethanolic extract of *P. amarus* at 12.5, 25, 50, 66.7 and 75 % concentrations resulted in percentage mean mortalities of 80.00, 76.00, 64.00, 72.00 and 91.60, respectively of the workers caste of *M. bellicosus* after the maximum contact exposure period of 140 minutes to the extract. Ethanolic extract of *P. amarus* at 12.5 % concentration outperformed the ethanolic extracts of *A. albida* and *T. diversifolia* at 12.5 % concentration by having a better kill of the test insect samples within the same exposure period to the extracts. Similarly, this trend of superior performance of *P. amarus'* ethanolic extracts at different concentrations which were significantly different ($P < 0.05$) in the resultant percentage mean mortality of *M. bellicosus* was recorded. All the plant extracts at different concentrations were significantly different ($P < 0.05$) from control and the standard. The toxicity ratings of the extracts ranged between low in 50 % aqueous extract of *A. albida* to very high in 75 % aqueous extract of *T. diversifolia* and 75 % ethanolic extract of *P. amarus*. Generally, aqueous extract of *P. amarus* rated moderately low while the ethanolic extract *P. amarus* rated high. There were similarities in the trend of toxicity ratings of the aqueous and ethanolic extracts of *T. diversifolia* except at 75 % aqueous extract concentration. 12.5 % ethanolic extract of *A. albida* outperformed extracts of *A. albida* of other concentrations.

DISCUSSION

From this study, aqueous and ethanolic leaf extracts of *P. amarus*, *A. albida* and *T. diversifolia* showed insecticidal activities via contact toxicity, with toxicity ratings that ranged between low (in 50% aqueous *A. albida*) and very high in 75% aqueous *T. diversifolia* extract. Similarly, ethanolic extracts had toxicity ratings that ranged between moderately low at 50 % *A. albida*, as well as 12.5 % *T. diversifolia* and very high in 75 % *P. amarus*, and this supports Stoll (2001) who reported that leaf extracts of several higher plants have been tested to be effective against insect pests and diseases of various crops on the field and in store. From this study, extracts of the selected tropical herbal plants possess some insecticidal properties against *M. bellicosus* but several variations occurred, based on the extracting solvents and the concentration of the extracts as these influenced the efficacy and/or biocidal activities of the plant materials. Overall, ethanolic extracts of *P. amarus* at 75 % v/v concentration resulted in highest percentage mean mortality of *M. bellicosus* (Table 2) and this was found to be significantly different from all other extracts of different concentrations used in this bioassay. This suggests that apart from antiviral potency, *P. amarus* also has some insecticidal properties. Similarly, aqueous extracts of *P. amarus* showed some insecticidal activities at different concentrations, but the activities were significantly lower when compared with insecticidal potency of ethanolic extracts of *P. amarus*. This is in consonance with the findings of Khanna *et al*. (2003) that showed that ethanolic extracts of aerial and root parts of *P. amarus* had some insecticidal activities against stored grain pest *Tribolium castaeneum*. Biocidal activity of extracts tested in this study against *M. bellicosus* via topical application test was not concentration-dependent as it resulted in an irregular patter of mortality rate, from the lowest to the highest concentrations. The sequential mortality rate in correlation with increase in concentration of plant extracts as reported by many earlier workers did not occur in this study and it suggests that aqueous extract of *P. amarus* can best be applied at 25 % v/v concentration in order to have a better kill of *M. bellicosus*. This may be due to the mixture of the active compounds that were in the best proportional mixture for insecticidal activities at 25 % v/v concentration, although the proportional mixture of the extracts were not analyzed. Also, the result of this study showed that concentration may not be a factor in topical application method for toxicity test in botanical insecticides, but rather the plant material's biocidal components (secondary metabolites) and the extracting solvents used might have contributed to the efficacy of the extracts. This suggests that herbal plants to be evaluated for insecticidal activities have to be in the right mixture and/or proportion of the active compounds, no matter the concentration, in order to have an optimum performance when being used in plant protection against insects. Likewise, all the plant materials extracted with alcohol followed the same haphazard mortality pattern following topical application on workers of *M. bellicosus*. Although, ethanolic extracts gave a relatively better kill especially from *P. amarus* extract than all other aqueous and ethanolic extracts, the extracting solvent and/or the proportional mixture of the active compounds in plant extracts could be a vital factor in exploiting these botanical insecticides, because crude extracts contain different active ingredients like insecticides, fungicides, nematicides, acaricides, bactericides etc. which may need to be in a synergistic combination in crude extract form in order to be effective as insecticides. Extracting solvents play an important role in the biocidal potency of plant crude extracts as shown in this study. Aqueous extract of *P. amarus* leaves did not give a better kill of *M. bellicosus* as ethanolic extract of *P. amarus* leaves while aqueous extract of *T. diversifolia* resulted in higher mortality rate of *M. bellicosus* than ethanolic extract of *T. diversifolia* within the same exposure period. This suggests that each herbal plant material has its peculiarity as to which extracting solvent(s) will best extract the biocidal active compounds to be used in insect control. This supports the findings of Manzoor *et al.* (2011) that activity of crude plant extracts against termites is often attributed to complex mixture of active compounds and that Ethyl acetate extract of *Ocimum sanctum* leaves resulted in higher mortality of termite (*Heterotermes indicola* (W.) than methanol, butanol, hexane, water and chloroform extracts in this order. However, aqueous extract of *T. diversifolia* at 75 %v/v, killed 88 % of the test insects within the maximum exposure period, which showed that aqueous extraction of *T. diversifolia* will be best to extract the insecticidal active compounds in this plant material. This is in support of a study as reported by Areekul *et al.* (1987) that water decoctions and volatile fractions of flower heads of *Tithonia diversifolia* showed 85 and 75 % mortalities respectively of flies within the first hours after spraying which demonstrated high knockdown effects of aqueous extracts of *T. diversifolia* on the flies. Aqueous extract of *P. amarus* (5 % concentration) showed some oviposition deterrent activities (more than 50 %) against *Callosobruchus maculatus* Shifa Vanmathi *et al*. (2010). Ethanolic extracts of *P. amarus* at all the tested concentrations resulted in more than 70 % mean mortality of the test samples, which may be due to knockdown effect of ethanol on insects (personal observation) and this suggested that *P. amarus* apart from its use in treating ailments also has insecticidal compounds present in it that are readily extracted in ethanol.

Hence, further investigation has to be carried out on the best extracting solvents for different herbal plant materials for effective use in insect pest management and control. Also, the fractional constituent of the herbal plants that is insecticidal has to be separated from the whole active compounds present in these plant materials in order to maximize the potentials of biocidal properties of the tropical plants.

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A. V. Oyedokun*, J. C. Anikwe, F. A. Okelana, I. U. Mokwunye and O. M. Azeez
Entomology Section, Crop Protection Department,
Cocoa Research Institute of Nigeria, P.M.B. 5244,
Ibadan, Oyo State, Nigeria.
*Email: gokman_d@yahoo.com

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