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Laboratory and Field Evaluation of Some Chemical and Biological Larvicides against *Culex* spp. (Diptera: Culicidae) Immature Stages

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ABSTRACT

Efficiency and efficacy of three insecticides i.e., temephos, *Bacillus thuringiensis* var. *israelensis* (Bti) and pyriproxyfen against the immature stages of *Culex* spp. were evaluated under the laboratory and field conditions. LC_{50} values for pyriproxyfen, temephos and Bti under the laboratory conditions were 0.00079, 0.0059 and 0.012 mg L^{-1}, respectively. Under field conditions, temephos was the most effective insecticide, which caused 100% mortality of the larval population followed by Bti (60%), after one week of the first application. However, the mentioned mortality percentages decreased gradually to reach their minimum at the fifth week and then fluctuated to be 74% for temephos and 0.43% for Bti at the tenth week. After the second application at the tenth week, the same trend of temephos and Bti toxicity against larvae was observed. Pyriproxyfen caused 59.77% emergence inhibition against pupal population at the first week and gradually increased to reach 74% at the tenth week. Its effect reached 100% at the eleventh week after the second application and then gradually decreased to 36% at the fifteenth week. The results indicated the needs for decreasing the intervals between the first and the second application of all three insecticides to be 5 days for Bti and three weeks for both temephos and Pyriproxyfen, to keep the population of *Culex* spp. larvae under control. © 2011 Friends Science Publishers

Key Words: Mosquitoes; Control; Temephos; Bti; Pyriproxyfen; *Culex* spp.; Larvicides

INTRODUCTION

Culex spp. (Diptera: Culicidae) are the most widely distributed mosquito species in the world. They transmit many serious pathogens (filaria, West Nile virus & others) to man and his animals (Turell *et al.,* 2001; CDC, 2002; Cui *et al*., 2006; Kasai *et al*., 2008). *Culex* spp. are abundant in Riyadh, Kingdom of Saudi Arabia (Alahmed *et al*., 2007; Al-Khereji *et al*., 2007). They were found to be potential vectors of bancroftian filariasis (Omer, 1996).

Control of *Culex* spp. in their own breeding sites (water pools, sewer system with sewage water, drainage water, marches & ponds, etc.) is one of the important methods to reduce its population (Kettle, 1995). Conventional insecticides are progressively losing control over insect pests, because of insect resistance and public concern over the health hazards resulting from their use (Georghiou, 1983; Rawlines & Wan, 1995; Al-Sarar *et al*., 2005; Al-Sarar, 2010). Therefore, it was necessary to design various management programs, which include monitoring of resistance level, rotation of insecticides with different modes of action and evaluation of new compounds and/or formulations to control such insect pests (Georghiou, 1994). A wide range of synthetic and bio-insecticides has been tested and evaluated, either under laboratory or field conditions, to control the immature and/or adult mosquitoes (Dong-kayu, 2002; Thavara *et al*., 2004; Vilarinhos & Monnerat, 2004; Lee *et al*., 2005; Liu & Dean, 2006; Al-Sarar & Al-Shahrany, 2008).

This study was conducted to evaluate the insecticidal efficacy of three different agents: *Bacillus thuringiensis* var. *israelensis* as biolarvicide, pyriproxyfen, as juvenile hormone mimic and temephos as organophosphorus insecticide against *Cx. pipiens* susceptible strain under laboratory conditions and against field population of *Culex* spp. under field conditions. These three insecticides have been evaluated in different regions worldwide (Mulla *et al*., 1986; Schaefer *et al.,* 1988; Kawakami, 1989; Gunasekaran *et al*., 2004). This is the first time to evaluate the efficacy of these three insecticides against field mosquito strains in the region of Riyadh city, Kingdom of Saudi Arabia. The trials were conducted during the period from August 2007 to January 2008.

MATERIALS AND METHODS

Mosquitoes: Laboratory strain of *Cx. pipiens* L. was obtained from the High Institute of Public Health,

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Alexandria University, Egypt and established in the insectaria of Plant Protection Department, King Saud University, for nine years under 25±2ºC and 12/12 h light/dark photoperiod. Larvae were provided rabbit pellets and adults were provided a 10% sugar solution *ad libitum* and blood meals for 30 min, two times per week.

Insecticides used: Three commercial insecticides were tested in this work i.e., *Bacillus thuringiensis* var. *israelensis* in form of Dispersible Wettable Powder (Bti® 1200 IU, DWP, Longxiang Chemistry Co., China), pyriproxyfen granules (Sumilarv® 0.5% G, Sumitomo Chemical Co. Osaka, Japan) and temephos formulated in Water-Dispersible-Granules (Abate® 5%,WDG, Sumitomo Chemical Co. Osaka, Japan).

Laboratory bioassay procedure: The selected insecticides were evaluated against the 4th instar larvae using the standard bioassay technique (WHO, 1981) performed in single use cups containing 100 mL aqueous media. Temephos, pyriproxifen and Bti formulations were tested at concentrations ranged from 0.1 to 0.00001 mg/L in pure water. Each bioassay was conducted in triplicate, 20 starved larvae in each replicate. After 24 h of exposure, the LC_{50} values were calculated according to the method of Finney (1971), using the computer program Sigma Plot for Windows, Version 2.0, based on the Abbott's formula corrected mortality percentage (Abbott, 1925).

Field evaluation procedure: The efficacy of the insecticides was evaluated against the field population of *Culex* spp. The results obtained by Al-Khereji *et al.* (2007) showed that more than 97% of adult mosquitoes in Riyadh City belonged to *Culex* spp. The tested insecticides were applied at the recommended rates i.e., 2.5 g/m^3 for Bti, 6.0 g/m^3 for pyriproxyfen and 1.8 g/m^3 for temephos. Applications of the mentioned insecticides were conducted in small screen-covered test plots as same as mentioned by Floore *et al.* (2004). Twelve test plots, three replicates plus one plot as control for each insecticide were built up. The dimensions of each test plot were (1.2 m × 1.2 m × 0.5 m) and the concrete was used to cover the internal walls, including the bottom. After filling the test plots with water, the level of water height was maintained all over the experimental period. The natural presence of *Culex* mosquito larvae was monitored and counted during 45 days before beginning the study. At the end of 45 days, all the plots were covered with nets. Ten samples of the test plots water were weekly collected randomly from each plot using standard dipper of 300 mL capacity. The temperatures during the experimental periods were ranged from 38°C (August, 2007) to 18°C (January, 2008).

In case of the plots treated with pyriproxyfen, 100 pupae were collected weekly from each test plot and maintained in larval breeder 1000 mL capacity. Survival in both treated and control containers were determined by counting the number of adults that had successfully emerged from the pupal exuviae. The efficacy of the formulation through time was assessed as percentage of emergence inhibition (% EI) in treatment adjusted for any pupal mortality in the control according to the following formula, which described by Floore *et al.* (1991).

$$\% \text{ EI} = 100 - \left(\frac{\text{CS - DA}}{\text{CS + PE + DP}} \times 100 \right)$$

Where (CS) the number of cast pupae skin, (DP) dead pupae, (PE) partially emerged adults and (DA) dead adults.

In the experiments of temephos and Bti, the mosquito larval densities were measured weekly through taking 10 dips using a standard larval dipper of 300 mL capacity. On the basis of the reappearance of the 3rd and 4th instar larvae or pupae, a second round of application was carried out. Data were analyzed to measure reduction in the average densities of 3rd and 4th instars in comparison with untreated controls using the following formula of Haq *et al.* (2004):

$$\% \text{ Reduction} = 100 - [(C_1/T_1) \times (T_2/C_2)] \times 100$$

Where, C_1 and C_2 are densities of 3rd and 4th instars in untreated control on day 0 and on subsequent days of monitoring, while T_1 and T_2 in treated habitats represent instars before and after treatment, respectively. After calculation of the reduction percentages, the resultant values were subtracted from 100 and proportioned to the control value to calculate the percentages of population.

Statistical analysis: For larval bioassay under laboratory conditions, the differences between the LC_{50} values are considered significant if their fiducial limits (95%) did not overlap as mentioned by Litchfield and Wilcoxin (1949). In addition, statistical analysis was carried out for all the estimated measurements of treatments and compared with the control values by Student's *t*-test using the computer program (Sigma Plot for Windows, version 2.0).

RESULTS

The efficiency of tested insecticides: Data in Table I shows the LC_{50} values for the tested insecticides, temephos, Bti and pyriproxyfen against the 4th instar larvae of *Cx. pipiens* susceptible strain. Temephos, Bti and pyriproxyfen showed LC_{50} values as 0.0059, 0.012 and 0.000079 mg/L, respectively. According to the mentioned results, the juvenile hormone mimic pyriproxyfen, was the most effective insecticide followed by temephos and Bti against mosquito larvae.

The field efficacy of the selected insecticides: Table II shows the means of the larval population percentage of *Culex* spp. at different periods of application of temephos and Bti and the percentage of emergence inhibition of pupae as effect of the juvenile hormone mimic, pyriproxyfen, under the field conditions. The larval population was significantly decreased after the first week of application by temephos and Bti to be 0.97 and 40.04% of the larval population in the untreated control, respectively. The larval population increased gradually to reach its maximum (43.27

Table I: The determined toxicity values (LC_{50}'s) expressed as (mg/L) of the tested insecticides against the 4th instar larvae of *Cx. pipiens* and their corresponding confidence limits at (95%), qui square (X^2) values and the slope of the plotted toxicity regression lines

Tested insecticide	LC_{50} (mg/L)	Confidence limit (95%)	Slope ± SD.	X^2
Temephos	0.0059	0.0051-0.0071	3.752 ± 0.476	6.57
Bti	0.012	0.0018-0.056	0.357 ± 0.023	232.38
Pyriproxyfen	0.000079	0.000055-0.0001	0.909 ± 0.121	9.649

& 95.11%) of the control value for temephos and Bti at the 4th and 5th week of application, respectively. After that, the population slightly decreased ranging between 39.54 to 30.33% for temephos and between 91 to 99% for Bti during the period of 5th to 9th week of application.

At the tenth week of the experiment, when the larval population reached to 25.97% in the plots treated with temephos and 99.57% in the plots treated with Bti, both of two plots were treated with the tested insecticides for the second time. After one week of the second application, the same trend was observed i.e., the larval population markedly reduced to be 0.00 and 34.32% for temephos and Bt., respectively. The larval population increased gradually with time to 64.22 and 99% at the 15th week for temephos and Bt., respectively. After that, the larval population was fluctuated, up to the 20th week, between 43.88 to 25% for temephos treatment and between 98 to 100% for the Bti treatment.

Pyriproxyfen caused a significant inhibition of the pupal population, which was 40.23% at the first week after application. It decreased gradually to reach 25% at the 9th and 10th weeks of application. When this compound was applied for the second time, the pupal population dramatically decreased to 0.00% at second week of the second application. At the 12th week, the pupal population started to increase (19.21%) and such increasing was elevated gradually and ranged between 63.24 and 25.76% of the control value from the 15th week to the 20th week. Fig. 1 and 2 give a quick idea about the population densities of mosquito larvae and the percentage of adult emergence throughout the 20 weeks as a result of the first and second treatments with the three tested insecticides.

DISCUSSION

The obtained results of the efficiency of tested insecticides were in agreement with those data published by other investigators, the LC_{50} of temephos against the 4th instar larvae of susceptible strain of *Cx. pipiens* ranged between 0.005 to 0.007 mg/L (Kawakami, 1989; Rey *et al*., 2003). In case of pyriproxyfen, the determined LC_{50} value was in agreement with Mulla *et al*. (1986) and Ali *et al*. (1999), who reported LC_{50} values of this compound ranging between 0.000018-0.00042 mg/L, while completely different LC_{50} values, ranging between 0.000021 to

Fig. 1: The mortality percent of the larval population of *Culex* spp. after different periods of application by the tested insecticides (temephos and Bti) during the period Aug 2007/Jan 2008. The arrows indicate to the second application (at the tenth week) of the tested insecticides. Each value represents an average of three replicates ± SD

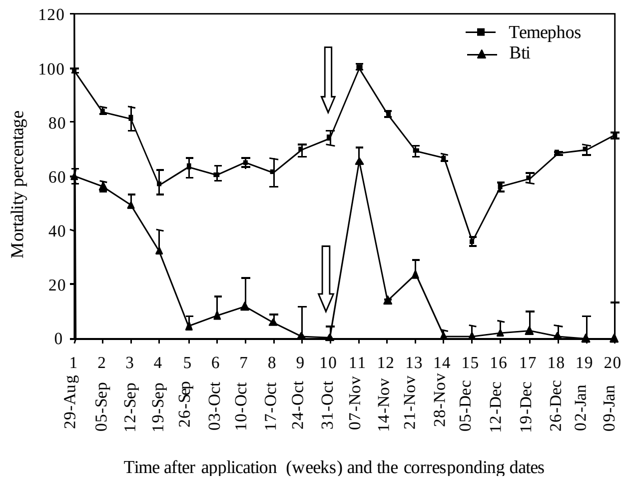
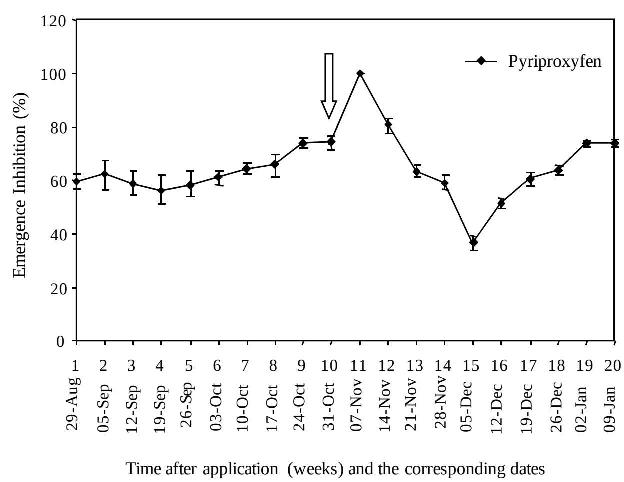


Fig. 2: The emergence inhibition percent of the pupal population of *Culex* spp. after different periods of application by the tested insecticide pyriproxyfen during the period Aug 2007/Jan 2008. The arrows indicate to the second application (at the tenth week) of the tested insecticides. Each value represents an average of three replicates ± SD



0.000029 mg/L against *Cx. Pipiens* were reported by other workers (Kawada *et al*., 1994; Schaefer *et al*., 1988; EL-Shazly & Refaie, 2002). As for the tested biolarvicide, Bti, various laboratories revealed that the LC_{50} value of this bioagent against larvae of *Cx. pipiens* ranged between 0.012 to 0.018 mg/L, which is in good agreement with our results (Hilmy & Medran, 1985; Poopathi *et al*., 2004).

Table II: Means of the larval population percentage of *Culex* spp. after different periods of application of temephos and Bti and the percentage of emergence inhibition of pupae as effect of the juvenile hormone mimic, pyriproxyfen in the enclosed tested plots

Time after application (Week)	Larval population (%)		Pupal population (%)	Control	Temperature (°C)
	Temephos	Bti	Pyriproxyfen		
1	0.97*** ± 0.90	40.04** ± 2.80	40.23** ± 2.80	100	38.4
2	15.97*** ± 1.20	43.70** ± 3.40	37.61** ± 5.20	100	35.2
3	18.72*** ± 4.40	50.49** ± 3.70	41.13** ± 4.50	100	34.3
4	43.27** ± 5.60	67.54* ± 7.70	43.86** ± 2.10	100	33.5
5	36.70** ± 3.70	95.11 ± 3.40	41.59** ± 4.40	100	33.5
6	39.54** ± 6.50	91.50 ± 7.10	38.89** ± 2.90	100	32.8
7	34.86** ± 1.60	88.03 ± 10.50	35.56** ± 1.40	100	30.1
8	38.70** ± 5.10	93.87 ± 2.80	34.10** ± 3.70	100	29.5
9	30.33** ± 2.30	99.18 ± 10.70	25.82** ± 1.90	100	28.3
10 (SA)	25.97** ± 2.40	99.57 ± 4.00	25.54** ± 4.80	100	27.0
11	0.00*** ± 0.00	34.32** ± 4.80	0.00*** ± 0.00	100	27.0
12	17.22*** ± 0.60	86.09* ± 0.70	19.21*** ± 2.80	100	23.5
13	30.77** ± 2.00	76.28* ± 5.20	36.54** ± 2.30	100	17.0
14	33.15** ± 1.30	99.00 ± 2.10	40.88** ± 2.55	100	17.2
15	64.22** ± 1.60	99.00 ± 3.80	63.24* ± 2.60	100	19.5
16	43.88** ± 1.70	98.00 ± 4.40	48.52** ± 1.80	100	16.5
17	40.91** ± 1.70	97.00 ± 7.00	39.26** ± 1.90	100	16.0
18	31.65** ± 0.70	99.00 ± 3.70	36.29** ± 1.70	100	12.5
19	30.42** ± 1.90	100.00 ± 7.90	25.83*** ± 0.90	100	18.0
20	24.89*** ± 0.20	100.00 ± 13.00	25.76*** ± 1.00	100	18.0

(SA): Second Application. Each value represents an average of three replicates ± S.D. Comparing to the control value, ***: Highly significant ($P \geq 0.001$), **: Moderately significant ($P \geq 0.01$), *: Significant ($P \geq 0.05$) (Student *t*-test)

Field efficacy of tested insecticides showed that temephos was the highest effective in reducing the larval population density in the treated plots. Such efficacy may be due to the fact that temephos is an organophosphorus insecticide, which has neurotoxic effect. Comparing with the other tested agents, it is expected that such compound exhibit the higher efficiency either under laboratory or field evaluation. On the other hand, such high efficacy was prolonged only through the first three weeks, which lead to the necessity to repeat the application for the second time at the tenth week. Actually, such observation may be due to its low persistence in the environmental components such as water, which may be attributed to the decomposition of the compound caused by hydrolysis reaction (Smith, 1993). In addition, in simulated tide pools the compound persisted for up to four days. It also persisted in oysters for two days after application.

In case of the tested biolarvicidal agent Bti, our results agree with Gunasekaran *et al*. (2004) who reported that Bti is effective against *Cx. quinqefasciatus* for up to 3 days only in drains. Formulations of Bti spores and crystals encapsulated in starch lost all spore viability and insecticidal activity within 4 days (Dunkle & Shasha, 1989). Therefore, we recommend, based on our results and the results of the above mentioned workers, that application of Bti to outdoor waters should be every 5 days for sustained control of mosquito larvae.

Pyriproxyfen is a juvenile hormone mimic which interferes with the hormonal balance of the metamorphosis of the targeted insect pest, which affects in turn the moulting process in form of inhibiting the adult emergence from the treated pupae. However, in highly polluted water, pyriproxyfen readily adsorbed onto organic matter and its biological activity persisted for two months after an initial application at rate of 0.1 lb/acre. Its persistence in water in the absence of organic matter declined as temperature and sunlight exposure increased (Schaefer *et al.,* 1988); this finding explains the results obtained in the current study. Pyriproxyfen was less effective at high temperatures (Table II), at 30-38^{o}C it caused only 57-65% inhibition of pupal emergence, while at low temperatures (23-28^{o}C) it caused 75-100% inhibition of pupal emergence. Jambulingam *et al.* (2008) reported that, in field experiments, pyriproxyfen was effective against *Culex* spp. Up to four weeks, which agrees with the present results.

In conclusion, our result revealed that the intervals between first and second application for all three insecticides should be 5 days for Bti and three weeks for both temephos and Pyriproxyfen to keep the population of *Culex* spp. larvae under control. Additionally, the high temperature in Riyadh city during the summer season should be considered when applying larvicides outdoors to control mosquito larvae.

REFERENCES

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.,* 18: 265–267

Alahmed, A.M., M.A. Al-Khereji and S.M. Kheir, 2007. Distribution and habitats of mosquito larvae (Diptera: Culicidae) in Riyadh region, Saudi Arabia. *J. King Saud Univ. Agric. Sci.,* 19: 39–55

Al-Khereji, M.A., A.M. Alahmed and S.M. Kheir, 2007. Survey and seasonal activity of adult mosquitoes (Diptera: Culicidae) in Riyadh City, Saudi Arabia. *Food Sci. Agric. Res. Centre,* 152: 5–17

Ali, A., M.A. Chowdhury, M.L. Hosain, U.A. Mahmud, D.B. Habiba and A.F. Aslam, 1999. Laboratory evaluation of selected larvicides against field-collected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *J. American Mosq. Cont. Assoc.*, 15: 43–47

Al–Sarar, A.S., 2010. Insecticide resistance in *Culex pipiens* (L.) populations (Diptera: Culicidae) from Riyadh, Saudi Arabia: Status and overcome. *J. Saudi Soc. Biol. Sci.*, 17: 95–100

Al-Sarar, A.S. and Z. Al-Shahrany, 2008. Laboratory evaluation of Some chemical and biological insecticides against the fourth instar larvae of *Culex pipiens* (Linn) (Diptera : Culicidae). *J. Adv. Agric. Res. Fac. Saba Basha*, 13: 597–603

Al-Sarar, A.S., H.I. Hussein, D. Al-Rajhi and S. Al-Mohaimeed, 2005. Susceptibility of Culex pipiens from different locations in Riyadh City to insecticides used to control mosquitoes in Saudi Arabia. *J. Pest Cont. Environ. Sci.*, 13: 79–88

CDC, 2002. Provisional surveillance summary of the West Nile Virus epidemic- US, Morb. Mort. *Week. Rep.*, 51: 1129–1133

Cui, F., L.F. Lin, C.L. Qiao, U. Xu, M. Marquine, M. Weill and M. Raymond, 2006. Insecticide resistance in Chinese populations of the *Culex pipiens* complex through esterase overproduction. *Entomol. Exp. Appl.*, 120: 211–220

Dong-kayu, L., 2002. Mosquito control evaluation of an insect growth regulator, pyriproxyfen against *Culx pipiens pallens* (Diptera: Culicidae) larvae in Marsh Area, Korea. *Korean J. Entomol.*, 32: 37–41

Dunkle, R.L. and B.S. Shasha, 1989. Response of starch-encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. *Environ. Entomol.*, 18: 1035–1041

EL-Shazly, M.M. and B.M. Refaie, 2002. Larvicidal effect of the juvenile hormone mimic pyriproxyfen on *culex pipiens*. *J. American Mosq. Cont. Assoc.*, 18: 321–332

Finney, D.J., 1971. *Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve*, p: 33. Cambridge University Press, London

Floore, T.G., J.L. Petersen and K.R. Shaffer, 2004. Efficacy studies of Vecobac® 12AS and Teknar® HP-D larvicides against 3rd instar *Ochlerotatus taeniorhynchus* and *Culex quinquefasciatus* in small plot field studies. *J. American Mosq. Cont. Assoc*., 20: 429–433

Floore, T.G., C.B. Rathburn, J.C. Dukes, B.W. Clementes and A.H. Boike, 1991. Control of *Aedes taeniorhynchus* and *Culex quinquefasciatus* emergence with sustained release Altoside sand granules and pellets in saltwater and fresh water test plot. *J. American Mosq. Cont. Assoc*., 7: 405–408

Georghiou, G.P., 1983. Management of resistance in arthropods. *In*: Georghiou, G.P. and T. Saito (eds.), *Pest Resistance to Pesticides*, pp: 761–791. Plenum Publishing Corp., New York

Georghiou, G.P., 1994. Principles of insecticide resistance management. *Phytoprotection*, 75: 51–59

Gunasekaran, K., P.S. Boopathi Doss and K. Vaidyanathan, 2004. Laboratory and field evaluation of Teknar HP-D, a biolarvicidal formulation of *Bacillus thuringiensis* ssp. *israelensis*, against mosquito vectors. *Acta Trop.*, 92: 109–118

Haq, S.R., M. Bhatt, K.G. Vaishna and R.S. Yadav, 2004. Field evaluation of biolarvicides in Surat city, India. *J. Vect. Borne Dis*., 41: 61–66

Hilmy, N.M. and A.I. Medran, 1985. Larvicidal activity of new isolation of *Bacillus thuringiensis* serotype H-14 on certain Egyptian mosquito species. *J. Egyptian Soc. Parasitol.*, 15: 263–271

Jambulingam, P., C. Sadanandane, P.S. Boopathi Doss, S. Subramanian and M. Zaim, 2008. Field evaluation of an insect growth regulator, pyriproxyfen 0.5% GR against C*ulex quinquefasciatus*, the vector of *Bancroftian* filariasis in Pondicherry, India. *Acta Trop.*, 107: 20–24

Kasai, S., O. Komagata, T. Tomita, K. Sawabe, Y. Tsuda, H. Kurahashi, T. Ishikawa, M. Motoki, T. Takahashi, T. Tanikawa, M. Yoshida, G. Shinjo, T. Hashimoto, Y. Higa, and M. Kobayashi, 2008. PCR-based identification of *Culex pipiens* complex collected in Japan. *Japanese J. Infect. Dis.*, 61: 184–191

Kawada, H., T. Kohama and Y. Abe, 1994. Larvicideing activity of a water-soluble granular formulation of the insect growth regulator, pyriproxyfen against *Culex* mosquito. *Japanese J. Environ. Entomol. Zool.*, 6: 77–86

Kawakami, Y., 1989. Insecticide resistance of *Culex pipiens molestus* Forskal collected in Shinjukuku, Tokyo. *Japan J. Sanit. Zool.*, 40: 217–220

Kettle, D.S., 1995. *Medical and Veterinary Entomology*, 2nd edition. CAB International, Cambridge, United Kingdom

Lee, Y.W., J. Zairi, H.H. Yap and C.R. Adanan, 2005. Integration of *Bacillus thuringiensis* H-14 formulations and pyriproxyfen for the control of larvae of *Aedes aegypti* and *Aedes albopictus. J. American Mosq. Cont. Assoc.*, 21: 84–89

Litchfield, J.T. and F.A. Wilcoxin, 1949. Simplified method of evaluating dose-effect experiment. *J. Pharm. Exp. Theor.*, 96: 99–103

Liu, X.S. and D.H. Dean, 2006. Redesigning *Bacillus thuringiensis* CryI Aa toxin into a mosquito toxin. *Protein Eng. Design Select.*, 19: 107–111

Mulla, M.S., H.A. Darwazeh, B. Kennedy and M. Dawson, 1986. Evaluation of new insect growth regulators against mosquitoes with notes on nontarget organisms. *J. American Mosq. Cont. Assoc*., 2: 314–320

Omer, M.S., 1996. A survey of bancroftian filariasis among South-East Asian expatriate workers in Saudi Arabia. *Trop. Med. Inter. Heal.*, 1: 155–160

Poopathi, H., M. Subbiah and B.K. Tyagi, 2004. Mosquitocidal toxins of spore forming bacteria: recent advancement. *African J. Biotech.*, 3: 643–650

Rawlines, S.C. and J.O. Wan, 1995. Resistance in some Caribbean populations of *Ae. aegypti* to several insecticides. *J. American Mosq. Cont. Assoc.*, 11: 59–65

Rey, D., D.J. Philipe and J.C. Meyran, 2003. Factors influencing the toxicity of xenobiotics against larval mosquitoes. Comp. Rend. *C.R. Biologies*, 326: 317–327

Schaefer, C.H., T. Miura, E.F. Dupras, F.S. Mulligan and W.H. Wilder, 1988. Efficacy, nontarget effects, and chemical persistence of S-31183, a promising mosquito (Diptera:Culicidae) Control Agent. *J. Econ. Entomol.*, 81: 1648–1655

Smith, G.J., 1993. *Toxicology and Pesticide Use in Relation to Wildlife: Organophosphorous and Carbamate Compounds*. U.S. Department of the Interior, C.K. Smoley, Boca Raton, Florida

Thavara, U., A. Tawatsin, W. Kong-ngamsuk and M. Mulla, 2004. Efficacy and longevity of a new formulation of temephos larvicide tested in village-scale trials against larval *Aedes aegypti* in water-storage containers. *J. American Mosq. Cont. Assoc*., 20: 176–182

Turell, M.J., M.L. O'Guinn, D.J. Dohm and J.W. Jones, 2001. Vector competence of North American mosquitoes (Diptera: Culicidar) for West Nile Virus. *J. Med. Entomol*., 38: 130–134

Vilarinhos, P.T.R. and R. Monnerat, 2004. Larvicidal persistence of formulations of *Bacillus thuringiensis var. israelensis* to control larval *Aedes aegypti*. *J. American Mosq. Cont. Assoc.*, 20: 311–314

WHO, 1981. *Instructions for Determining the Susceptibility or Resistance of Mosquito Larvae to Insecticides*. WHO/VBC/81, 807, World Health Organization, Geneva, Switzerland

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