THE POTENTIAL OF NEEM SEED OIL (*AZADIRACHTA INDICA*) AS NATURAL HOUSEHOLD INSECTICIDE.

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ABSTRACT.

Use of synthetic insecticides has resulted into health hazards, ecological imbalance, resistance by pests and environmental pollution. Neem seed oil is reported to be a natural pesticide that has excellent insecticidal properties whose usage will reduce the risks associated with the use of synthetic insecticides.
In this study, attempts were made to formulate neem seed oil-additive emulsions to be used as insecticide on household pests. Solvent extraction method was used for extracting oil from neem seed while acetone and calcium oxide were used as additives (inert ingredients) to formulate emulsifiable medium which were tested on four common household pests (cockroaches, black ants, soldier ants and millipedes).
It was observed that the additive samples to which 5 ml of neem seed oil were added were the most effective and active of all the tested mixture samples. The insects were either killed or their movement retarded within the shortest time compared to other samples with lesser amount of neem seed oil
The results of the study showed that extracted oil from neem seed in emulsions with additives can be used as natural household insecticides. Also, acetone and calcium oxide used as additives (inert ingredient) readily improve the insecticidal properties of the neem seed oil with acetone being the most effective.

Keywords: Neem Seed Oil, Acetone, Calcium Oxide, Solvent Extraction, Household Insecticides.

INTRODUCTION.

Insecticides are substances used to kill insects (IUPAC, 2006). They can be categorized into three types which are natural, organic and inorganic insecticides. Natural insecticides consists of extracts and essential oils extracted from plants such as nicotine, pyrethrum and neem oil secreted by plants as defences against insects. Neem (*Azadirachta indica*) is a tree in the mahogany family *Meliaceae* common in India subcontinent. It is a fast-growing tree that can reach a height of 15-20 m (about 50-65 feet). All parts of neem tree (root, seeds, leaves, flowers and bark) are used for preparing many different medical and industrial products. Neem has been the most traditionally used plant as environmentally friendly botanical pesticide in India, Pakistan and Africa to protect grains and cereals from pests. Fresh neem leaves are mixed with grains and cereals before storage. Also, fumigating the house with smoke of dried neem leaves in evenings for 1-2 minutes is an excellent ayurvedic method to keep mosquitos' away (Girizi and Shankara, 1998).

Neem seed was found to have high concentration of oil. A Pakistani scientist (Salimuzzaman Siddiqui) identified three bitter compounds from neem oil, which he named *nimbin*, *nimbinin*, and *nimbidin* respectively (Mongkholk *et al.,* 2004). Neem oil is used for preparing cosmetics (soap, shampoo, balms and creams) which are useful for skin care such as acne treatment and for keeping skin elasticity. The oil is also widely used as lubricants and drugs for variety of diseases such as diabetes and tuberculosis (Puri, 1999; Ganguli, 2002). The seeds also contain a complex secondary metabolite called azadirachtin. Apart from azadirachtin, salannin, gedunin, azadirone, nimbin, nimbidine, nimbicidine and nimbinol are other important limonoids of neem.

There are several methods of obtaining neem oil from the seeds such as mechanical pressing, supercritical fluid extraction and solvent extraction (Sohail, 2009). Mechanical extraction is the most widely used method to extract oil from neem seed. However, the oil produced by this method usually has a low price, since it is turbid and contains a significant amount of water and metals contents (Liauw *et al*., 2008). Extraction using supercritical fluid produced high purity oil but the operating and investment cost is high. Extraction using solvent has several advantages such as higher yield, less turbid oil, and relatively low operating cost compare to other methods (Liauw *et al*., 2008).

Therefore, solvent extraction was used in this study to extract oil from neem seed and the oil was subsequently mixed with additives and tested for its insecticidal potentials.

MATERIALS AND METHODS.

Materials

Ripe neem fruits were harvested from neem trees in the premises of Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The fruits were de-pulped to obtain the seeds which were washed thoroughly with water to remove dirt and sand. Pure grade ethanol (67 %), acetone and calcium oxide of analytical grade were procured from Yetkal laboratory, Isale General, Ogbomoso, Nigeria.

Equipment

Electro- mantle, soxhlet extractor, condenser, what Mann filter paper, pipes for oil collection, electric oven.

Methodology

Extraction of Neem Seed Oil.

The neem seeds were decorticated to remove the hull from the seeds and then dried at 50 ^{o}C to constant weight in the electric oven. The dried seeds were crushed using mortar and pestle, and then screened using sieve screen to obtain a particle size of 0.550- 0.625 mm which was used for oil extraction. For each extraction run, 70 g neem seed powder was mixed with 300 ml 67% pure ethanol and extraction carried out for 6 h by total reflux in a soxhlet extractor. At the end of the extraction process, the obtained mixture of neem oil and solvent (ethanol) was poured into a 500 ml beaker and placed inside an electro mantle and heated. The heating process was maintained at 40 ^{o}C to ensure that the extracted neem oil does not evaporate with ethanol and after 5 h, the solvent had evaporated, leaving the extracted neem oil in the beaker. The neem oil was then cooled and poured into a clean bottle.

Characterization of Neem Seed Oil

The extracted neem seed oil sample was characterized using infra-red spectrometer. Infra-red spectroscopy provides valuable information concerning the functional groups present in neem oil molecules. This was obtained by irradiation of the oil sample with light from the infra-red region 5000 to 500 cm $^{-1}$ of the electromagnetic spectrum. The results of sample analysis were presented in Table 1.

Mixing of Neem Seed Oil with Additives (Inert Ingredients)

Pesticides formulations consist of active and inert ingredients. The inert ingredients play key roles in pesticide effectiveness and product performance. The additives (inert ingredients) employed for this study were acetone and calcium oxide powder. The extracted neem seed oil was mixed with additives to boost its performance and efficiency.

20 ml acetone was measured into four separate beakers each labelled A, B, C, and D. Varying quantities of the extracted neem seed oil was added to each sample as follows: 0.25 ml, 0.3 ml and 0.5 ml while sample D (without neem seed oil) served as control. The contents of each beaker was mixed thoroughly on gyratory shaker at 200 rpm for 30 minutes.

5g of calcium oxide granule was dissolved in 1 litre of distilled water forming calcium hydroxide from which 20 ml calcium hydroxide was measured into four separate beakers each labelled E, F, G and H. The extracted neem seed oil was added to each sample in the following quantities, 2 ml, 3 ml and 5 mlwhile sample H (without neem seed oil) served as control. The contents of each beaker was mixed thoroughly on gyratory shaker at 200 rpm for 10 minutes forming emulsion.

Testing of Neem Seed Oil - Additives Mixture on Different Insects.

Each of the samples A, B, C, D, E, F, G and H were tested separately on each group of insects (10 insects each) and their activities observed. Each of the prepared neem seed oil and additive mixture were sprayed separately using plastic bottle sprayer on each group of insects inside big, flat, white plastic bowl. The tested insects were cockroaches, soldier ants, millipedes and black ants.

RESULTS

The result of infra-red characterization of the extracted neem seed oil gave the functional groups present in neem seed oil as presented in Table 1:

Table 1: Results of Infra-red Analysis of Extracted Neem Seed Oil Sample

Wavelength Peak (cm^{-1})	**Compound Type**	**Functional Group (Bond)**
2920	Alkane	C-H
2380	Alkynes	C≤H
2100-2070 (broad)	Alkenes	C=H
1689	Esters	C-O
1738	Saturated Aldehyde	C-O
1580	Benzeoid compound	C-C

Results of sample activities on different insects:

The following results were obtained after the samples were tested on different insects.

Table 2: Effect of Neem seed oil + Acetone Mixture on Cockroaches

Neem oil (ml)	Acetone (ml)	Sample	Activities
2	20	A	Kills after 30 seconds
3	20	B	Kills after 20 seconds
5	20	C	Kills after 15 seconds
Nil (Control)	20	D (Control)	No effect

Table 3: Effect of Neem seed oil + Acetone Mixture on Soldier Ants

Neem oil (ml)	Acetone (ml)	Sample	Activities
2	20	A	Kills after 40 seconds
3	20	B	Kills after 35 seconds
5	20	C	Kills after 25 seconds
Nil (Control)	20	D (Control)	Kills after 68 seconds

Table 4: Effect of Neem seed oil + Acetone Mixture on Black Ants

Neem oil (ml)	Acetone (ml)	Sample	Activities
2	20	A	Kills after 18 seconds
3	20	B	Kills after 14 seconds
5	20	C	Kills after 10 seconds
Nil (Control)	20	D (Control)	Retard movement after 30 seconds

Table 5: Effect of Neem seed oil + Acetone Mixture on Millipedes

Neem oil (ml)	Acetone (ml)	Sample	Activities
2	20	A	Retard movement after 27 seconds
3	20	B	Retard movement after 20 seconds
5	20	C	Retard movement after 12 seconds
Nil (Control)	20	D (Control)	No effect

Table 6: Effect of Neem seed oil + Calcium Hydroxide Sample on Cockroaches

Neem oil (ml)	Calcium hydroxide (ml)	Sample	Activities
2	20	E	Retards movement after 10 seconds
3	20	F	Retards movement after 8 seconds
5	20	G	Retards movement after 5 seconds

Nil (Control)	20	H (Control)	No effect

Table 7: Effect of Neem seed oil + Calcium Hydroxide Sample on Soldier Ants

Neem oil (ml)	Calcium hydroxide (ml)	Sample	Activities
2	20	E	Retards movement after 20 seconds
3	20	F	Retards movement after 14 seconds
5	20	G	Retards movement after 10 seconds
Nil(Control)	20	H (Control)	No effect

Table 8: Effect of Neem seed oil + Calcium Hydroxide Sample on Black Ants

Neem oil (ml)	Calcium hydroxide (ml)	Sample	Activities
2	20	E	Kills after 42 seconds
3	20	F	Kills after 20 seconds
5	20	G	Kills after 13 seconds
Nil(control)	20	H (Control)	Retard movement after 80 seconds

Table 9: Effect of Neem seed oil + Calcium Hydroxide Sample on Millipedes

Neem oil (ml)	Calcium hydroxide (ml)	Sample	Activities
2	20	E	Retard movement after 98 seconds
3	20	F	Retard movement after 76 seconds
5	20	G	Retard movement after 54 seconds
Nil(control)	20	H (Control)	No effect

DISCUSSION OF RESULT

Effect of Neem seed oil +Acetone Mixture on Cockroach.

The acetone sample to which 2 ml of neem oil was added was discovered to be the least effective of all the samples that contain neem oil because it takes longest time (30 seconds) to kill the cockroaches. This is followed by the sample that contains 3 ml neem oil. The sample that contains 5 ml neem oil is the most effective since it kills cockroaches within the shortest time. The control sample had no effect on cockroaches.

Effect of Neem seed oil + Acetone Mixture on Soldier Ants.

All the samples had effects on soldier ants by killing them but at different rates. The sample that contains 5 ml neem oil was the most effective followed by the sample that contains 3 ml with the sample that contains 2 ml neem oil being the least effective. The control sample also killed soldier ants but after a very long time.

Effect of Neem seed oil +Acetone Mixture on Millipedes.

All the samples that contain neem seed oil had effect on millipedes by retarding their movement but at different rates. The sample that contains 5 ml was the most effective followed by other samples with lesser amount of neem oil. The control sample had no effect on millipedes.

Effect of Neem seed oil + Acetone Mixture on Black Ants.

The mixture of neem seed oil and acetone killed black ants at different time intervals. The sample that contains 5 ml neem seed oil was the most effective followed by others. The control sample only retarded the movement of the insects after 24 seconds.

Effect of Neem seed oil + Calcium Hydroxide Mixture on Cockroach.

Slight differences were observed in the rate at which the samples that contains various quantities of neem oil retarded the movement of the cockroaches. The control was observed to have no effect on the insects.

Effect of Neem seed oil + Calcium Hydroxide Mixture on Soldier Ants.

All the sample mixtures were found to retarded the movement of the soldier ants but at different rates. The sample that contains 5 ml neem oil was the most effective followed by others. The control sample was observed to have no effect on the insects.

Effect of Neem seed oil + Calcium Hydroxide Mixture on Black Ants.

The sample that contains 5 ml neem oil was the most effective by killing black ant insects after 13 seconds, followed by the sample that contains 3 ml neem oil which kills the insects after 20 seconds. The sample that contains 2 ml neem oil killed the insects after 42 seconds while the control sample retarded the movement of the insects after 80 seconds.

Effect of Neem seed oil + Calcium Hydroxide Mixture on Millipedes.

All the samples that contains neem oil affected the millipedes by retarding their movement at different time intervals with the sample that contains 5 ml neem oil being the most effective. The control sample had no effect on the movement of millipede.

It was observed that the additive samples to which 5 ml of neem seed oil were added were the most effective and active of all the tested mixture samples. These samples acted faster on tested insects than other samples with lesser amount of neem seed oil. The insects were either killed or their movement retarded within the shortest time compared to other samples with lesser amount of neem seed oil. The insecticidal properties of neem seed oil was reported in previous studies (Girizi and Shankara, 1998; Munoz-Valenzenla *et al*., 2007; Asogwa *et al*., 2010) and its active ingredient azadirachtin has been reported to be the main ingredient for fighting insects and pests (Eleonora, 2009). Azadirachtin contains OH group which readily activate the unsaturated ring towards electrophilic substitution which enhances insecticidal repellent properties of the neem seed oil. Peter (2000) reported that the most effective preparations of neem oil for insecticidal properties are the emulsion based on combining neem oil with other additives. The tested additives in this study (acetone and calcium oxide) which were components (inert ingredients) of most commercial household synthetic insecticides nationwide were observed to also enhance the performance of the neem seed oil as household insecticides.

CONCLUSION

Results of this study showed that neem seed oil can serve as an important natural household insecticide whose usage can reduce the risks associated with the use of synthetic insecticides.
Also, acetone and calcium oxide used as additives (inert ingredient) in this work were found to readily improve the insecticidal properties of the neem seed oil.

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