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TWO INDIGENOUS AQUATIC WEEDS *LEMNA MINOR* AND *SPIRODELLA* SPP., GAVE PROMISING BIOLOGICAL CONTROL OF MOSQUITO LARVAE WITH RAINBOW FISH ON FIELD LEVEL IN KARACHI, SINDH, PAKISTAN

RAJPUT MUHAMMED TARIQ[1], S. NAIMUL HASAN NAQVI[2] AND S.M. NAUSHAD ZAFAR[2]

[1]M.A.H. Qadri Biological Research Centre, University of Karachi, Karachi-75270, Pakistan,
[2]Department of Zoology, University of Karachi, Karachi-75270, Pakistan.

Abstract

Two indigenous aquatic weeds *Lemna minor* (Fam: Lamnaceae) commonly called as common duckweed and *Spirodella* spp., commonly called as large duckweed were used for the biological control of mosquitoes with local guppy fish also called Rainbow fish *Poecellia reticulata* in stagnant and polluted dirty water containing commonly *Culex* spp., and in few cases *Anopheles* spp., and in very few cases the *Aedes* spp., where the water became clear after some time or in overhead flown water and/or in pipeline leakage water. Such type of cases has been recorded in Karachi University Campus at BRC building. The plant was used @ 1kg/100m^2 and fishes @ one fish/m^2 were released in experimental areas. The fishes started their work of predating the mosquito larvae just after few minutes of their release in water. The plants *Lemna*, *Spirodella* and fishes supported to each other in symbiotic condition. The plants *Lemna* and *Spirodella* covered the surface of water reducing the oviposition area for egg laying mosquitoes; at the same time reducing area for respiration for already existing mosquito larvae in that area and reducing the water covered area by converting dirty water into water vapours through the process of transpiration and used as alternate food for fishes, especially for mosquito controlling fishes. The best and satisfactory results were obtained in 5 weeks depending on area of water, successful plant growth, quantity of plant introduced and number of fishes released in that area. This method not only avoids the use of synthetic chemicals, side effects of the chemicals where used, increase of pollution in water, resistance in the mosquitoes, inducement of chemicals in food chain, wastage of money and time but also provides the less smell, beautiful and pleasant environment for our health by providing plenty of fresh oxygen by the plants in day time. By this way a complete sound and safe control of mosquitoes may be achieved.

Introduction

Mosquitoes are the worst enemies of mankind. They not only disturb our sound sleep but also they suck our blood and are cause of different diseases. People use different methods to repell or to kill them such as mats, coils, repellents, spraying chemicals, fumigation and netted windows, doors and mosquito nets but even these they are not being controlled and instead of reducing they are increasing day by day, because all these methods are temporary and less effective. The reason is this that we are trying to kill only the adult mosquitoes which are coming in our houses or sleeping places but we are not destroying their breeding points which are the permanent mosquito reservoir from where they are coming in millions on daily basis. On the other hand, all these methods (except spray and fumigation) do not kill the mosquitoes, instead of killing they repell the mosquitoes, whereas when spray is done mostly the mosquitoes get shifted from that area to another area. Only in fumigation, which is done in closed and air tight places such as

rooms, offices, ships, aeroplanes, buses etc., you can kill mosquitoes, but the question arise how much mosquitoes 600 or 1000 and how many times you will fumigate daily. If you will spray daily, fumigate daily even then you can not control the mosquitoes, because they are coming from permanent breeding points daily not in hundreds, thousands but in millions. So how you can control them? On the other hand have you thought about the expenses? Many hundred rupees are required for this purpose and even then there is no solution, no control of mosquitoes. Further more each of us can not spend a huge amount. For instance a pair of coils is used daily in our house, one pair of coil cost mostly Rs. 7 therefore 7x30 = Rs.210 p.m. and 210x12 = Rs.2520/year. By spending this amount yearly which is a least amount, you are not killing a single mosquito and similar is the case for other repellents. By spray we may kill mosquitoes partly but it is very expensive and have many hazardous effects. Such as coils, mats, repellent, skin lotions cause breathing and allergy problems in children. So now a days all these methods have failed to control the mosquitoes mainly due to the development of resistance in them against commonly used pesticides/insecticides/chemicals. (Anon., 1970; Naqvi, 1987; Azmi *et al*., 1991; Naqvi, 1992; Tariq & Zafar, 2000; Tariq *et al*., 2005). Only among mosquitoes 96 species of mosquitoes have been found resistant against Organo-chlorine, Organo-phosphate, Carbamate, Synthetic Pyrethroid and Botanical Pesticide, i.e. 36 v/s OC, 32 v/s OP, 19 v/s CB, 08 v/s SP and only 01 v/s BP (Georghiou & Mellon, 1983).

Now the scientists have diverted their thinking towards the biological control and biopesticidal control, because least resistance has been reported against biopesticides. Fishes act as predators of mosquito larvae, dragon flies also act as predators of adult mosquitoes, dragon flies nymphs for mosquito larvae control (Qadri *et al*., 2007). *Bacillus thuringiensis* (B.T) is famous as biological control agent in the world. Some work has been done in Pakistan on rating system and selection of appropriate indigenous fish species for mosquito control by Ahmed *et al*., (1986; 1988). In this paper we have used local weeds *Lemna minor* and *Spirodella* spp., for the biological control of mosquitoes with fish commonly called as guppy or Rainbow fish in Karachi, Sindh, Pakistan as *Gambusia* spp., has been reported in sewage ponds by Emerick (1942).

Materials and Methods

Culturing and collection of weeds and breeding and collection of fish

a. *Lemna minor* (Fam: Lemnaceae) collected from North Karachi was identified by the authorities of Department of Botany, University of Karachi, and it was confirmed from net as well. This plant was cultured for field trials in the Campus University of Karachi near Jama Masjid Ibraheem, in the road side channel. The *Spirodella* spp., was collected from Nausheroferoz District Nawab Shah and cultured in Biological Research Centre, University of Karachi and then introduced on targeted experimental place.

b. The guppy fish was purchased for the 1st time in hundreds and were released in the breeding pond especially for this purpose, and from there, they were taken to the experimental area. Some times directly purchased fishes were also released in the experimental areas in emergency or due to the shortage of time or the quantity of the required fishes.

Application of plant and fish: The mosquito breeding points were identified, in different areas in Karachi. These experimental places were measured and the area was calculated. The number of average larvae were calculated by taking 100ml water from five different places at experimental area. The plant was spread @ 1kg/100m^2 whereas the guppy fishes were released @ one fish/m^2.

Observation before and after application: The water bodies were investigated for the presence and absence of the mosquito larvae. The average value of the mosquito larvae was calculated by taking 5 observations from 5 different points of the mosquito breeding place area. This average mosquito larvae value was called as pretreatment value. Then the plant was spread and the fishes were released according to the calculated estimate. The first observation was noted after one week, the other on second week. In this way continuously 5 observations were noted and the increase and growth of the plant was noted by its population. The presence of the fishes either they are alive or not was also taken into consideration. The post-treatment weekly observation was noted in the same way as stated above. The data was recorded and analyzed accordingly as shown in Tables 1 & 2. The duckweed was thrown in the water in such a way that it can grow and spread easily in all existing water area.

Table 1. Showing the place, area, DW-quantity and number of fishes released.

Experimental place	Area calculated in m^2	Duckweed kg/m^2	Fish/m^2
Near Ibrahim Masjid, u.o.k.	1.5x100 = 150	1.5 kg	15 fishes
Near Shaikh Zayed Islamic Centre	10x15 = 150	1.5 kg	15 fishes
Civic Centre	0.5x500 = 250	2.5 kg	25 fishes
Yaseenabad	20x15 = 300	3.0 kg	30 fishes
Hassan Square	0.5x700 = 350	3.5 kg	35 fishes
Korangi Town	18x33 = 594	6.0 kg	60 fishes
New Karachi, Power House	10x100 = 1000	10.0 kg	100 fishes
Orangi Town	2x600 = 1200	12.0 kg	120 fishes
Enrolment Building, u.o.k.	30x50 = 1500	15.0 kg	150 fishes
Landhi Town	35x60 = 2100	12.0 kg	120 fishes

Table 2. Pre-treatment and reduction, average value of mosquito larvae in five weeks.

Experimental place	Pre-treatment value	Post treatment value					
		1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	Control %
Near Ibrahim Masjid, u.o.k.	415	377	290	181	88	15	96.38
Near Shaikh Zayed Islamic Centre	491	389	271	136	67	11	97.75
Civic Centre	600	538	302	190	58	02	99.66
Yaseenabad	518	412	278	121	40	05	99.03
Hassan Square	622	561	408	216	25	00	100.00
Korangi Town	396	301	213	11	37	01	99.75
New Karachi, Power House	473	400	312	203	78	12	97.46
Orangi Town	380	299	215	103	26	00	100.00
Enrolment Building, u.o.k.	457	323	219	101	19	01	99.78
Landhi Town	719	591	378	13	07	00	100.00

Results and Discussion

The observation noted and the results obtained give a clear picture that the Duckweed and guppy fish collectively are very effective in the biological control of the mosquito larvae as shown in (Fig. 1A-F). This method is sound, safe, easier and cheapest one. It also provides an anti-bad smell, beautiful and pleasant healthy environment. There are no chances of side effects of the chemicals because no chemical is used in this method and so no chance of resistance, no water pollution and no air pollution. This method have also proved fast and the most effective in controlling the *Culex* spp., of the mosquito, mostly and abundantly found in dirty, stagnant and polluted water. Beside this sometimes the *Anopheles* spp., of the mosquito, responsible for malaria, were also found breeding in the stagnant water where there was overflow of water from the overhead tanks and where there was leakage of pipeline. Therefore the malaria vector mosquitoes were also controlled by this method. Similarly in some overhead tank flown water, leakage pipeline water or in the gutters, ditches, the pure clean water was found. In one case the *Aedes* spp., of the mosquito, which is known as Dengue vector, were also found breeding in University Campus at Biological Research Centre (BRC) building. There also, the *Lemna* and *Poecelia* controlled the breeding of the *Aedes* mosquitoes as reported by Eigenmann (1923). Bannerman (1910) reported about *Aplocheilus lineatus,* which was kept in a fernery (water storage place) at Parel cleaned the water of mosquito larvae. In the present investigation the same result has been noted by the use of *Poecilia reticulata* fish.

De Vertevil (1922) reported that *Rivulus harti* fish was found to be a voracious feeder of Anopheline larvae, as were also *Poecelia reticulata* and *Polycentrus schomburgki*. The effectiveness of *P. reticulata* against Anopheline is also confirmed by the present work. Balfour (1928) reported about *Fundulus bermudae* fish which kept down *Culex pipiens fatigans* and *Aedes* spp., a natural balance having been established. Fish and predatory arthropod larvae may have been a factor in preventing establishment of *Anopheles*. In the present research work more or less same type of results have been achieved. The *Poecilia reticulata* fish gave maximum control of *Culex* spp., *Anopheles* spp., and *Aedes* spp., here in Karachi, Sindh, Pakistan.

Gribben (1933) reported that large numbers of small fish *Rivulus harti* (Cyprinodontidae) preyed on mosquito larvae (*Anopheles* and *Aedes* species) in Wrinles (small water bodies separated from lake after lowering water level) on Pitch Lake, Trinidad. The present investigation also involved the small fish *P. reticulata* and confirms the earlier report.

Afridi & Majid (1938) reported a local fish *Aphanius* (*Lebias*) *dispar*, recommended for *Anopheles stephensi* control. This fish occurs in Ethiopia, Israel and the Red Sea area and was established in a spring and a brackish drain at Karachi, Pakistan. No mosquito larvae were found in the drain. In the present work near Jama Masjid Ibrahim and near C-category of the residence houses, due to the pipeline leakage the *Anopheles* spp., was breeding as a large population. It was controlled in two weeks only by means of guppy fishes with a common duckweed. The present work confirms the previous work.

De Carvalho (1940) reported that fishes are commonly used in Brazil for the control of *Ae. aegypti* but are not considered reliable against Anophelines; principally because Anopheline larvae and fish often occur together which may be due to no contact in between mosquito larvae and fishes, though waters are frequently found to contain abundant fish and no larvae. In the present work with the fish (*Poecelia*) the plant *Lemna* and *Spirodella* spp. has been also used which decreases not only the surface area for

existing Anopheline, Adenine or Culex larvae, but also the larvae of any mosquito species get together in a limited area due to the increment.of *Lemna* or *Spirodella* spp., population on the water surface, therefore the fishes easily get more chances to find and consume the mosquito larvae. It has also been recorded and observed against Anopheline larvae in Karachi University Campus residential area. Were the satisfactory results were achieved in two weeks time. Furthermore the fishes should be small, strong, prolific and be surface feeders with larvae as their preferred food. This has all been also considered in the present investigation.

Bay (1965) reported an instant fish as a new tool for mosquito control. He described the fishes from the high lands of Argentina and Brazil and other from Africa showed promise for mosquito control. Among these *Aphyosemion* and *Nothobromchius* from Africa, *Cynolebias* from Argentina and Brazil and *Simpsonichthys* from Brazil in the South-Western U.S. was under study at the time of reporting. In the present investigation *Poecilia reticulata* was used only in this work.

Buranarerk & Camarillo (1968) reported the comparative efficiency of four fishes predatory on mosquito larvae in Araneta University Foundation Compound, in laboratory and field conditions. *Tilapia* gave the highest percentage of wrigglers consumed with 97.80% followed by *Mollienesia* (*Poecilia*) with 97.33%, Sword tail fish with 97.10% and Gold fish gave the lowest number of wrigglers consumed with 69.56%. In the fields or ponds there had been apparent reduction of wriggler population in ponds and stagnant unavoidable water where the fishes were released as compared to control pond. In the present work, *Poecelia* gave 95-99% reduction of mosquito larvae in five weeks in ponds unavoidable waters with *Lemna*. Thus, in ornamental ponds around residential houses and in a community where stagnant water is unavoidable, the introduction of *Poecelia, Tilapia, Cyprinus, Barbus, Gambusia* or any other larvivorous fish available locally in country or where needed may be practiced effectively. For best and long term results the *Lemna* spp., *Spirodella* spp., of the plant may also be used, as in the present investigation, in which *Lemna, Spirodella* spp., and fish supports each other.

Dubitskij & Abil'Daev (1975) reported laboratory and field trials of a larviphage in control of malaria mosquito larvae by means of *Oryzias latipes* foreign to USSR, characterized by small size, flattened head, dorsally-directed mouth and great mobility penetrates water with dense weeds in penetrable to other kinds of fish. This fish eradicated *Anopheles maculipennis* from an area of over 800m^2 in 8 to 10 days, whereas in the present paper, the eradication of the *Anopheles* spp., in University Campus was achieved in two weeks, which is also in line with the above report but it may be possible that they may have used more number of fish as compared to the present work, but the satisfactory results have been found in one to five weeks depending upon area, fish numbers and depth of water in experimental place.

Angerilli (1980) reported the laboratory experiments to identify possible effects of different shapes of vegetation on the rates at which an aquatic predator could find and consume mosquito larvae. The fathead minnow *Pimephales promelas* (the predator) required lesser time as compared to control to find and consume the light coloured prey (mosquito larvae) against the dark coloured plants. In addition predator-prey encounters were increased because the plants caused the fish search systematically in plant filled tanks, whereas, they swam at random in the plant-less tanks. In the present work, the *Lemna* covered the surface of water due to which the surface area for mosquito larvae decreased and the mosquito larvae gathered where there were no plants, in this way the fishes were easily able to catch the larvae and predate or consume them easily.

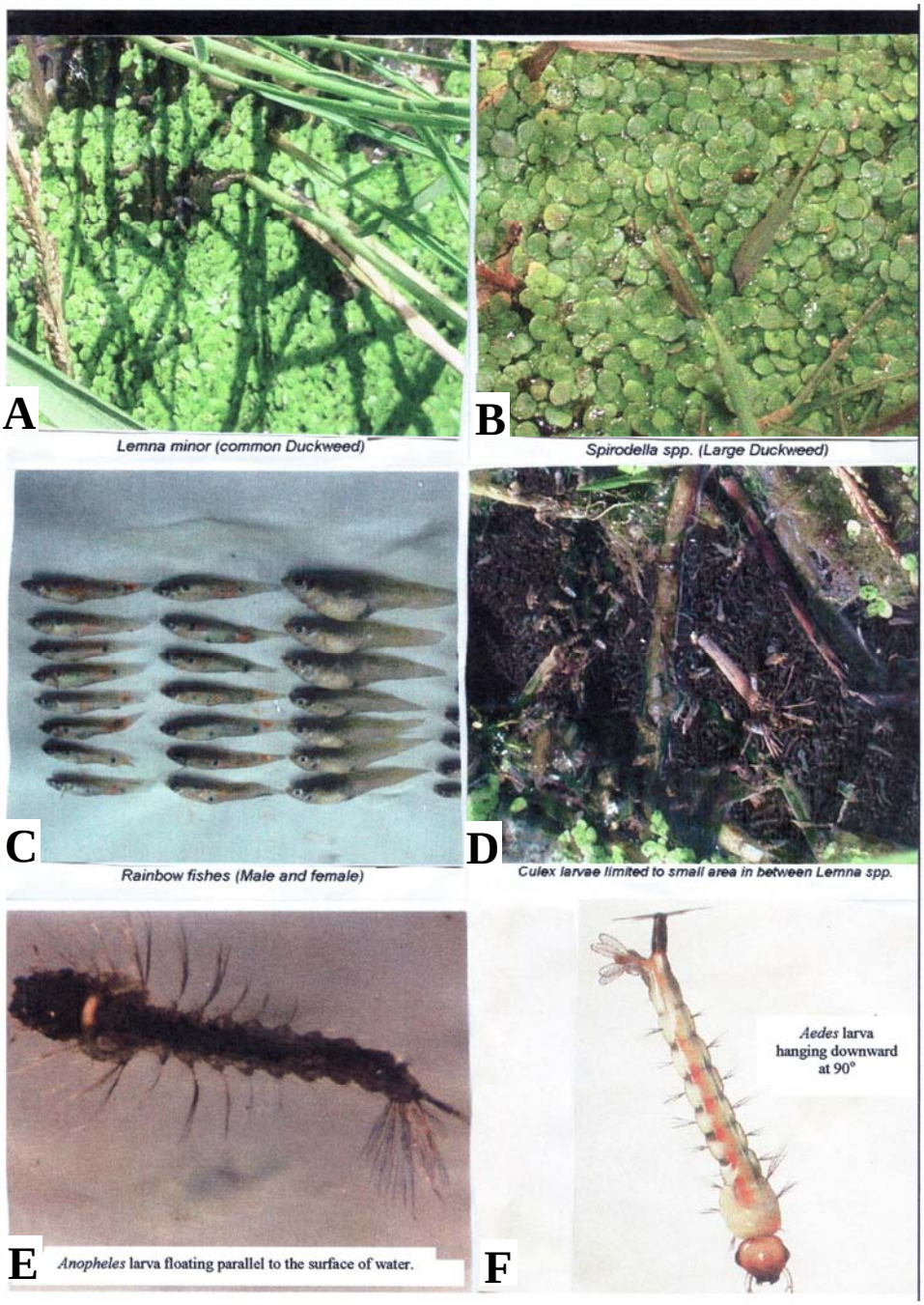

Fig. 1. A, *Lemna*; B, *Spirodella* spp.; C, Guppy fishes; D, *Culex* mosquito larvae surrounded by weed(s); E, Malaria vector mosquito larva and F, Dengue vector mosquito larva.

Attaur-Rahim (1981) reported a larvivorus fish, the Cyprinodontid fish. *Aphanius dispar* (Rupel) which is an omnivore and eat mosquito larvae in unused water storage tank. The species is indigenous and common in Riyadh, Saudi Arabia. In the present investigation the *Poecilia reticulata* from Poeciliidae was used, but *Cyprinus carpio* (fam: cyrinodontidae), may also be used which is available locally and is also an omnovore fish. It may also be used in Pakistan, but it is very sensitive fish so it can only live in fresh and pure water and may be effective for water storage tanks and ornamental ponds in Pakistan.

Bay (1985) reported a list of larvivorous fishes in Biological Control of Mosquitoes. Among the listed larvivorous fishes (other than *Gambusia* and *Poecilia*), *Aphanius dispar*, *Cyprinus carpio* and *Tilapia mosambica* are also found locally in Pakistan and they may also be used in the biological control of mosquitoes, but in the present study, *Poecilia reticulata* was used and excellent (promising) results were achieved in five weeks time. So, not only *Poecilia* and *Gambusia* but also *Tilapia, Cyprinus* and *Aphanius* may also be introduced to meet the quantity requirements of the fishes in Biological Control.

Connor (1992) reported the control of yellow fever vector mosquito, also called the Dengue fever vector mosquito in Merida, Yucatan, Mexico. When the anti-larval campaign began the *Ae. aegypti* index was nearly 50%. By the end of October anti-larval measures included stocking of containers with bottom-feeder perch the *Ae. aegypti* index was reduced. In the present work *Poecelia reticulata* was used in some containers at laboratory level in the beginning before going in field they gave good and satisfactory results against *Ae. aegypti* in 100 litre water drum in which 10 fishes were released. They gave 100% control till the fishes were in drum for few months.

Conclusion

Lemna and *Spirodella* spp. supports the breeding of *Mansonia uniformis* (Chow *et al*., 1955) abundantly found in Sindh, Pakistan and is the predatory on *Anopheles, Aedes* and *Culex* spp. On the other hand *Lemna* and *Spirodella* spp., do not support other *Anopheline, Aedinine* and *Culex* spp., such as *An. culicifacies*, *An. subpictus*, *An. stephensi*, *Ae. aegypti, Ae. albopictus*, confirmed malaria and dengue vectors respectively and common *Culex* spp. *Lemna* or *Spirodella* and fish collectively creates an excellent biological control environment of mosquito larvae, giving beautiful scene of these weeds and Rainbow fishes.

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