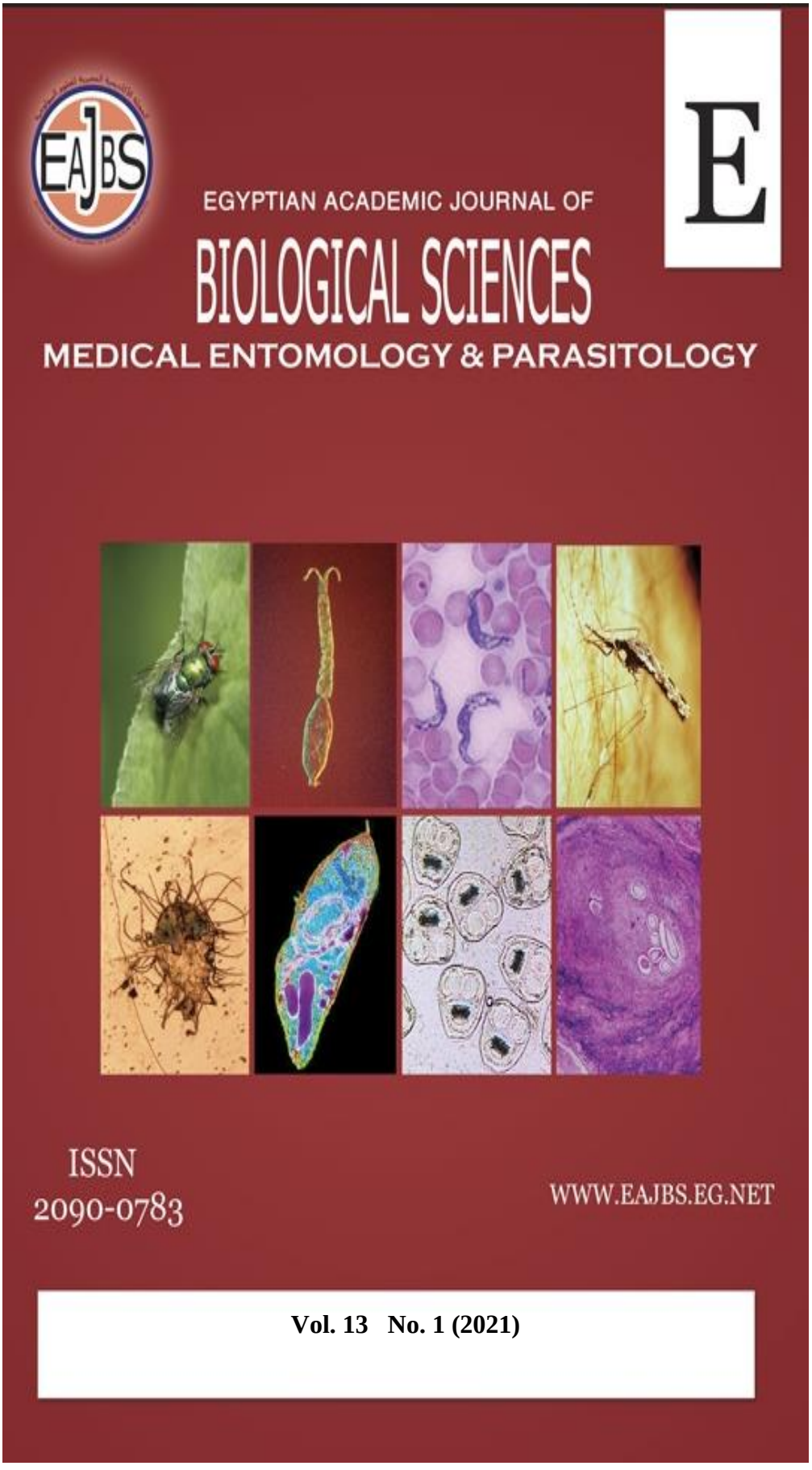

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The Insecticidal Activity of Entomopathogenic Bacteria Isolated from *Musca domestica, Chrysomya albiceps* and *Lucilia sericata* against the Mosquito Larvae of *Culex pipiens*

Amr fouda[1], M. A. Awad[2], and M. M. Kabadaia[2]
1-Botany and Microbiology Department, Faculty of Science, Al-Azhar University.
2-Zoology and Entomology Department, Faculty of Science, Al-Azhar University.
E-mail : mohamed_awad@azhar.edu.eg

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ABSTRACT

The present study evaluated the toxicity of different bacterial strains isolated from the outer surface of adult flies associated with carrions at Al – Mansoureya, Giza Governorate, Egypt against *Culex pipiens* larvae. 11 different bacterial strains were isolated and identified from the adults of *Musca domestica*, *Chrysomya albiceps* and *Lucilia sericata*. Only five strains caused mortality in *C. pipiens* larvae more than 50 %. The highest larval mortality was caused by the bacterial strains; *Klebsiella oxytoca* and *Staphylococcus sciuri*. The results indicated that entomopathogenic bacterial strains could have the potential for the biological control of mosquitoes.

INTRODUCTION

The mosquito vector, *Culex pipiens* has a wide distribution in Egypt and is the main vector of Rift valley virus (Meagan *et al.,* 1980; Darwish and Hoogastrall,1981), *Wuchereria bancrofti* (Khalil *et al.,* 1930 and Gad *et al.,* 1996) and the western Nile virus (Pelah *et al.,* 2002).

Insecticide applications, although highly efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding vectorial capacity (Liu *et al.,* 2006). Furthermore, they are responsible for substantial hazards to a variety of non-target organisms and the environment in the form of biomagnification (Gold *et al.,* 2001).

Recently, a great reduction in the use of chemical insecticides as the development of biological control agents has intensified (Alves *et al*.,1998; Polanczyk *et al.,* 2003; Pamplona *et al.,* 2004; Cuo *et al.,* 2015). In particular, entomopathogenic bacterial strains have enormous potential for biological control of insects (Palma *et al.,* 2014; Peralta and Palma, 2017; Lobo *et al.,* 2018).

Hundreds of bacterial strains may affect insects, but only a few can be used effectively for biological control of the vectors that cause tropical diseases (Lobo *et al.,* 2018).

The present study evaluates the toxicity of different bacterial strains isolated from the outer surface of some flies associated with carrions against the mosquito vector, *Culex pipiens* for the first time.

MATERIALS AND METHODS

1. Collection of Non-Biting Flies:

House flies, and other non-biting flies (blowflies) were captured by a nylon net from carrions of different animals (e.g.; horse, donkey, dog) at Al – Mansoureya, Giza Governorate, Egypt. The collected flies were placed into sterile tubes. Then fly tubes were placed in the freezer for killing the flies were identified to species level. After identification of flies, 1 ml of sterile physiological saline solution was added to each vial, which was shaken vigorously for 5 min. with the fly remaining inside. The fly was then removed from the saline solution and was checked for bacteria dislodged from the external surfaces of the fly.

2-Bacterial Isolation and Identification:

2.1- Bacterial Isolation:

Different types of enrichment and selective media were used for counting and isolation of bacteria that occurred on the external body surface of different flies. After killed of flies in the freezer, merged in sterilized saline solution, shacked for about 5 min. and serial dilution was made. Plate count agar media was inoculated from serial dilution for counting different bacterial species found on the external body surface of flies and nutrient agar media and Trypticase soy agar (TSA) were also inoculated for isolation of different bacterial species. Bacteria were isolated by agar streaking method onto surface plates of nutrient agar, the plates were incubated aerobically at 37◦C for 24h, after growth, bacterial colonies were subjected to purification processes. (Cowan, 1985). The chemical constituents of these media are as the following:

2.1.1. Trypticase Soy Agar (TSA) (Leavitt *et al.* 1955 &Labour and welfare, 2009):

This medium is used for the cultivation of a wide variety of microorganisms and is commonly used as a maintenance medium for culture collections, and testing bacterial contaminants in cosmetics (Curry *et al.,* 1993). It consists of (g/l): 15 Tryptone (pancreatic), 5 Soybean meal, 5 Sodium chloride and 15 Agar. pH adjusted to 7.4 ± 2.0 before sterilization.

2.1.2. Nutrient Agar Medium (**Eaton *et al.*, 1995):**

Nutrient Agar continues to be a widely used general-purpose medium for growing non-fastidious microorganisms. If required, enrichments can be added to this medium. It consists of (g/l): 3 Beef extract, 5 Peptone, 5 NaCl and 20 Agar. pH adjusted to 7.0 before sterilization.

2.1.3. Plate Count Agar Medium:

All media were manufactured by Oxoid (Cambridge, UK), Difco (USA), or Britannia (Buenos Aires, Argentina) and prepared according to the manufacturer's instructions mostly by dissolving the solid ingredients in distilled water and then heating to 60-70 °C with stirring. Then prepared media were sterilized by autoclaving at121°C for 15 minutes and when cool poured in sterile Petri-dishes or sterile culture tubes. For maintenance of stock culture, 5ml tryptic soy agar poured in slants, autoclaved, and then tilted to provide slopes for stock cultures. Or by pouring 1ml of tryptic soy broth medium supplemented with 15% glycerol in eppendorf's tubes, autoclaved, and then preserved in -80°C freezer (Curry *et al.,* 1993).

2.2-Bacterial Identification:

Primary identification (morphological, physiological and biochemical tests) was used. In addition, automated identification by using the Biomerieux vitek 2 system was used for confirmation. (Saad, 2016).

3.Laboratory Maintenance of the Mosquito's Colony:

Mosquitoes used in this study were *Culex pipiensL.* reared for several generations in the insectary of medical entomology at the Department of Zoology, Faculty of Science, Al-Azhar University under controlled conditions of temperature 27 ± 2 ^{O} C, relative humidity of 70 ± 10 % and 12 -12 light – dark region.

4.Susceptibility Bioassays:

In order to evaluate the toxicity of the isolated bacterial strains against *Culex pipiens* larvae. Each tested bacterial strain was performed in 100 ml of dechlorinated tap water (Conc. 50%) contained in 200 ml plastic cups. Then 25 third instar larvae of *Culex pipiens* were put immediately into each plastic cup containing the suspension of bacteria. For each bioassay, a replicate with no bacteria was prepared as a control. Meanwhile, three replicates of each bacterial strain were usually used. All plastic cups were incubated under control conditions at a temperature of 27 ± 2 ^{O} C, relative humidity of 70 ± 10 % and 12 -12 light–dark region. Larval mortality was recorded daily for 4 days, moreover, the pupated larvae were recorded. All bacterial strains that caused larval mortality higher than 50 % were applied in a concentration of 75 %.

RESULTS

The isolated bacterial strains from the external body surface of *Musca domestica*, *Chrysomyaalbiceps* and *Luciliasericata* collected from carrions during the study period from 1/2018 to 12/2018 are given in table (1).

Toxicity of isolated bacterial strains against *Culex pipiens* larvae:

As shown from table (1) and Figure (1) when the different bacterial strains were tested against the 3 rd larval instar of *Culex pipiens*at conc. of 50 % of the bacterial strains; *Klebsiella oxytoca, Proteus vulgaris, Morganella morganii, Lactobacillus delbrueckii* and *Staphylococcus sciuri*, caused larval mortality percent more than 50 %. The higher larval mortality 72 % in *Culex pipiens*larvae was caused by the bacterial strain; *Klebsiella oxytoca.*On the other hand, when the bacterial strains were tested against the 3 rd larval instar at conc. Of 75 % they caused larval mortality rate ranging from 68 to 88 %. The highest mortality rate (88 %) was found to be caused by the bacterial strains; *Staphylococcus sciuri* isolated from *Musca*, *Chrysomya*and *Lucilia*flies. (Table 2 and Fig. 2). However, these bacterial strains of bacteria increased the mortality rate of *Culex* larvae and decreased the pupation rate (Fig. 3).

Table 1: Toxicity of different bacterial strains against the 3 rd larval instar of *Culex pipiens* at conc.50%**.**

Bacterial strain	Larval mortality on the First day	Larval mortality on the Second day	Larval mortality on the Third day	Larval mortality in Four day	Total Larval mortality	Larval mortality %	Pupation %
Staphylococcus sciuri	4	4	2	3	13	52	48
Staphylococcus lentus	2	3	2	1	8	32	68
Staphylococcus oxylosus	2	3	2	3	10	40	60
Kocuria rosea	1	1	0	1	3	12	88
Klebsiella pneumonia	6	4	4	6	20	80	20
Staphylococcus lentus	1	2	3	4	10	40	60
Enterobacter aerogenes	1	2	1	1	5	20	80
Staphylococcus sciuri	1	1	1	2	5	20	80
Staphylococcus simulans	1	2	1	1	5	20	80
Staphylococcus sciuri	2	3	3	5	13	52	48
Lactobacillus delbrueckii	3	3	3	4	13	52	48
Morganella morganii	3	3	4	3	13	52	48
Klebsiella oxytoca	5	4	4	5	18	72	28
Proteus vulgaris	3	3	4	3	13	52	48
Water control	0	1	0	1	2	8	92

M., Ch., L. between brackets indicate that the bacterial strain isolated from *Musca domestica, Chrysomya albiceps* and *Lucilia sericata*.

No. of *Culex pipiens* larvae = 25 in each tested experiment.

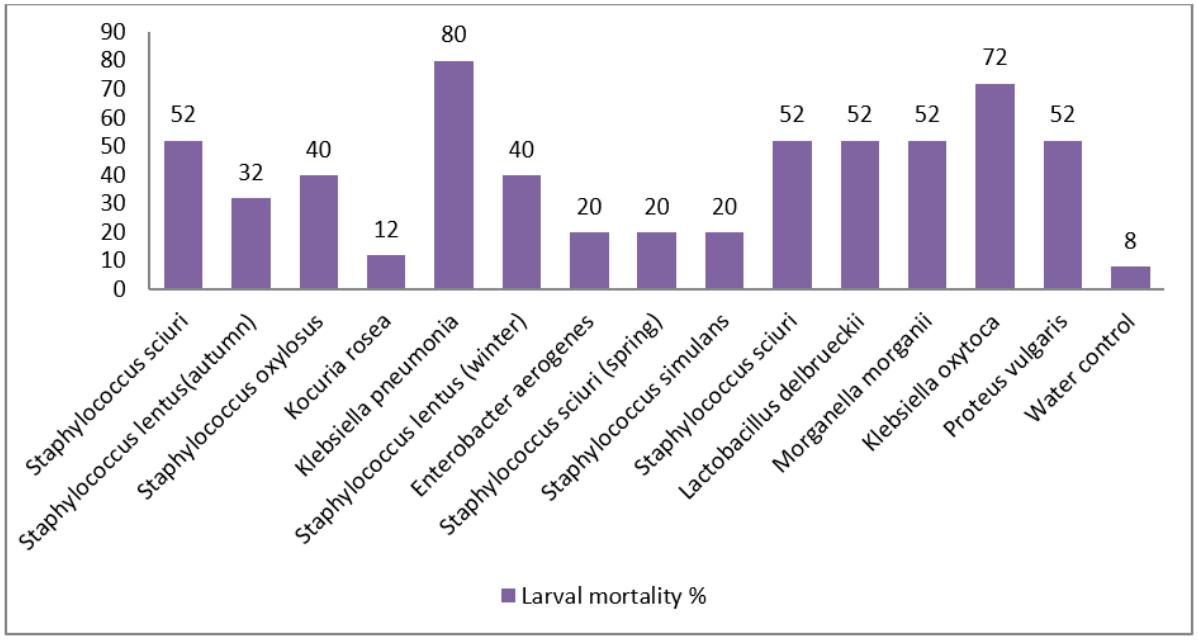

Fig. 1: Larval mortality percentage of 3 rd larval instar of *Culex pipiens*treated by different bacterial strains at conc. 50%.

Table 2: Toxicity of different bacterial strains against the 3 rd larval instar of *Culex pipiens* at conc.75%**.**

Bacterial strain	Larval mortality on the First day	Larval mortality on the Second day	Larval mortality on the Third day	Larval mortality in Four day	Total Larval mortality	Larval mortality %	Pupation %
Staphylococcus sciuri	6	5	6	5	22	88	12
Staphylococcus oxylosus	6	3	3	5	17	68	32
Klebsiella pneumonia	6	4	4	6	20	80	20
Staphylococcus lentus	6	3	5	4	18	72	28
Lactobacillus delbrueckii	6	4	3	5	18	72	28
Morganella morganii	6	4	5	4	19	76	24
Klebsiella oxytoca	5	4	5	6	20	80	20
Proteus vulgaris	5	4	4	5	18	72	28
Water control	0	1	0	1	2	8	92

M. , Ch. , L. between brackets indicate that the bacterial strain isolated from *Musca domestica*, *Chrysomya albiceps* and *Lucilia sericata*.

No. of *Culex pipiens* larvae = 25 in each tested experiment.

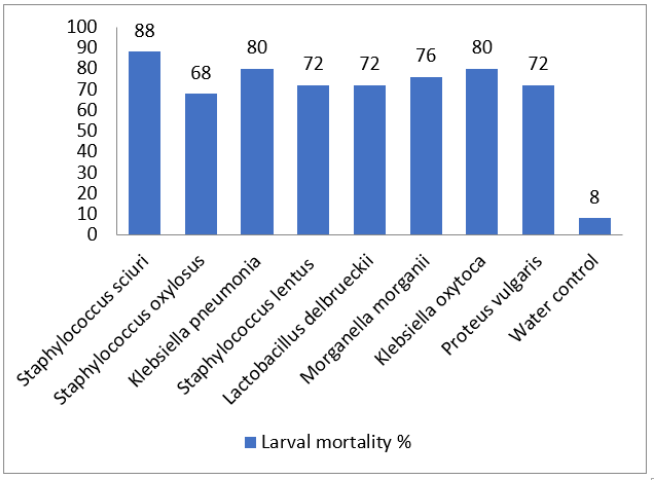

Fig. 2: Larval mortality percentage of 3 rd larval instar

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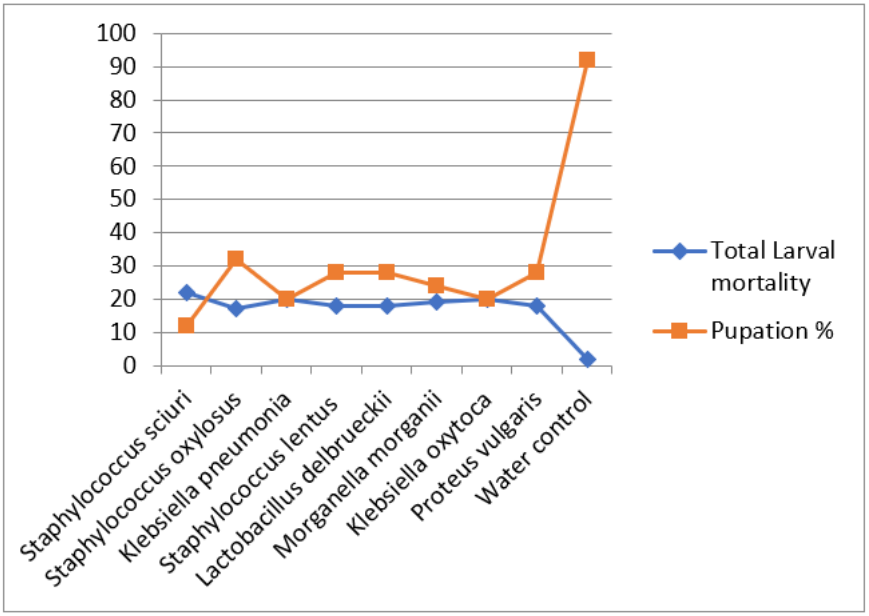

Fig. 3: Relation between larval mortality and pupation of 3 rd larval instar of *Culex pipiens* treated by different bacterial strains at conc. 75%of *Culex pipiens* treated by different bacterial strains at conc. 75%

DISCUSSION

In recent decades, the development of biological control agents has intensified (Alves,1998; Polanczyk *et al.* 2003; Pamplona et al.2004 and Cuo et al. 2015). Entomopathogenic bacterial strains have enormous potential for the biological control of insects (Schnepf *et al.*, 1998; Palma *et al.*, 2014; Peralta and Palma, 2017; Lobo *et al.*, 2018).

In this study different entomopathogenic bacterial strains isolated from *Musca*, *Chrysomya*and *Lucilia*flies associated with carrions were evaluated as biological control of *C.pipiens* . Out of 14 bacterial strains isolated from the outer surface of flies collected from carrions and tested against the 3 rd larval instar of *C.pipiens*, Only 5 strains caused larval mortality higher than 50 %.*Klebsiella oxytoca* caused the highest larval mortality. As shown from the present study the bacterial strains caused a high mortality rate in *C.pipiens* larvae as compared to Lobo *et al.* (2018) who stated that *Bacillus thuringiensis* isolated from soil in Brazil caused mortality in *Aedes aegypti* larvae about 4 %. They considered that this rate was higher than that recorded in many other insecticidal trials involving mosquito larvae (Dias et al. 2002; Praca *et al.*, 2004; Ootani *et al.*, 2011; Pereira *et al.*, 2013). However, bacterial strains with mosquitocidal action tend to be rare in comparison their effect against other orders, such as the Lepidoptera (Polanczyk *et al.*, 2004; Silva *et al.*, 2012; Silva – Werneck et al., 2000) and Coleoptera (Martins *et al.*, 2003; Silva, 2008). On the other hand, Suryadi *et al.* (2016) showed that four toxic isolates of *B. sphaericus* caused mild toxicity against the larvae of *C. quinquefaciatus*, *An. Aconitus* and *Ae. aegypti*.

In conclusion, most of the isolated bacterial strains from the adult flies which harbor carrions showed potential for the control of mosquitoes. However, further research is needed to identify the toxins o these strains.

REFERENCES

Abdel-Hamid, Y.M., Soliman, M.I. and Kenawy, M.A., 2011. Mosquitoes (Diptera: Culicidae) in relation to the risk of disease transmission in El Ismailia Governorate, Egypt. *Journal of Egyptian Society of Parasitol*ogy, *41*(2), pp.347-56.

Alves, S.B., Moino Jfnior, A., and Almeida, J.E.M., 1998a, Produtos fitossanitdrios entomoparogenos, ln: Controle Microbiano de lnsetos, s. B, Alves, ed., piracicaba, Fealq, pp, 2l7-238.

Darwish, M. and Hoogstraal, H. (1981): Arboviruses infesting human and lower animals in Egypt., A review of thirty years of research. *Journal of Egyptian Public Health Association*,56: 1 – 112.

El-Sheikh, T.M., Hassan, M.I., Moselhy, W.A., Amer, M.S. and Shehata, A.Z., 2011. Evaluation of the biological activity of some Cupressus semprevirens (Cupressaceae) extracts against the mosquito vector Culex pippins. (Diptera: Culicidae). *Egyptian Academic Journal of Biological Sciences. A, Entomology*, *4*(1), pp.33-48.

Fiuza, L., Polanczyk, R. and Silva, R. (2004): Isolamento DE Bacillus thuringiensis Berliner A partir de amostras de solos e sua patogenicidade para *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae). *Current Agricultural Science and Technology*, *10*(2).

Gad, A.M., Hammad, R.E. and Farid, H.A., 1996. Uptake and development of *Wuchereria bancrofti* in Culex pipiens L. and Aedes caspiuspallas. *Journal of the Egyptian Society of Parasitology*, *26*(2), pp.305-314.

Gold, L.S., Slone, T.H., Ames, B.N., Manely, N.B., (2001): Pesticide residues in food and cancer risk; a critical analysis. In: Krieger, R. (Ed.), Hand book of Pesticide Toxicology, 2nd ed. Academic Press, San Diego, C.A., pp.799-843.

Khalil, M, Halawani, A, Hilmi, I. S. (1930: the transmission of Bancroftian 559 filariasis in Egypt. *Journal of the Egyptian Medical Association*, 15:315-32.

Liu, N., Xu, Q., Zhu, F. and Zhang, L.E.E., 2006. Pyrethroid resistance in mosquitoes. *Insect Science*, *13*(3), pp.159-166.

Meegan J.M., Khalil G.M., Hoogstraal H, Adham F.K. 1980. Experimental transmission and field isolation studies implicating *Culex pipiens* as a vector of Rift Valley fever virus in Egypt. *American Journal of Tropical Medicine and Hygiene*, vol. 29pp 1405-1410.

Pamplona, Lde G., Lima JW., Cunha JC., and Santana, EW., 2004. Evaluation of the impact on Aedes aegypti infestation in cement tanks of the Municipal District of Canindé, Ceará, Brazil after using the Betta splendens fish as an alternative biological control Luciano de Góes Cavalcanti. *Revista da Sociedade Brasileira de Medicina Tropical*, 37(5):400-404.

Palma, L.; Muñoz, D.; Berry, C.; Murillo, J.; Caballero, P. Draft genome sequences of two Bacillus thuringiensis strains and characterization of a putative 41.9-kda insecticidal toxin. *Toxins*, 2014, 6, 1490–1504.

Pelah, D., Abramovich, Z., Markus, A. and Wiesman, Z., 2002. The use of commercial saponin from Quillajasaponaria bark as a natural larvicidal agent against Aedes aegypti and Culex pipiens. *Journal of Ethnopharmacology*, *81*(3), pp.407-409.

Peralta, C. and Palma, L., 2017. Is the insect world overcoming the efficacy of Bacillus thuringiensis?. *Toxins*, *9*(1), p.39.

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