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First report on insecticides susceptibility of restricted foci of *Aedes aegypti*, a viruses' vector, in Gezira state, Sudan

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Abstract
First attempt using the available adult traps (Light trap; Pyrethroid indoor-spray; window trap and clay pots) showed no encouraging results due to the absence of attractants. Therefore, susceptibility of adults *Aedes aegypti* was evaluated by using field-collected larvae. The larval surveillance of seven locations eastern and western banks of the Blue Nile north and south of Wad Madani, the state capital, resulted in observing *Aedes aegypti* larvae in Barakat nursery only. The larvae were reared in climatic rooms at a temperature of 25±2 °C and 80% relative humidity until reaching adulthood. The confirmed adults were placed in cages supported by sugar and blood fed on rabbits for continuous generation production. Testing for females adult susceptibility to Bendiocarb (1%); DDT (4%) and Permithrin (0.75%) was carried out with standard WHO impregnated papers according to the WHO (2013) method. Overall, all, the adult females were fully susceptible (1OO% mortality) to I hour of exposure to the diagnostic dosages of Permithrin (100% death after 40 minutes); Females were relatively less susceptible to DDT (74% and 93% death in one hour and 24 hours, respectively), however, the examined *Aedes aegypti* were scored resistant (100% alive after 24 hours) to Bendiocarb. According to our knowledge, the present study is the first report of resistant *Aedes aegypti* in Gezira state and could be in the Sudan. The implementation of such findings were discussed.

Keywords: *Aedes aegyptii*, insecticide susceptibility, Bendiocarb, DDT, Permithrin, Gezira Sudan

Introduction

Arboviruses, such as Rift Valley virus, infection can cause severe disease in both humans and animals including sheep, goats, cattle and camels by mosquito bite and contact with animals or their infected tissues [1]. Such infection could result in significant economic losses via restricting or banning the domestic movement of livestock and additionally affects animals' exportation. In Sudan such mosquito-borne viruses were reportedIt was stated that Yellow Fever had been detected in 26 localities in Sudan's Darfur region from November 16, 2012, to January 16, 2013 and the Centers for Disease Control and Prevention (CDC) confirmed that 849 cases and 171 deaths had been reported [2]. In Sudan, the first report of the Rift Valley fever virus (RVF) was in1973 in domestic animals and later in livestock and in humans in Khartoum, Kassala, El Gezira, Sennar, and White Nile states [3-7]. However, *Culex* and *Anopheles,* in general, were incriminated as vectors in the 2007 Rift Valley outbreak in Sudan [8]. Moreover, dengue epidemics have been reported in the Eastern part of Sudan [9-11].

Vectors play an important role in arboviruses infection and circulation. As reported, *Aedes aegypti* is a competent vector for several arboviruses such as yellow fever, Chikungunya fever, Dengue fever [12-14] and for Zika [15]. *Aedes aegypti* is a container-inhabiting mosquitoes, thrive in urbanized areas. Females feed primarily on humans making them an exceptionally successful vector. Such vector plays an important role in arboviruses infection and circulation and hence, viruses' ecology. For instance, dengue-3 virus could be persist through transovarial transmission passage in successive generations of *Aedes aegypti* [16]. Therefore, the seasonal distribution of the vector and how such virus disease case distribution correlates with climate should also be undertaken and hence, public health preparedness should be focused during peak months [17]. To our knowledge, no information is available about the exact vector (s) that involved in such viruses infection in Sudan during viruses outbreaks and in subsequent years.

It is important, however, to know the virus' vector in each geographical region within the Sudan for better understanding of the virus ecology. This work, however, is a contribution not only to our knowledge of the biology, ecology and management of mosquito species, with special focusing on *Ae aegypti*, but as a step toward better investigating and describing more appropriate indices for improved surveillance with special reference to Sudan. Furthermore, as reported, [18], *Ae aegypti* can develop resistance to most of the insecticide classes in the market. Therefore and because of the very limited budget, the objectives of the present study were: to evaluate current adult field-collected populations' susceptibility to the Bendiocarb (the most commonly used insecticide), DDT and Permithrin to monitor potential changes in susceptibility in the foreseeable future and to get brief information about the life cycle of *Aedes aegypti* collected.

Materials and Methods

Study Area

Seven locations were selected for this trial in central Sudan, around Madani, the capital of Gezira state. The locations were on the eastern and western banks of the Blue Nile. On the eastern bank were: Elguneid Sugar cane Facory (Elguneid 1); Elguneid Sugar cane agricultural section (Elguneid 2); Abu Haraz and from the western bank were; Madani-Agricultura Research Corportion; Elmadina Arab; Barakat town and Elrabwa village.

Mosquito Populations

First attempt was performed to evaluate the efficiency of the available adult traps (Light trap; Pyrethroid indoor-spray; window trap and clay pots) in trapping adult mosquito. The preliminary tests showed no encouraging results because of the absence of attractants. Therefore, the tests were conducted using collected larvae. Within each of these seven locations, larval samples were collected from some most heavily larval infested sites during August and September, 2015. A total of 50 dips were combined as one collection per location, brought to the Reference Center, and placed in dishes (30 x l0 cm), containing 1 liter of tap water. The larvae were separated in different dishes based on the genus (*Anopheles*, *Culex* or *Aedes*). The larvae were reared in a climatic chamber at a temperature 25 °C ± 2 °C and 80% ± 10% relative humidity and a photoperiod of 16:8 h light:dark until reaching adulthood. The confirmed adults of *Anopheles* and *Culex* species were freezed for future precise identification. However, *Aedes aegypti* adults, which were found in Barakat nursery only, were placed in cages supported by sugar and blood fed on rabbits for continuous generation production. *Aedes aegypti* is a holometabolous insect, therefore, to have a general idea about its life cycle, time durations from each stage to another were recorded.

Insecticide Susceptibility Tests

Testing for *Aedes aegypti* females adult susceptibility to Bendiocarb (1%); DDT (4%) and Permithrin (0.75%) was carried out with standard WHO impregnated papers according to the WHO method [19]. Six replicate samples of 25 females 5 days old per tube, fed on sugar solution, were used. Four replicates females exposed for 1 hour to impregnated papers of each insecticide plus two untreated controls, were then checked for mortality every 15 minutes up to one hour and finally after 24 hours. A mosquito is classified as dead if it is immobile or unable to stand or fly in a coordinated way.

Results and Discussion

Mosquito Collection (adults)

First attempt performed to evaluate the efficiency of the available adult traps (Light trap; Pyrethroid indoor-spray; window trap and clay pots) in trapping adult mosquito. The collected adults were classified for *Anopheles*, *Culex* and *Aedes* species and to blood non-fed; fed; half gravid and gravid. Very few numbers of mosquito species were collected however, the light trap collected good numbers of sand flies. This preliminary tests showed no encouraging results because of the absence of attractants. Therefore, field larval mosquito populations were collected from seven locations on eastern and western banks of the Blue Nile around Madani, reared in the lab, classified and reported (Table 1). Table 1, showed that *Culex* genera dominated by 71% of the total collected mosquito species. The *Anopheles* genera dominated in only one location (Madani-Agric. Res. Corporation, western bank). Interestingly, out the seven location on the two banks of the Blue Nile, *Ae. Aegypti* was collected from only one location (Barakat nursery).

Table 1: Total number of mosquito larvae collected (two collections) from seven locations, east and west of the Blue Nile, Gezira state, during the rainy season (August and September 2015)

Location	Anopheles Spp.	Culex spp.	Aedes spp.	Total	% From Total	Percent of An: Cx
Elguneid Sugar cane 1	23	60	0	83	3	27.7:72.3
Elguneid Sugar cane 2	109	191	0	300	10	36.3:63.7
Abu Haraz	25	865	0	890	30	2.8:97.2
Medani (Agricultural Research Corporation)	444	76	0	520	18	85.4:14.6
Elmadina Arab	44	734	0	778	27	5.7:94.3
Barakat village	15	11	150 (85% of the site's collection)	176	6	57.7:43.3 (15% of the total)
Elrabwa village	49	120		169	6	29:71
Total	709	2057	150	2916		
%	24%	71%	5%			

Aedes aegypti Life Cycle

In the current study, the larvae of *Ae. Aegypti* were collected from containers in a nursery in Barakat, Gezira state. As known, *Aedes aegypti* is a holometabolous insect, it goes through egg, larva, pupa, and adult stage and the environmental conditions affect the adult life span. Therefore, the *Aedes aegypti* larvae were reared in a climatic chamber until reaching adulthood and took complete blood meals from rabbits. The laid eggs, were placed in the water for hatching. The newly hatched larvae spent a short time in the first three larval stages, up to three to four days in the fourth instar, entered the pupal stage and took approximately two days to developed to adults. These results were found coincided with the reported statement [20]. The importance of such result is that such *Ae. Aegypti* population could be spread all over the region from this town in very short time. This could be exaggerated with the lack of commitment and financial backing necessary to maintain an eradication program. Additionally, regarding the oviposition, female mosquitoes select the oviposition sites by the influence of various factors such as visual, tactile, and olfactory stimuli. For the immatures' growth, development, and survival the gravid females choose the potential larval habitat by a chemical evaluation of the medium before depositing their eggs. As a consequence, the reproduction successes [21-23]. Based on the results obtained in this study, the oviposition of *Ae Aegypti* in such plastic and iron containers, normal clean water and under such nursery conditions in Barakat opens the possibility of evaluating species-specific local oviposition attractants that could be evaluated for *Ae Aegypti* in a foreseeable future. Additionally, the GIS-based survey that provided basis for planning a strategy for reducing malaria risk by elimination of the malaria vector breeding sites in northern Sudan [24] could be studied for viruses' vectors. In general, it is important to plan for precise investigation in biology and ecology of such important vector (s). Therefore, as future work regarding the biology and ecology of such vectors in Sudan, *Anopheles, Aedes* and *Culex* species, in general, were incriminated as vectors for dengue, RVF and Yellow fever [8]. However, literature survey revealed no information about the exact vectors and its role in such enzootic cycle. Therefore, as it was reported [25, 26] that in Houston, Texas, the West Nile virus (WNV) investigations stated the year-round existence of *Culex restuans*, the main WNV vector in California and *Culex nigripalpus*, the known WNV vector in Florida however, in Houston, Texas instead *Culex quenquefasiatus* is the main vector and none of the aforementioned species is a WNV vector in Houston. This could indicate that precise studies has to be performed for each region in a country for identifying the exact vector and hence, better understanding of such virus ecology. Additionally, vector competence of selected African mosquito species were studied for Rift Valley fever virus [27]. Such study should be performed in Sudan to confirm the exact vector (s) of each virus in a certain region. The current study proposed to make the first step in achieving such goal.

Susceptibility Tests

Overall, the results of the susceptibility tests showed that all the *Aedes aegypti* adult females were susceptible (1OO% mortality) to I hour of exposure to the diagnostic dosages of Permithrin (100% death after 40 minutes); Females were relatively less susceptible to DDT (74% and93% death in one hour and 24 hours, respectively). However, the examined *Aedes aegypti* were scored 100% alive after 24 hours to Bendiocarb. In fact, such result is a strong suspicion of resistance and must be investigated further. Apparently, in Sudan, Bendiocarb 80% ((2,2-Dimethyl-1,3-benzodioxol-4-yl) *N*-methylcarbamate) has been used as a residual effect insecticide against the vector of malaria, *Anopheles arabiensis*, since 2007, with 62.5 g a.i./10 liter for 250 meter square and 3-4 month duration. The susceptibility of the malaria main vector, *Anopheles arabiensis*, was reported [28]. However, according to our knowledge, the present study is the first report of could-be resistant *Aedes aegypti* in Barakat town, Gezira state, and could be in the Sudan. Therefore, such results suggest that first: such vector could be a timed-bomb any time such viruses exist in the area. Secondly, continuous application of Bendiocarb could lead to operational failure. Consequently, such results suggest that Permithrin is effective for management programs during mosquito-borne viruses' outbreaks. Additionally, the results showed that such population were relatively less susceptible to DDT comparable to Permithrin. Nevertheless, DDT was banned in Sudan long time ago however, the results indicate that further investigation of the mechanisms and distribution of resistance should be undertaken. Additionally, when comparing the present data on adult susceptibility for the three insecticides, it appears that the excessive and continuous use of Bendiocarb in the area to control malaria vector, *An arabiensis*, might have induced a slow yet progressive decrease in susceptibility in *Ae aegypti*. Hence, alternative strategies should be developed and implemented to reduce the probability of selection pressure. Furthermore, resistance detection and mechanisms in addition to rotation programs for resistance management should be implemented immediately. Moreover, for a long run, to halt or minimize viruses' infections, recommended vectors' management strategies including: well-managed insecticides rotation; breeding sites reduction and Community awareness should be implemented in cooperation with the public health campaigns.

Finally, the successful control of such mosquito species depends on a knowledge of the biology, ecology and evaluating and improving the existent and developing new surveillance methods. It is, therefore, could be recommended that drying up of the breeding sites and insecticide treatment could be a temporary important measures. In addition, detailed proposals for studying the ecology, biology and management of *Ae aegypti* to monitor periodically the susceptibility to insecticides would be important. According to our knowledge this is the first report about *Ae aegypti* in Gezira state. However, the implications of these findings as a step toward combating such vectors over the medium-and long-term and the reduction of breeding sites would be effective.

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