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THE POTENTIAL REPELLENCY OF AQUEOUS EXTRACTS OF BASIL (OCIMUMBASILICUML.) LEAVES AND NEEM (AZADIRACHTAINDICAA.JUSS.) SEEDS ON ANOPHELES ARABIENSIS(DIPTERA: CULICIDAE) FEMALES IN GEZIRA STATE - SUDAN - 2019

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ABSTRACT

Background: Malaria remains a disease of global importance. The World Health Organization (WHO) estimated that 3.2 billion people are at risk of malaria, of which 1.2 billion are at high risk. Vector control interventions include the use of bed nets, indoor residual spraying (IRS), larviciding, larvivorous fish, space spraying and mosquito repellents among others. Insecticide treated bed nets (ITNs) and IRS are the most widely used vector control (VC) methods in Africa. (Alessandro *et al.*, 1995, and Nevill *et al*., 1996). **Objectives: 1.4.1** To study the potential of basil (*O. basilicum*) leaf and Neem (*A. indica*) seeds aqueous Extracts (aq-E) as repellents for *Anopheles* adults. **Methodology:** This study was conducted as a cross sectional using *An. Arabiensis* mosquitoes adults taken from the Insectary of the Blue Nile National Institute for Communicable Disease (BNNICD), University of Gezira Wad Medani, the capital of the Gezira State. The materials used: plastic bag to conserve neem seed and basil leafs, carpet to drying the leaves and seed in the shadow, blender-mixer to prepare powder and dark glass jars to conserve powder until to using. **Were collected:** Basil leaves and neem seeds from Eltalha village, South Gezira Locality, around 20 km west of Wad Medani. **Results:** The results showed that in the NSE (old and new)there areno big difference between those resting on the surface of the cage (treated net) and others sites. BLE results showed that the number of mosquitoes resting on the treated net were even higher than those resting on the other sites. It wasconcluded that more detailed investigations are required forboth extracts to verify the presence or absence of the repellency property. **Conclusion:** The study recommends to More studies are needed, Development of better extraction methods, Isolation of the active ingredients, determining their identities and concentrations in each extracted fraction. and Revising the bioassay method.

1. INTRODUCTION

Historically, several insecticides have been used in the VC of malaria, with varying success. Dichlorodiphenyltrichloroethane (DDT) was extensively used in IRS in the 1940's and late 1970's (Curtis, 2002). DDT has the longest residual efficacy of any insecticide. When sprayed on walls and ceilings, it can last approx. 6-12 months, dependent on the dosage and nature of substrate (WHO, 1997). In addition, its spatial repellency and irritant effect on malaria vectors strongly limits human-vector contact. However, the observed decrease in malaria infection following the introduction of DDT for malaria control was short- lived, due to the emergence of resistance in malaria vectors. The WHO now recommends four classes of insecticides for use against adult mosquitoes in public health programs: pyrethroids, organochlorines (OCs), organophosphates (OPs) and carbamates (WHO, 2013). Of these four classes, pyrethroids have become the insecticides of choice in treating mosquito bed nets to prevent malaria transmission. In 2009, pyrethroids accounted for 75% of IRS worldwide and the treatment of long lasting insecticide bed nets (LLINs), while carbamates and OPs represented a small percentage of global usage (WHO, 2012). Pyrethroids are considered safer, cheaper and have a longer residual action than other insecticides. In addition, pyrethroids are used in IRS as an alternative to DDT and the OP malathion, where mosquito vectors have become resistant to both insecticides (WHOPES, 2011). Examples of pyrethroids include alpha-cypermethrin, cyfluthrin, bifentrin, cypermethrin, cyphenothrin, deltamethrin, etofenprox, lambda-cyhalothrin and permethrin (WHOPES, 2014).

1.1 Justifications and Problem state

Anopheles mosquitoes are major vectors of malaria in the Sudan. Most mosquito repellents today might cause some side- effects, especially the synthetic chemicals (e.g. DEET and permethrin).These side- effects could appear after a prolonged use, e.g. occasional skin rashes, blisters, skin and mucus membrane irritation and others. Repellents are one of the major personal protective agents. Therefore, the search for new sources of active ingredients (a.i.) is required. The Sudanese fauna and flora is a rich source of such (a.i.). A variety of plant-based products are currently needed to protect people from mosquito bites and disease transmission, especially during the rainy-season in the Sudan.

1.2 Objective

To investigate the possibility of having the of repellency property or the presence of repellent active ingredients in the leaves basil (*Ocimum basilicum*) and the seeds of neem tree (*Azadirachta indica*) aqueous – extracts (aq-E).

2. MATERIAL AND METHOD

2.1 Study Design and Study area

This study was conducted as a cross sectional using An. Arabiensis mosquitoes female adults taken from the Insectary of the Blue Nile National Institute for Communicable Disease (BNNICD), University of Gezira (U of G), Wad Medani, the capital of the Gezira State (latitude 13-15.20 N and longitude 32.5 – 34.0 E.), central Sudan.

2.2 Materials

2.2.1 Neem Seed and Basil leaves collection, Drying and Powder preparation

Plastic bags for co-action and preservation of neem seeds (NS) and basil leaves (BL), blender-mixer for the preparation of the powder and dark glass jars for storing the powder.

2.2.2 Preparation of the stock solutions

Distilled water (DW), sieves, plastic bottle (2.5), dishes, sensitive balance, aluminum foil and refrigerator.

2.2.3 Impregnated net

Dishes, test tubes (100 ml), bed net fabric (40 x40 cm2), and stock solution.

2.2.4 Bioassay

Cage (Plate), rabbits, forceps, cotton, aspirators, stop watch and paper cups (500 ml).

2.3 Methods

2.3.1 Collection of basil Leaves and Neem seeds

BL and NS were collected from El Talha village, South Gezira Locality, around 20 km west of Wad Medani town.

2.3.2 Preparation of basil leaf powder(BLP)

The collected BL were kept under shade for 7 days to dry, powdered by using to an electric blender–mixer (Moulinex LM 241), and stored in dark glass jars surrounded with aluminum foil and left in the laboratory at room temperature of 25 °C until used.

2.3.3 Preparation of neem seeds powder (NSP)

The collected seeds were dried under shade for 45 days, powdered and stored as above.

2.3.4 Extraction

3.3.4.1 Basil: BLP (250 g) was transferred to a plastic bottle (2.5 L) and completed with DW to 2 L; shaken for 30-60 seconds, then every 10 minutes for 1 hour, and left in the laboratory for 48 hours; sieved and the volume was completed to 2 L again. The sieved powder was dried under shade, weighed and the measured difference was considered as the material stayed (extract) in the water as the extracting solvent (highly polar). This was considered the BLE stock solution, which was kept in the refrigerator until needed.

2.3.4.1 Neem

NSP was treated as above with DW following the same steps in to obtain the stock solution. This was also kept in the refrigerators until needed. Two batches of NSE were prepared, i.e. 3 months stock (old) and fresh extract.

2.3.5 Impregnated net

According to WHO for impregnated net through saturating the fabric net (40 x40 cm2) was immersed in a dish that contains measured volume of the stock solution of each extract and removed. The amount taken by the net was measured, i.e. 30 ml/ net; dried under shade and used for the bioassay.

2.3.6 Concentrations tested

2.3.6.1 Old neem batch

125 g of the old NSP was added to 1 L of water (125 g/L), the extracted weight was 42.5 g/L (stock solution). Exactly 30 ml (i.e. 1.275 mg) was taken from the stock solution to saturate the net (i.e. 42.5 mg/ml; 0.8 mg/cm2)

2.3.6.2 New neem batch

125 g of the new NSP was treated as above; the extracted material weight was 61.7 mg/L (stock solution). Again 30 ml (1.85 mg) was taken from the stock solution to saturate the net (61.7 mg/L; 1.15 mg/cm2).

2.3.6.3 Basil

125 g powder of basil leave also treated as NSP and the extracted weight was 31.55 g/L (stock solution). The 30 ml (946.5 mg) taken from the stock solution was used to saturate net (0.6 mg/cm2).

2.4 Bioassay

The above- mentioned concentrations from NSE and BLE were tested by placing the net on the top of the cage; 20 unfed Anopheles females were transferred to each cage in the presence of Rabbit for feeding and in the absence of the rabbit. The exposure period was 1hr. The 36 situation inside the cage (resting place of the adults) was taken each 10 minutes (bottom, sides, top/ surface, exit/entrance point).

2.5 Data Analysis and Interpretations

Data was entered into excel sheet and analyzed by SPSS (version 16)

3. RESULTS

Table 1: Mosquitoes exposed inside the cage to the impregnated fabric (40x40 cm in the top) treated with the old neem solution (3months) in the absence of the rabbit.

Time (Min)	*No. Exposed/ Cage	Resting place inside the cage (%)				Moving %
		Bottom	Surface	Side	Exit	
0-10	20	40.0	5.0	21.7	31.7	1.6
10-20	20	23.3	6.7	33.3	23.3	13.3
20-30	20	25.0	0.0	38.3	28.3	8.4
30-40	20	20.0	8.3	30.0	35.0	6.7
40-50	20	23.3	8.4	30.0	28.3	10.0
50-60	20	20.0	6.7	31.7	30.0	11.6
Mean±S.E.	20	25.3±3.1	5.9±1.3	30.8±2.2	29.4±1.6	8.6±1.7
Control	20	7.5	26.7	10.4	20.4	35.0

*Each treatment was replicated 3 times.

Table 2: Mosquitoes exposed inside the cage to the impregnated fabric (40x40 cm in the top) treated with the fresh neem solution in the absence of the rabbit.

Time (Min)	No. Exposed	Resting place (%)				Moving %
		Bottom	Surface	Side	Exit	
0-10	20	6.7	10.0	21.7	45.0	16.6
10-20	20	3.3	8.4	20.0	48.3	20.0
20-30	20	1.7	8.3	23.3	51.7	15.0
30-40	20	5.0	6.7	18.3	55.0	15.0
40-50	20	0.0	8.3	21.7	58.3	11.7
50-60	20	5.0	5.0	21.7	55.0	13.3
Mean±S.E.	20	3.6±1.0	7.8±0.7	21.1±0.7	52.2±2.0	15.3±1.2
Control	20	7.5	26.7	10.4	20.4	35.0

*Each treatment was replicated 3 times.

Table 3: Mosquitoes exposed inside the cage to the impregnated fabric (40x40 cm) treated with the old neem solution (3months) in the presence of the rabbit.

Time (Min)	No. Exposed/ cage	Start Feeding %	Resting Place In %				Moving %
			Bottom	Surface	Side	Exit	
0-10	20	0.0	11.7	8.3	15.0	18.3	46.7
10-20	20	21.7	15.0	8.3	16.7	30.0	8.3
20-30	20	46.7	1.7	3.3	10.0	31.7	6.6
30-40	20	55.0	3.3	5.0	10.0	25.0	1.7
40-50	20	70.0	1.7	1.7	5.0	11.6	10.0
50-60	20	76.7	0.0	1.7	5.0	16.6	0.0
Mean±S.E	20	45.0±12.0	5.6±2.5	4.7±1.2	10.3±2.0	22.2±3.3	12.2±7.0
Control	20	71.7	5.0	6.7	10.4	0.4	5.8

*Each treatment was replicated 3 times.

Table 4: Mosquitoes exposed inside the cage to the impregnated fabric (40x40 cm) treated with the freesh neem solution in the presence of the rabbit.

Time (Min)	No. Exposed	Start Feeding %	Resting place (%)				Moving (%)
			Bottom	Surface	Side	Exit	
0-10	20	0.0	11.7	11.7	13.3	43.3	20.0
10-20	20	8.3	10.0	6.7	11.7	58.3	5.0
20-30	20	23.3	1.7	8.3	6.7	51.7	8.3

30-40	20	36.7	5.0	10.0	3.3	35.0	10.0
40-50	20	58.3	1.7	3.3	16.7	16.7	3.3
50-60	20	60.0	0.0	5.0	11.7	20.0	3.3
Mean±S.E.	20	31.1±10.3	5.0±2.0	7.5±1.3	10.6±2.0	37.5±6.9	8.3±2.6
Control	20	71.7	5.0	6.7	10.4	0.4	5.8

*Each treatment was replicated 3 times.

Table 5: Mosquitoes exposed inside the cage to the impregnated fabric (40x40 cm in the top) treated with basil extract in the absence of the rabbit.

Time (Min)	No. Of Mosquito Exposed	Resting Place %				Moving %
		Bottom	Surface	Side	Exit	
0-10	20	0	13.3	0	18.3	68.4
10-20	20	0	18.3	0	18.3	63.4
20-30	20	0	23.3	0	11.7	65.0
30-40	20	0	16.7	0	5.0	78.3
40-50	20	0	28.3	0	18.3	53.4
50-60	20	0	23.3	0	13.3	63.4
Mean±S.E	20	0	20.5±2.2	0	14.2±2.2	65.3±3.3
Control	20	0.3	27.2	6.1	11.4	50.0

*Each treatment was replicated 3 times.

Table 6: Mosquitoes exposed inside the cage to the impregnated fabric (40x40 cm in the top) treated with the basil solution in the presence of the rabbit.

Time (Min)	No. Exposed	Start Feeding %	Resting place %				Moving %
			Bottom	Surface	Side	Exit	
0-10	20	33.3	1.7	13.3	10.0	1.7	40.0
10-20	20	11.7	0.0	21.7	13.3	5.0	48.3
20-30	20	10.0	0.0	26.7	18.3	5.0	40.0
30-40	20	6.7	1.7	28.3	38.3	18.3	6.7
40-50	20	0.0	0.0	18.3	63.3	6.7	11.3
50-60	20	0.0	0.0	20.0	65.0	10.0	5.0
Mean±S.E	20	10.3±5.0	0.6±0.4	21.4±2.3	34.7±10.1	7.8±2.4	25.2±8.0
Control	20	5.0	0.3	27.2	6.1	11.4	50

*Each treatment was replicated 3 times.

Table 7: Comparison of mosquito population exposed inside the cage to the impregnated fabric (40x40 cm in the top) treated with the basil solution in the absence and presence of the rabbit.

Resting position	Present	Absent
Start to feed	10.3	
Bottom	0.6	0.0
Surface	21.4	20.5
Side	34.4	0.0
Exit	7.8	14.2
Moving	25.2	65.3

4. DISCUSSION

Over the last 5 decades the indiscriminate use of synthetic insecticides in public health programs and agriculture for the control of pest species has created multifarious problems, *viz*. insecticide resistance, environmental pollution, toxic hazards to humans and other non-target organism, etc. In attempt to overcome these problems, great emphasis has been recently placed on the research and development of forms of pest control using natural products of plant origin. Studies on plant products as Insect Repellent have indicated that they could provide possible alternative solutions to synthetic insecticides.

There are different studies important on the neem in the field effectiveness of neem cake borne compounds for causing negative impacts on the reproductive behavior of the Asian tiger mosquito. Interestingly, neem cake methanol and ethyl acetate fractions have been also proved as effective against larvae of A. albopictus (Nicoletti *et a)l.* 2010. Furthermore, neem cake powder applied in rice fields at the dose of 500 kg/ha, either alone or coated over urea, was able to exert a striking reduction in the abundance of Culex quinquefasciatus late instars larvae and pupae (Rao *et al.* 1992).

Other study it used neem oil nano emulsion was found to be effective in controlling mosquito larvae. The reduced

size and uniform spreading of these fine particles increased the larvicidal efficacy. The nano emulsion is easily affordable, economically feasible and moreover less toxic than synthetic pesticides, and may be used as an alternative for control of vector-borne diseases. It has the advantage of being eco friendly and effective, and has shown promising larvicidal activity. However, further work is going on in the authors' laboratory to evaluate the topical application of neem-based nano emulsions on human beings for mosquito repellency (Anjalian *et al.* 2011).

In this study the result analysis showed in movement and resting in the old solution most of mosquitoes resting in the bottom and the number of mosquitoes in the exits increase with the time that means most of mosquitoes wear annoying from the weather inside the cage, after 20min the mosquitoes started affecting to repellent but in the 30min reaming the mosquito wear adapting with the repellent but in new solution the mosquitoes moving rather than old solution.

This study dependent on different techniques and two sections through trials, in the section one through first technique has old neem solution without rabbit showed significant different between surface and others sites such as surface (5.9), bottom (25.3), side (30.8), Exit (29.4) and moving (8.6) besides control.

In the second technique it had been contain new neem and absence rabbit here it showed doesn't different clear enough between bottom and surface but it so simply, then there are different between surface and others sites that through observation the means such as bottom (3.6), surface (7.8), side (21.1), exit (52.2) and moving (52.) besides control In third technique it contained old neem solution and a rabbit it showed doesn't large different between surface and others site except exit such as bottom (5.6), surface (4.7), side (10.), exit (22.2) and moving (12.2) besides control.

In the fourth technique contained new neem solution and a rabbit here it showed also doesn't large different between surface and others sites except exit such as bottom (5.6), surface (7.5), side (7.5), exit (37.5) and moving (8.3) besides control then through different techniques observed the surface impregnated had small different than others sites through old and new neem solution with rabbit absences and present, besides control except with absence rabbit and old.

Another study carried out different results from this study it depended on landing of *An. culicifacies*, total anophelines, and total *Culex* on neem oil concentration were significant ($P < 0.05$) that an increase in concentration of neem oil up to 1% increases protection from *An. culicifacies (r2* = 0.88), anophelines (r2 = 0.83), and *Culex* (r2 = 0.90). Anophelines were more sensitive to the repellent action of neem oil than *Culex* (Sharma, 1994).

Also different study depended on different repellents *with neem* The complete protection time of the commercial repellents was different, 8 hrs for DEET and 6 hrs for MyggA. For the first 6 hrs both DEET and Mygg A gave 100% protection but after 2 hrs, protection of MyggA was decreased to < 80% while DEET gave 100%. Neem provided 100% protection for 3 hrs and then protection was dropped to 20- 60% after 7 hrs. But, 20% chinaberry gave 90-100% protection for the first 1 hr, and thereafter, dropped to 50-80% after 11 hrs (Sharma, *et al.*, 2015).

This it used basil through two Sections had two technique through trials, the first technique it contained basil and absence rabbit it showed negative results all mosquitoes resting on the impregnated surface of the cage, when compared surface and others site, the surface it above than others sites, because it had highest main than others. The second technique of trials contained basil and a rabbit also it showed resting on the impregnated surface with basil it highest than others sites except exit and moving but it so simply different through two techniques observed the basil doesn't affect to repel mosquitoes, through different two section this showed basil hadn't affectivity that observed through trials. But there were different studied showed different results for basil such as a study was to determine efficacy of sweet basil (*Ocimum basilicum)* on *Aedes aegypti* adults. The test substances formulated into a jelly were applied on the rabbit skin and tested with starved *Aedes aegypti* adult females in a mosquito cup. Synergized crude oleoresin extract of pyrethrum and Vaseline pure petroleum jelly included as a positive and negative control respectively. The Kd50 was estimated to be 369 ppm and 0.027ppm for *Ocimum* oil and pyrethrum extract respectively. 1000ppm of Pyrethrum extract was comparable to 30000 ppm of *Ocimum* oil ($P > 0.05$). The combination of *Ocimum* and *Eucalyptus* oil led to a more superior product with knockdown effect. *Ocimum basilicum* can be formulated with other plant compounds and applied in mosquito control (Peter Koech. 2018).

Other study carried out different results from this study as one study was to investigate mosquito repellent activities of Sweet Basil (*O. basilicum*), Neem (*A. indica*) and Lemon Eucalyptus (*Eucalyptus citriodora)* extracts. Different concentrations of the extracts were tested for mosquito repellency on rabbit skin as the host of *Ae. aegypti*. Laboratory reared starved females were used for the tests and data collection was done by observational parameters based on frequency of mosquito landing and blood engorgement. Synergized Crude oleoresin extract of Pyrethrum and Ballet mosquito repellent® were included as positive test controls and Vaseline pure petroleum jelly® as a negative test control. The results showed that synergized Pyrethrum oleoresin showed complete protection at 0.1% as compared to Lemon Eucalyptus oil and Sweet Basil oil at 2% and 3% respectively($p<0.05$). Neem oil and Ballet did not provide complete protection. The mean

percent repellency of 5% Neem oil was 84.21 and that of Ballet was 66.84 ($p<0.05$). Sweet basil and Lemon Eucalyptus oils can be alternative to pyrethrum as natural mosquito repellents from plant origin.

CONCLUSIONS

Neem seeds extract showed some promising repellency effect on Anopheles mosquitoes, but the extraction technique, concentration and application to the net might need some modifications for verification and improvement.

The Basil leaf extracts in water with the tested concentration showed wear repellency effect.

Recommendation

The following recommendations were made:

1. More studies are needed
2. Development of better extraction methods.
3. Isolation of the active ingredients, determining their identities and concentration in each extracted fraction.
4. Revising the bioassay method.

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