INSECTICIDAL ACTIVITY OF LEAF POWDER AND ETHANOLIC EXTRACTS OF AZADIRACHTA INDICA, OCIMUM GRATISSIMUM AND DRACAENA ARBOREA AGAINST NYMPHS OF THE DOMESTIC PEST PERIPLANETA AMERICANA (DICTYOPTERA: BLATELLIDAE)

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ABSTRACT: Laboratory studies aimed at assessing the efficacy of leaf powders and ethanol extracts of Azadirachta indica, Ocimum gratissimum and Dracaena arborea applied at various concentrations of 10-50 in controlling the nymphs of Periplaneta americana through contact toxicity test via filter paper and by topical application with five replications. The results showed significant differences (p≤0.05) between the treatments and the controls with mean mortality values from leaf powder as: 00 (control): 0.5±0.20, 0.6±0.20, 0.5±0.20; 10g: 9.8±2.60, 9.5±2.60, 5.3±1.80; 20g: 14.5±3.30, 12.8±2.90, 7.0±2.30; 30g: 17.8±3.60, 15.5±3.30, 8.8±2.60; 40g: 20.3±2.90, 17.0±3.60, 10.3±2.60, 50g: 23.5±4.10, 22.0±3.30, 12.5±2.90; Contact toxicity by topical application of ethanolic extracts gave control: 0.6±0.20, 0.25±0.1, 0.6±0.20; 10ml: 16.3±3.40, 12.0±2.80, 10.3±2.60; 20ml: 19.5±3.60, 16.3±3.40, 12.8±2.90; 30ml: 23.3±4.00, 19.3±3.80, 14.5±3.30; 40ml: 26.5±4.60, 22.3±3.90, 17.0±3.60, 50ml: 27.3±4.60, 24.8±4.20, 21.3±3.90 while contact toxicity on filter paper, recorded mortality values of control: 0.6±0.20, 0.0±0.00, 0.6±0.20; 10ml: 16.3±3.40, 12.0±2.80, 9.8±2.60; 20ml: 19.0±3.80, 17.0±3.60, 12.0±2.80; 30ml: 24.0±4.20, 19.3±3.80, 14.3±3.30; 40ml: 26.0±4.60, 20.8±3.90, 16.5±3.50, 50ml: 27.0±4.60, 25.0±4.30, 21.0±3.90 respectively for A. indica, O. gratissimum and D. arborea treatments. All treatments proved effective and nymph mortality was concentration dependent, however A. indica ethanolic extract treatments were most significantly bioactive. Phytochemical screening revealed the presence of active ingredients such as saponins, alkaloids, tannins, glycosides, flavonoids, terpenoids and phenols with larvicidal and insecticidal properties when utilized in powder or extract forms. Finally, botanicals as reported by literature, have no toxic effects on man, are eco-environmental friendly and locally available, hence should be utilized in biological pest management practices and control systems.

Keywords: Azadirachta indica, Ocimum gratissimum, Dracaena arborea, Periplaneta americana, Nymphs, Mortality, Contact Toxicity.

1. INTRODUCTION

Cockroaches are the most abundant insect pest of public health importance; they infest hospitals, food manufacturing industries, kitchens and residential apartments [1, 2]. Cockroach (Periplanata americana) infestation has always raised safety concerns, especially as carriers of food-borne pathogens and food spoilage organisms [3]. As they feed on materials, cockroaches leave filth and secrete offensive and sickening oily liquid having odor that ruin food [4]. Cockroaches feed on human excreta as well as human food, thus are potential transmitters of diseases such as dysentery, typhoid, cholera and other food-borne infections which have been experimentally confirmed [3-6]. Dust containing cockroach excreta triggers allergic reaction such as wheezing in many individuals, making it particularly harmful to asthmatic patients [7]. A robust association has been established between the presence of cockroaches and increase in the severity of asthma symptoms in individuals sensitive to cockroach allergen [8-10]. Not only do cockroaches present variety of health hazard when found in our homes, but are also a threat in commercial places, spreading diseases through any food source hence cause food poisoning as they come in contact with food [8, 10]. About twenty two species of bacteria, viruses, fungi, protozoa and five species of worms have been reportedly isolated from the body of P. americana [11]. Cockroaches are
confirmed as vectors of poliomyelitis virus, enteropathogenic bacteria, amoeba cyst, eggs of worms and the fungus Aspergillus species [12]. The ancient man had deployed different methods of control, including prayers, magic spells, cultivation systems, mechanical practices as well as application of organic and inorganic substances [13] including pesticides, with developing countries and Nigeria in particular been faced with the most challenges in achieving the sound management of pests. However, the best pest control method is that which is non-toxic and environment friendly, hence the use of natural plant parts/products as bio-pesticides to overcome the problems of synthetic chemical hazards, is considered the best control measure which has become popular due to their degradability, least persistence and least toxicity to non-target organisms, economical and easy availability [14]. About two hundred plants with insecticidal properties including neem have proven successful in the control of over five hundred and fifty insect species in the orders Dictyoptera (cockroaches and mantis), Coleoptera, Isoptera, Homoptera, Heteroptera, Diptera, Orthoptera and others [15] [16]. Evidences abound that botanical pesticides are generally safe and effective [17], their use in Nigeria as in other parts of Africa is still hampered by some challenges such as most data gathered are obtained from laboratory trials; field data are rare. There is still hardly any developed appropriate technology for the application of botanicals, especially the oil and dust formulations [18]. Also compared with synthetic insecticides, the effects of natural insecticides are short-lived thus frequent applications are required to obtain reasonable degree of control. Furthermore, bio pesticide formulations are yet to be available in usable forms to farmers in commercial quantities so as to serve as alternatives to synthetic pesticide.

**Plate 1. Life cycle of the American cockroach (P. americana) showing nymphs**

Source: Authors

**1.1. Statement of Problem**

Owing to the problems associated with the usage of the first to third generations of pesticides on human health, animals and the environment, researchers over the years delved towards the discovery of the fourth generation of pesticides which is plant and plant products based (termed biopesticides) for the control of insect pests of field, storage, domestic and structural importance.

Plant active ingredients called phytochemicals are now been screened, extracted, identified, discovered and developed into formulations designed specifically for the control of insect pests. According [16, 19], these plant derived products are bio-degradable, target specific, eco-friendly, inexpensive, locally available, and generally safe, thus offering potential for quality and effective control of insect pests, hence the rationale behind this research since P. americana is a target specie for control as reported by [20] indicting it as food spoilage organism, vector of pathogenic organisms of human diseases, triggers of allergies and asthmatic attacks in sufferers, destruction of valuable assets with its filthy oily discharge and its offensive smell amongst others. However, the development and or availability of these plants and plant products in commercial quantities especially in Africa in general and Nigeria specifically is lacking, hence absolute reliance on information and products from the United States of America. Also, information if available locally are based on laboratory trials rather than field tests and research due to the absence of branded products sold commercially.
Therefore, in response to the above problems, this study seeks to use the extract forms (leaves) of the test plants *Azadirachta indica*, *O. gratissimum* and *Dracaena arborea* in controlling adult *P. americana*. Furthermore, to identify, extract and screen the test plants qualitatively for phytochemicals. Finally, to recommend that the active ingredients (secondary metabolites or phytochemicals) identified from these plants be discovered, developed, and branded into biopesticide formulations that can be readily commercialized for sales to the teeming populace who are in dire desperation of controlling the menace associated with *P. americana* infestation in homes, hotels, hospitals and food preparation areas.

Neem tree, *A. indica* Juss of the Meliaceae family, is a tropical evergreen plant related to mahogany and found in tropical nations [21-23]. It originated from South East Asia and is very well known worldwide because of its biological control properties [24]. The name is derived from the Persian word *Azadiracta* meaning noble tree. Neem is cultivated in many regions of Africa, Australia and Latin America, because it adapts very well to the soil and semi-arid climates in tropical and sub-tropical countries [16]. Its medicinal properties present a potential for treating animals and humans. The specific use of Neem in India for natural crop potential has extended worldwide over the past number of years, including a great number of countries where it is being sold commercially [25]. The production of insecticides of Neem leaves can be done in a relatively simple, artisanal way or else as an industrial process. Studies indicate that the active substances of Neem (*Azadirachtin, Salannin, Nimbin, Nimbidin* and *Meleniatril*) have repellent, antifeedant and insecticidal effects against a number of insect pests [16, 26-28]. Upon ingestion, they have a very special effect on the metamorphosis of insects, preventing their growth and development. These substances are not toxic to human beings, mammals, birds, reptiles and fishes when applied in the indicated concentrations and do not affect the beneficial flora or fauna in the cultivated fields [16].

*A. indica* pesticides play vital roles in pest management and hence have been widely used in Agriculture for its safety and efficacy as biopesticides [29, 30]. There has been evident shift all over the world from synthetic pesticides to non-synthetic ones; largely due to the wide spread awareness of the side effects of these synthetic pesticides on plants, soil and other living organisms. Neem oil is a naturally occurring pesticide found in seeds from the neem tree. It is yellow to brown, has a bitter taste, and a garlic/sulfur smell. It has been used for hundreds of years to control pests and diseases. The biologically active compound *azadirachtin* (extract of *A. indica* seed and leaf), act as repellents for a broad spectrum of agricultural and household insects. This plant is traditionally used as pesticidal agent in India [31]. *Azadirachtin* reduces insect feeding and acts as a repellent. It also interferes with insect hormone systems, making it harder for insects to grow and lay eggs [24]. Neem oil has been reportedly used to control the following pests: garden snails and slugs, cockroaches, bed bugs, scabies mites, dust mites, ants, fleas, ticks, fruit fly, whiteflies, Japanese beetles, cherry slug and pear slug [32]. Also, neem products such as neem soaps, shampoo, toothpaste, hand and body lotions, face wash, food supplements, agricultural and veterinary products are marketed the world over by many companies. Agroneem is a formulation containing *azadirachtin* (0.15%) as the active ingredient, 15% neem biomass (neem lipids), thus utilizing the complete spectrum of neem’s potential as a pest management tool [22, 31]. It attacks insects at different stages of growth as anti-feedant, insect repellent, growth regulator and insecticide [26]. It is not toxic and does not harm the workers. It is bio-degradable and protects the environment. It is compatible with most commonly used fertilizers, fungicides and insecticides. In a research by [33], the wetting of banana corm or pseudo-stem with neem cake extract, aqueous neem seed powder, neem kernel powder or with emulsified neem oil disrupted the settling response, egg laying and larval feeding of *Cosmopolites sordidus* (Banana corn borer).

As repellents, *Azadirachtin* prevents insects from initiating feeding [34]. As feeding deterrent, it causes insects to stop feeding after first taste due to secondary hormonal or physiological effects of the deterrent substance. As growth regulator, neem disrupts normal development interfering with chitin synthesis. Susceptibility to the various effects of neem by insects differs by species [35]. Studies have shown that the neem compound *Azadirachtin* is a more effective insect repellent than the widely used synthetic chemical known as DEET (N, N-diethyl-meta-toluamide) a carcinogen with long periods of use [36]. Extracts from the neem tree are also used as repellents (the wood of the neem tree is strong and resistant to termite damage, used as mosquito repellents and as firewood for charcoal making), fumigant (neem oil act as fumigants), pesticides (the leaves act as a natural pesticide), fertilizers, manure (neem leaves and the cakes gotten when oil has been removed from seeds can improve soil structure and add to the plant nutrient base), diabetic food and animal feed, urea coating agent and soil conditioner (neem leaves can be used to make soil less acidic [37, 38, 39] reported that the toxic effect of *A. indica* is ephemeral in nature disappearing within 14-21 days. They also investigated the successful use of *A. indica* in the control of migratory locust, army worm whitefly and even head lice and found it to be safe to other
beneficial organism such as earthworms. [40] stated that powdered seed kernel of *A. indica* mixed with harvested rice significantly reduced insect pest infestation. Also mixing *A. indica* leaves with harvested rice, treating storage bugs with *A. indica* extract or putting its leaves between the bags and storage floor achieved similar results [41].

Plant extracts have been used worldwide as an alternative method to control pests unlike synthetic pesticide which have one active compound, and exhibit only one type of biological effect. Single plant derived compound may have more than one biological effect [29]. Detailed investigations have revealed that several plant species have more than one compound and have diverse biological effects. The chances of quick development of resistance to different chemical is minimal in extracts responsible for biological effect or repelling of some natural plant as with the neem plant (*A. indica*). Various parts of the neem tree, (bark, leaves, flowers, seeds and fruits pulp) are used mostly in the powder or extract form having medicinal [42] and pesticide properties resulting from its various active components, including Azadirachtin which is the main ingredient used in the manufacture of biopesticides that are been exported to various countries as lots of research are been conducted to test its safety and efficacy for use as pesticides [43, 44]. *Azadirachta indica* oil and seed extracts are known to possess germicidal and antibiotic properties which are useful to protect the plants from different pests. The greatest advantage of *A. indica* based pesticides and insecticides are that they do not leave any residue on the plants.

Laboratory experiments have been conducted by several researchers evaluating the insecticidal properties of neem extract (Powders, liquid) prepared from various neem parts (seed, leaf, stem, and root) against various insect pests with successful level of control obtained [45] [46] [43, 47-49]. Hence, neem plant contents have been reported as effective bioinsecticide found useful in controlling over 400 insect species of medical and veterinary importance in various Orders of Insects [15, 32, 43, 47, 50].

*Ocimum gratissimum* is found throughout the tropics and subtropics and its greatest variability occurs in tropical Africa and India. It is widely distributed throughout Central America, West African Coast and used in Trinidad and Tobago and Nigeria. *O. gratissimum* Linnaeus (Scent leaf) plant of the genus *Ocimum* also called clove basil and commonly known as scent leaf, consist of shrubs whose plant materials serve as spices, condiments and for medicinal purposes dated back to the history of mankind [51]. Recently, the exploitation of wild plants for medicinal purposes has gained major acceptance in many countries of the world. To further underscore the importance of herbal medicine, most national governments have established the traditional medicine regulatory council under the supervision of their various health ministries’ to tap the numerous potentials of herbs. This may be because traditional medicine has long been practiced even before the orthodox medical practice appeared [52]. *Ocimum gratissimum* belongs to the group of plants known as spices. The plant is an erect small plumb with many barnacles usually not more than one meter high [53]. It is of the family Lamiaceae, genus *Ocimum* and species *gratissimum*. It is cultivated as a home garden crop and also grown on commercial scale. It is used for a variety of reasons such as culinary for the preparation of salads, soups, pastas, vinegars, jellies and food flavoring. In traditional medicine, the leaves have been used as a general tonic and antidiarrheal agent and for the treatment of conjunctivitis by instilling directly into the eyes [54]. The leaf oil when mixed with alcohol is applied as a lotion for skin infections, and taken internally for bronchitis. The dried leaves are snuffed to alleviate headaches and fever among other uses. In addition, various extracts of *O. gratissimum* have been tested in vitro and shown to be active against some bacterial and fungal isolates and used in the treatment of various ailments [55, 56].

Biopesticides or plant based fumigants have long been reported as attractive alternatives to synthetic fumigants for the management of arthropods because botanicals pose little threat to the environment or to human health [57]. *Ocimum* is known for its pesticide properties due to diverse group of compounds in its essential oil [58]. *O. gratissimum* essential oil is reported to contain bioactive constituents that are insecticidal and repellent [59]. The toxicity is due to the effects of the compounds eugenol, monoterpenoids and sesquiterpenoids found in the plant extract. [60], studied the larvicidal activity of *O. gratissimum* on larvae of *Aedes aegypti* Linnaeus and found that *O. gratissimum* oil exhibited 100% mortality at 300 mg/L concentration at twenty four hours of exposure. Leaves and extracts of leaves of *Ocimum* species are reportedly useful as grain protectants in Eastern Africa [61] and as insect repellents [62]. [63, 64], reported in their separate studies that the use of leaves of *Ocimum* species exhibited larvicidal action and thus proved successful in controlling the life stages of mosquito.

*Dracaena arborea* is evergreen with simple leaves that are alternate, lanceolate with parallel venation providing a spiky, topiary look and thrives well in light, well-drained soil or dry soil. Most dracaena prefer bright indirect light, many adapt to lower light levels while others need direct sun to keep their colour, thus thrive best in warm temperature [65]. Its ability to adapt to poor growing conditions made it very popular and important as boundary and ornamental plant. Dragon blood as it is commonly
called among the Ibibios is used to settle land dispute among people and as varnish for violin and photoengraving [66]. [67] reported the presence of anti-parasitic and antifungal compounds in Dracaena. *Dracaena* species have been shown to possess insecticidal properties. [66] in his studies, reported the use of root, bark and leaf powders of *D. arborea* in controlling two storage pests of bean (*C. maculatus*) and maize (*S. zeamais*). According to [68], cut leaves of *D. steudneri* placed in a box with caterpillars of Charaxes (Nymphalidae) led to their death. Larvicidal activity of steroidal saponins from *D. arborea* on *Aedes albopictus* has been proven ([69]. [66] stated in his work that ethyl acetate and aqueous fractions of leaf extract of *D. arborea* demonstrated insecticidal activity against *Sitophilus zeamais* (Motsch.) and *Callosobruchus maculatus* (Fab.) and offered protection to stored grains. [59, 65] reported the repellency quality of *D. arborea* against *C. maculatus* while [70] confirmed the successful botanical insecticidal properties of *D. arborea* and *Vitex doniana* leaf extracts on the larvae of *Anopheles* mosquito hence concluded that the test plants have larvicidal property.

**Plate 2-4. A. indica tree, O. gratissimum shrub, D. arborea tree**

**Source:** Authors

2. MATERIALS AND METHODS

2.1. Study Setting

The research was carried out in the Postgraduate Laboratory, Faculty of Science, Niger Delta University, Bayelsa State located between latitudes 4°45’N and 4°60’N and longitudes 6°50’E and 8°00’E.

2.2. Study Period

The study period of this research work was from November, 2016 to September, 2018.

2.3. Sample Collection

2.3.1. Collection of *P. americana* Nymphs

A total of one thousand eight hundred same age progeny of *P. americana* nymphs collected from the stock that hatched from eggs in the laboratory were used to establish the main test sample. The nymphs were placed ten in a trough in readiness for the various bioassays as stated by [71].

2.3.2. Collection of Plant Material

Fresh leaves of *A. indica* were collected from forests in Amassoma town in Southern Ijaw Local Government Area of Bayelsa state, Nigeria. The plants were identified by laboratory technologist and botanist Mr Dimie Otobo, of the department and taken to the Postgraduate laboratory of the Faculty of Science for processing and usage in the various bioassays.
2.4. Sample Preparation

2.4.1. Preparation of Powder

Using the methods employed by [72, 73], the fresh leaves of the test plant *A. indica*, *O. gratissimum* and *D. arborea* were separated manually and washed briefly under running tap water to remove sand and debris, then sun-dried for a period of three days until crispy but seven days for *D. arborea*. 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°C for eight hours. 50g of the powdered materials were bagged and labeled separately for further usage.

2.4.2. Preparation Of Ethanolic Extract

Adopting the procedures of [49, 71], leaf the leaf powdered forms of *A. indica* was separately weighed as follows: 0g (control), 10g, 20g, 30g, 40g and 50g into different Bama bottles labelled A, B, C, D, E and F according to the measured weight in grams respectively. Equal volume of 10ml of 70% ethanol was added into each of the bottles in the ratio 0g:10ml, 10g:10ml, 20g:10ml, 30g:10ml, 40g:10ml and 50g:10ml respectively and allowed to stand for 24 hours. The crude ethanolic extract was filtered using Whatman No.1 filter paper through a plastic funnel to a 50ml beaker respectively. The filtrate was transferred into a round bottom flask and heated in water bath at 40°C 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 dessicator. The resultant yield was 60g of the extract.

2.5. EXPERIMENTATION

To examine the effects of the leaf powder and extracts of the test plant on the nymphs, the treatments were applied both as contact poison for leaf powder and contact toxicity by topical application for ethanolic leaf extract as was used by [68, 74-76]. All treatments were arranged in completely randomized design (C.R.D.)

2.5.1. Contact toxicity of leaf powder

To evaluate contact toxicity of *A. indica* leaf powder on nymphs, the methodology of [68, 70] was adopted where 10g, 20g, 30g, 40g, and 50g respectively of leaf powder of the test plants were measured into different transparent plastic boxes with perforated lids and labeled. Thirty nymphs were randomly introduced into each box. Boxes were covered with vent nettings held by rubber stopper. No leaf powder was added to the control and the treatment replicated five times. Mortality rate was recorded at 24, 48, 72 and 96 hours respectively. After three blunt probes using a dissecting probe, the insects were considered dead.

2.5.2. Contact Toxidty by Topical Application of Ethanolic Leaf Extract

Using the procedure spelt out by [73, 75] to evaluate contact toxicity by topical application, thirty adult cockroaches were placed randomly in boxes lined with moist filter paper. Using a pipette, 10ml, 20ml, 30ml, 40ml and 50ml, respectively of the ethanolic leaf extracts was applied to the dorsal surface of the thorax of each nymph. Distil water was used as control and each treatment was replicated five times. Insects were examined daily for mortality within 24-96 hours and any insect that did not respond to three probing with a blunt probe at five minutes recovery period was considered dead.

2.5.3. Contact Toxicity on Filter Paper

Adopting the method employed by [71], Whatman No. 1 filter paper was placed in plastic boxes, where 10ml, 20ml, 30ml, 40ml and 50ml, respectively of the ethanolic leaf extracts were applied and allowed thirty minutes to dry off. Thirty nymphs were introduced randomly into each box. Distil water was used as control and each treatment was replicated five times. Insects were examined and recorded daily for mortality within 24, 48, 72 and 96 hours and any insect that was immobile and did not respond to three probing with a blunt dissecting probe at five minutes recovery period was considered dead.
2.6. Qualitative test

The leaves of the test plant were screened qualitatively for phytochemicals according to standard procedures used by [66, 77, 78], 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 by excel for windows program version two Microsoft office 2010. Data obtained were expressed by calculation of the mean and standard error (Mean±SE) statistically. Student T-test and pair wise multiple comparisons were used for significant differences at alpha level, p≤ 0.05. All graphs were plotted using Microsoft excel.

3. RESULTS AND DISCUSSION

The results of the effects of contact toxicity by spreading and contact toxicity by topical application of leaf powder and ethanolic extracts of *A. indica*, *O. gratissimum* and *D. arborea* on the nymphs of *P. americana* are presented on figures 1-3. At the end of 96 hours duration (four days), significant mean mortality of nymphs was observed among treated nymphal population over the control (p≤0.05). Generally, mean total mortality of nymphs was concentration dependent as nymphal deaths increased with increase in concentration of the treatment and exposure time (figs. 1-3).

The leaf powder treatments also affected death of the nymphs significantly (p≤0.05), with the highest mean values compared to the control (fig.1). 00 (control): 0.5±0.20, 0.6±0.20, 0.5±0.20; 10g: 9.8±2.60, 9.5±2.60, 5.3±1.80; 20g: 14.5±3.30, 12.8±2.90, 7.0±2.30; 30g: 17.8±3.60, 15.5±3.30, 8.8±2.60; 40g: 20.3±2.90, 17.0±3.60, 10.3±2.60; 50g: 23.5±4.10, 22.0±3.90, 12.5±2.90 respectively for *A. indica*, *O. gratissimum* and *D. arborea* treatments with respect to contact toxicity.

Nymphal mortality was highest in topical application of ethanolic leaf extract of *A. indica* (27.3±4.60) and lowest in contact application of *D. arborea* aqueous (9.3±2.60) extracts. Mean total mortality of nymphs using ethanolic leaf extract was recorded as follows: 00 (control): 0.6±0.20, 0.25±0.1, 0.6±0.20; 10ml: 16.3±3.40, 12.0±2.80, 10.3±2.60; 20ml: 19.5±3.60, 16.3±3.40, 12.8±2.90; 30ml: 23.3±4.00, 19.3±3.80, 14.5±3.30; 40ml: 26.5±4.60, 22.3±3.90, 17.0±3.60, 50ml: 27.3±4.60, 24.8±4.20, 21.3±3.90 respectively for *A. indica*, *O. gratissimum* and *D. arborea* treatments with respect to topical toxicity (fig. 2).
Figure 2. Effects of contact toxicity treatments of ethanolic leaf extracts applied topically against *P. americana* nymphs

There were significant differences (p≤0.05) in mean total nymph mortality regarding ethanolic leaf extracts of the test plants over the control with recorded values as follows: 00 (control): 0.6±0.20, 0.0±0.00, 0.6±0.20; 10ml: 16.5±3.50, 12.0±2.80, 9.8±2.60; 20ml: 19.0±3.80, 17.0±3.60, 12.0±2.80; 30ml: 24.0±4.20, 19.3±3.80, 14.3±3.30; 40ml: 26.0±4.60, 20.8±3.90, 16.5±3.50, 50ml: 27.0±4.60, 25.0±4.30, 21.0±3.90 respectively for *A. indica*, *O. gratissimum* and *D. arborea* treatments with respect to contact toxicity on filter paper (fig 3).

Figure 3. Effects of contact toxicity of ethanolic leaf extracts on *P. americana* nymphs

Nymphs of cockroach were more susceptible with *A. indica* treatments than *O. gratissimum* and *D. arborea* though all were also successful in controlling the nymphs at various levels of concentration. *Azadirachta indica* extracts proved more bioactive recording total mean nymph mortality of 27.3±4.60 at highest concentration for topical and 27.0±4.60 for contact toxicity using ethanolic leaf extract after four days as against *D. arborea* which was least with 21.3±3.90 and 21.0±3.90 for topical and contact toxicity respectively. From the result, both contact and topical applications of all treatment types proved effective in controlling the nymphs, though the ethanolic extract treatments recorded more mortality values than the powder.

The asterisks on the graphs represent controls that show significant difference from the treatment (p≤ 0.05).

Table 1 depicts the result of the qualitative phytochemical constituents of ethanolic leaves extracts of the three study plants. *Azadiracta indica* ethanolic leaf extract had moderate (+++) levels of tannins, cardiac glycosides, alkaloids, flavonoids, terpenoids and trace (+) amounts of phenols. *Dracaena arborea* had high (+++) level of alkaloids, moderate (+) level of phenols and cardiac glycoside and trace (+) for
tannins, saponins, flavonoids and terpenoids. *Ocimum gratissimum* had high (+++) level of alkaloids, moderate (+) for cardiac glycoside, flavonoids and trace (+) for tannin, saponins, terpenoids and phenols.

Table 1. Phytochemical screening of the aqueous and ethanolic leaf extracts of *A. indica*, *D. arborea* and *O. gratissimum* plants

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>A. indica</th>
<th>O. gratissimum</th>
<th>D. arborea</th>
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<tbody>
<tr>
<td>Tannins</td>
<td>++</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Glycoside</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Saponin</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
<td>+</td>
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*Source:* Authors

The increasing usage of synthetic pesticides is of serious concern as they accumulate in the environment. Bio-pesticides are good alternatives to the synthetic ones as they cause little environmental pollution, have low toxicity level to humans and several other advantages [79]. Biopesticides or plant based chemicals have long been reported as attractive alternatives to synthetic pesticides for the management of arthropods because botanicals pose little threat to the environment or to human health [80]. Bio insecticidal activities of leaf powder and ethanolic extracts of *A. indica*, *O. gratissimum* and *D. arborea* were investigated for the control of the nymphs of *P. americana* (cockroach) which resulted in the reduction of the number of treated cockroach nymphs after a period of four days. This result is in agreement with earlier observations of [75, 76]. There was positive correlation between the concentration of extracts and nymph mortality values. A remarkable increase in the mortality was observed and the increase was treatment concentration dependent. Total mean mortality of nymphs was concentration dependent, progressing as the concentration of the treatments increased and highest with the highest concentration confirming the observation of [5, 75, 76, 81]. Total mean mortality of the cockroach nymphs treated with ethanolic extracts of all three plant parts were observed highest at 50ml concentration of *A. indica*, followed by *O. gratissimum* and *D. arborea*. The leaf extract showed higher efficacy than the leaf powder and highest with ethanolic extracts applied topically. All treatments groups proved successful in controlling the nymphs of *P. americana*. The treatments were very much effective at higher concentration giving rise to higher number of nymphal deaths. Finally, the ethanolic extract treatments constituted from *A. indica* plant parts was most bioactive in achieving nymph control.

The nymphal deaths recorded in the treatment groups were significantly different over those of the controls. Student T-test and pair wise multiple comparisons for mean mortality between the treatment groups and the control, showed significant difference at alpha level, (p≤ 0.05) with increase in concentration of leaf powder and extracts and the exposure time reflecting in higher nymphal deaths. This is in conformity with earlier reports of [5, 75, 76] who worked independently on the biocidal effects of various plants on *P. americana* adults.

Topical application of the extracts of the three plants generally indicated high potency for mortality of nymphs. This is in agreement with the result obtained by [75, 82, 83], who confirmed that topical application of plant extracts on different insect species necessitated direct contact of the toxicants or active ingredients on the insect’s bodies with resultant death. The mortality rate of nymphs observed in this study is also in consonance with the findings of [45] who reported toxicity of both the powder and extracts of leaves and stem of *A. indica*. Also extracts in the ethanolic form gave better effects compared to the aqueous and powdered forms because active materials in plants are more soluble in ethanol. Furthermore, using pairwise multiple comparisons at the highest concentration of the tested botanicals, significant difference at p≤0.05 existed among the treatments and between the treatments and the controls.

The success in the larvicidal activities of the test plants are attributed to the presence of the biological active ingredients termed phytochemical that are contained in the plants [75, 76]. Therefore, the larvicidal activity of *O. gratissimum* leaves (powder and ethanolic extract) against *P. americana* is attributed to the principal constituent of Eugenol responsible for biological activities observed on the cockroach [48, 59]. This was further confirmed by [60, 64] who attributed the toxicity of *O. gratissimum* treatments on the insect species to the toxic effects of the compounds eugenol, monoterpenoids and sesquiterpenoids found in the plant extracts. [30, 84] in their various studies revealed that the major components of *O. gratissimum* species such as P-lymene, A-thujene, myrcene and thymol in addition to phenol were responsible for the control of insect pests. Topical application of the ethanolic extracts...
recorded high mortality compared to the contact. The active compounds extracted from Ocimum plants parts are found to be rich in camphor, citral, geranool, linoolool, linacalyacetale and methyl charicol whose metabolites acted as larvicide, insect growth regulators, repellent and oviposition deterrent on the cockroaches as reported by [30, 85].

The larvicidal properties recorded by D. arborea treatments as by [67] was attributed to the presence of secondary metabolites such as mannispirostan A and Spiroconazole A which are penogenin triglycosides components, hence, the usefulness of the plant in traditional insect pest management systems [85-87]. Furthermore, contact action was stronger against P. americana nymphs using A. indica and O. gratissimum compared to D. arborea. This recorded success of mortality is attributed to the fact that P. americana nymphs though having wings, are poor fliers and so were always in contact with the treated surface as observed earlier by [88]. The insects avoided contact with the treatments and were seen hanging around the breeding troughs. The behaviour of the cockroaches was due to the presence of phytochemicals in the extract that repelled the insects thus influencing their locomotion, feeding behaviour, developmental and behavioral patterns with resultant deaths which is in agreement with earlier reports obtained by various scholars working independently who disclosed that contact toxicity caused abrasive effects on insect cuticle with resultant interference in the respiratory mechanisms of the insects and the end effect of death [2, 74, 89-91].

The possible pathway through which A. indica killed the insects as reported by [92] was that Azadirachtin, an insect growth disruptor, known to be an antagonist in the juvenile hormone and 20-hydroxyecdysone (20E) interfered with the insect’s hormones and stopped them from breathing resulting into death while the nymphs that survived were found weak and feeble due to inadequate contact with the extracts and powdered forms of A. indica treatments which is also in agreement with results obtained by [45] [32]. A. indica is able to kill insects by acting as antifeedant, thus when the insects were hungry and tried to feed on the treated powder or extracts of A. indica plant, the presence of azadirachtin, salanin and melandriol, initiated anti-peristaltic wave in the alimentary canal of the insect resulting to repellency activity [5, 84]. This induced vomiting sensation deterring feeding in the insects and inability to swallow by interfering with the insect’s hormone thereby stopping them from breathing and subsequent death as confirmed by [37]. Feeding deterrence as revealed by [93] was possibly due to the changes induced in the physiology and behaviour of the insects by the botanicals.

The result of the present study showed that the mortality of P. americana nymphs exposed to leaf powder and extract treatments of all test plants increased with increase in concentration of treatments and exposure time. The result compared favourably with results from similar studies. [46] Who reported that A. indica leaf powder has pest control agent that caused the mortality of insects compared to the control. [75, 94-98] in their respective studies using A. indica obtained similar results and all reported that A indica was a potential botanical pesticide which caused antifeedancy, deterrence and mortality of insects.

The result of this study also confirmed that O. gratissimum leaf powder and ethanolic extracts are effective biocides for against control. It also indicated that O. gratissimum ethanolic extract performed better than the leaf powder which is in agreement with the observations of [5, 25, 58, 76] 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 [99], 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. Contact application showed the presence of insecticidal properties in the plant but higher potency was observed with topical application of the extracts especially ethanolic extracts of leaves. Direct contact of the toxicants or active ingredients in the test plants with the insect’s bodies was facilitated by topical application and resulted in more mortality of nymphs. These results are in agreement with those obtained by [82, 83, 100, 101].

Phytochemical screening of the leaves of A. indica gave the presence of alkaloids, glycosides, terpenoids, flavonoids, tannins and saponins as secondary metabolites which is in agreement with the report obtained by [22, 102, 103]. These secondary plant chemicals attract or repel insects thus influencing their locomotion, oviposition, feeding behavior, developmental/physiological processes and behavioral patterns as reported by [91, 104, 105]. This is also in concordance with earlier reports by [106-108] which stated that Neem extracts comprised complex mixture of molecules, including normal hydrocarbons, phenolic compounds, terpenoids, alkaloids and glycosides that act on various phases of insects life cycle, making it difficult for insect pests to resist the physiological effects of the extract such as insecticidal and feeding deterrent.

According to [95], phytochemicals produce various effects in insects which suggest the diversity of physiological interference caused by them with resultant disruption of growth and inhibitory effects on
reproduction in insects by causing delay or inhibition of oviposition, fecundity, deformities in oocytes, reduced mating and alterations of biochemical contents in the reproductive system. The effectiveness of the test plants against P. americana was mostly due to the presence of phytochemical constituents and or compounds found in them. According to [23, 109, 110], over 135-140 phytochemicals and other compounds have been isolated from different parts of A. indica which possessed insecticidal properties. [11, 45, 111, 112] all recorded that plant extracts disrupt the cellular structure of the cockroach midgut epithelium, gastric caeca, columnar cells, peritrophic membrane, striated border and longitudinal muscles leading to mortality of the insects.

Ocimum gratissimium extracts revealed the presence of saponins, tannin, flavonoids, terpenoid, alkaloid, and phenols in various quantities which is in agreement with earlier studies by [113-116], who further stated that these compounds possessed insecticidal, antihelmintic, nematocidal and antimicrobial properties.

D. arborea analysis revealed the presence of saponins, tannin, flavonoids, terpenoid, alkaloid glycoside and phenols in trace to high levels. The aforementioned compounds being secondary metabolites and their detection arose from the reaction of the functional groups(s), they contain with chemical reagents to produce different colours and changes in physical nature which is in conformity with results indicated by [66, 71], [117] reported that the presence of the above mentioned phytoconstituents instilled natural defense on the plants against herbivory. He disclosed that these secondary metabolites consisted of mixtures of closely related compounds rather than a single toxicant. He further concluded that synthetic pesticides example rotenone is a combination of six insecticidal isoflavonoids, glycosides and tannins which caused death of insects. The presence of the phytochemicals justified the insecticidal properties exhibited by A. indica, O. gratissimium and D. arborea. This is also in agreement with the observation by [88] which stated that secondary constituents of plants such as alkaloids, saponins, cardiac glycosides are compounds that jointly or synergistically contribute to produce insecticidal activity against insects.

Tannin present in all test plants as revealed by the phytochemical screening are phenolic compounds containing hydroxyl and carboxyl groups which form strong complexes with proteins and other macromolecules thus hindering the growth and afterwards development into adults by the immature stages owing to the presence of ovicidal and larvicidal properties of the plants as confirmed by [32, 55, 91, 108, 118, 119]. Tannins hinder development of organisms as it precipitates and inactivates adhesion enzymes and cell development proteins with resultant effect of antifeedant properties in the insects and eventual death thus functioning as pesticides and growth regulators [120]. According to the [42], tannins possess astringent properties and thus drag the insect tissue together, restricting the flow of blood with resultant death of insects. Tannins also turn insect skin into leather by binding proteins to form water insoluble substances resistant to proteolytic enzymes.

Terpenoids are compound of tetranortriterpenoid that act as insect repellents and feeding inhibitor and as hormone ecdysone, thus controlling the process of metamorphosis by inhibiting the development of immature insects from nymphs to adult cockroaches as confirmed by [80, 121-123][63].

Flavonoids and terpenoid which are phenolic compounds are most important as antioxidants in dietary. Thus their molecules delayed and prevented oxidation reduction reaction catalysed by radicals thus inhibiting feeding in the cockroaches [122]. The presence of flavonoids in the test plants also acted as repellent and reproduction controller in the cockroach thus inhibiting oviposition and egg hatchability in the cockroaches as confirmed by [37, 124] reported that flavonoids play vital role in plant resistance serving as natural pesticides, thus having the capacity to modulate the feeding behavior of insects. Flavonoids aid plant normal growth and development against injury by pests thus plants with high flavonoids and tannins content possess the greatest insect resistance.

Saponins which are glycocides with soapy characteristics are reported to possess bioactive agents due to their bitter and acrid taste resulting in feeding deterrents in the insects [69, 70, 125]. Saponins cause hemolysis of blood and poisoning in animals hence interfering with the replication of Deoxyribonuclei acid (DNA) with eventual death of the insects [126].

Glycosides are plant steroids and are poisonous derivative from plants. They are stored in inactive form conjugated with sugars. These poisons are activated by enzyme hydrolysis causing the sugar part to be broken off making the chemical available for use as the insects feed on the plant [127]. [128] reported that insect antifeedants are mainly triterpenoids, which is on a 30-carbon skeleton, occurring as glycosides and highly oxygenated.

Alkaloids are known sources of potent insecticides, acting in synergy with saponins and tannins as repellents and antifeedant, thus deter insect pests with resultant adverse effects [127, 129]. Alkaloids function as neurotoxins and attack the acetylcholinestarase receptor site of the insects, altering the
permeability of the neuromuscular juncture, generating new nerve impulses that lead to spasmodic contractions, delay larval development, convulsions and finally death of the nymphs and adults as confirmed by [129, 130].

The presence of phenols is synonymous with toxicity as they play important roles in plant herbivore and pathogen interaction. Phenols exhibit antioxidant properties which are pesticidal [124]. The presence of secondary metabolites in these tested plants as previously reported by [67], acted as both insecticides and antifeedants [68, 88].

Phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Plant bioactivity depends on chemical compounds which may inhibit insect feeding. Toxic effects to insect pests are produced by the compounds terpenoids, steroids, phenols, flavonoids, tannins, alkaloids and cyanogenic glycosides [107, 124]. This agrees with the report of [111], who disclosed that primarily, phytochemicals affect the midgut epithelium and secondarily affect the gastric caeca and malpighian tubules of insects initiating anti feedant properties and eventual death of insects. Thus, their presence in the test plants further confirmed their potential for use as bio pesticides.

4. CONCLUSION

The findings of this study showed that A. indica, O. gratissimum and D. arborea leaf powder and extracts all adequately controlled the (P. americana) nymphs. A general analysis of the data for the effectiveness of treatments on the nymphs of the test cockroach species revealed an increase in total mean mortality with number of days, with most of the surviving insects being weak and less motile. Thus, the potency of the test plants against the household insect pest of health and epidemiological importance cannot be over emphasized. Therefore, there is the recommendation for the discovery and development of the plant chemicals into potent biopesticides that can replace the synthetic products. Finally, the plants are cheap and locally available however, further research is required to determine the efficacy of these plants using higher concentrations and on a wide range of other common insect pests.

5. RECOMMENDATION

The authors recommend the Government establishment and funding of research institutions where more studies on the usefulness of plants and plant products that are cheap and locally available as biopesticides in insect pest control can be carried out on a wide range of other common insect pests. Also Research centers for the culturing, breeding and rearing of insect pests for research purposes should be established by Government and non- governmental bodies aimed at facilitating the ease of carrying out similar research on insect pest control where new and same age stocks are required.

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