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Research article

Repellency and larvicidal activities of *Azadirachta indica* seed oil on *Anopheles gambiae* in Nigeria

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ABSTRACT

Despite the recent decline in the global prevalence of malaria, the disease continues to be one of the major causes of morbidity and mortality among pregnant women and under-five children in Nigeria. The adoption of an integrated approach to malaria control including the use of bio-insecticide will further reduce the burden of malaria. This study determined the repellency and bio-insecticidal effects of *Azadirachta indica* oil on *Anopheles gambiae* in Ibadan, Nigeria. The study was experimental in design. Oil was extracted from the ground seed kernel of *Azadirachta indica* plants using N-hexane as a solvent. Larvicidal tests were carried out on 600 third and fourth instar stages of *Anopheles gambiae* using an aliquot of extracted oil emulsified with a surfactant (Tween 80) at concentrations ranging from 100 to 500 ppm. Mortality was recorded every 24 h for five days. Repellency tests were carried out by exposing Guinea pigs that were previously treated with the oil mixed with paraffin at 10–40% v/v concentrations, to 70 adult female *Anopheles gambiae* in netted cages. Data were analysed using descriptive statistics and ANOVA. The oil yield accounted for 40.0% weight of the ground seed kernel. The larvicidal effect was significant across the concentration of the emulsified *Azadirachta* oil ranging from 91.6-100.0%, compared to the control experiment ranging from 5-15% (LC50 and LC90: -1666.86 ppm and -2880.94 ppm respectively). A 100.0% larval mortality of *Anopheles gambiae* was recorded within three days at 500 ppm. All the concentrations of the oil solution also caused 100% inhibition of pupae formation. The repellent effect of adult *Anopheles* was significant ($p < 0.05$) across the concentrations but with varying degrees of protection. The highest repellent effect was observed at 40.0% (v/v). The possibility of using *Azadirachta indica* as bio-insecticide against *Anopheles gambiae* was established in this study.

1. Introduction

Malaria continues to be a primary cause of morbidity and mortality in Nigeria (Morakinyo et al., 2018; WHO, 2018). In year 2017, an estimated 219 million cases of malaria occurred worldwide (WHO, 2018). About 92% of these cases occurred in the World Health Organization (WHO) African Region where Nigeria accounted for 25% of the global burden. Children under the age of five are the most vulnerable with a child dying every 2 min from malaria infection (WHO, 2018).

Mosquitoes are important vectors of several diseases (An et al., 2020). *Anopheles gambiae* Giles commonly referred to as the African malaria mosquito, is the most common vector of human malaria in the Afro tropical Region (CDC, 2010). *Anopheles gambiae* are recognised malaria vectors due to their inclination to humans as a host, proneness to the *Plasmodium* parasite, and their indoor-feeding pattern (CDC, 2010). *Anopheles gambia* are known to have a short life cycle, anthropophilic (White, 1974), and active at night, mostly from midnight to 4:00 am (Gillies and de Meillon, 1968).

Vector control is a crucial prevention tool for reducing the burden of malaria. In the past, mosquito eggs, larvae and pupae are mostly controlled in tropical countries with organophosphates, and indoor residual spraying (Lees et al., 2014). Nevertheless, the negative effects of these methods on the environment and human health, physiological resistance to vectors, high operational cost, community acceptance and rising occurrence of insecticide-resistant mosquito vector species has rendered the use of organophosphates, and indoor residual spraying unsustainable (Benelli, 2015a; Dua et al., 2009).

In recent years, the search for newer products and alternatives for mosquito control that are environmentally safe, target-specific and easily degradable is on the rise (Ohia and Ana, 2015). Botanical metabolites are increasingly been realised as potential substitute for chemical insecticides (Vivekanandhan et al., 2018). Quite a number of plant

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products have been identified to be effective, eco-friendly, biodegradable, user-friendly, inexpensive and pose little or no risk to human and environmental health (Azizullah et al., 2014; Benelli et al., 2015b; Murugan et al., 2015). These plant products have previously been used as insecticides for controlling larvae, adult mosquitoes or as repellents for reducing human-mosquito contact through biting (Venkatachalam and Jebanesan, 2001; Dua et al., 2009; Prabhu et al., 2011).

Neem trees, *Azadirachta indica*, belongs to the family Meliaceae are well-known in the tropical and subtropical regions of the world (NRC, 1992). Neem seeds contain numerous biologically active compounds including nimbin, nimbidin, and nimbolides (Locantoni et al., 2006; Sharma and Dhiman, 1993; Su and Mulla, 1998a). The most potent of which is azadirachtin, present in the seeds at a concentration of about 5 mg/g of the kernel (Schmutterer, 2002).

Neem seeds extracts have demonstrated a range of insecticidal properties on a broad range of insect species (Isman, 2006; Schmutterer, 2002), including repellence and anti-feeding (Locantoni et al., 2006), ovicidal activity (Isman, 2006), fecundity suppression (Isman, 2006; Su and Mulla, 1998), inhibition of metamorphosis and disruption of growth and reproduction (Schmutterer, 2002), insect growth regulation (Mordue and Blackwell, 1993; Mordue and Nisbet, 2000), and deterrence of egg-laying (Schmutterer, 2002). The main product of neem is the oil extracted from its seeds (Benelli et al., 2015c). Moreover, neem seed extracts have larvicidal ability against vectors of diseases of public health significance such as malaria, filaria, dengue, dengue haemorrhagic fever, and yellow fever (Dua et al., 2009). The use of neem seed extract as a bio-insecticides could be complementary to other malaria control methods (Gianotti et al., 2008).

The incessant and haphazard use of conventional insecticides for the control of mosquito vectors has led to recent increase in the development of resistance and negative impacts on non-target organisms and the environment. Therefore, there is a need for development of biological effective mosquito control tools (Govindarajan et al., 2016). With Nigeria being one of the 10 countries with the highest burden of malaria in the year 2017 (WHO, 2018), the development and adoption of alternative methods of integrated vector management remain the key. However, there is a dearth of information on the bio-insecticidal effects of the seed oil of *Azadirachta indicia* on *Anopheles gambiae* in Nigeria. This study determined the repellent and larvicidal potential of emulsified *Azadirachta indica* seed oil formulation as a suitable alternative for commercially available insecticides against *Anopheles gambiae* in Nigeria.

2. Methods

2.1. Seed collection and preparation

Neem seed collection and handling was carried out according to the method described by Vyas and Mistry (1996). Fully matured fruits were plucked from Neem trees located within the University of Ibadan premises. The collected fruits were washed thoroughly under a running tap and thereafter soaked in a container for 3-days to remove the outer skin of the seed. The seeds were later air-dried under shade for 3 days. The coats covering the seeds were removed by a decortication process by carefully exerting pressure on the seeds using local mortar and pestle to separate the kernel from seeds. The mixture was winnowed and sieved to obtain pure reddish-brown seed kernels. The seed kernel was pulverized using an electric blender. The full description of the procedure used for seed preparation was reported in our previous work (Morakinyo et al., 2015).

2.2. Extraction of neem seed oil

The ground Neem seed kernel was subjected to oil extraction with about 800 ml Analar grade hexane in a Soxhlet apparatus following the method described by Vyas and Mistry (1996). The organic solvent was used for the extraction because earlier studies have reported that organic solvents have the ability to remove oil from Neem paste (Gahukar, 1996; Koul, 1996). Hexane and ethanol were the only solvents used in the extraction of neem oil because they have been proven to be effective in the extraction of the active ingredient Azadirachtin (Govindarajan et al., 2016). This is followed by ethanol and other solvents such as methanol, water, methyl ethyl ketone but the later solvents have been reported to enhance the degradation of azadirachtin content (Koul, 1996). A rotary vacuum evaporator was used to remove the solvent from the extract.

2.3. Neem oil formulations

2.3.1. Emulsified neem oil formular (stock solution)

This was prepared by mixing 2 drops of Tween 80 (Polyethylene glycol sorbitan monooleate, a non-ionic surfactant, and oil-in-water emulsifier) to 1ml of Neem oil mixed with 10ml of distilled water in a sample bottle. The solution was shaken vigorously to ensure thorough dissolution of oil in water. This was then made up to 1 L with the addition of more distilled water to obtain a 1000 ppm stock solution (Gbolade et al., 2000; Oyedele *et al.*, 2005).

2.3.2. Repellent oil formulation

Different concentrations (10–40%) of Neem oil in a solvent (liquid paraffin B.P.) were prepared following the procedures described by Oyedele et al.(2000) and Tawatsin et al. (2006). The prepared formulation were placed in screw-cap vials and kept at room temperature until repellency tests were carried out (Gbolade et al., 2000; Oyedele *et al.*, 2005).

2.4. Test organism

The *Anopheles gambiae s.s* used for this study were larvae and *Kisumu* adults (as locally called) obtained from the colony reared in greenhouse conditions (25–30 °C, relative humidity 60–70%) following standard operating procedures for mosquito maintenance (WHO, 1975). The mosquitoes were identified at the Department of Zoology, Faculty of Science, University of Ibadan, Nigeria. The female adult *Anopheles gambiae* (Kisumu) were fed with blood from exposed skin of an experimental animal (Guinea pigs) in a netted cage (37 × 30 × 28 cm) at ambient temperature overnight in a dark room. A moistened filter paper placed on moistened cotton wool that was mounted on Petri dishes were placed in the cage to facilitate the laying of eggs. After 24hrs, the moistened filter papers were filled with batches of brown-dark coloured eggs. The filter paper containing the eggs were then carefully transferred into large bowls of water. Within 48hrs, the eggs were hatched into larvae and were floating parallel to the water surface. The larvae obtained from *Anopheles* mosquito were fed *ad libtum* with baby fish meal.

2.5. Breeding of adult Anopheles mosquito

The procedure described above for breeding of mosquito larva was carried out for adult culture. The larvae were allowed to complete their life cycle to the adult stage. As the adults emerged they were immediately transferred into mosquito cage (30 × 30 × 30 cm) following the standard procedure for indoor mosquito collection (WHO, 2003). The adult mosquito were fed with honey solution every 24 h. Honey is one of the oldest known medicine. In animal experimentation, absolute feeding with honey for 2 weeks caused marked elevation in serum iron and haemoglobin that was associated with a reduction in white blood cells (Al-Waili, 2003).

2.6. Larvicidal test

The method adopted for larvicidal experiment was modified from Okumu et al. (2007) and Gbolade et al. (2000). The larvicidal effect of Neem oil emulsion was carried out under greenhouse conditions. Several preliminary tests were carried out to determine the range of lethal doses

for the used formulation. Five aliquots from the stock solution 1000 ppm (0.1%) i.e. 100, 200, 300, 500 ppm respectively were prepared by serial dilution method. The same procedure was carried out for the Tween 80 solution as positive control while ordinary water served as a negative control.

Actively swimming *Anopheles* mosquitoes of third and fourth instar larvae stages were introduced into each bioassay cups using a dropping pipette. Each bioassay cup contained 20 larvae (WHO, 1996). Each concentration of the experimental formulation contained dilution with water from the breeding medium i.e. 100 ml by serial dilution method and supplemented with little quantity of baby fish meal and covered with a net (Omobuwajo et al., 2005). Mortality was monitored and recorded at every 24 h by probing larvae with a needle and all moribund larvae were counted as dead for 5 days.

2.7. Repellency test

The repellency of Neem oil at different concentrations in liquid paraffin was assessed using the animal-bait technique (Omobuwajo et al., 2005). The method adopted was modified from a similar method used by other researchers (Dual et al., 1996; Oyedele et al., 2000; Tawatsin et al., 2006). The experimental animals used were female Guinea Pigs of moderate sizes. The adult female mosquitoes were fed regularly for 4–5 days (from the date of adult emergence) in a cage (30 × 30 × 30 cm) but were blood starved prior to their use for the repellency test. The experimental animals were prepared by removing the hair from their abdomen (dorsal and lateral portion) to afford the mosquito's direct access to their blood veins.

The bare part of the body of the experimental animal in scoop net (to minimize the animal's mobility) was exposed to the mosquitoes for 10s in triplicates. Thereafter, 2–5ml of the repellent solution of each concentration (10–40%) was topically smeared on the exposed skin of the animals and placed in the cage containing the female mosquitoes for 60s. The number of mosquitoes biting on the treated portion of the animal was recorded every minute (at 1, 2, 3 min) for a 3-minute exposure. The repellency was determined at an hourly interval for up to 4 h after the application of oil solution. Percentage repellency (+/-SD) was determined using the established method. The results were compared with that of a commercial repellent wipe containing N–N-Diethyl Benzamide 12% w/w cream base.

2.8. Statistical analysis

The data obtained were analysed using descriptive statistical analyses. Means were compared by measured ANOVA and where statistical significance was observed, the means were ranked by Turkey's HSD to determine the level of significance using SPSS software. Log–probit analysis was carried to determine the median (LC50) and 90% lethal concentration (LC90). The susceptibility test/effectiveness of *Azadirachta indica* oil as larvicide on *Anopheles gambiae* larvae was obtained using Abott's formular (WHO, 2003).

The mortality rate in this study was obtained as % mean mortality for across the concentration at each given exposure period. The percentage mean protection time was used as a standard of measure of repellency for *Azadirachta* oil and commercial repellent cream against adult female *Anopheles gambiae*. A comparison of repellency for each test repellent derived from different exposure periods was carried out using ANOVA. Means were ranked by Turkey's Honestly Significant Different Test.

3. Results

3.1. Larvicidal effect

The study showed that Neem oil obtained by hexane extraction was lethal to third and fourth instar stages of *Anopheles gambiae* larvae. The preliminary trials revealed that all the concentrations of emulsified Neem oil above 1000 ppm i.e. 0.5–10% elicited absolute (100%) mortality against the third and fourth instar stages of larvae within 24 h. All the preparations beyond 1000 ppm gave rise to 100% mortality within 24 h.

Generally, all the concentrations of emulsified neem oil used (100–500 ppm) in this experiment showed a reduction in the wriggling rate of the larvae and complete inhibition of pupa formation for the period of exposure. The moribund larvae sank to the bottom of the solution but when touched with dropping pipette, they responded with little body wriggling to move away from the area of disturbance. In the control experiment, however, larvae activities were not different from those in ordinary water solution. They floated and fed on the fish meal normally. The rate of mortality was highly reduced even compared with ordinary water medium, there was pupa formation and adult emergence until the test was terminated.

Table 1 shows the mean mortality from 100-500 ppm concentrations of the emulsified Neem oil and Tween 80 solutions for 120 h of exposure time. The maximum mortality was first observed in 500 ppm at 72 h and this effect continued with lower concentrations as exposure time increased. This implies that the pattern of mortality over the 120 h of exposure was not the same for each treatment.

The trend of the effectiveness of Neem (*Azadirachta indica*) oil as larvicide at different concentrations (100–500 ppm) are shown in Figure 1. The graph shows at 24 h the rate of larvae mortality across the concentrations was less than 20%, and the effect increased progressively afterwards. At 48hours, the effect in 500 ppm was almost as thrice (56%) as those that occurred in 100 ppm and 200 ppm (20%) respectively. The optimum (100%) effect was observed at 72 h in 500ppm whereas other concentrations could not attain this level. At the end of the experiment, the least concentration (100 ppm) was able to produce a 90% larvicidal effect. Figure 2 shows that the larvicidal effect was due to the action of Neem oil which increased with increase in concentration whereas the increase in surfactant had a protective effect on the larvae.

The results of Turkey's HSD multiple comparison tests that were used to identify significantly pairwise comparisons are summarised in Table 2. Mean values with same letters are not significantly different from one another while those with different letters differ significantly.

In Table 3, the lethal concentrations for the whole of the exposure period taken as one for the treatment group using probit analysis was presented. The median anti–larva potency (LC50) of the emulsified Neem oil was 723.257 ppm while the lethal concentration to cause 90% mortality of the population (LC90) was 1971.51 ppm, as projected by the logarithm of the concentrations in base 2. This was quite very low for Tween 80 that gave negative values. This implies that Tween 80 did not have any significant effect on larvae mortality ($p > 0.05$).

3.2. Mosquito repellency

The average number of mosquitoes landing to bite the bare skin of the animal were 25.72 ± 8.36. The positive control (Odomos cream) however gave absolute repellency against female *Anopheles* mosquito (100%) throughout the exposure period (4 h) which was similar to the result obtained for all other concentrations used within 1 h of exposure. The blank controls gave no repellency against the mosquitoes. The result in Table 4 shows that the repellency of Neem oil was inversely proportional to the exposure period ($p < 0.05$). The maximum repellent effect was observed at 40% v/v concentration and lowest at 10%v/v.

4. Discussion

Despite the recent decline in the global prevalence of malaria, the disease continues to be responsible for high morbidity and mortality in Africa south of Sahara (Wassmer and Grau, 2017). Obviously, there is the need to seek alternative ways to combat menace particularly in Nigeria with one of the highest-burden in Africa (WHO, 2018). Recently, the adoption of environmentally friendly biodegradable insecticides of plant origin to control malaria vector is gaining importance (Ohia and Ana,

Table 1. Mean mortality of *Anopheles gambiae* larvae exposed to emulsified Neem oil (N) and Tween 80 (T) solutions for a period of 120 h.

Treatments (ppm)	24 h	48 h	72 h	96 h	120 h
N100	2.00 ± 2.00	4.67 ± 1.16	10.67 ± 1.53	16.33 ± 3.79	18.33 ± 2.08
N200	1.00 ± 1.00	4.67 ± 1.15	12.67 ± 2.88	17.33 ± 1.15	18.67 ± 1.15
N300	1.67 ± 0.56	6.33 ± 0.58	14.33 ± 2.31	17.00 ± 1.00	19.00 ± 1.00
N400	2.67 ± 2.08	8.00 ± 1.73	15.33 ± 0.58	17.67 ± 2.08	20.00 ± 0.00
N500	3.00 ± 1.00	11.33 ± 4.93	20.00 ± 0.00	20.00 ± 0.00	20.00 ± 0.00
T100	-	0.67 ± 1.15	1.00 ± 1.00	1.33 ± 0.58	3.00 ± 1.00
T200	0.33 ± 0.58	0.67 ± 0.33	1.00 ± 1.00	1.33 ± 0.58	1.33 ± 0.58
T300	-	0.33 ± 0.57	1.33 ± 1.15	1.67 ± 1.15	2.67 ± 1.15
T400	-	-	1.00 ± 1.00	1.00 ± 1.00	1.33 ± 0.58
T500	0.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	0.33 ± 0.58	1.00 ± 1.00

All concentrations measured in parts per million (ppm) at 95% confidence intervals. Five concentrations were tested in both treatment groups and replicated three times with 60 mosquitoes per concentration making 600 mosquitoes in both cases.

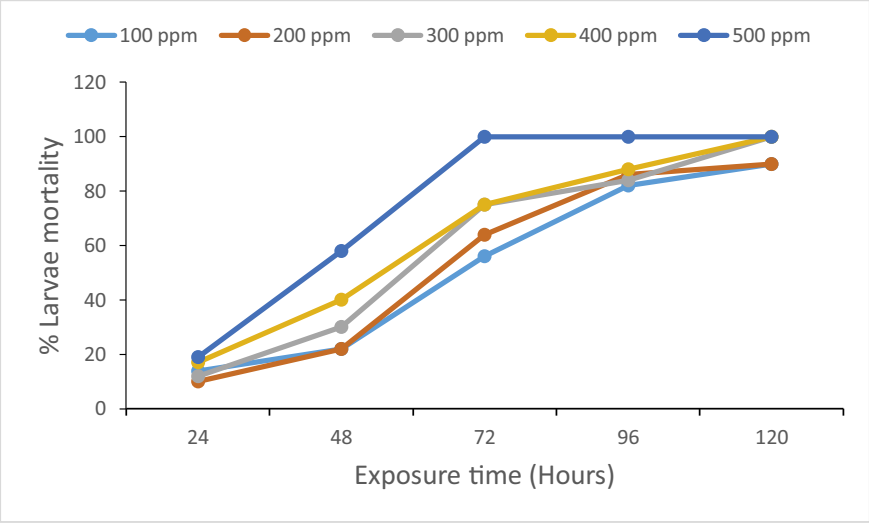

Figure 1. The larvicidal effectiveness of *Azadirachta indica* oil at different concentrations in 120 h. The figure shows that at 24 h the rate of larvae mortality for the different concentrations was less than 20%, while the effect increased progressively afterwards. At 48hours, the effect in 500 ppm was almost as thrice (56%) as those that occurred in 100 ppm and 200 ppm (20%) respectively. The optimum (100%) effect was observed at 72 h in 500 ppm whereas other concentrations could not attain this level.

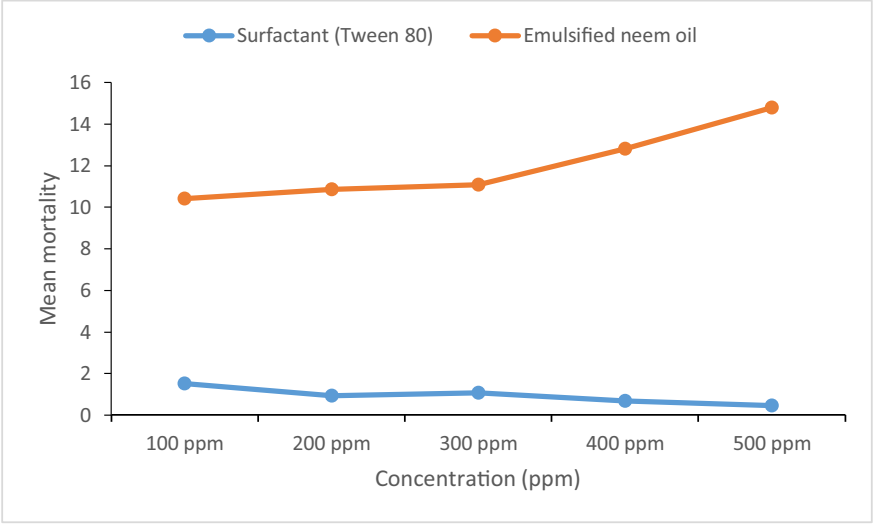

Figure 2. The trend of larval mortality in Neem oil solutions compared with surfactant solutions. The figure shows that the larvicidal action of the Neem oil increased with increase in concentration whereas the increase in surfactant had a protective effect on the larvae.

Table 2. Comparison of significance across different concentrations of Neem oil and Tween 80.

Treatment	T500	T400	T300	T200	T100	N100	N200	N300	N400	N500
Mean mortality	0.47[a]	0.67[a]	1.07[a]	0.93[a]	1.53[a]	10.40[b]	10.87[b]	11.07[b]	12.80[bc]	14.80[c]

Df: Degree of freedom; SS: Sum of Squares; MS: Mean Square; N: Emulsified Neem oil; T:Tween 80 (Polyethylene glycol sorbitan monooleate); Ppm: Concentrations in Part per Million; Superscripts: a<b < bc < c. Superscripts with the same letters are not significantly different.

Table 3. Probit analysis indicating the LC50 and LC90 between Neem oil (EC) and Tween 80.

Treatments	Intercept	Slope (S.E)	LC50 (ppm)	LC90 (ppm)	X^2 (Df)	P-Value	Exposure period (hrs.)
Neem oil	-0.74255	0.00103 (0.0032)	723.257	1971.51	288.92 (66)	0.00	24–120
Tween 80	-1.75950	-0.0106 (0.166)	-1666.86	-2880.94	76.132 (73)	0.373	24–120

Keys: *S.E: Standard Error; L.C50 - Lethal concentration to cause 50% mortality in population; L.C90 - Lethal concentration to cause 90% mortality in population;* X^2 *- Chi-Square; Df - degree of freedom in the bracket.*

2015). In this study, we determined the larvicidal and repellent potential of emulsified neem oil formulation against *Anopheles gambiae*.

4.1. Extracted neem oil

This study demonstrated that Neem kernel is rich in oil. The oil yield obtained by organic solvent (hexane) extraction was approximately 40% which gave credence to the value of 25–47% reported in other studies (Panhwar, 2005; Vietmeyer, 1992). Anya et al. (2012) and Ismadji et al. (2012), reported that the oil yield from Neem seeds varies from 25 to 45%. Neem seed oil is well recognised for its high medicinal and insecticidal importance (Lokanadhan et al., 2012).

4.2. Larvicidal effectiveness of neem oil

This study showed that Neem oil is an effective larvicide against *Anopheles gambiae* larvae. It was highly toxic to mosquito larvae and inhibited the development of pupae. The high rates of larval mortality observed within 72 and 120 h at lower concentrations indicated its high toxicity to mosquito larvae. It has been reported that under field conditions, emulsified formulations of *A. indica* oil showed an excellent larvicidal potential against different mosquito genera, including Aedes, *Anopheles* and Culex (Dua et al., 2009; Benelli et al., 2015c).

The absolute pupal inhibition in this study was consistent with that reported by Okumu et al. (2007) in their study on testing the larvicidal effects of *Azadirachta indica* oil formulation on the malaria vector *Anopheles gambiae*. A 50% inhibition of adult emergence was achieved when neem oil formulation was applied on the third and fourth stage *Anopheles gambiae s.s.* larvae at a concentration of 6 ppm (Okumu et al., 2007). The demonstration of the sublethal-effect of Neem oil in this study through inhibition pupal and adult formation is an indication that Neem oil does not only have to kill the mosquito larvae to be considered effective for malaria control, but rendering them inactive or limiting their growth to non-vector stage of the life cycle is also another advantage. Also, rendering the larva as prey for other organisms for food is a way of managing the ecosystem. This is indeed a better alternative to synthetic insecticides.

The 5 days period to achieve larval toxicity reported in this study was lower than the 12 and 15 days reported by Singh and Srivastava (1984) and Scott and Kaushik (1998), respectively. Neem oil has been reported to possess the ability to inhibit can the midgut epithelium, the respiratory system, gastric caeca and the malphigian tubules of mosquito larvae (David et al., 2002; Rey et al., 1999). It works by inhibiting the production of ecdysone, an enzyme that allows for the moulting of the larva, thus ensuring that the larva failed to moult, remains in the larval stage and ultimately died. If the larva manages to enter the pupal stage, there is the likelihood that it remains absolutely sterile without any capacity for reproduction (Prajapti, 2005).

Neem oil also possesses the ability to weaken the defence system of a larva thus allowing easy infiltration of the pathogenic organisms into the insect system (Su and Mulla, 1998a,b). Anopheline larva did not develop resistance or change in susceptibility to exposure to emulsified neem oil over a three months period (Awad and Shimaila, 2003). The resistance of mosquitoes to neem-based compounds will likely occur with a larvicide that is based on a lone active ingredient than the one with a multitude of compounds (Mulla and Su, 1999; Okumu et al., 2007).

4.3. The repelling potential of neem oil against mosquito

Repellency is one of the proven ways of preventing vector-borne illnesses by reducing man-vector contact. Neem products are effective mosquito repellants (Govindarajan et al., 2011a,b; Nagpal et al., 2001) are capable of offering about 90–100% protection against malaria vectors (Nagpal et al., 2001). The repellent action of Neem oil as assessed by this study showed the multifunctional insecticidal activity of Neem oil for mosquito control at the household level. The repellency action of Neem oil observed in this study gave better protection than that reported for *Citrus sinensis* oil and Hemizygia oil (Tawatsin et al., 2006; Omobuwajo et al., 2005; Oyedele et al., 2000). This could be attributed to the fact that active ingredients of Neem oil in liquid paraffin tend to be more stable and possess a longer duration of action in terms of repellency than *Citrus sinensis* oil and Hemizygia oil which are essential oils and evaporates readily into the air. This effect thus explained that a repellent compound

Table 4. Mean Repellency (%) of Neem oil against *Anopheles gambiae*.

Concentration (V/V)	Exposure Time (Hour)				
	0	1	2	3	4
N 10%	100.0	96.190 ± 0.48	93.810 ± 1.26	95.714 ± 0.82	92.857 ± 0.82
N 20%	100.0	97.143 ± 0.82	96.666 ± 1.26	95.238 ± 0.95	94.286 ± 1.65
N 30%	100.0	98.095 ± 0.48	95.714 ± 0.82	93.333 ± 0.48	93.333 ± 0.48
N 40%	100.0	99.524 ± 0.48	98.571 ± 0.82	96.667 ± 1.25	95.238 ± 0.95

is influenced by its evaporation rates and the nature of formulation (Oyedele et al., 2000).

The high protection rate (92–100%) exhibited by Neem oil in paraffin in this study is an indication that Neem oil in comparison to some commercial synthetic repellent creams (e.g. 12% N–N Diethyl benzamide) could be an alternative repellent ingredient if formulated into creams. An improved repellency of plant-derived topical repellents has been reported after formulation with some bases or fixative materials such as liquid paraffin, vanillin, and salicyluric acid (Stuart and Estambale, 2003; Vatandoost and Vaziri, 2004).

5. Conclusion

The neem oil formulations tested in this study were effective larvicide against *An. gambiae* larvae and inhibits adult emergence at very low concentrations. Also, the Neem oil formulations possesses repellent activity against *An. gambiae* larvae. Repeated applications over a period of time would ensure better protection such as obtained from some commercial synthetic mosquito repellants. The ability of Neem oil formulations to cause mortality to *An. gambiae* larvae at relatively low concentrations signify the possibility of their usage as alternatives to synthetic insecticides in the control of malaria vectors. The need for the incorporation of complementary techniques for integrated vector management in addition to reducing adult biting remains a key to reducing malaria prevalence among under-five children and pregnant women.

Declarations

Author contribution statement

Ayinde, A.A.: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Morakinyo, O.M.: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Sridhar, M.K.C: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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