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Research Paper

Insecticidal effects of various neem preparations against some insects of agricultural and public health concern

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Abstract

Neem (*Azadirachta indica*) has some medicinal and pesticidal properties resulting from its various active components, including azadirachtin. Laboratory experiments were conducted to study the insecticidal properties of extracts and powders prepared from different neem parts (seed, leaf, stem and root) against the *Macrotermes spp.*, *Phaseouslus spp.*, *Periplaneta spp.* and larvae of *Anopheles spp.*, which are important pests of agricultural and public health sectors. The lethal effect was more pronounced with the seed extract (40-55 %), followed by the leaf extract (30-45 %), the stem extract (30-40 %), and the root extract (10-30 %), as compared to the control (distil water) which registered 0 % mortality. The termites and the weevils were seen to be more susceptible to the various extracts, compared to the cockroaches and mosquito larvae. Oil extracted from the neem seed kernel showed even greater lethal properties on the insects, with a minimal lethal concentration, in all cases, being 0.50 % ^{v}/v. Again the termites and the weevils responded faster, recording total deaths within 2-5 minutes, compared to the cockroaches and the mosquito larvae where total deaths were experienced only after 30-90 minutes. It was also found out that there was a direct relation between the concentration and degree of lethal effectiveness of the oil. The neem, especially the seed oil, has great potential as natural biocide against termites and weevils.

Keywords: Neem, azadirachtin, insecticidal, lethal, kernel, pests.

Introduction

The neem tree has over centuries been used as a herbal plant, including its effects against insect pests. Various parts of the neem tree: the bark, leaves, flowers, seeds and fruit pulp are used, mostly in the powdery or the extract form. The powdery form is normally used for the preservation of stored seed-bean grains against weevil attacks or mixed with dry grounded clay or sawdust and sprinkled over young plants, such as maize and sorghum, against pest infestation. Rain water or dew helps dissolve the extract active substance, which get into the plant through translocation. The extracts, however have some application problems including their effect period, lasting for 4-8 days, thus there is a need for many applications in a season. The aqueous extracts are sprinkled on field crops against various pests. The increasing demand for high quality food, free from chemical residues, makes it imperative that non-chemical means of protecting stored products or crops against insect damage be used. Investigations on the effectiveness of neem extracts as organic pesticide is vital, but the development of standardized formulation and certification of the neem-pesticide products is even more crucial.

Many people have expressed concerns about harmful effects of chemical pesticides and show interest in organic farm products. Thus to address these concerns there is the need to reduce the use of chemical pesticides and supplement with relatively lesser toxic ones. The neem extracts could play this role, but need to be assessed against various pests. Neem (*Azadirachta indica*) is a tropical or semi-tropical evergreen, drought resistant tree in the mahogany family, Meliaceae. It grows to an average height of 15-20 m, with wide branches. Though known to have great medicinal (anthelmintic, antifungal, anti-diabetic, antibacterial, antiviral, anti-fertility and sedative) properties, the neem extracts do not normally kill pests right away rather they repel them or affect their growth, and it's repellent and pesticide properties are broad spectrum in nature [1]. Also neem cake, a residue after oil is extracted from the seed kernel, is used as an insect repellent in rice paddies[2], as well as being effective on soil-borne fungal pathogens and plant parasitic nematodes [3,4] .The neem has bitter compounds including nimbin, nimbinin and nimbidin, capable of providing relief from minor muscle to joint pain, as well as a complex secondary metabolite, azadirachtin, a mixture of seven isometric compounds, labelled as azadirachtin-A to–G, with the azadirachtin-A greatest in quantity and the azadirachtin-E being the most effective insect growth regulator [5].

A part from these there are over twenty other compounds with some biological activities, including salannin and meliantriol [6]. In an enclosed environment or when the oil coats the insect their respiratory system is blocked, preventing breathing and causing suffocation. It has been found that the effectiveness of the neem extract depends not only on the dosage but also increases with earlier larval stage treatment. The neem extract should be used not more than three months after preparation. It is found that the azadirachtin level is relatively lower in the seed after 8-10 months storage and extracts from such seeds are less effective as a pesticide. The authors also found that the neem extracts have no killing effect on some organisms, including the honeybee workers, spiders, butterflies, ants and ladybugs, at rates of 500 ppm, and that earthworms actually benefit from soil application of neem by-products [4]. Fine extracts (using alcohol) are more active than crude water extracts. Activity is dosage-dependent. Generally 50-75 g seed / liter of water give optimal control of most pests [7]. Some natural additives such as garlic (*Allium sativum*) and hot pepper (*Capsicum frutescens*) exhibit synergistic effect on the neem product. Studies also reveal some relatively great effectiveness when these extracts are mixed with or applied alternatively with bio-pesticides such as *Bacillus thuringiensis* (Bt) – [8]. Emulgators, such as soap cake powder and "rimulgan" are normally used to aid in the application of neem seed oil, as a pesticide. The neem oil and the emulgator are mixed in the rate of 4:1, and then diluted between 0.5 to 1.0 % in water depending on the type of plant or pests to be controlled. The 0.5 % is used for plants with soft leaves, like many vegetables, and 1.0 % can be used for plants with hard leaves, like many fruit trees or ornamentals [9].

Termites greatly destroy wood and wood products, damage crops, destroy books and corks of stored bottles, damage fabric and plastics [10]. Weevils, such as the boll weevil (*Anthonomus grandis)* and grain weevils, *Sitophilus granaries*, attack crops, stored grains, other dry foods and flours. Cockroaches are mostly nocturnal omnivorous, from the order *Blattaria*, found everywhere including homes and hospitals and feed on human and pet's food. They create offensive odour and allergic relations and passively transport microbes, which are potentially dangerous to human [11, 12]. Chemical trails in their feaces help them find their routes and air-borne pheromones aid them find their mating partners [13]. Mosquitoes are from the *Culicidae* family and undergo complete metamorphosis. The common ones are the *Anopheles*, *Aedes* and *Culex* types. Adults have piecing mouth parts, feeding typically on nectar and plant juice, but the female, equipped with an elongated proboscis collect blood from their host for the development of her eggs [14]. In so doing they could pick up and transmit plasmodium, a causal agent of malaria, into the blood streams of human being. Out of its 72 odour receptors on its antennae at least 27 are tuned to detect chemicals found in perspiration. Bio-controls, using dragon flies nymph and fishes, are effective on mosquitoes[15,16]. Preventive measures, such as drainage of stagnant water (breeding sites), as well as biological control agents, using *Bacillus thuringiensis,* are used against mosquitoes. This study aimed to assess the insecticidal efficacy of various neem preparations against some agricultural and public health pests under laboratory conditions.

Materials and Methods

Various parts of neem tree (roots, leaves, bark, stem, seeds) and insect-pests – *Macrotermes spp.*, *Phaseouslus spp.*, *Periplaneta spp.* and larvae of *Anopheles spp.* were collected into separate containers.The various selected parts of the neem tree were dried in trays, grounded in crucibles, before being extracted. Three forms of neem products were prepared (powder, aqueous extract and oil emulsifiable concentrate): Three hundred (300) grams of each of the grinded neem parts were dried and further ground to produce very fine powder, to treat the various insect-test samples.

Another set of 300 grams of each of the grounded neem parts were properly mixed with 1 l of distilled water, allowed to stand for 24 hours, then sieved through a 2 mm mesh and further filtered through a filter paper. The filtrate, the aqueous extract was then used to treat the various selected insects.

Ripped fruits collected from neem trees were pulped to remove the flesh. The seeds were then dried, carefully cracked and the kennels removed. The latter were washed and dried to avoid fungal infection. One kilogram of seed kernel was roasted in an oven at 110^{o} C for 30 minutes for easy extraction and maximum yield of oil. The kernels were blended and a little water added to it, mixed to form paste, transferred into a saucepan kneaded and heated for 3 hours until enough oil was collected on the surface of the mixture. Further heating and pressing was done to obtain more oil and evaporate any possibly "free" water from the oil.

Dilution of Oil
Various volumes of the oil (0.1 cm^3, 0.5 cm^3, 3.0 cm^3, 6.0 cm^3 and 10.0 cm^3) were each pipetted into a 100 ml volumetric flask and topped to the 100 ml mark with 99.8 % ethanol, producing 0.1 % v/v, 0.5 % v/v, 1.0 % v/v, 6.0 % v/v and 10.0 % v/v concentrations, respectively.

Collection of Selected Samples
Various methods were used to collect each of the selected insects for analysis. Termite-infested wood and termite moulds were collected to the laboratory and the termites shaken out, cockroaches were trapped from isolated buildings into a net mesh, mosquito larvae were scoop out from stagnant water, and the bean weevils were collected after infested beans were stirred, shaken and sieved out into a pan. Infested bean plant was also used as a source for weevils.

Treatment of Selected Samples
Fifty (50) ml of powder made from each of the five neem parts – seed, bark, root, stem, leaf were sprinkled over the insect-test samples, 10 in each glass beakers, which had the tops sealed with a perforated parafilm, to prevent any insect escaping. Water was however poured half-way full in the containers before the mosquito larvae samples were placed on the surface of the water and treated. The control variants had selected insects treated with distilled water. Labelling was done and aseptic measures taken.

The same measurements (50 ml of each of the aqueous neem parts extracts were sprayed on selected insect samples, 10 of each type, with two replicates) and procedure used for the powdery extracts was adopted.The same procedure was used for the neem seed oil treatment. Various concentrations - (0, 0.1, 0.5, 1, 3, 6, 10) all in percentage volume per volume (% v/v) were prepared and used to spray the insect in their respective containers and the time and nature of their movement observed.

Results

The aqueous suspensions expressed more inhibitory effect on termites than the powder, for all test-samples (Figure 1). Among the extracts, that from the seed had the greatest effect whilst that of the roots and stem had the least. The control (distilled water) showed no effect. Figure 2, 3, and 4 below, show the effect of the various extracts on the weevils, mosquito larvae, and cockroaches, respectively. The results of these, though varied in terms of magnitudes, took a similar trend as that of the termites, in Figure 1. In

all cases it is detected that the maximum effectiveness of these preparations were mostly attained 48 hours after their application.

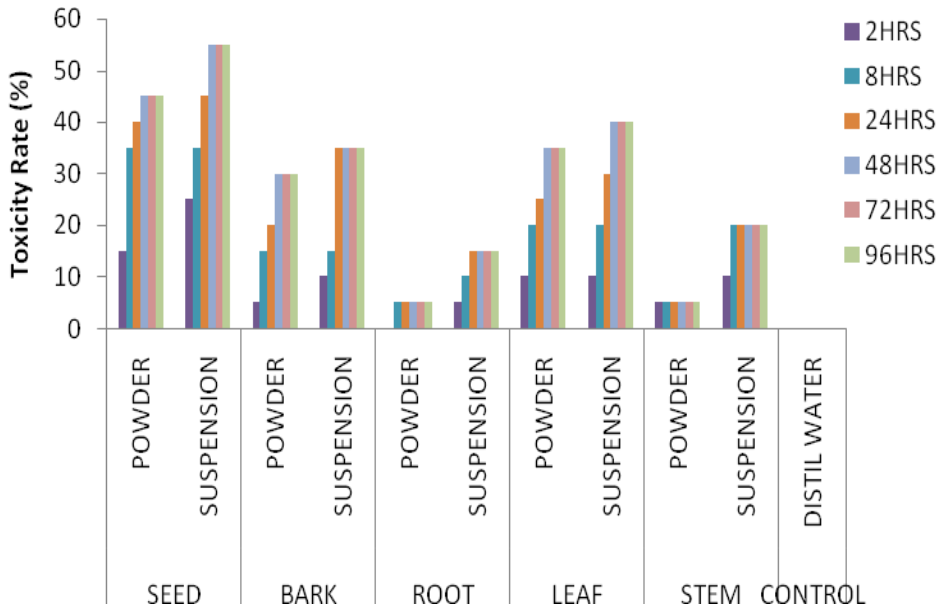

Figure 1: Effects of Various Powdery and Aqueous Neem Extracts on Termites

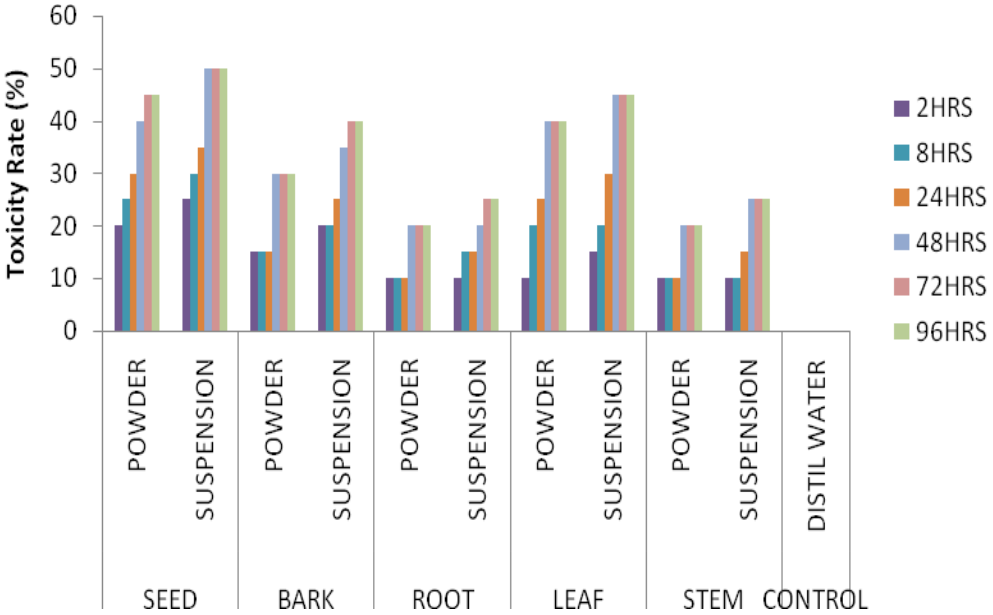

Figure 2: Effects of Various Powdery and Aqueous Neem Extracts on Weevils

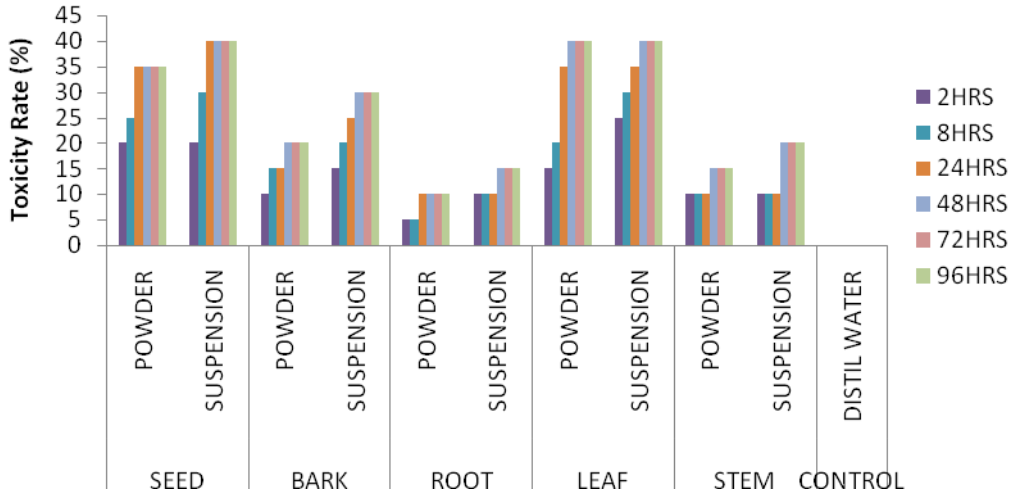

Figure 3: Effects of Various Powdery and Aqueous Neem Extracts on Mosquito Larvae

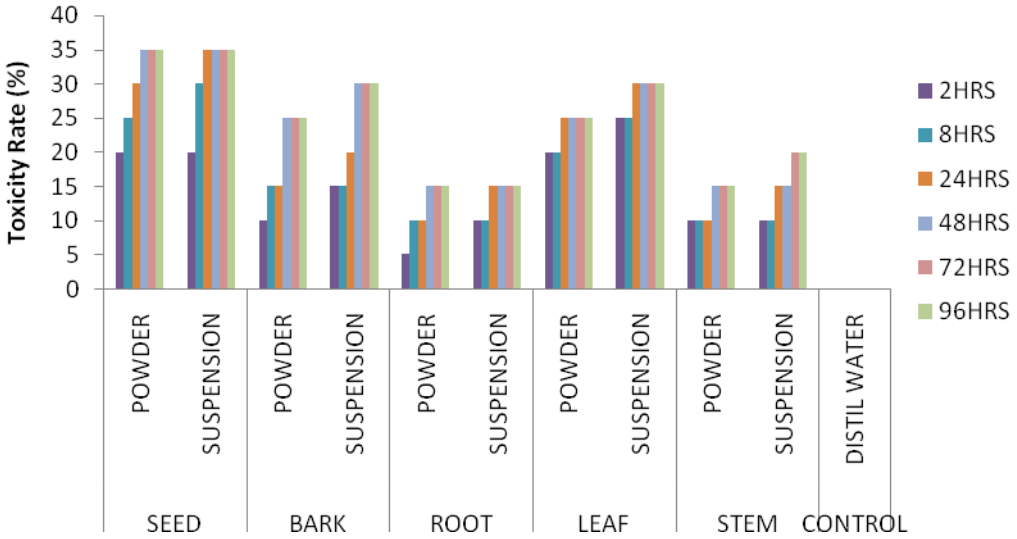

Figure 4: Effects of Various Powdery and Aqueous Neem Extracts on Cockroaches

Generally the toxicity rate of both the powder and suspension extracts ranged from 15-55%, with the neem seed extracts being the most effective against the insects (35-55% mortality), followed by the neem leaf (25-45 %), then lastly the bark, stem and roots (15-40% effectiveness). The control (distil water) was not effective (0%). Extracts in the aqueous forms gave better effects, compared to the powdered forms. The effects of the extracts were more pronounced against the weevils and termites (50% and 55% mortality, respectively) and relatively lesser for the cockroaches and the mosquito larvae (35% and 40%, respectively). Based on relatively better insecticidal effects of the neem seed extracts (Figure 1- 4), hence the activities of various concentrations of neem oil were evaluated, to ascertain the minimum lethal dose and the time taken for such doses to be effective. The findings are illustrated in Figures 5-8.

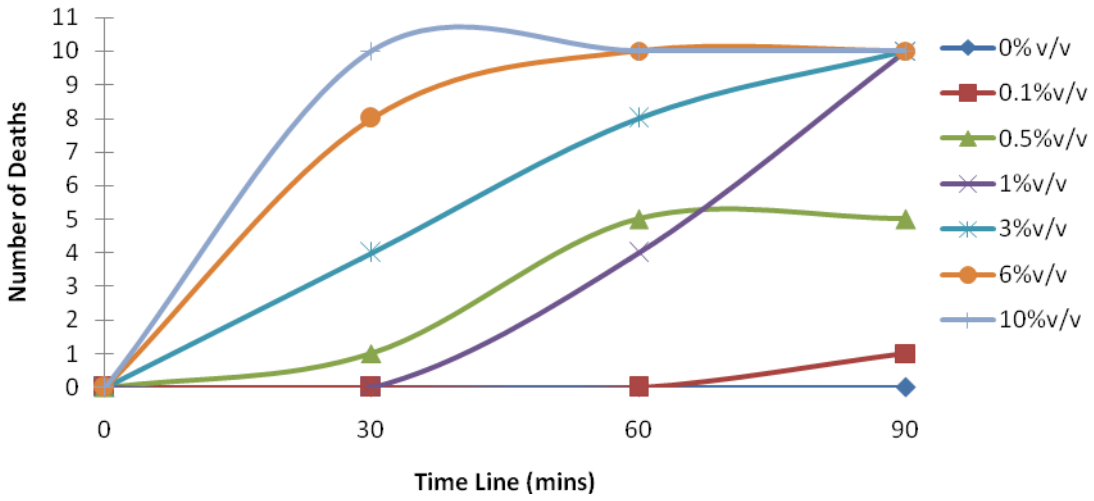

Figure 5: Effect of Neem Seed Oil on Cockroaches

The death rates per time for the cockroaches were increased with respect to concentration levels used (Figure 5). The higher the concentration of the neem seed oil, the faster it's killing effect on the cockroaches. The minimum lethal dose here is 0.50 % ^{v}/v as it killed exactly half of the insects in 60 minutes, as compared to the controls (0 %) which had no effect on the insects.

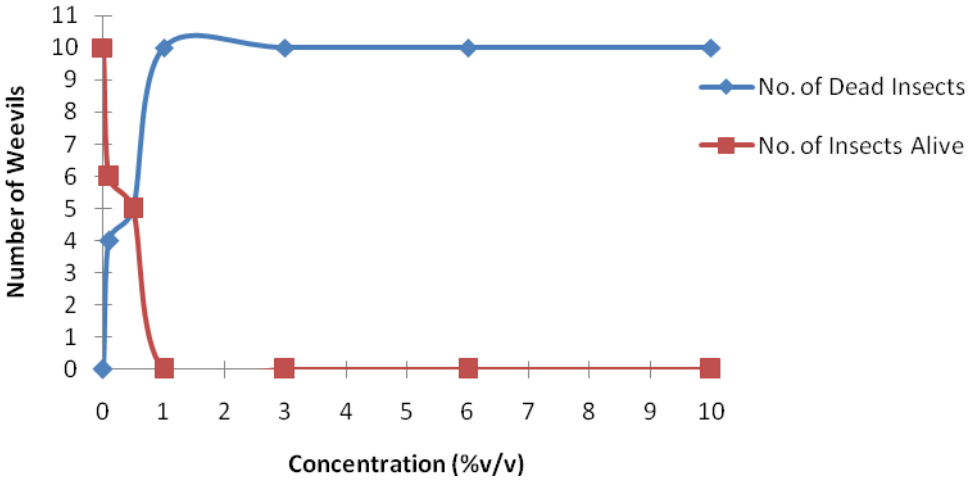

Figure 6: Effect of Neem Seed Oil on Life Expenctancy of Weevils, within 120 seconds

From Figure 6 above, the lethal effect of the oil on the weevils was relatively fast and effective especially with the increase in concentration of the neem oil. Concentrations of 0.50% ^{v}/v and above were seen to be effective since they were able to kill half or more of the total number of the weevils. The sample concentrations of 1 % ^{v}/v and above killed all the weevils within the 120 seconds. The control (0 %) did not experience any deaths of the insects.

From (Figure 7) below, the test carried out for a period of three minutes had fewer numbers of recorded deaths of the termites compared to the number of deaths recorded for the tests that used five minutes.

The number of death of the termites increased as the concentration for the neem oil increased. For the 3 % ^{v}/v concentration of the neem oil, a drop in the number of deaths for the termites was recorded for less than 3 minutes. This could be attributed to some factors including mutagenic ones. The minimum lethal concentration of the neem oil on the termites was 0.50 %, within five minutes reaction time period and 1.0 % for three minute period, the control (0 %) did not record any deaths.

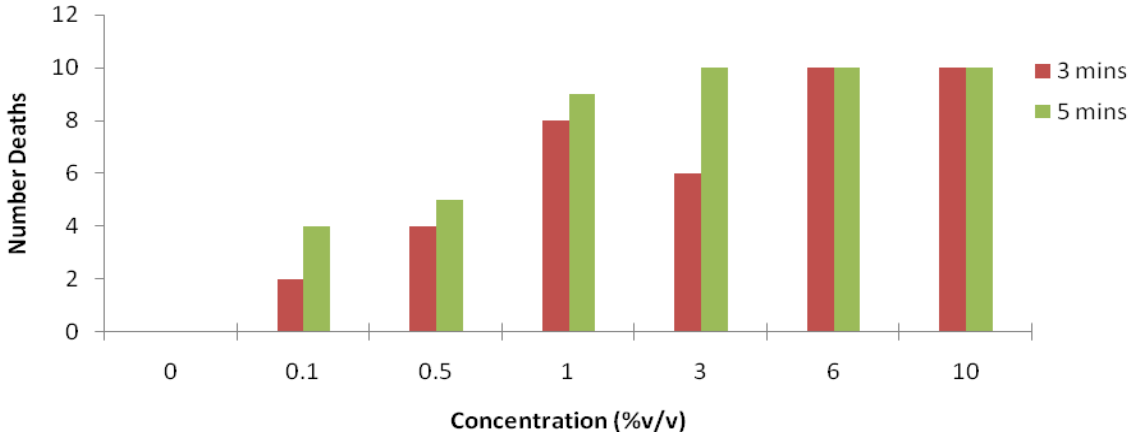

Figure 7: Lethal Effect of Neem Seed Oil on Termites

From Figure 8 below, it took a longer period of time (60 to 120 minutes) before the mosquito larvae died with respect to the concentrations of the neem oil used. The minimum lethal concentration for the neem oil on the mosquito larvae was 0.50 % ^{v}/v.

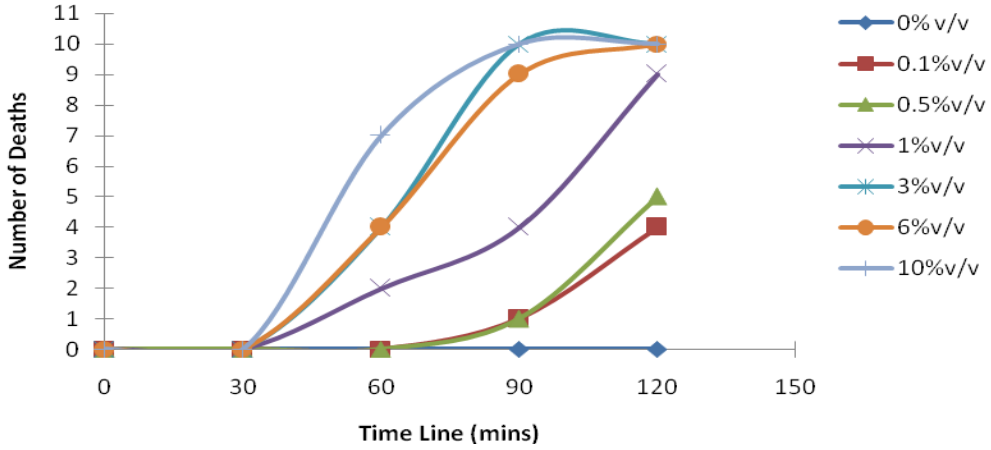

Figure 8: Lethal Effect of Neem Seed Oil on Mosquito Larvae

Discussion

Researchers indicate that with increasing use and overuse of chemical pesticides many insect-pests develop resistivity [18]. Neem pesticides, relatively less toxic, environmentally friendly and effective are suitable substitutes for an integrated pest management. Though with varied degrees of effectiveness, the various neem extracts were seen to be effective against the selected insects, and this makes it better a product for agricultural use, especially for mix-cropping practices, as a field could be infested with more than one of these insects under study. A general analysis on the data for the effectiveness of both the powdered and aqueous neem extract on the selected insects revealed an increased in toxicity rate from

first day to second day and generally stabilized from the second to the fourth day, with most of the survived insects being weak and less motile. The neem seed kernel oil also showed some correlations between the death rates for the insect-pests as against the time and concentration levels. The higher the concentration of the neem seed oil, the faster it killed the pests. The minimum lethal concentration of the neem oil on the termites is 0.50 % ^{V}/v for five minutes reaction period and 1.0 % for the three minute reaction period. Generally, the minimum lethal dose in all cases is 0.50 % ^{V}/v as it killed exactly half of the insects. In the case of the cockroaches the time duration was 60 minutes. That of the mosquitoes took 60 to 120 minutes and 2-5 minutes for the termites and weevils, as compared to the controls which had no effect on the insects. Results of a research by[19]. Recommended a 3% concentrated Neem Azal as it was effective at laboratory conditions. However, this dosage, according to them revealed poor performance in the field. It was found that laboratory trials recorded relatively high effectiveness than field trials, since the latter is subjected to other prevailing environmental factors.

Conclusion

Production of neem pesticides is technically feasible. All the various selected neem extracts exhibited some degrees of insecticidal properties and have great potential to be used in agricultural and industrial sectors. The broad spectral nature of the neem extracts has great benefit to farmers, especially with mix-cropping. Among the extracts tested, the seed kernel oil was the most effective, followed by the aqueous and powder preparations. The minimal lethal concentration of the neem seed kernel oil for the test samples is 0.50 % ^{V} / V.

The termites and the weevils were relatively more susceptible to the extract preparations compared to the cockroaches and the mosquito larvae. There is some direct correlation between concentration and time on one side and lethal effect on the other. There is an inverse-relationship between the concentration of a sample and the time required for the effects of the extracts to be felt.

Recommendation

A comparative study on laboratory and field trials is recommended to ascertain the respective needed doses of the extracts and reaction periods for each of the two conditions. For example in this study both the 3-minute and 5-minute reactions periods were effective at laboratory conditions, using 0.50 % ^{V} / V concentration. Economic-wise the former is preferable, provided that it performed similar effect under field condition. Studies should also be done to improve the smell of the seed oil so that it can comfortably be used on the skin as a repellent-pomade against mosquitoes and other nuisance insects.

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