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Short Communication

Larvaecidal effects of aqueous extracts of *Azadirachta indica* (neem) on the larvae of *Anopheles* mosquito

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The effect of crude aqueous extracts of *Azadirachta indica* (neem) against the larvae of *Anopheles* mosquito was investigated. Exposure of the larvae to undiluted extracts of seed oil, leaf and bark for 12 hours led to 100, 98, and 48% mortality, respectively. Dilution of these extracts also resulted in mortality of the larvae. We suggest that the seed oil and leaf extract of neem contain properties that could be developed and used in the control of mosquitoes in the tropics.

Key words: *Anopheles* mosquito, neem, *Azadirachta indica*.

INTRODUCTION

Azadiracta indica A. Juss (neem tree) is a member of the family meliaceae in the order Fagales of the Dicotyledonous class in the in plant kingdom. When fully grown, it can reach 24m in height with dense wide spreading crown. The crown bears large number of compound leaves each with 5-9 pairs of leaflets. Flowering is twice a year (April/May and August/September). When fertilized one seeded fruits are produced with fibrous cover. According to Radwanski (1977), a tree can yield between 20-50 kg of fruits annually.

The neem has been reported to contain several biologically active constituents such as azadirachtin (Naganishi, 1975), meliantriol (Lavie et al., 1965), salanin (Warthen et al., 1978), as well as nimbin and nimbidin (Shin-foon, 1984). Garg and Bhakuni (1984) reported salanolide (a meliacin) as one of the bitter principles in neem seed oil. Furthermore, Raman and Santhanagopalan (1979) reported tignic acid (5-methyl-2-butanoic acid) as part of the seed constituents. This compound is believed to be responsible for the distinctive odour of the neem oil. The limonoids are freely soluble in organic solvents such as hydrocarbons, alcohol and ketones, but are sparingly soluble in water.

Azadirachtin, a limonoid, has been reported to have adverse effect on endocrine system of a bean beetle, *Epilachna varivestis*, and to cause sterility in the female insects (Schmuthere et al., 1981). Schluter and Schulz (1983) also reported this compound to cause degradation in larval epidermis preventing the larvae from molting. Insect larvae treated with meliantriol with concentration as low as 0.2 µg/larva were observed to cease to feed. In recognition of this important properties of neem on insects, and in realizing the abundance of the plant in the northern part of Nigeria, this preliminary effort is made to test the effect of crude extract of neem seed oil, leaf and root on the larvae of anopheles mosquito that is responsible for transmitting the malarial parasite, *Plasmodium* sp. The choice of crude extract was to ensure adaptability by the local populace.

MATERIALS AND METHODS

Extracts of seed kernel, leaf and bark of neem were prepared from plant material collected at Arkilla plantation along Kalambaina road in the southwestern part of Sokoto, Nigeria. Extraction of all the parts was carried out using local methods. This is to allow for easy adoption by the local population. Seed kernel extract was prepared from sundried seeds that were pounded in a wooden motor into a paste. The paste was compressed to extract oil which was collected in a round bottom conical flask. To prepare the leaf extracts, fresh leaves were collected, washed and crushed in a motor into a watery paste. This was then gradually passed on wire gauze on top of a conical flask and carefully pressed to extract the liquid which collect in a flask placed below the wire gauze. The flask containing the liquid was then sealed for further use. Bark extract was prepared from fresh pieces of stem bark removed from a full-grown neem tree. The pieces were dried, powdered and sieved to separate finer particle from granules and fibres. From the finer material, 10 g

Table 1. Mortality rate of different quantity of neem seed oil extract on anopheles mosquito larvae.

Seed oil Concentration (ml)	Time (hours)															
	1	2	3	4	5	6	7	8	9	10	11	12	Total	%	Mean	SE (±)
20	8	10	2	5	3	4	8	2	4	5	4	5	60	100	5	5.53
10	2	1	8	6	9	9	3	8	7	3	3	3	59	98	4.92	0.57
5	5	6	8	4	2	6	1	4	11	2	1	1	51	85	4.25	0.89
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. Mortality rate of different quantity of neem leaf extract on anopheles mosquito larvae.

Extract concentration (ml)	Total (hours)															
	1	2	3	4	5	6	7	8	9	10	11	12	Total	%	Mean	SE (±)
20	8	6	5	2	2	4	6	1	4	2	0	1	50	83	4.12	0.94
10	1	3	6	5	5	7	8	4	4	0	2	0	45	75	3.75	0.76
5	0	0	8	8	1	1	4	5	4	5	4	1	41	68	3.42	0.11
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3. Mortality rate of different quantity of neem bark extract on anopheles mosquito larvae.

Bark Concentration (ml)	Time (hours)															
	1	2	3	4	5	6	7	8	9	10	11	12	Total	%	Mean	SE (±)
20	4	2	1	1	3	2	2	5	4	4	1	0	29	48	2.42	0.45
10	0	0	3	1	0	4	2	0	2	0	1	1	14	23	1.17	0.39
5	0	0	0	3	0	2	2	0	2	0	1	1	12	20	1.0	0.30
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

was measured and dissolved in 100 ml distilled water in a conical flask.

Mosquito larvae were collected in a plastic bucket from a pool of stagnant water near the students' block of hostels at the Main Campus, Usmanu Danfodiyo University, Sokoto. The bucket containing the larvae was kept in netted enclosure in the Laboratory at room temperature (30 ± 2^0C).

Three sets of four petri dishes were laden with moist filter paper and placed on a laboratory bench. Using a metal loop, 20 mosquito larvae were transferred from the plastic bucket into each of the twelve petri dishes. The sets of petri dishes were labeled a, b, c and d. In the first set, 20 mls of the seed oil extract were added into dish (a), 10 mls in (b), and 5 mls in (c). In petridish (d), 20 ml of distilled water was added as control. In the second and third sets of petri dishes, leaf and bark extracts were added in the same manner as for seed oil in the first set. The effects of the three extracts were monitored by counting the number of dead larvae at one-hour interval for 12 h. Three replicates of the experiments was conducted and the mean taken. The date obtained was statistically assessed to determine variance and standard error of the mean.

RESULTS AND DISCUSSION

The effects of the plant extracts on mosquito larvae are presented in Tables 1, 2 and 3. When 20 ml of the seed oil extract was applied, all the larvae died within 12 h. Decreasing the amount of the seed oil extract to 10 and 5 ml, reduced the mortality to 98 and 85%, respectively, after 12 h. For the leaf extract (Table 2), 83% mortality was recorded when the larvae were treated with 20 ml extract, while 75 and 68% mortality was recorded with 10 and 5 ml extracts, respectively, after 12 h. The highest mortality for the bark extract is 48% with 20 ml.

This work demonstrates the potency of *A. indica* in the control of mosquito larvae. Seed oil appeared as the most lethal among the various parts tested. The high mortality recorded for seed oil extract might be attributed to deficiency of dissolved oxygen in the water. Previous studies have shown that extracts of some

plant parts do possess insecticidal effects. This result compare favourably with that from other species, for example *Ambrosia meritima* require application of 1000 ppm to achieve result similar to that of leaf extract (Sharif and El-Sawy 1962). Extracts of *Vernonia amygdalina* leaves can cause about 70% mortality in *Streptococcus marcescencs* and *S. pyogenes* (Dangoggo el al., 2002).

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