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ENTOMOLOGICAL SURVEILLANCE OF *AEDES AEGYPTI* AND ARBOVIRUSES OUTBREAK OF DENGUE FEVER IN THE RED SEA GOVERNORATE, EGYPT

By

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Abstract

Red sea Governorate coordinates: N 25^{o} 32' 1", E 33^{o} 26' 18" and one of the borders Governorates. The Red Sea Coast length of 1080 km, from the Gulf of Suez, latitude 29 north, to the border of Sudan on latitude 22 north with about 306,000 living in area of about 203,685km^2(January 2018), and it is divided into seven cities include Hurghada (capital), Safaga, Al-Kuseer, Ras Ghareb, Marsa Alam, Shalatin, Halaib. Four Cities Hurghada, Safaga, Al-Kuseer, Ras Ghareb were selected for entomological surveys where dengue fever was anticipated to be occurred at November 2017. A total number of 469 houses were participates survived, positive adult houses were 20.47% (96/469), while positive larvae houses were 10.23 % (48/469). The stogmoyia indices for the parameters of house index (HI), container index (CI) and Breteau index (BI) were (10.2, 7.0 &16) respectively. The survey yielded a total number of immature stages of *Aedes aegypti* of 92.22% (960/1041), or 92.5 % (888/960) for larvae and 7.5% (72/960) for pupa. The immature stages of other species were recorded 7.78% (81/1041), *Aedes detritus*, *Culex pipiens*, *Cx. antennatus* and *Cx. perexiguus* and represented 86.42% (70/81) for larvae and 13.58% (11/81) for pupa. Adults *Ae. aegypti* were 20.36% (90/442), but adult mosquito of other species were 79.64% (352/442). Adults *Ae. aegypti* at Hurghada showed resistant to deltamethrin, cyflothrin and bendiocarb, and suggested resistance to lambda-cyhalothrin, but they were susceptible to malathion. At Safaga City showed resistant for bendiocarb and susceptible to cyflothrin. Larvae of *Aedes aegypti* at Hurghada City showed resistant to chlorpyrifos, but susceptible to temephos. Also, at Safaga City showed susceptible to temephos insecticide.

Key wards: Red Sea, *Aedes aegypti*, Dengue virus, Entomological indices, Insecticide bioassay.

Introduction

Vector-borne infectious diseases are re-emerged due to changes in the public health policy, insecticidal and/or the drug resistance, shift in emphasis from the prevention to emergency response, demographic, and the changes in social, climatic and pathogenicity (Gubler, 2009). The climate change is expected to cause extensive shifts in the infectious and vector-borne diseases epidemiology (WHO, 2003). Scenarios on the effects of climate change typically attribute altered the distribution of communicable diseases to the rise in average temperature and altered incidence of infectious diseases to weather extremes (Canyon *et al*, 2016).

In Egypt, Kirkpatrick (1925) reported *Aedes aegypti*. Gad (1963) identified *Ae. aegypti*, *Ae. caspius* and *Ae. detritus*. Holstein (1967) stated the complete eradication of the *Ae. aegypti* from Egypt. But, Heikal *et al.* (2011) reported the re-emergency of *Ae. aegypti* in southern Egyptian border as introduced from the Sudan. Shoukry *et al.* (2012) detected larvae of *Ae. aegypti* in water bodies in the Toshka Project. Saleh (2012) reported *Ae. aegypti* in Aswan Governorate.

Generally, *Ae aegypti* is the main vector of zoonotic arboviruses; Yellow fever (CDC, 2010), Dengue fever (El-Bahnasawy *et al*, 2011), Chikungunya viruses (Mostafa *et al*, 2002) and Zika fever (Morsy, 2018). Also, blood transfusion and needlestick injury must be in mind (Abdel-Motagaly *et al*, 2017).

Burdino *et al*. (2011) in North West Italy identified two imported cases of DENV infections from the South Egypt in patients travelling together, confirming the importance of returning travelers as sentinels of the rapidly changing epidemiology in specific geographic areas. They added that there must a careful evaluation and follow-up of febrile travellers back from dengue endemic areas. El-Bahnasawy *et al.* (2011) reported that dengue (DF) and dengue hemorrhagic fevers (DHF) occur in the urban and suburban areas in the Americas, South-East Asia, the Eastern Mediterranean and the Western Pacific, but mainly in rural areas of Africa. They added that *Ae. aegypti* and endemicity of DF & DHF in the neighboring regional countries must be in mind of the public health authorities.

Dengue fever cases have been registered earlier in Egypt; the last outbreak was in 2015 in Dairut, but there are no data about outbreaks in cities on coast of the Red Sea (e.g., Hurghada, Sharm El-Sheikh, & Dahab), which have become resort destinations for Russian citizens (WHO, 2017). Two cases of dengue fever were imported from Hurghada, Egypt, where the DF was not considered endemic, to Moscow. These cases showed how emergence of DF in popular resort regions on the coast of the Red Sea can spread infection to countries where it is not endemic (WHO, 2017).

In October 2017, the health department of the Red Sea Governorate reported cases of dengue fever in El-Qoseir City 145 km south of Hurghada, with a population of about 50,000. The preliminary results suggested that 1,200-2,500 persons were infected (El-Sheikh, 2017; Saifullin *et al*, 2018).

This study aimed to investigate *Aedes aegypti* presence in the Red Sea Governorate, to calculate vector infestation indices, and intervention efficacy, as a marker of transmission and/or sudden outbreak, also to evaluate the efficacy of some insecticides against the yellow fever or tiger mosquito *Ae. aegypti* under field & laboratory conditions.

Materials and Methods

Larvae collection: for 5-20% of premises according to density HI, CI, BI, Pupal demographic surveillance (WHO, 2017). Data achieved by certain parameters:

$$\text{House (premise) index (HI)} = \frac{\text{Infested house x 100}}{\text{Inspected house}}$$

$$\text{Container index (CI)} = \frac{\text{Positive container x 100}}{\text{Inspected container}}$$

$$\text{Breteau index (BI)} = \frac{\text{Positive container x 100}}{\text{Inspected house}}$$

Larval density did not or poorly indicate adult density & transmission potential (AHI).

$$\text{AHI} = \frac{\text{House infested by adult x 100}}{\text{House inspected}}$$

Adult surveillance: For all premises indoor and outdoor collection using hand aspiration and spay sheet collection. Samples for bioassay from every area were collected, and mosquitoes were identification by international keys (Kent and Chester, 1966; WHO, 2018).

Insecticides susceptibility: The bioassay determined different susceptibility or resistance levels of insecticides on collected mosquitoes' populations. Adult unfed *Aedes aegypti*, 1-3 days old, reared from collected larvae were used. They were aspirated by a hand aspirator into paper cups to assess the insecticides susceptibility by diagnostic concentration, they were exposed to diagnostic doses for certain times (WHO, 1981) in the plastic chambers and transferred to the clean chambers. Insecticides were 5% malathion, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 0.15% cyfluthrin & 0.1% bendiocarb.

Larval bioassay: 200 larvae were exposed to diagnostic doses of 0.01ppm chlorpyriphos & 0.02ppm temephos in water for 24hr at four replicates for each diagnostic concentration and 25 larvae was used as control. Tests were done at 25± 1ºC & 70-80% relative humidity. Mortality were recorded 24hr post exposure, by summing dead mosquitoes across all exposure replicates & expressed as a total number% of exposed mosquitoes. Control mortality was also calculated. If control mortality was ≥20%, test was discarded, but if mortality was <20%, mortality was corrected by Abbott's formula (1925). If control mortality was <5%, no correction needed, but in control mortality of ≥5%, must be corrected.

Statistical analysis: Data collected, tabulated and analyzed using the suitable statistical computer software package (SPSS15.0).

Results

Table 1: Entomological parameters and indices for cities at Red Sea Governorate

City	House count	Positive adult	Positive larvae	HI	CI	BI	AHI
Hurghada	333	70	30	9.0	6.0	14.1	21.6
Safaga	16	3	4	25	15.4	37.5	18.8
Al-Kuseer	94	21	12	12.8	10.5	21.3	22.3
Ras Ghareb	26	2	2	7.7	4.0	7.7	7.7
Total	469	96	48	10.2	7.0	16	20.9

HI = (house index) CI = (container index) BI = (Breteau index)

Of 469 houses, positive adults were 20.47% (96/469), while positive larvae were 10.23% (48/469). Stogmoyia indices for HI parameters, CI, & BI were 10.2, 7.0 & 16, respectively. Safaga gave high indices (25, 15.4 & 37.5 for HI, CI & BI, respectively), Ras Ghareb gave lower indices (7.7, 4.0 & 7.7 respectively) and Hurghada & Al-Kusser showed (9.0, 6.0 & 14.1 & 12.8, 10.5 & 21.3 respectively).

Table 2: Immature mosquito collected from cities of Red Sea Governorate during autumn 2017

City	*Ae. aegypti*			*Ae. detritus*			*Cx. pipiens*			*Cx. perexiguus*			*Cx. antennatus*		
	L	P	T	L	P	T	L	P	T	L	P	T	L	P	T
Hurghada	509	26	535	0	0	0	36	3	39	6	1	7	0	0	0
Safaga	173	23	196	0	0	0	10	1	11	2	0	2	6	4	1
Al-Kuseer	206	23	229	4	1	5	5	1	6	1	0	1	0	0	0
Total	888	72	960	4	1	5	51	5	56	9	1	10	6	4	10

L = larvae P = pupa T = total

Immature stages were 92.22% (960/ 1041), 92.5% (888) larvae & 7.5% (72) pupae. Immature stages of others were 7.78% (81/ 1041), *Ae. detritus*, *Cx. pipiens*, *Cx. antennatus* and *Cx. perexiguus* as 86.42% (70) larvae and 13.58% (11) pupae. Immature stages from Hurghada were high 55.73% (535/960) than those from Al-Kuseer 23.85% (229) and Safaga 20.42% (173).

Table 3: Adult mosquito collected from cities of Red Sea Governorate during autumn 2017

City	*Ae. aegypti*			*Ae. detritus*			*Cx. pipiens*			*Cx. perexiguus*			*Cx. antennatus*		
	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
Hurghada	12	54	66	0	0	0	63	131	194	6	18	24	0	0	0
Safaga	2	7	9	0	0	0	3	20	23	0	1	1	6	6	12
Al-Kuseer	2	13	15	1	4	5	30	63	93	0	0	0	0	0	0
Total	16	74	90	1	4	5	96	214	310	6	19	25	6	6	12

M = Male F = Female T = Total

Ae. aegypti was 20.36% (90/442). Other adults were 79.64% (352/442), for *Ae.detritus*, *Cx. perexiguus* and *Cx antennatus*. *Ae. aegypti* from Hurghada were 73.33% (66/90) more than that from Al-Kuseer 16.67% (15/90) and Safaga 10% (9/90).

Table 4: Adults insecticides bioassay.

Area	Insecticide group	Insecticide used	Diagnostic dose %	No. adults	Corrected mortality %	Susceptibility & resistant
Hurghada	Pyrethroid	Deltamethrin	0.05	50	88.75	Resistant
		Lambada cyhalothrin	0.05	50	91.25	Suggested resistant
		Cyflothrin	0.15	50	83.75	Resistant
	Carbamate	Bendiocarb	0.1	50	24.4	Highly resistant
	Organophosphorus	Malathion	5.0	50	100	Susceptible
Safaga	Pyrethroid	Cyflothrin	0.15	50	100	Susceptible
	Carbamate	Bendiocarb	0.1	50	19.4	Highly resistant

At Hurghada, adults showed resistant to deltamethrin, cyflothrin and bendiocarb, and showed suggested resistance to lambda cyhalothrin, but susceptible to malathion. At Safaga they showed resistant to bendiocarb and susceptible to cyflothrin insecticides.

Table 5: Larvae insecticides bioassay

Area	Insecticide group	Insecticide used	Diagnostic dose PPM	Larvae	Corrected mortality %	Susceptibly & Resistant
Hurghada	Organophosphorus	Chlorpyriphos	0.01	100	8	Highly resistant
		Temephos	0.02	100	100	Susceptible
Safaga		Temephos	0.02	100	100	Susceptible

Larvae at Hurghada resisted chlorpyrifos, but were susceptible to temephos. At Safaga larvae showed susceptible to temephos.

Discussion

Generally, application of fogging usually is not effective in reducing the density of adult *Aedes* mosquito due to the indoor nature of such vector, so intimate application and community mobilization is the very important to encourage the people to open the windows during fogging. Other application of adult mosquito pesticide (e.g.; residual spray) is also not effective and not recommended hence the behavioral nature of resting places of such vector, but aerosol domestic spraying may be effective. The AHI/ HI indices indicated that more than 50% of infested houses come from neighbors' breeding places and the mosquitoes visited these houses for feeding and resting places hence, use of doors and windows specific mosquito netting was very indicated.

As to mosquitoes, Gad and Salit (1973) in Ras-Ghareb, Hurghada, Safaga and Koseir reported *Anopheles multicolor*, *Ae. caspius* and *Cx. pipiens*. Mostafa *et al.* (2002) reported that *Culex* species were the commonest; *Cx. pipiens*, *Cx. antennatus* and *Cx. univittatus*. *Cx. thelerei* was found only in El-Kharga oasis. *Aedes detritus* was found in Assiut, El-Fayium, Giza, El-Wady El-Gadeed and South Sinai, and *Ae. caspius* was coomon in Assiut and Aswan Governorates. Mikhail *et al.* (2009) in Egypt reported that mosquitoes were six species of *Culex*, 13 *Anopheles* species, only *Aedes caspius, Ae. detritus,* and *Culiseta longiareolata.*

WHO (2015) reported that a DF outbreak with at least 253 cases in Assiut Governorate. Abozeid *et al.* (2018) showed that *Ae. aegypti* caused dengue fever outbreak of more than 680 cases in the Red Sea Governorate. Abdelkader (2018) reported the PCR positivity of 101 people suffered from the dengue fever in both the Red Sea and Qena Governorates.

The abuse of insecticides for mosquito larvae might be the reason for resistance. WHO (1992) reported that in the Eastern Mediterranean Countries *Cx. pipiens* larvae were resistant to the organophosphorus insecticides. Thavaselvan *et al.* (1993) in Indian reported that *An. stephensi, Cx. quinquefaciatus* and *Ae. aegypti* larvae were resistant to malathion and fenitrothion. Mazzarri and Georghiou (1995) in Venezuela found *Ae. aegypti* resisted temephos, malathion and pirimiphos methyl, and propoxur, but with moderate resisted to permethrin and lambda-cyhalothrin. Zayed *et al.* (1997) in Giza, Beheira, Demiatta & Assiut Governorates reported that *Cx. pipiens* larvae were resistant to chloropyrifos, fenitrothion, fenthion, malathion and temephos. Mostafa and Allam (2001) in El-Fayiom Governorate found that *Cx. pipiens* larvae resisted temphos, fenitrothon, bromophos and fenthion, but were susceptible to malathion, permethrin and diazinon. Zayed *et al.* (2006) in Qalyobia Governorate found that *Cx. pipiens* larvae were susceptible to malathion but resisted lambada-cyhalothrin, permethrin, cyfluthrin, propxur, fenitrothion and bendiocarb. Mikhail *et al.* (2007) in Qatar reported that the chloropyrifos and cyfluthrin were highly effective larvicides for the *Cx. pipiens* complex, cyphenothrin was moderate resistant, but propetamphos, and etofanprox was the least ones.

Al-Sarar (2010) in Riyadh found that two *Cx. pipiens* populations from Wadi Namera were highly resistant to deltamethrin, the third one from Al-Wadi District had low resistance to lambada-cyhalothrin and moderate resistance to betacyfluthrin, and bifenthrin.

Abd-El-Samie and Abd-El-Baset (2012) in Sharkia and Assiut Governorates studied the efficacy of organophosphorus, carbamate, and synthetic pyrethroid as well as the insect growth regulator against *Cx. pipiens* field and laboratory populations. They found that the laboratory mosquitoes were highly susceptibility to insecticides than the field ones. Alsheikh *et al.* (2016) in Saudi Arabia found that *Ae. aegypti* adult and larvae were susceptible to cyfluthrin, with variable resistances to lambda-cyhalothrin, deltamethrin, permethrin, fenitrothion, bendiocarb and DDT. The larvae was resistant to temephos, but high susceptibility to the methoprene than diflubenzuron (IGRs).

Conclusion

Generally speaking, the dengue virus in fection in humans is often unapparent but can lead to a wide range of clinical manifestations, from mild fever to potentially fatal shock syndrome. Meanwhile, its vector is difficult to controlling as having wide range of breading places.

The outcome results showed that the Red Sea Governorate has high *Ae. aegypti* density and consequently dengue fever suspected.

Meanwhile, the extensive abuses of the insecticides pave the way to the mosquitoes' as well as other insects for insecticidal resistance. Consequently, the *Aedes aegypti* regular periodical control of both adults and larvae, as well as the development of new friendly control measure are recommended.

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