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Full length Research paper

The role of *Gambusia affinis* (exotic fish) as a Biocontrol measure against Malaria Vector (*Anopheles arabiensis*), Gezira state, Sudan

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Malaria is still one of the most widely stressing diseases today. A lot of efforts were made, especially biological control using fish to reduce the malaria vectors density. This research was carried out at South Gezira locality, Gezira state, Sudan, during the period (2009-2012). This research aimed to find out the role of the *Gambusia affinis* (exotic fish) as a biological control measure against malaria vectors (Anopheles arabiensis). Out of the seven agricultural sectors, El Hosh agricultural sector was selected as the study area due to it accessibility. El Gubshan sector was selected randomly as the control area. The method adopted include monthly entomological surveys to assess the density of adult and larvae of *Anopheles arabiensis* in both the study and control villages for three years. The data were analyzed by SPSS System (Statistical Package of Social Sciences). The results obtained showed that, the use of *Gambusia affinis* has been shown to be effective in controlling mosquito larvae ($p<0.001$). The study revealed that, the reduction in Anopheles larval densities a month after fish seeding reached 93.6%. The seasonal fluctuation of water in the irrigation canal causes changes in the habitats which is determinant to population of *Gambusia affinis*.

Keywords:*Gambusia affinis*, Biological control, Malaria Vector, *Anopheles arabiensis*, Mosquito larval.

INTRODUCTION

Resistance among arthropods to chlorinated hydrocarbon, organo phosphorous and carbamate insecticides appeared to have started since 1950s; it resulted in an unexpected turn towards suitable alternative and especially to biological control (Anonymous, 1960).Interest in biological control of aquatic Diptera actually began in the late 1800s (Lambron, 1890).At that time dragon flies as natural enemies for the control of mosquitoes were clearly recognized. Meish (1985) reviewed that, predatory fish that eat mosquito larvae, have been used for mosquito control for at least 100 years. The mosquito fish, *Gambusia affinis* is the best known biological mosquito control agent as suggested by Lloyd, (1987). The biological attributes of the *Gambusia affinis* are a high reproductive capacity, high survivor ship small size, relatively high tolerance to variance in temperature, salinity and organic waste (Bay et al., 1976). *Gambusia affinis* was stressed as a biological control because it is easily manipulated than dragon flies and it was quickly utilized and transported throughout the world during the early attempts to control mosquitoes (Legner and Sjogren, 1984). It was economical and environmentally a sound method (Kent, 2006, net1). It was purposely introduced from its native Texas (South USA) to Hawaiian Island in 1903 and in 1921 it was introduced in Spain, then from there into Italy during 1920s, and Later to 60 other countries (WHO, 2003). It came from Egypt to Sudan in 1928. The fish was first bred in concrete tanks at Wad Medani civil hospital for fewer years. In 1937, the fish was stocked in many minor canals in Gezira irrigation system. During 1973-1974, 1000 fish were released in each of 121 minor canals (Haridi, 1979). In 1981, 43 minor canals were checked. The assessment revealed that, fish flourished in some canals and disappeared in others (El Safi, 1983). In 2002, *Gambusia* fish was initiated in six States, covering limited areas (NMCP, FMOH, 2002). Till now it was still persists in

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many canals and drains in Gezira irrigation scheme. Many trials and studies were conducted worldwide and generally explained that, using of fish as biological control tools leading to remarkable reduction in larvae of malaria vector and hence reduction on malaria transmission was occurred (Bay et al., 1976; El Safi, 1983; Kramer et al., 1987; Louis and Albert, 1988; Fletcher et al., 1992; Rajinkant, 1993 and Shamo, 2001).

The Problem

Malaria is a public health problem, at present, 109 countries in the world are considered malarious region. Therefore, an estimated 350-500 million malaria cases among 3.3 billion people at risk, causing nearly a million deaths, mostly of children <5 years about 90% of these occurring in sub- Sahara Africa.(WHO,2000, 2004, 2005, 2008). Several studies documented an increase in malaria incidence as a consequence of irrigation and agricultural development in many countries including Sudan (Ijumba, et al., 2002 and Gratz, 1979) .In Sudan, the disease constitutes around 40% of all infectious diseases burden and it is cause of hospital consultation in about 50% to 70% of patients. Malaria cases are estimated as 7.5-10 million cases and 35000 deaths every year. These figures bring Sudan on the top of WHO/ EMERO countries (Malik and Khalafalla, 2004).
The main Objectives of this research was to determine the effects of *Gambusia* fish on the adults and larvae densities of malaria vector.

MATERIALS AND METHODS

The study area

Gezira State is the one of the most productive States in Sudan. It is situated at the central of the Sudan.

The methods

Study design

The study is primarily designed to find out from field trial study the efficiency of *Gambusia affinis* as a biological measure against malaria vector (*Anopheles arabiensis*) in El Hosh area,Gezira State, Sudan.

Sampling design

Cluster random sample was selected in order to obtain information to determine the effects of biological control using fish in malaria vector control. Out of the seven agricultural sectors, El Hosh agricultural sector is selected as the study area due to its accessibility. It comprises of about 26 villages, three of them are selected as the study villages by random sample, named Rewaina, Fauida Ewaida and Fauaida Eziazat (Figure 3.1). El Gubshan sector was selected as the control area it comprises of about 18 villages; three of them are selected by random sample as the control villages, named Wad Elmahi, Zananda Fadul El Seid and Zanada Jubara (Figure 3.2).

Field assessment

Following the WHO procedures during the period of December 2004 through December 2006 as follows:
Monthly adults and larvae surveys were initiated in both the study and control villages and continued for 24 months to assess the densities of adults and larvae of *Anopheles arabiensis* in both the study and control villages.

Adult collection by space spray-knock down

10 rooms were selected randomly in each of the studied villages taking into account their building types, presence of a number of people and their sites in relation to the breeding sites. The indoor resting mosquitoes were collected by knockdown space spray on sheets, a team of three collectors usually under takes this collection, white sheets measuring 2 m were spread so as to cover the entire floor area and other horizontal surfaces such as the top of tables, beds and other places where mosquito may hide. All opening in the rooms including doors, windows, eaves were also closed. The collection was under taken in the early morning. A hand sprayer with dilute solution of pyrethrum was used for collection in door resting mosquitoes. Ten minutes after spraying, and mosquitoes that are knocked down were collected on the floor sheets. The mosquitoes were picked off from the sheets and put in Petri dishes for sorting and recording. The total number of mosquitoes collected from all houses was divided by the number of rooms sprayed to give the average density per room Appendix (1). Plate (3.1) shows the indoor resting mosquitoes by the sheet space-spraying (knock down collection).

Larval collection

This method was used to collect larvae and pupae from breeding sites, by lowering the dipper greatly into water at an angle 45 degree until one side is just below the surface while dipping. Care was taken not to disturb the larvae and cause them to sink downward.The dipper was then held steadily until the larvae and pupae were rise to the surface of the water, and then the larvae and pupae are counted to determine their density per dip, Ten sites were sampled in both the study and control villages, each site was scooped three times (WHO, 1992). Plate (3.2) shows the mosquito larval collection by the use of dipper. 300 dips from different fixed sites were made every month for 24 month. All aquatic stages of

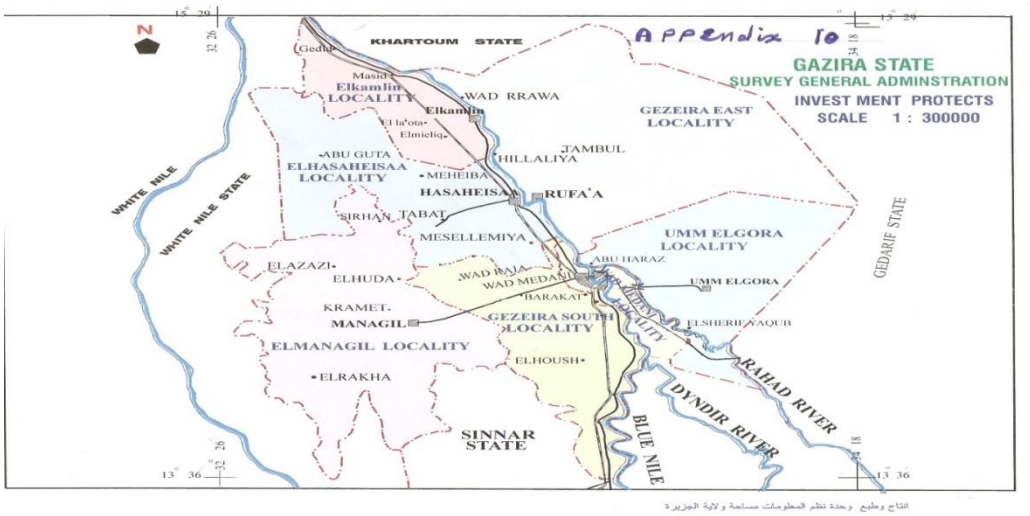

Fig **(3.1): Gezira State Map**

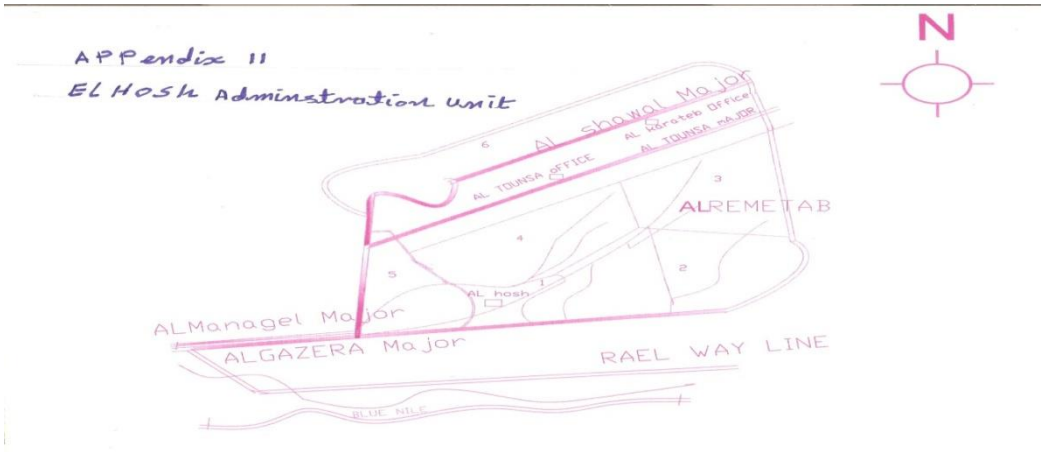

Fig (3.2): El Hosh administration Unit

mosquitoes were sorted (WHO, 1992). The average larvae per dip were calculated by using a special formula (Appendix 2).

Fish production, collection, transportation and stocking

Mass production of *Gambusia affinis* was achieved by rearing these fish in specially constructed hatcheries. The fish breeds throughout the year and therefore, a large stock was produced within a short time. About four months the number was exceed more than 100000 fish .The fish was captured using a standard bait trap. After capture, the fish were transported in plastic buckets about 20 liter, each one contain about 100 gravid *Gambusia affinis*. The ice was used to cool the water during extended holding periods in hot weather. The time needed to receive the study area is about 1/2 hour. 600 fish were transported daily in open container by vehicle until were reach the study zone. Introducing fish into a pond was done by placing the container with fish directly into the pond for 5-10 minutes or until the pond water and container water are near the same temperature. Fish application was carried out at the rate of 20 fish per 100 m2 surface area of the water bodies. The fish was seeded in the early morning. Thus, a stock of over 100000 *Gambusia affinis* was raised during 2005-2008 and introduced into different breeding habitats, covered area at about 16 hectare. Plate (3.3) shows the introducing of *Gambusia affinis* into a new environment.

Monitoring and redistribution of fish

area at about 16 hectare. Plate (3.3) shows the introducing of *Gambusia affinis* into a new environment.

Table (4.1): Explains the average densities of adult and larvae of *Anopheles arabiensis* on both the study an control villages before the intervention

	Study	Control
Larval density	4.7/dip	7.6
Adult density	2.6/room	4.4

Table (4.2): Average larvae densities of *Anopheles arabiensis* in the study and control villages, year (2009-2012)

Month	Yea2009 Average density per dip		Year 2012 Average density per dip	
	Study	Control	Study	Control
January			0.10	2.2
February	0.3	4.0	0.29	2.57
March	2.1	1.7	0.26	2.97
April	0.2	0.2	0.08	4.12
May	0.0	0.0	0.0	0.7
June	0.0	0.09	0.0	0.77
July	0.2	0.03	0.6	1.11
August	0.4	0.53	0.9	4.02
September	0.6	1.04	0.13	1.31
October	0.96	2.2	0.47	2.03
November	0.37	2.6	0.6	2.17
December	0.60	2.2	0.86	1.8

No. of villages 3 (study), 3 (control)
No. of site inspected 30 (study), 30 (contro
Type of breeding sites canals, drains, Abu eshriens and pools

Paired sample test

	Paid differences							
				95% confidence				
	Mean	St. dev.	St. err.	Lower	Upper	T	d.f.	Sig. (2 tailed)
Pair 1	-5.22	1.55	.429	-1.46	.412	-1.22	12	0.247

Paired sample test

	Paid differences							
				95% confidence				
	Mean	St. dev.	St. err.	Lower	Upper	T	d.f.	Sig.(2 tailed)
Pair 2	-1.77	1.04	.289	-2.42	-1.16	-6.19	12	0.00

health workers were selected to redistribute and to convert fish to its potential breeding sites where it's seen. This was done by stocking large number of fishes near the target breeding sites to enable good coverage. Monthly fish application was carried out in newly created or natural habitats and those found with mosquito breeding. Also regular and weekly visiting was obtained. The average daily fish that were converted to the created breeding sites is about 5000 fish and reach more than 500000 specimens during the study period.

Gambusia affinis assessment

Gambusia affinis were sampled using standard dip net. Another method was used by irritation the fish and then divided the total number seen by the area covered expressed as meter squire (WHO, 1975). 5 sites were inspected in each water bodies and the density was expressed as fish per meter squire (Appendix 3). Plate (3.4) shows the method of *Gambusia affinis* assessment by the use of mosquito net.

Table (4.3): Average adult densities of *Anopheles arabiensis* in the study and control villages, year (2009-2012)

Month	Year 2009		Year 2012	
	Average density per room		Average density per room	
	Study	Control	Study	Control
January			0.83	3.3
February	1.7	4.3	0.67	4.8
March	0.8	2.1	0.40	3.37
April	0.13	0.87	0.10	3.6
May	0.0	0.0	0.0	1.03
June	0.0	0.03	0.0	0.77
July	0.07	0.07	0.0	1.17
August	0.27	1.9	1.03	3.83
September	1.8	6.6	0.77	4.3
October	2.1	14.7	1.63	5.27
November	2.5	18.3	1.77	3.33
December	4.3	10.9	1.5	2.37

No. of villages 3 (study), 3 (control)
No. of room searched 30 (study), 30 (control)

Paired Samples Test

	Paired differences					t. test	d.f.	Sig.(2-tailed)
	Mean	St. dev.	St. err.	95% confidence				
Pair 1	-3.805	5.13	1.42	-6.907	-.703	-2.673	12	0.020

Paired Samples Test

	Paired differences					t. test	d.f.	Sig.(2-tailed)
	Mean	St. dev.	St. err.	95% confidence				
Pair 2	-2.37	1.18	0.326	-3.08	-1.659	-7.268	12	0.00 *

RESULTS

Effect of *Gambusia affinis* in the reduction of larvae densities of *Anopheles arabiensis*

Entomological assessment in the study and control villages were both began in December 2004. Results from (Table 4.1) showed that, the first average larval densities of Anopheles arabiensis recorded before the intervention occurred showed 4.7 and 7.6 per dip for the study and control villages, respectively. On the other hand, the average density of the adult *Anopheles arabiensis* was 2.6 and 4.4per room for the study and control villages respectively.

The breeding sites for *Anopheles arabiensis* were abundant in the study and control villages. Breeding always occurred in pools, ditches, drains, minor canals and small water bodies near the irrigated farms.

The larval density in the first count had been found to be 4.7 and 7.6 per dip on both the study and control villages, respectively. In the second count, a month after the intervention of the year 2005, where fish was seeded, a dramatic drop on mosquito larvae was observed in the study villages as compared to the control,(93.6%). Thereafter, observable increase on larval density was observed in March in the study villages as compared to the control. This followed by highly reduction on larval density from April to June on both the study and control villages. Although a gradual increase on larval density was recorded in the study group, but the average was higher in the control villages (Table 4.2). Statistical analysis showed that, there is no difference significant between the two average densities.

In January of the next year 2009, the average larval densities were 0.10 and 2.2 per dip in both the study and control villages respectively, (Table 4.2). A gradual increase on larval density was observed in the study

villages, while more was founded in the control. However, no larvae were detected during May and June in the study. Where as, lower density was reported in the control. Thereafter, higher larval density was observed during August to October in the control villages as compared to the study. The statistical analysis suggested that, there is a highly significant differences (P<0.001) between the two average larval densities (t = 6.19).

Effects of *Gambusia affinis* in the reduction of adult density of *Anopheles arabiensis*

The monthly surveys of adult densities of *An. arabiensis* on both the study and control villages showed that, a gradual decrease in mosquito density per room was recorded in the study villages during February, March and April. On the other hand, low density was obtained in the control. Adult mosquitoes were observed during May, June and July on both the study and control villages. This was followed by a gradual increase in the mosquito density but the average was higher in the control compared to the study villages (Table 4.3). Statistical analysis showed that, there is a significant differences (P<0.05) between the two average densities (t =2.673).

The study was continued for another year. It was revealed that, a sharp decrease in ***Anopheles*** adult average density in the study villages was obtained as compared to the control (Table 4.3).

Statistical analysis showed a highly significant difference between the average densities (P< 0.00), t = 7.268).

DISCUSSION

The study showed that, a sharp decrease in larval density had occurred in the study villages as compared to the control. The reduction on ***Anopheles*** larvae density after a month of fish seeding was 93.6% in the study villages and 47.3% in the control. Similar to results by Sabatinelli (1991) in the Grand Comoros who noted that, the percentage of breeding places positive for *Anopheline gambiae* decreased from 41% to 6% after one year. Thereafter, a gradual increase on larval density was observed during July to December in the study villages but the average was higher in the control. This was attributed to the effects of turbidity, and high water current which hamper the fish to flourish and then reduce their numbers. This result is compatible with Young (2001) who noted that, high turbidity and sediments reduces light, which can limit plant growth and can interfere with visual hunting by fish and other aquatic organisms.

Monthly surveys were conducted after the introduction of *Gambusia affinis*; this indicated that, a gradual reduction on adult density was recorded in the study villages as compared to the control. The reduction after two month of fish seeding was 69.2% as compared to 52.2% in the control. This significant decrease on adult populations was reflected by the statistical analysis that showed significant difference, t = 2.673 (P<0.05) between the average densities in both villages. Due to the end of the irrigation season. Gradual build up in adult mosquito density was observed in September, October, November and December in the study villages, however; the average was higher in the control. Mahmoud (1985) suggested that, seasonal fluctuation of water in the Gezira irrigation canals causes changes in the habitat which is detrimental to populations of the fish. A potential impact of *Gambusia affinis* on mosquito population was observed during the study period. However, the total number of the mosquitoes collected in December during the first year of the study was 470 and 1924, per room in the study and control villages, respectively. Indicating the role and mass effects of *Gambusia affinis* in reduction of larval density and hence decrease the average density of adult ***Anopheles*** *arabiensis*. In contrast, Service (1983) and Mahmoud (1985) both have indicated that, *Gambusia affinis* has not been effective in the control of malaria. Our findings indicated that, the reduction in adult ***Anopheles*** in the two years of the study was 72% as compared to the 29% in the control. Such studies on mosquito breeding and its control through *Gambusia affinis* was conducted in India during June to October 1991 and provided 87.8% mosquito larval control (Prasad et al., 1993). The study was concluded that, in Sudan, Gezira Irrigation Scheme, the use of *Gambusia affinis* has been shown to be effective in controlling mosquito larvae.The study revealed that, the reduction in ***Anopheles*** larval densities a month after fish seeding reached 93.6%.It was recommended that, support and strengthen the use of larvivourous fish in malaria control programme.

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Plate (3.1): The indoor resting mosquitos' collection by the sheet space- spraying.

Plate (3.2): Mosquito larval collection by the use of dipper.

Plate (3.3): Introducing of *Gambusia affinis* into newly environment.

Plate (3.4): *Gambusia affinis* assessment by the use of mosquito net