



Original article

Influence of the mixtures composed of slow-release insecticide formulations against *Aedes aegypti* mosquito larvae reared in pond water

Naser A. Alkenani*

Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, P.O. Box 80303, Jeddah 21589, Saudi Arabia

ARTICLE INFO

Article history:

Received 1 January 2017
 Revised 21 February 2017
 Accepted 23 February 2017
 Available online 27 February 2017

Keywords:

Aedes aegypti
 Larvicides
 Bactimos briquets
 Altosid
 Dudim

ABSTRACT

Vector borne diseases remain the major source of illness and death worldwide. *Aedes aegypti* is the primary carrier of dengue and dengue haemorrhagic fever in many developing countries in the tropical world. Because *A. aegypti* populations are becoming more and more resistant to conventional and non conventional insecticides, alternative strategies have to be rapidly implemented in the future for dengue vector control. The present study aimed to evaluate the larvicidal efficacy of slow-release formulations (SRFs) of bacterial insecticide Bactimos briquets blended with tow insect growth regulators (IGRs), Altosid XR – briquets and Dudim DT tablet respectively, against mosquito larvae of *A. aegypti* the primary vector of dengue fever in Jeddah governorate, Saudi Arabia. Semi-field trials were conducted at dengue mosquito research station, Dept. of Biological Sciences, faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. The efficacy of the test formulations was calculated as the number of emerging adults compared to the initial number of larvae added or the inhibition of emergence (IE%). The assessment of effectiveness was made at weekly intervals until the level of efficacy decrease to $\leq 50\%$ IE. The inhibition percentage of emergence of adult for each mixture weekly in addition to the calculation of the cycle of the effective centers for each mixture. Collectively, the results of the present investigation indicate that the combination of Bactimos with Altosid or Dudim maybe promising for controlling *A. aegypti* mosquito larvae provided that treatments persist at least during the whole dengue transmission season.

© 2017 The Author. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Mosquitoes are medically and economically significant groups of insects among dipterans. Mosquitoes are the vectors for various number of human and zoonotic disease pathogens affecting human and animal hosts, including those that cause malaria, filariasis, Japanese encephalitis, chikungunya, and dengue and yellow fevers (Govindarajan et al., 2013; Shivakumar et al., 2013; Jayapriya and Gricilda, 2015). Currently, there are more than 300 mosquito species in the world grouped in 39 genera and 135 subgenera. *Aedes aegypti* is a very important disease vector, transmitting the

arbovirus causing dengue haemorrhagic fever and chikungunya in human (Ringu-Perez et al., 1997). Dengue is an important mosquito transmitted viral disease, which is prevalent in more than 100 endemic countries (Marques and Kaplan, 2015). *A. aegypti* is generally recognized as the primary vector of dengue viruses causing 50 million cases of infection and 300,000 deaths each year in tropical and subtropical areas (Darriet et al., 2010). Over the last 25 years there has been a global increase in both the distribution of *A. aegypti* and the epidemic dengue virus activity (Jansen and Beebe, 2010). Additionally, dengue fever has become a pandemic threat and a major public health challenge to the health officials and policy makers in nearly all the tropical areas of the world (WHO, 2011; Bhatt et al., 2013). Dengue fever is endemic in kingdom of Saudi Arabia, especially in the western and southern regions (Khan et al., 2008; El-Badry and Al-Ali, 2010; Al-Azraqi et al., 2013; Gamil et al., 2014).

To prevent proliferation of mosquito borne diseases and to improve quality of environment and public health, mosquito control is essential. The major tool in mosquito control operation is the application of synthetic insecticides such as organochlorine

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

* Corresponding author.

E-mail address: nkenani2016@gmail.com<http://dx.doi.org/10.1016/j.sjbs.2017.02.006>

1319-562X/© 2017 The Author. Production and hosting by Elsevier B.V. on behalf of King Saud University.
 This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

and organophosphate compounds. But this has not been very successful due to human, technical, operational, ecological, and economic factors. Excessive use of synthetic pesticides causes emergence of pesticide resistance and harmful effect on non-target organisms and environment (Arivoli et al., 2015). In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health, and other non-target populations, their non biodegradable nature, higher rate of biological magnification through ecosystem, and increasing insecticide resistance on a global scale (Brown, 1986; WHO, 1996). Moreover, the widespread use of synthetic insecticides has led to many negative consequences (Pavela, 2008), resulting in increasing attention to natural products (Pirali-Kheirabadi and da Silva, 2010).

Bio-insecticides or bio-pesticides are based on natural products like naturally occurring chemicals such as plant derived biochemicals (plant extracts) and pheromones or microorganisms and microbial pathogens like bacteria, they are used to target various mosquito vector species and have been long touted as alternatives to synthetic chemical insecticides for vector and pest management because they pose little threat to human and environmental health (Murray, 2006; Chandler et al., 2011). With the current trend in dengue incidence worldwide and without an effective vaccine, it is expected that the widespread use of insecticides will continue. Many insecticide formulations have been developed and tested for their efficacy against a wide spectrum of mosquito vectors. In this concern, a great impetus has been given to the use of slow-release insecticide formulations against mosquito larvae (WHO, 2005). Such formulations are likely to enhance residual larvicidal activity via greater stability and maximized contact with the target mosquito larvae (Mulla et al., 1988; Cornel et al., 2000; Bond et al., 2004; Seng et al., 2008; Jacups et al., 2014). The objective of this study was to evaluate the larvicidal efficacy of slow-release formulations (SRFs) of bacterial insecticide Bactimos briquets blended with two insect growth regulators (IGRs), Altosid XR – briquets and Dudim DT tablet respectively, against mosquito larvae of *A. aegypti* the primary vector of dengue fever in Jeddah governorate, Saudi Arabia.

2. Material and methods

2.1. Tests of formulation mixtures

The evaluation of the active larval fatality for some mixtures that are made from 2 of slow-release preparations and at the same level of concentration that in special for each preparation for the tested mixtures are follows:

- The bacterial insecticide Bactimos + Tablets of the insects growth regulator Altosid.
- The bacterial insecticide Bactimos + Tablets of the insect growth regulator Dudim.

2.2. Mosquito material

The strain of *A. aegypti* in this study was collected from Al-Ajwad District, Jeddah Province, Saudi Arabia (N213531.22, E39168.10). The mosquitoes were maintained in the insectary under laboratory conditions of 27 ± 1 °C, $70 \pm 5\%$ relative humidity (RH) and a period of 14 h. of light and 10 h. darkness. The larvae were reared in Dengue Mosquito Research Station (DMRS), at King Abdulaziz University until pupation and adult emergence took place for maintaining the stock culture.

2.3. Insecticide (compounds tested)

- (1) The bacterial insecticide: *Bactimos briquets* (*Bacillus thuringiensis* Israelensis, 7000 ITU; 10% a.i., Baltimore, N.D., USA).
- (2) Two slow-release formulation of insect growth regulator: Altosid XR-briquets (methoprene 2.1% a.i., briquet weight 48gm, provided by Zoecon, USA) and Dudim DT tablets (Difubenzuron 2% a.i., tablet weight 2 g, supplied by DGM Italia Srl.)

2.4. Experiments

Semi-field trials were conducted at dengue mosquito research station, Dept. of Biological Sciences, faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Experiments were carried out in white plastic pools (50 × 50 × 30 Cm) containing 30 L of pond water. Pools were placed in shade under a roof and were kept covered with muslin cloth sheets to prevent debris and oviposition by wild mosquitoes. Each pool received a batch of 25 third instar larvae of *A. aegypti* and the test formulation. The dosage of each formulation required for larval treatments (A quarter pieces, ~3.2 g of Bactimos briquet; 7 g of Altosid briquet; and 0.3 g of dude tablet) was determined according to the recommended dosage for field control pool without formulations were used as control. The larvae were given the usual larval food during the tests. Pond water was slowly added to the pools every other day to compensate evaporation. New live batches of 3rd larval instar of *A. aegypti* were added weekly to the test pools. All the mixtures of the above mentioned preparations has been tested against the 3rd instar larvae of the mosquito *A. aegypti* in the experimental troughs which contains 30 L of pond water with 4 replicates and the control. Any pupae produced were transferred to small plastic cups containing water and placed in adult cages for emergence. The efficacy of the test formulations was calculated as the number of emerging adults compared to the initial number of larvae added or the inhibition of emergence (IE%). The assessment of effectiveness was made at weekly intervals until the level of efficacy decrease to ≤50% IE. The inhibition percentage of emergence of adult for each mixture weekly in addition to the calculation of the cycle of the effective centers for each mixture.

2.5. Statistical analysis

Percentage of larval mortalities and inhibition of adult emergence were corrected for control mortalities using Abbott's formula (Abbott, 1925).

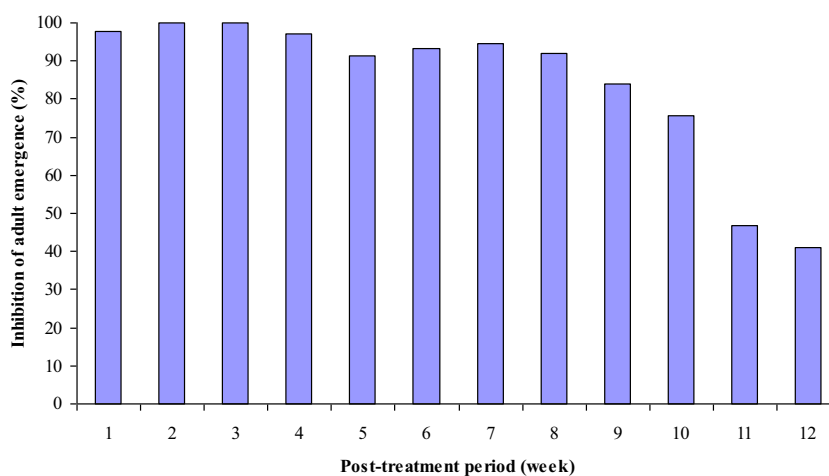
3. Results

Table 1 showed the recovered outcome of the treatment of the 3rd larval instars of *A. aegypti* mosquito reared in pond water with a mixture made of two slow release formulations including the bacterial insecticide Bactimos plus tablets of the insect growth regulator Altosid. The result proved that the larval fatalities of the mixture continued for a number of weeks with 90–100% inhibition of the emerging adult stage (Fig. 1). The obtained result in Table 2 and Fig. 2 showed that the treatment with the mixture made of Bactimos tablets with those of the insect growth regulator Dudim against 3rd instar larvae of *A. aegypti* in the pond water started to lose its efficacy to give less than 90% inhibition of adult emergence 10 weeks post-treatment when compared with 2 or 7 weeks of the effective control (90–100% IE).

The present result indicate that the mixture of Bactimos plus Dudim tablets continued its fatal activity against the 3rd instar

Table 1The efficacy of Bactimos briquets plus Altosid tablets mixture as slow-release formulations against 3rd instar larvae *A. aegypti* mosquito reared in pond water.

Post-treatment (weeks)	Dead larvae ^a (%)	Pupae produced (%)	Adult emerged (%)	IE ^b (%)	Duration of effective control with 90–100%IE (weeks)
1	86	14	2	97.9	
2	97	3	0.0	100	
3	98	2	0.0	100	
4	70	30	3	97	
5	67	33	8	91.3	8
6	59	41	6	93.4	
7	63	37	5	94.7	
8	52	48	7	92.1	
9	49	51	16	84	
10	42	58	22	75.8	
11	31	69	48	46.7	
12	33	67	55	40.9	

^a Four replicates, 25 larvae each; control mortalities ranged from 4 to 11% IE.^b IE = Inhibition of adult emergence, corrected for control mortalities (Abbott, 1925).**Fig. 1.** The inhibition percentage in the hatchability of the emerging from larvae reared in pond water after treatment with a mixture of Bactimos briquets plus Altosid tablets as slow-release formulations.**Table 2**The efficacy of Bactimos briquets plus Dudim DT tablets mixture as slow-release formulations against 3rd instar larvae *A. aegypti* reared in pond water.

Post-treatment (weeks)	Dead larvae ^a (%)	Pupae produced (%)	Adult emerged (%)	IE ^b (%)	Duration of effective control with 90–100%IE (weeks)
1	65	35	7	92.5	
2	94	6	0.0	100	
3	92	8	0.0	100	
4	83	17	5	100	
5	81	19	2	97.8	10
6	67	33	0.0	100	
7	57	43	4	95.7	
8	51	49	6	93.2	
9	43	57	7	93	
10	39	61	9	90.1	
11	38	62	17	81.1	
12	27	73	19	79.6	
13	30	70	37	60.6	
14	16	84	45	40.2	

^a Four replicates, 25 larvae each; control mortalities ranged from 4 to 11% IE.^b IE = Inhibition of adult emergence, corrected for control mortalities (Abbott, 1925).

larvae for a long period of effective control and gave an increase of 5–1.4 folds than the treatment with Bactimos or Dudim tablets each alone respectively. The present results of the investigation have proved the possibility of limiting the problem of the efficacy reduction of the slow-release formulations when treating the pond water larvae of the mosquito *A. aegypti* however the water might

be accumulated from rains, or nursery, or garden irrigation or stagnant water with house precincts especially those under construction by mixing 2 slow-release formulations from the non-conventional insecticides which differ in their effect like the biological bactericides or the insect growth regulators. Hence the mixing of 2 compounds leads to the increase of their residual effect of the

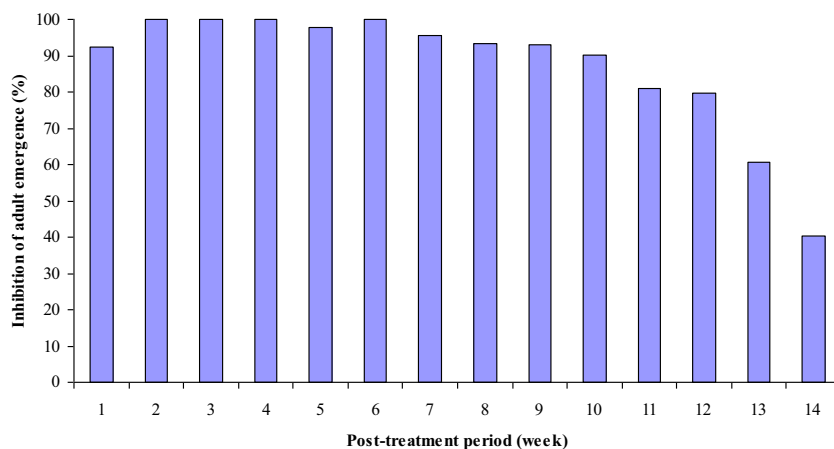


Fig. 2. The inhibition percentage in the hatchability of the emerging from larvae reared in pond water after treatment with a mixture of Bactimos briquets plus Dudim DT tablets as slow-release formulations.

active material within their breeding sites which might increase the period of effective control of the mixture better than using each compound alone.

4. Discussion

The result showed that the use of this mixture of Bactimos and Altosid has yielded a 1.3–4 folds increase in the effective *A. aegypti* control when compared with the treatment of Bactimos alone (2 weeks) and with Altosid (6 weeks) in the pond water *A. aegypti* 3rd instar larvae. (Alkenani et al., 2015). This was really assured from the results of this study especially when the mixture made of the bactericidal insecticides Spinosad which is characterized by its rapid effective action against the larval stage plus the insect growth regulator Dudim (which affect the molting process and pupation) which lead to a synergistic action. Our results were in agreement with Darriet et al. (2010) who assured that under field conditions when the mixture made of the bacterial insecticides Spinosad and the insect growth regulator Pyriproxyfen gave more efficacy against larvae of *A. aegypti* for continued 8 month giving effective control for periods of 3 months or 5 months when used singularly by Spinosad tablets or Pyriproxyfen tablets respectively.

From another perspective the mixture of 2 unconventional insecticides with different mode of action is considered one of the effective methods in the programs of mosquito abatement especially those species that has resistance to conventional insecticides because it is yet known that the use of an insecticide alone (especially the conventional ones) for longer intervals of time in the control program could lead to the increase of the dosages of that particular insecticide which evidently lead to the building of resistance in the mosquito strains for that insecticides and for any other insecticide from the some chemical group.

Generally we believe that this investigation had proved that the slow-release unconventional insecticides like the bacterial insecticides or the insect growth regulators against the larvae of the mosquito *A. aegypti* had produced effective control at 90–100% in the inhibition of the emerging adults which continued for a number of weeks when treated with the tested preparation for one treatment in the breeding site without the need for the repetition of the frequency of control when compared with what happens through the use of the conventional chemical insecticides, however this might lead to the rational use of the insecticides which automatically reduces the cost of control and reduces environment pollution (Darriet et al., 2010).

Considering the good performances and the complementary action of Spinosad and Pyriproxyfen against the dengue vector *A.*

aegypti, a mixture of both compounds was tested against third instars of the Bora strain. The insecticide mixture showed very good efficacy against *A. aegypti* in terms of larvicidal effect and adult emergence inhibition. Similarly, Lee et al. (2005) have recently demonstrated that addition of pyriproxyfen to Bti formulations enhanced larval control and prevented adult emergence. In our study, the rapid killing effect of Bactimos on mosquito larvae allows to overcome the slow and specific action of Altosid or Dudim on larvae and adults. Larvae that emerged later and had not been affected by Bactimos were subsequently killed at the pupal or adult stage by the Altosid or Dudim. This combined action of Bactimos with Altosid or Dudim probably explains the strong synergism observed with the mixture at recommended dose. Such phenomenon may then improve the efficacy of the treatments in the field while substantially reducing the cost and toxicity as a result of a reduction of insecticide amounts. So far, synergism between bacterial and/or biological agents has been demonstrated in literature (Wirth et al., 2000; Corbel et al., 2002, 2003; Darriet et al., 2005) and is considered as an important parameter for controlling resistant pests and for slowing down the evolution of resistance (Curtis, 1985). Because *A. aegypti* populations are becoming more and more resistant to conventional and non conventional insecticides, alternative strategies have to be rapidly implemented in the future for dengue vector control. The combination of insecticides such as Bactimos with Altosid or Dudim maybe promising for controlling *A. aegypti* mosquito larvae provided that treatments persist at least during the whole dengue transmission season. Investigations should now be conducted under simulated field conditions to assess the efficacy and residual activity of such combinations in natural breeding sites of *A. aegypti*, as for example in domestic water storage containers, and pond water might be accumulated from rains, nursery, garden irrigation or stagnant water with house precincts especially those under construction which represent a primary larval habitat for *A. aegypti* in many countries affected by the disease.

References

- Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18, 256–269.
- Al-Azraqi, T.A., El Mekki, A.A., Mahfouz, A.A., 2013. Seroprevalence of dengue virus infection in Aseer and Jizan regions, Southwestern Saudi Arabia. *Trans. R. Soc. Trop. Med. Hyg.* 107, 368–371.
- Alkenani, N.A., AL-Ghamdi, K.H.M., Saleh, M.S., Mahyuob, J.A., 2015. Semi-field evaluation of some slow-release insecticide formulation against the dengue mosquito *Aedes aegypti* (L). *Alex. Sci. Exch. J.* 36, 157–162.

- Arivoli, S., Raveen, R., Samuel, Tennyson, Sakthivadivel, M., 2015. Adult emergence inhibition activity of *Cleistanthus collinus* (Roxb.) (Euphorbiaceae) leaf extracts against *Aedes aegypti* (L.), *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae). *Int. J. Mosq. Res.* 2, 24–28.
- Bhatt, S., Gething, P.W., Brady, O.J., Messina, J.P., Farlow, A.W., Moyes, C.L., Drake, J. M., Brownstein, J.S., Hoen, A.G., Sankoh, O., Myers, M.F., George, D.B., Jaenisch, T., Wint, G.R., Simmons, C.P., Scott, T.W., Farrar, J.J., Hay, S.I., 2013. The global distribution and burden of dengue. *Nature* 496, 504–507.
- Bond, J.G., Marina, C.F., Williams, C.F., 2004. The naturally derived insecticides Spinosad is highly toxic to *Aedes* and *Anopheles* mosquito larvae. *Med. Vet. Entomol.* 18, 50–56.
- Brown, A.W., 1986. Insecticide resistance in mosquitoes: a pragmatic review. *J. Am. Mosq. Control Assoc.* 2, 123–140.
- Chandler, D., Bailey, A.S., Tatchell, G., Davidson, G., Greaves, J., Grant, W.P., 2011. The development, regulation and use of biopesticides for integrated pest management. *Phil. Trans. Roy. Soc. B* 366, 1987–1998.
- Corbel, V., Darriet, F., Chandre, F., Hougard, J.M., 2002. Insecticide mixtures for mosquito net impregnation against malaria vectors. *Parasite* 9, 255–259.
- Corbel, V., Raymond, M., Chandre, F., Darriet, F., Hougard, J.M., 2003. Efficacy of insecticide mixtures against larvae of *Culex quinquefasciatus* (Say) (Diptera: Culicidae) resistant to pyrethroids and carbamates. *Pest Manag. Sci.* 60, 375–380.
- Cornel, A., Stanich, J., Farey, D., Mulligan, F.S., Hyde, G., 2000. Methoprene tolerance in *Aedes nigromoculis* in Fresno country, California. *J. Am. Mosq. Control. Assoc.* 16, 228–233.
- Curtis, C.F., 1985. Theoretical models of the use of insecticide mixtures for management of resistance. *Bull. Entomol. Res.* 75, 259–265.
- Darriet, F., Duchon, S., Hougard, J.M., 2005. Spinosad: a new larvicide against insecticide resistant mosquito larvae. *J. Am. Mosq. Control Assoc.* 21, 495–496.
- Darriet, F., Marcoinbe, S., Etienne, M., Yebakima, A., Agenew, P., Yptcha, M., Corbet, V., 2010. Field evaluation of pyriproxyfen and spinosad mixture for the control of insecticide-resistant *Aedes aegypti* in Martinique (French west Indies). *Parasit. Vectors* 3, 88.
- El-Badry, A.A., Al-Ali, K.H., 2010. Prevalence and seasonal distribution of dengue mosquito, *Aedes aegypti* (Diptera: Culicidae) in Al-Madinah Al-Munawwarah, Saudi Arabia. *J. Entomol.* 7, 80–88.
- Gamil, M.A., Eisa, Z.M., Eifan, S.A., Al-Sum, B.A., 2014. Prevalence of dengue fever in Jizan area, Saudi Arabia. *J. Pure Appl. Microbiol.* 8, 225–231.
- Govindarajan, M., Rajeswary, M., Amsath, A., 2013. Larvicidal properties of *Caesalpinia pulcherrima* (family: Fabaceae) against *Culex tritaeniorhynchus*, *Aedes albopictus* and *Anopheles subpictus* (Diptera: Culicidae). *Int. J. Pure Appl. Zool.* 1, 15–23.
- Jacups, S.P., Paton, C.J., Ritchie, S.A., 2014. Residual and pretreatment application of starycide insect growth regulator (triflumuron) to control *Aedes aegypti* in containers. *Pest Manag. Sci.* 70, 572–575.
- Jansen, C.C., Beebe, N.W., 2010. The dengue vector *Aedes aegypti*: what comes next. *Microbes Infect.* 14, 272–279.
- Jayapriya, G., Gricilda, S.F., 2015. Bioefficacy of leaf extracts of *Rhinacanthus Nasutus* Linn (Acanthaceae) against *Aedes Aegypti* Linn and *Culex Quinquefasciatus* Say. *IJPRBS* 4, 219–233.
- Khan, N.A., Azhar, E.I., El-Fiky, S., Madani, H.H., Abuljadal, M.A., Ashshi, A.M., Turkistani, A.M., Hamouh, E.A., 2008. Clinical profile and outcome of hospitalized patients during first outbreak of dengue in Makkah, Saudi Arabia. *Acta Trop.* 105, 39–44.
- Lee, Y.W., Zairi, J., Yap, H.H., Adanan, C.R., 2005. Integration of *Bacillus thuringiensis* H-14 formulations and pyriproxyfen for the control of larvae of *Aedes aegypti* and *Aedes albopictus*. *J. Am. Mosq. Control Assoc.* 21, 84–89.
- Marques, A.M., Kaplan, M.A.C., 2015. Active metabolites of the genus *Piper* against *Aedes aegypti*: natural alternative sources for dengue vector control. *Univ. Sci.* 20, 61–82.
- Mulla, M.S., Darwazeh H.A., Axelrod H., 1988. Activity of slow release formulations of IGRs fenxycarb and Altosid against mosquitoes and non-target aquatic organisms. In: *Proceeding and Papers of the 56th Ann. Conf. of California Mosquito and Vector Cont. Assoc.* pp. 184–191.
- Murray, B.I., 2006. Botanical Insecticides, Deterrents and Repellents in modern and an increasingly regulated world. *Annu. Rev. Entomol.* 51, 45–66.
- Pavela, R., 2008. Larvicidal effects of various Euro-Asiatic plants against *Culex quinquefasciatus* Say larvae (Diptera: Culicidae). *Parasitol. Res.* 102, 555–559.
- Pirali-Kheirabadi, K., da Silva, J.A.T., 2010. *Lavandula angustifolia* essential oil as a novel and promising natural candidate for tick (*Rhipicephalus (Boophilus) annulatus*) control. *Exp. Parasitol.* 126, 184–186.
- Ringu-Perez, J.G., Clark, G.G., Gubler, D.J., 1997. Dengue and Dengue hemorrhagic fever. *Lancet* 7, 352–971.
- Seng, C.M., Setha, T., Nealon, J., Socheat, D., Nathasn, M.B., 2008. Six months of *Aedes aegypti* control with a novel controlled-release formulations of pyriproxyfen in domestic water storage containers in Cambodia. *South. Asian J. Trop. Med. Pub. Health.* 39, 822–826.
- Shivakumar, M.S., Srinivasan, R., Natarajan, D., 2013. Larvicidal potential of some indian medicinal plant extracts against *Aedes aegypti* (L.). *Asian J. Pharm. Clin. Res.* 6, 77–80.
- Wirth, M.C., Federici, B.A., Walton, W.E., 2000. Cyt1A from *Bacillus thuringiensis* synergizes activity of *Bacillus sphaericus* against *Aedes aegypti*. *Appl. Environ. Microbiol.* 66, 1093–1097.
- World Health Organization, 1996. Report of the WHO informal consultation on the evaluation on the testing of insecticides, CTD/WHO PES/IC/96.1. WHO, Geneva, pp. 69.
- World Health Organization, 2005. Prevention and control dengue and dengue hemorrhagic fever. WHO, Regional publication, Serial no. 29, pp. 134.
- World Health Organization, 2011. World malaria report: 2011. WHO, Geneva.