THE INTERNATIONAL JOURNAL OF SCIENCE & TECHNOLEDGE

Bioefficacy of Extracts of *Ocimum Gratissimum* Leaves on Dry Wood Termites *Cryptotermes Brevis* (Isoptera: Rhinotermitidae)

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Abstract:
The bioefficacy of leaf powder and ethanolic extracts of Ocimum gratissimum were evaluated against the dry wood termite Cryptotermes brevis in concentrations of 20-60g (powder) and 20-60ml (extract) all in three replications. The investigation revealed that mortality of termites was directly proportional to concentration of extracts and exposure time. The leaf extract proved more potent than the powder. The leaf powder also brought about repellency of the termites as they were seen to gather away from the powder. In all, there was 100% mortality of the termites in both the powder and extract treatments though the extracts brought about optimal mortality of termites in recorded time of four hours as against the powder of six hours respectively. Phytochemical screening of O. gratissimum leaves revealed the presence of the secondary metabolites such as tannins, flavonoids, terpenoids, glycosides, phenols, alkaloid and saponins working independently or synergistically to bring about the positive control of the termites. Therefore O. gratissimum should be included in traditional pest management and control systems especially in the effective control of structural insect pests since it is locally available, affordable, not toxic and environment friendly.

Keywords: *Bioefficacy, leaf powder, extract, ocimum gratissimum, cryptotermes brevis*

1. Introduction

Cryptotermes brevis is the dry wood termite belonging to the order Isoptera, class rhinotermitidae, family kalotermitidae and genus Cryptotermes and commonly called the powder post termite. It is the most widely and frequently introduced termite in the world. It is an invasive and widespread pest species Edward & Mill, (1986) known to be ubiquitous on wooded structure where they commonly infest. C. brevis are social insects living entirely within the infested wood chamber and obtain water and absorbed from wood fibers and by metabolic processes. The specie has cylindrical, stud phragnotic head and short mandibles adapted for mandibulate feeding (biting and chewing wood). It also has shorter legs, are slow moving, have well developed tarsal claws for gripping surfaces, moniliform (beadlike) antenna and blindness except in the reproductive caste. This specie of termite undergoes holometabolous metamorphosis exhibiting all developmental life stages of egg, larva, pupa and adult (Thorne, 1999). Dry wood termites are structural destructive insect pests found around buildings (inside and outside) where they make line marks or tunnels upwards into the ceiling/roof causing serious damage and destruction to rafters. They are also found infesting dry tree trunks and logs of wood abandoned in the open. Structure-infesting drywood termites are heat tolerant and can survive extreme temperature (Scheffrahn, 1997b). They attack household items like clothings, books, rugs and other valuables Okunade, (2004) especially raffia knitted mats, purses, files, caps and bags. They are also pests of wood and wood products such as felled timbers and their products, structural timbers, furniture, fabrics, storage structures and other wood work articles which are completely devastated by these beetles tough they were originally highly priced Lale, (2002). According to Termite web (2009), *C. brevis* infest household furniture such as wooden frames, beams, roofing sheets/ceilings, and roofs of buildings, hence are adapted to human habitation where they build galleries (tunnels) on these wooden surfaces thus residing as a colony and blocking the entrance against intruders thereby completely destroying the wood and wooden structures in homes and leaving their frass as the destruction continues. *C. brevis* control cost in the United States of America is estimated to range between 120-300 million dollars annually with the greatest losses occurring in California peninsula, Florida and Hawaii and untold amount World Wide (Scheffrahn *et al*., 1997a, 1998, Invasive Species, 2013).

Huge amounts of money are continuously spent by Government of countries, private individuals, corporate bodies and other organizations aimed at controlling the incidence of *C. brevis*infestation using control methods chemicals (insecticides), acoustic emissions detection, microwave, xray, infrared metabolic gas and canine detection Scheffrahn *et al*., (1993, 1997a, 1998; Thorne, 2000) which still have such setbacks of residue problems, health hazards, cost of production

and procurement (Levis & Havesty, 1996; Quarles, 2004), affect natural enemies and develop pesticide resistant insect strains (Georgiou, 1991; Deedant, 1994). There is the increasing demand for the development of safe alternatives to replace synthetic pesticides (Lale, 1995; Grainge & Ahmed, 1998) thusresearchers have advanced towards the use of natural organically base biopesticide from plants in our locality that are easily cultivated, with no cost of production and absence of residue problems. This study was undertaken to evaluate the bioefficacy of the powder of *O. gratissimum* with the view to developing an environmental, health safety and effective compound for the positive control of this destructive structural insect pest.

Plant powders and extracts in recent times have been studied as alternative source of materials for insect control due to their broad based bioactive constituents (Isman, 2006; Bindurani *et al*., 2012), resulting into prompt research efforts at the use of natural plant products as potential sources of commercial pest control agents.*Ocimum gratissimum* is a valuable perennial herbal tree native to India but widely distributed in the tropics of Africa, subtropics and in Asia where it is grown wild or cultivated as home garden crop or on commercial scale for its multiple traditional and clinical potentials (Sulistirini *et al*., 1999).It is known for its ethnopharmacology, morphology, phytochemistry, pharmacological, clinical and toxicological studies (Prabbu *et al*., 1994; Dubey *et al*., 1997; Nakamura, *et al*., 1997; 2004, Lemos*et al*., 2005; Edeoga *etal*., 2006).*O. gratissimum*components have been proven to possess a broad range of properties including antibacterial (Nakamura, *et al*., 1997, Oussalah *et al*., 2007, Prabbu*et al*., 2009), antifungal (Dubey *et al*., 2000, Nakamura *et al*., 2004, Terezinba *et al*., 2006, Matasyoh *et al*., 2007), antiviral (Schnitzler *et al*., 2011), insecticidal (Essam, 2001; Sosan *et al*., 2001) and antioxidant properties (Chu *et al*., 2002, Ganiyu, 2006). Previous studies on the chemical constituents of Ocimum species by various authors have revealed the presence of numerous bioactive components called phytochemicals on all parts of the plants viz: stem, root, flowers and leaves (Middleton & Kandaswanmi, 1992, Prabbu *et al*., 2009).

2. Materials and Methods

2.1. Collection of Test Insect

Dry wood termite specie *C. brevis* used in the study were collected from the earthen tunnels constructed by the specie in a house in Yenagoa metropolis employing methods for insect collection (Lale, 2006; Udo, 2008). Colonies of eggs, larvae, pre pupae, pupae and adults were collected and used for the bioassay, but observations was recorded on adults basically.

2.2. Collection and Preparation of Leaf Samples

Fresh leaves of *O. gratissimum* were collected from residential area at Agudama-Epie Yenagoa, Bayelsa State and taken to the laboratory of Biological Sciences, Niger Delta University for processing and experimentation. The leaves were washed thoroughly in running tap water to remove sand and debris. Leaves were later air dried at room temperature for a period of 6 days and later sundried for a day to the point of crispy Briyai, (2012). The leaves were ground using the hand mill and later blended to obtain finely divided powder which was further dried in a hot air oven at 60^0C for eight hours. 20g, 40g and 60g of the powdered materials were bagged and labeled separately for further usage in the bioassay.

2.3. Preparation Of Ethanolic Extract

Adopting the procedures of Obeng-Ofori and Akuamoah, (1998); Udo *etal*., (2012), leaf the leaf powdered forms of *A. indica was* separately weighed as follows: 00g (control), 20g, 40g, and 60g into different transparent rectangular boxes labelled A-D. Equal volume of 10ml of 70% ethanol was added into eachbox by concentration in volume to volume (ml) ratio. The crude ethanolic extract was filtered using a Whatman No.1 filter paper through a plastic funnel to a 50ml beaker respectively. The filtrate was transferred to a round bottom flask and heated in a water bath at 40^oC for 72 hours to allow for ethanol evaporation. Using a rotary evaporator, the extracts were concentrated to dryness and used for the bioassay. Stock solution was prepared with 2.8 liters of ethanol in 1.5kg of the powder which was allowed to stand for 24 hours. The mixture was filtered and the filtrate evaporated in a desiccator. The resultant yield was 60g of the extract.

2.4. Experimental Design

To examine the effects of the leaf powder and extracts of the test plant on the termites, the treatments were applied as contact poison both for leaf powder and ethanolic leaf extract. All treatments were arranged in completely randomized design (C.R.D.)

2.5. Methodology

Employing the methodology of Udo, *et al*., (2004) with modification, the entire content of *C. brevis*colony in tunnel was emptied into different concentrations of *O. gratissimum* leaf powder (20g, 40g and 60g) and ethanolic extracts (20ml, 40ml and 60ml) respectively. No leaf powder was added to the control while distil water was used as the extract control. The experiment was replicated three consecutive times allowing for the termites to build up the earthen tunnels within the period of 7 days interval. Time duration of the observation for deaths was two to six hourswith the number of insect mortality recorded for the period understudy.

2.6. Qualitative Test

The leaves of the test plant were screened qualitatively for phytochemicals using standard procedures documented by Okwu, (2001); Trease and Evans, (2002); Udo, (2013), where the leaf powder was extracted at room temperature with 75% ethanol by maceration and concentrated to dryness in a rotary evaporator.

2.7. Data analysis

All results were tested and analyzed using one way ANOVA. Data obtained were expressed in number of deaths. Student T-test and pair wise multiple comparisons were used for significant differences at alpha level, $p \leq 0.05$. All graphs were plotted using Microsoft excel.

3. Result

The result obtained showed that mortality of termites was directly proportional to concentration of treatments (leaf powder and extracts) and time of exposure. The rate of termite death increased with concentration of the treatments and exposure time and was more in the leaf extracts than the powder. There were no termite deaths recorded in the control within the period under study. The leaf powder also brought about repellency of the termites as they were seen to gather away from the powder. In all, there was 100% mortality of the termites in both the powder and extract treatments though the extracts brought about optimal mortality in recorded time of 4 hours as against the powder of 6 hours respectively.

Figure 1 below gives the result showing the contact effect of leaf powder of *O. gratissimum* on the subterranean dry wood termite *C. brevis*. Death of termites was concentration of treatment dependent and was seen to increase with increase in the concentration of the treatment powder. Again, the termite mortality increased with the increase in the exposure time of the treatment. There was significant difference between the hours of exposure and the mortality of the termites at alpha level $p<0.05$. Also significant difference at $p<0.05$ was recorded between the mortality of cockroaches and the concentration of the leaf powder.

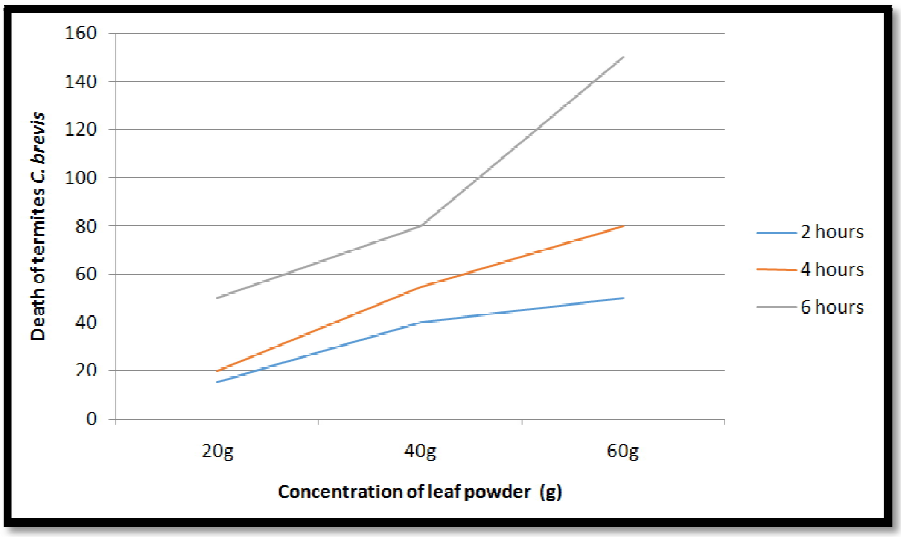

Figure 1: Effect of Leaf Powder of O. Gratissimumon the Termite C. Brevis Showing Number of Deaths Recorded within Six Hours.
Source: Authors

There was significant difference in termite mortality between the hours of exposure and also between the concentrations of the leaf powder at $p<0.05$.

The charts below gives the result showing the contact toxicity effect of ethanolic leaf extracts of *O. gratissimum* on the subterranean dry wood termite *C. brevis*. Death of termites was directly proportional to concentration of the treatment and was seen to increase with increase in the concentration of the extracts and the exposure time (Fig. 2). There was significant difference between the hours of exposure and the mortality of the termites at alpha level $p<0.05$. Also significant difference at $p<0.05$ was recorded between the mortality of cockroaches and the concentration of the leaf extracts.

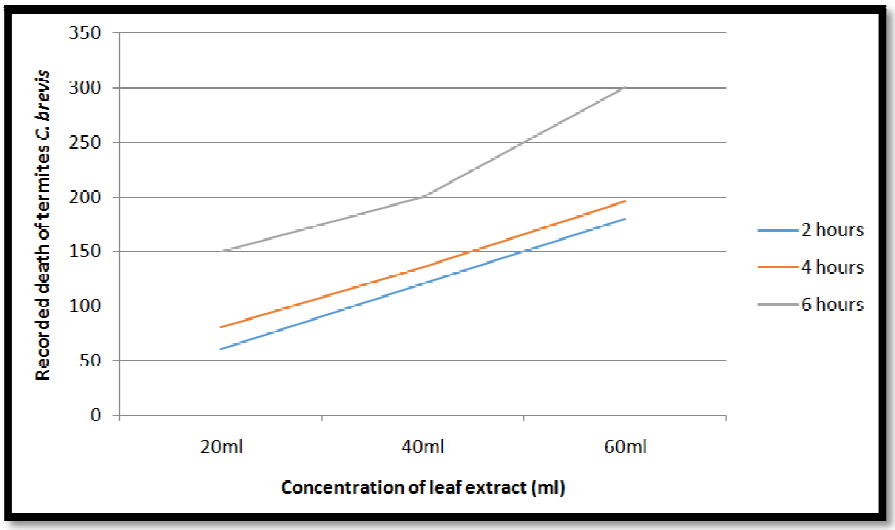

Figure 2: Effect of Ethanolic Leaf Extracts of O. Gratissimum on the Termite C. Brevis Showing Number of Deaths Recorded within Six Hours
Source: Authors

There was significant difference of termite mortality between hours of exposure and also between the different concentrations of ethanolic extracts at $p<0.05$.

Result of the phytochemical analysis of the *O. gratissimum* leaves revealed the availability of the following active metabolites: tannins, glycoside, saponin, alkaloids, flavonoids, terpenoids and phenols in various degrees of strength viz: present (+) for saponin, tannins, terpenoid and phenols; moderately present(++) for glycoside and flavonoids and highly present (+++) for alkaloids.

4. Discussion

Insect are the major causative pests of wood deterioration and nearly 90% of the total dry matter loss in storage are due to insect pests (Bindurani *et al*., 2012). Insect pests have largely been controlled using synthetic pesticides which eventually pollute the environment with toxic residues, affect natural enemies of pests and developed insecticidal resistant insect strains (Deedant, 1994, Georgiou, 1991; Pallpane, 1993). The result obtained showed that mortality of termites was directly proportional to concentration of treatments (leaf powder and extracts) and time of exposure. The rate of termite death increased with concentration of the treatments and exposure time and was more in the leaf extracts than the powder. No termite deaths were recorded in the control. There was 100% mortality of the termites in both the powder and extract treatments though the extracts brought about optimal mortality in recorded time of 4 hours as against the powder of 6 hours respectively. However, all the treatments proved successful in controlling the population of the termite *C. brevis*

The bioefficacy of *O. gratissimum* leaves (powder and ethanolic extract) against *C. brevis* is attributed to the principal constituent of Eugenol responsible for biological activities observed on the insects (Nwanyi*et al*., 1995; Keita *et al.,* (2000, 2001). This was further confirmed by Sosan *et al.,* (2001); Jinq *et al*., (2002) who attributed the toxicity of *O. gratissimum* treatments on insect species to the toxic effects of the compounds eugenol, monoterpenoids and sesquiterpenoids found in the plant extracts. According to Obeng-Ofori *et al*., (1998), Janine *et al*., (2005); Matasyoh, *et al*., (2007), *O. gratissimum* leaves contained Eugenol, methyl eugenol, cis-ocimene, pinene, camphor germacrene-D, trans-carypohyllene, farnesene and I-bisaboline which earned the plant its insecticidal ability. Furthermore, Nadkarini, (1999); Oparaocha*et al*., (2010); Venketachalam and Jabasen, (2001) in their various studies revealed that the major components of *O. gratissimum* species such as P-cymene, A-thujene, myrcene, methyl chavical and thymol in addition to phenol were responsible for the control of insect pests. The active compounds extracted from *Ocimum* plants parts are found to be rich in camphor, citral, geranoil, linalool, lincalyacetale and methyl charicol whose metabolites acted as biocide, insect growth regulators, repellent and oviposition deterrent on insects as reported by Lawrence, (1997); Babu & Muragan, (1998); Venketachalam & Jabasen, (2001); Essam (2001). Other components of *O gratissimum* derived from the leaves include oleanoic acid (Njoku *et al*., (1997), citrate, ethyl cinnamate, linalool (Dubey *et al*., (1997), Y-terpinene, limonene, terpinolene and 1, 8- cineole (Jirovetz *et al*., (2005).

The leaf powder also brought about repellency of the termites as they were seen to gather away from the powder. The repellency property of *O. gratissimum* on the termites is in conformity with that obtained by Lale, (1995); Grainge & Ahmed, (1998) who reported that *O. gratissimum* are used in the production of insect repellents as it gives out a sweet scent of camphor that deters and repels insects.

The result of this study also confirmed that *O. gratissimum* leaf powder and ethanolic extracts are effective biocides for controlling the termites. It also indicated that *O. gratissimum* ethanolic extract performed better than the leaf powder which is in agreement with the observations of Deshpande & Tipnis, (1997), Keita *et al.,* (2000); Orwa*et al*., (2009); who independently reported that *O. gratissimum* contained diverse group of compounds or essential oil that contain bioactive constituents with pesticidal, insecticidal and repellent properties, hence the positive outcome obtained

in this study. According to Cristana *et al*., (2006), extracts of Ocimum specie, resulted in increase in the brain lipid and cholesterol content as the biomolecule of the plant extract enters the brain of the insect which actively becomes the site of action with resultant biochemical changes.

Ocimum gratissimum extracts screened for phytochemicals revealed the presence of saponins, tannin, flavonoids, terpenoid, alkaloid, and phenols in various quantities which is in agreement with earlier studies by Lemos*et al*., (2005); Edeoga *etal*., (2006); Akinmoladun, (2007); Ladipo *et al*., (2010); Justina & Solomon, (2017); Oladosu-Ajayi*et al*., (2017). They further stated that these compounds possess insecticidal, antihelmintic, nematocidal and antimicrobial properties.

5. Conclusion

The present study on the bioefficacy of leaf powder and extracts of *O. gratissimum* on the subterranean dry wood termite *C. brevis*has proven that the above test plant has both repellent and insecticidal properties. The population of termite was drastically controlled to the barest minimum using this botanical which is locally available, easily affordable and extracted, target specific, not toxic and without the problems of residue.

6. Recommendation

The authors do herein recommend the use of *O. gratissimum* extracts as substitute for chemical pesticides. This approach to insect pest control is economically viable, target specific, has broad spectrum of control, possesses little or no threat to man and other animals, is environment friendly and highly affordable as the plants are locally available in Nigeria. Furthermore, that *O. gratissimum* extracts be applied on a wide range of insects to establish its inclusion in pest control system and approach.

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