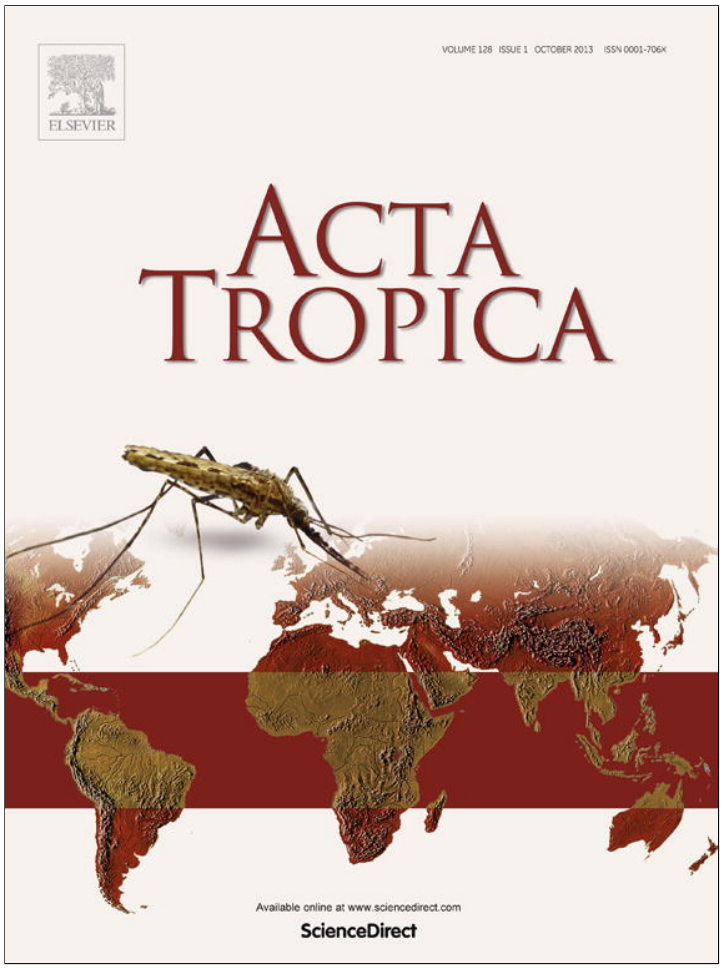

Acta Tropica 128 (2013) 103–109

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Ovicidal activity of three insect growth regulators against *Aedes* and *Culex* mosquitoes

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ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 21 June 2013
Accepted 30 June 2013
Available online 13 July 2013

Keywords:
Mosquitoes
Egg hatching
Insect growth regulators
Ovicidal activity
Egg desiccation
Interspecific variations

ABSTRACT

Interspecific variations in the susceptibility of freshly and embryonated eggs of *Aedes albopictus*, *Ae. aegypti*, *Ae. atropalpus* and *Culex pipiens* were tested against three classes of insect growth regulators (IGRs) including ecdysone agonist (azadirachtin), chitin synthesis inhibitor (diflubenzuron) and juvenile hormone analog (pyriproxyfen) at 0.001, 0.01, 0.1 and 1.0 ppm concentrations. Egg hatching inhibition was dose dependent, the highest being at 1.0 ppm concentration for freshly laid eggs of *Ae. albopictus* (pyriproxyfen: 80.6%, azadirachtin: 42.9% and diflubenzuron: 35.8%). *Aedes aegypti* showed lower egg hatching inhibition when exposed to pyriproxyfen (47.3%), azadirachtin (15.7%) and diflubenzuron (25.5%). Freshly laid eggs of *Cx. pipiens* were most susceptible to diflubenzuron. *Aedes atropalpus* eggs were tolerant to all three classes of IGRs. Embryonated eggs of *Ae. albopictus*, *Ae. aegypti*, *Ae. atropalpus* and *Cx. pipiens* were resistant to pyriproxyfen, azadirachtin and diflubenzuron than freshly laid eggs. The median desiccation time (DT_{50}) of *Ae. atropalpus* eggs was maximum (5.1 h) as compared to *Ae. aegypti* (4.9 h), *Ae. albopictus* (3.9 h) or *Cx. pipiens* (1.7 h) eggs. Insignificant relationship between the rates of desiccation and egg hatching inhibition suggests other factors than physical providing eggs the ability to tolerate exposures to various IGRs. Egg hatching inhibition was due to the alteration in embryonic development caused by IGRs. Changes in the egg shell morphology and abnormal egg hatching from the side of the egg wall instead of operculum, was observed at higher concentrations of diflubenzuron. Morphological and physiological variations in eggs may be the key factor to influence the ovicidal efficacy of IGRs. The present data provide a base line for the improvement of the ovicidal efficacy of the insecticide and its formulation.

1. Introduction

Mosquitoes are important vectors of dengue, malaria, chikungunya, yellow fever and many other diseases, causing more than a million deaths worldwide (WHO, 1995, 2012; AMCA, 2012). Globally, vector borne diseases control is focused on the management of the adult and larval populations using insecticides with other components in various management regimes (WHO, 1995). However, due to extensive use of these insecticides, mosquito vectors have developed resistance against many classes of insecticides (Brogdon and McAllister, 2004). As a result, many mosquito borne diseases re-emerged resulting in increased morbidity and mortality in human population (Nauen, 2007).

Insect growth regulators are comparatively safer to non-target organisms (Mulla, 1995) and have been recommended for mosquito control (WHO, 2006). Growth regulators include chemicals with unique mode of actions such as juvenile hormone analog, chitin synthesis inhibitor, ecdysone agonist (Mulla and Su, 1999; Mordue et al., 2005; Soin et al., 2010). For instance, some insect growth regulators such as methoprene, pyriproxyfen and diflubenzuron are registered as mosquito larvicides (WHO, 2006). Insect growth regulators have shown significant larvicidal efficacy against *Aedes albopictus* (Skuse) mosquito at low lethal doses as compared to microbial, organophosphates and synthetic pyrethroids insecticides (Ali et al., 1995). Few studies have also shown disrupted hormonal balance inside developing embryo (Berger and Dubrovsky, 2005). Partial exposure of *Culex quinquefasciatus* (Say) and *Aedes aegypti* (L.) eggs were observed to various doses of insect growth regulators (Miura et al., 1976; Vasuki, 1990; Su and Mulla, 1998; Umar et al., 2007). Several other groups (Zahiri and Mulla, 2006; Albernaz et al., 2009; Govindarajan et al., 2008) tested low doses of microbial, fungal and plant products to inhibit egg hatch but none of these products were effective as ovicide.

In eggs, multiple chorionic layers are developed during embryonic development. Some layers are rigid that provide mechanical support and protection whereas others provide adequate nourishment to the developing embryo (Clements, 1992). Egg morphology reflects different oviposition behaviors and habitats of mosquitoes

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http://dx.doi.org/10.1016/j.actatropica.2013.06.025

(Carpenter and LaCasse, 1955; Hawley, 1988; Clements, 1992; Suman et al., 2008, 2011). The surface morphology, physical structure and chemical composition of the eggs determine the ability of eggs to adapt and tolerate adverse conditions such as desiccation (Jagadeeshan and Singh, 2007; Sota and Mogi, 1992). In order to control surge in mosquito populations freshly laid and/or early developing eggs can be treated with and tested against insect growth regulators.

Since their introduction to agrochemical market, insect growth regulators have been widely tested for their larvicidal properties against various insect pests, in both the laboratory and field conditions (Charmillot et al., 2001; Sihuincha et al., 2005). Although, several studies were made to determine the effects of insect growth regulators on mosquito larvae (*e.g.*, *Ae. aegypti*; *Ae. albopictus*) (Ali et al., 1995; Chism and Apperson, 2003; Sihuincha et al., 2005) but the research on ovicidal efficacy against mosquitoes is still lacking.

For the first time, we evaluated ovicidal efficacy of three classes of insect growth regulators against mosquito eggs. Ovicidal efficacy of the three classes of insect growth regulators, *e.g.*, chitin synthesis inhibitor (diflubenzuron), juvenile hormone analog (pyriproxyfen) and ecdysone agonist (azadirachtin) were tested against freshly laid and embryonated eggs of *Ae. aegypti*, *Ae. albopictus*, *Ae. atropalpus* (Coquillett) and *Culex pipiens* (L.). The former two species lay individual eggs at the edges of the water surface (Hawley, 1988), whereas, *Ae. atropalpus* deposits egg in patches at the sides of its habitat (Hedeen, 1953). *Culex pipiens* lays eggs which form egg raft to float on the water surface (Carpenter and LaCasse, 1955; Clements, 1992). We hypothesize that variations in egg morphology and oviposition behaviors determines ovicidal efficacy of insect growth regulators and that the embryonated eggs are less susceptible to insect growth regulators than the freshly laid eggs. We have also tested our secondary hypothesis that highly water permeable eggs are more susceptible to insect growth regulators.

2. Materials and methods

2.1. Mosquito rearing

All experiments were conducted with freshly laid and embryonated eggs obtained from four species of mosquitoes, *i.e.*, *Ae. aegypti*, *Ae. albopictus*, *Ae. atropalpus* and *Cx. pipiens*. Mosquito colonies were maintained at 26 ± 2 °C, 65% RH, and 16L: 8D photoperiods. Mosquito larvae were reared into dechlorinated tap water in 2 L enamel tray and rat chow provided as a larval food. Once emerged the adults were transferred to cages (1 m^3) holding 10% aqueous sugar solution, a food source for adults. After 5–7 days of emergence, adult individuals of *Aedes* spp., were blood fed on guinea pig whereas *Cx. pipiens* on quail. During blood feeding the animals were cared for as per Animal Use Protocol #86–129 of the Rutgers University.

2.2. Insect growth regulators

Ovicidal efficacy of the three classes of insect growth regulators, *i.e.*, a chitin synthesis inhibitor (diflubenzuron: Adept®, WP 25%, Chemtura USA Corp., Middlebury, CT, USA), a juvenile hormone analog (pyriproxyfen: NyGuard® EC 10, MGK®, Minneapolis, MN, USA), and an ecdysone agonist (azadirachtin: Azatin XL®, EC 3, OHP Inc., Mainland, PA, USA) was determined. The fresh 1% stock solution of each class of insect growth regulator was prepared in dechlorinated tap water. Eggs were exposed to 1.0, 0.1, 0.01 and 0.001 ppm concentrations.

2.3. Egg exposure to insect growth regulators

Freshly laid eggs were exposed to insect growth regulators by allowing seven gravid females in an oviposition chamber containing previously insect growth regulator treated water. The chamber was constructed by joining two deli cups (450 mL capacity and 9 cm diameter) with glue. Two holes, one for aeration and another to hold the cotton roll were made on the cover of the chamber. Cotton roll soaked with 10% sugar solution served as adult mosquito food. Whatman® filter paper #1 (4 cm in diameter) was oviposition substrate for all *Aedes* spp., whereas, none was used for *Cx. pipiens*, since it lays eggs on the water surface directly. The eggs were left in the water until they hatched. Dried brewer's yeast powder (15 mg/chamber) served as the food for the emerging larvae. The same batches of mosquitoes were allowed to lay eggs in untreated water for the control experiments.

Freshly laid eggs of *Aedes* and *Culex* species were incubated at 24 ± 1 °C and 75–80% RH for 48 h to obtain embryonated eggs. Embryonated eggs were treated with different concentrations (0.001–1.0 ppm) of insect growth regulators. Embryonated eggs from the same batch unexposed to any chemicals served as control.

The ovicidal effects of insect growth regulators were observed on freshly laid and embryonated eggs of *Aedes* spp. (50–100 eggs/replicate) and *Cx. pipiens* (100–170 eggs/raft/replicate). Control experiments were conducted in dechlorinated tap water. All experiments were performed in three replicates; each experiment was repeated three times. All experimental conditions were the same unless reported otherwise.

2.4. Egg hatching inhibition

The first instars emerged from the eggs were counted under a dissecting microscope before removing them from the chamber with the help of a pipette on a daily basis. The unhatched freshly laid and embryonated eggs were bleached with 20% Clorox® Regular-Bleach (6% sodium hypochlorite) (Clorox®, Corporate Head Quarters, Oakland, CA, USA) and observed under dissecting microscope for any morphological abnormalities that may have been caused from the exposure of insect growth regulators. The eggs that did not hatch even after seven days of exposure test samples were considered as dead.

Egg hatching inhibition was calculated as the percentage of unhatched eggs. The percentage of unhatched eggs in control experiments was adjusted with treatments by using Abbott's formula (Abbott, 1925). Control experiments with more than 20% egg mortality were discarded. One-way ANOVA (significant at $p \leq 0.05$), Fisher's least significant difference (LSD) and *T*-test were applied to compare the results ($p \leq 0.05$).

2.5. Effects on embryonic development and hatching pattern

To determine the effects of insect growth regulators on embryonic development, both treated and untreated freshly laid and embryonated eggs were exposed to 20% Clorox® Regular-Bleach for 10–20 min to make egg shell transparent. The bleached eggs were examined under dissecting microscope for any morphological changes in egg shell, abnormal egg hatching or deformed developmental patterns.

2.6. Effects of water permeability on eggs hatching

Approximately, 2 h old eggs of *Ae. aegypti, Ae. albopictus*, *Ae. atropalpus*, and *Cx. pipiens* were obtained by the methods as described above. A highly dense area of *Aedes* eggs was located on the paper substrate (>50 eggs/cm^2), blotted with paper towel for 5 min to remove extra water (Bounty® Paper Towels, USA). Then,

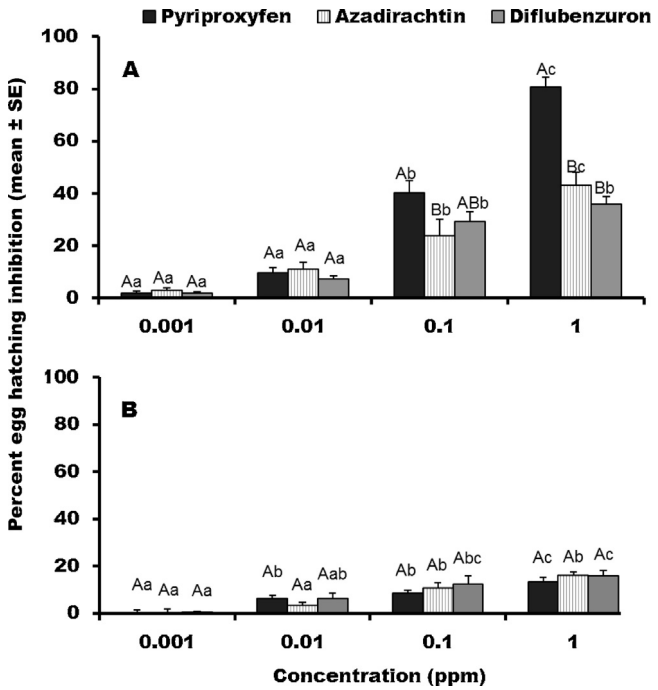

Fig. 1. Effects of pyriproxyfen, azadirachtin and diflubenzuron insect growth regulators on egg hatching inhibition of freshly laid (A) and embryonated eggs (B) of *Aedes albopictus*. Significant differences among different insect growth regulators for individual concentrations are denoted by upper case and among various concentrations by lower case letters at $p \leq 0.05$.

the paper was cut into 1 cm × 1 cm paper pieces. Egg raft of *Cx. pipiens* was placed on the blotted filter paper in a similar manner. Then, eggs were placed in a desiccator at 75–78% RH and 24 ± 1 °C temperature. Control eggs were kept in water. In order to show the mechanism that intake water replaces evaporated water; control experiments were conducted at the room temperature of 26 ± 2 °C and RH 50–60%. The degree of egg desiccation was observed under dissecting microscope by counting shrunken eggs at 30 min interval until all the eggs were collapsed.

Data were subjected to probit analysis (PASW statistics 18, Release 18.0.0, SPSS Inc., USA). Median desiccation time DT_{50} (defined as the duration at which 50% eggs collapsed) and median desiccation time DT_{90}, (defined as the duration at which 90% eggs collapses) of the eggs were calculated for the four mosquito species. Fiducial limits were determined for DT_{50} and DT_{90}. Differences in the DT_{50} and DT_{90} were compared by One-way ANOVA at $p \leq 0.05$ using Fisher's least significant difference (LSD). The relationship between water permeability and hatching inhibition at 1 ppm concentration was determined by Pearson's coefficient of correlation (r).

3. Results

3.1. Egg hatching inhibition

3.1.1. Aedes albopictus

Egg hatching inhibition in freshly laid eggs increased with an increase in concentration of insect growth regulators. Most eggs hatched at lower concentrations of pyriproxyfen, azadirachtin and diflubenzuron with inhibition rates ranging between 1.7–2.8% and 7.2–10.8% at 0.001 and 0.01 ppm, respectively ($p > 0.05$) (Fig. 1A). Freshly laid eggs exposed to higher concentrations of pyriproxyfen failed to hatch up to 40.1% at 0.1 ppm and 80.6% at 1.0 ppm. Egg hatch inhibited by pyriproxyfen was significantly higher than the eggs exposed to 0.1 ppm (23.6%) and 1.0 ppm (42.8%) concentrations of azadirachtin (Fig. 1A). Similarly, egg hatching was lower in diflubenzuron treatment than pyriproxyfen at the same concentrations (29.2% at 0.1 ppm and 35.8% at 1.0 ppm) ($p < 0.05$).

Pyriproxyfen, azadirachtin and diflubenzuron inhibited hatching of embryonated eggs lower than the freshly laid eggs ($p < 0.05$) (Table 1). There was no significant difference in hatching inhibition of embryonated eggs at 0.001 or 0.01 ppm concentrations when exposed to three classes of insect growth regulators ($p > 0.05$) (Fig. 1B). Pyriproxyfen inhibited hatching of embryonated eggs at 1.0 ppm was significantly higher than 0.1 ppm ($p < 0.05$) (Fig. 1B). The overall hatching inhibition was high in freshly laid eggs.

Table 1
T-test analysis for the significant difference of inhibition of egg hatching against different insect growth regulators between freshly laid and embryonated eggs of different mosquito species.

Conc. (ppm)	Mosquito species			
	Aedes albopictus	*Aedes aegypti*	*Aedes atropalpus*	*Culex pipiens*
Pyriproxyfen				
0.001	NS	NS	NS	NS
0.01	NS	NS	NS	NS
0.1	***	**	*	NS
1.0	***	***	NS	NS
Azadirachtin				
0.001	NS	NS	NS	**
0.01	*	NS	NS	NS
0.1	NS	*	*	NS
1.0	***	NS	**	*
Diflubenzuron				
0.001	NS	NS	*	NS
0.01	NS	NS	NS	NS
0.1	**	NS	*	NS
1.0	***	**	**	***

NS, non-significant.
* $p < 0.05$.
** $p < 0.01$
*** $p < 0.001$.

3.1.2. Aedes aegypti

Hatching inhibition of freshly laid eggs was dose dependent. Pyriproxyfen inhibited hatching of freshly laid eggs more than azadirachtin or diflubenzuron at 0.01 (10.4%), 0.1 (26.2%) and 1.0 ppm (47.3%) ($p < 0.05$) (Fig. 2A). Most hatching in freshly laid eggs (89.6–95.7%) was observed at lower concentrations (0.001 and 0.01 ppm) of pyriproxyfen, azadirachtin and diflubenzuron (Fig. 2A).

Embryonated eggs of *Ae. aegypti* were less affected when exposed to the three classes of insect growth regulators (Table 1 and Fig. 2B). No significant difference was found in hatching inhibitions ($p > 0.05$) except when the embryonated eggs were exposed to 0.1 and 1.0 ppm of pyriproxyfen where egg inhibition increased slightly as compared to azadirachtin and diflubenzuron ($p < 0.05$) (Fig. 2B).

3.1.3. Aedes atropalpus

Freshly laid eggs of *Ae. atropalpus* did not show significant difference when exposed to pyriproxyfen, azadirachtin and diflubenzuron ($p > 0.05$) (Fig. 3A). The hatching inhibition increased with the increase in the concentration of insect growth regulators. The highest inhibition of egg hatch was observed at 1.0 ppm of pyriproxyfen followed by azadirachtin and diflubenzuron (Fig. 3A).

Embryonated eggs of *Ae. atropalpus* showed significant amount of tolerance for the three classes of insect growth regulators. Egg hatching inhibition remained the same for pyriproxyfen,

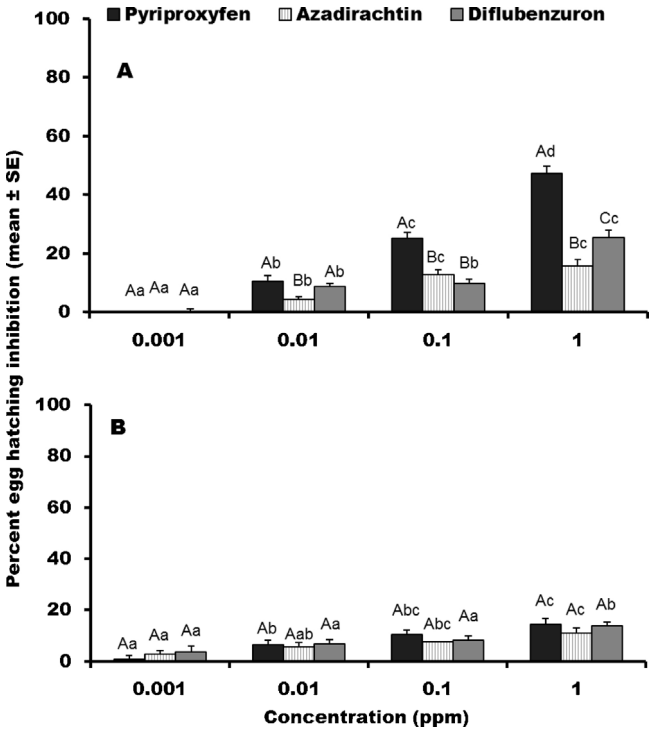

Fig. 2. Effects of pyriproxyfen, azadirachtin and diflubenzuron insect growth regulators on egg hatching inhibition of freshly laid (A) and embryonated eggs (B) of *Aedes aegypti*. Significant differences among different insect growth regulators for individual concentrations are denoted by upper case and among various concentrations by lower case letters at $p \leq 0.05$.

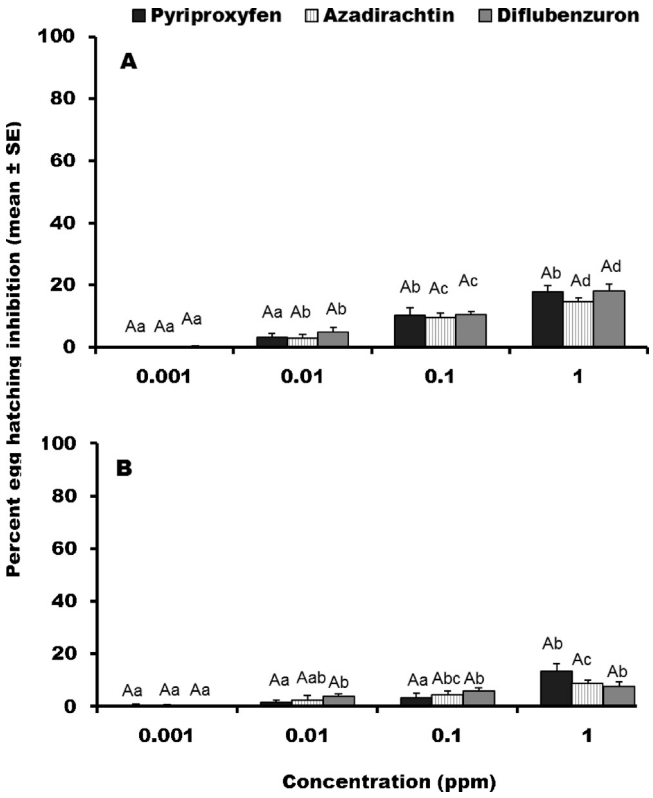

Fig. 3. Effects of pyriproxyfen, azadirachtin and diflubenzuron insect growth regulators on egg hatching inhibition of freshly laid (A) and embryonated eggs (B) of *Aedes atropalpus*. Significant differences among different insect growth regulators for individual concentrations are denoted by upper case and among various concentrations by lower case letters at $p \leq 0.05$.

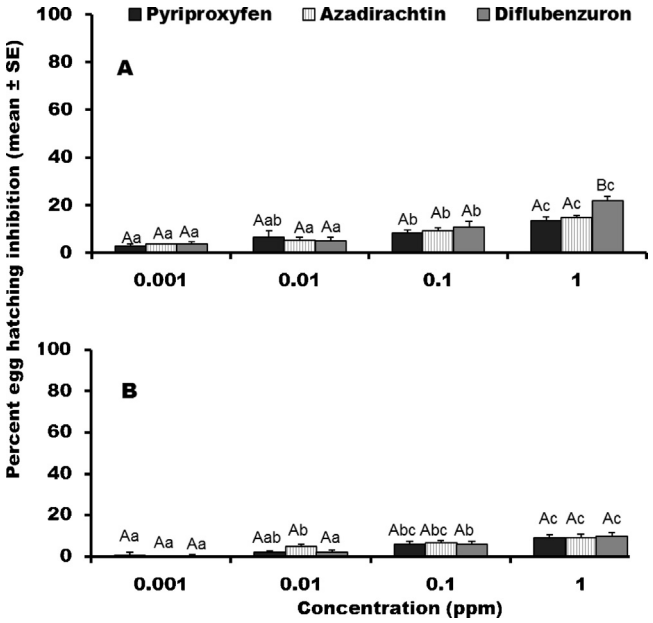

Fig. 4. Effects of pyriproxyfen, azadirachtin and diflubenzuron insect growth regulators on egg hatching inhibition of freshly laid (A) and embryonated eggs (B) of *Culex pipiens*. Significant differences among different insect growth regulators for individual concentrations are denoted by upper case and among various concentrations by lower case letters at $p \leq 0.05$.

azadirachtin and diflubenzuron ($p > 0.05$). The effects of higher concentrations of growth regulators on egg hatching inhibition were more than lower concentrations ($p < 0.05$) (Fig. 3B).

3.1.4. *Culex pipiens*

The response of freshly laid eggs of *Cx. pipiens* to various insect growth regulators was also dose dependent. Egg hatching inhibition increased with the increase in the concentrations of insect growth regulators. The inhibition of *Cx. pipiens* eggs hatch was higher in diflubenzuron treatment (21.8%) than pyriproxyfen and azadirachtin treatment at 1.0 ppm concentration ($p < 0.05$) (Fig. 4A).

Embryonated eggs of *Cx. pipiens* were more tolerant to insect growth regulators than freshly laid eggs ($p < 0.05$) (Table 1 and Fig. 4B). Although, higher concentrations, *i.e.*, 0.1 ppm and 1.0 ppm of pyriproxyfen, azadirachtin and diflubenzuron inhibited egg hatching but the percent of eggs hatched (90.0%) was higher than other mosquito species ($p < 0.05$).

3.2. Effects on embryonic development and hatching patterns

We found that 0.1 and 1.0 ppm concentrations of diflubenzuron dismantled egg raft of *Cx. pipiens* into individual eggs and induced larval emergence from the egg sidewall instead from operculum (Fig. 5). However, we did not see a similar effect on mosquito eggs at any concentration of pyriproxyfen or azadirachtin. Interestingly, the morphological abnormalities, altered body organization, disoriented cervical and abdominal regions, and chitinization during embryo development were observed in eggs exposed to pyriproxyfen, azadirachtin and diflubenzuron (Fig. 6). Eggs with such abnormalities did not hatch and died.

3.3. Water permeability of the eggs and it's relation with egg hatching inhibition

Culex pipiens eggs ($DT_{50} = 1.72$ h; $X^2 = 376.0$; df = 51; $p < 0.05$) retained water for shorter duration than

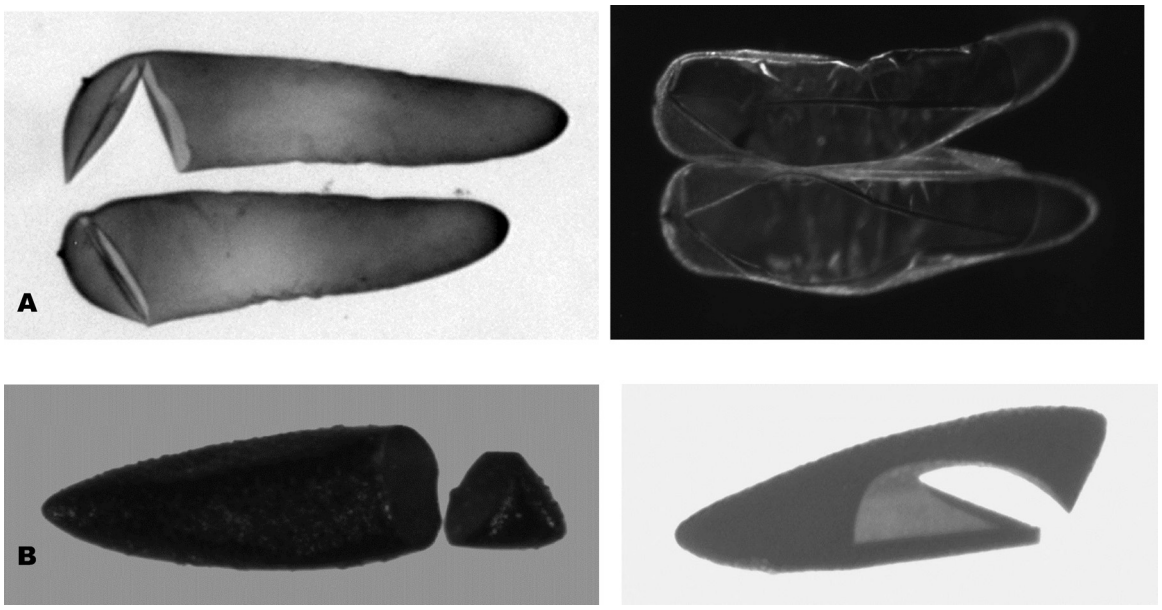

Fig. 5. Abnormal hatching of *Culex pipiens* (A) and *Aedes albopictus* (B) eggs due to diflubenzuron exposure. Controls (right) and treatments (left) are showing egg hatching from operculum and from side wall, respectively.

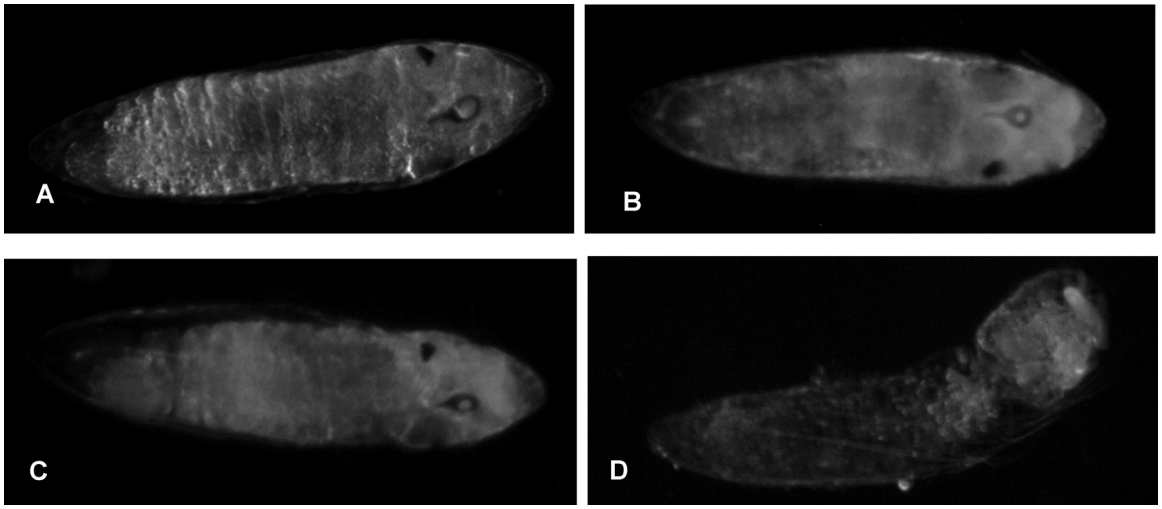

Fig. 6. Effects of insect growth regulators: control (A), pyriproxyfen (B), azadirachtin (C), and diflubenzuron (D) on embryonic development of *Aedes albopictus* mosquito.

Ae. albopictus (DT_{50} = 3.93 h; X^2 = 355.8; df = 37; $p < 0.05$), *Ae. aegypti* (DT_{50} = 4.85 h; X^2 = 587.7; df = 40; $p < 0.05$) and *Aedes atropalpus* (DT_{50} = 5.11 h; X^2 = 225.3; df = 37; $p < 0.05$). The median desiccation time for all the species were significantly different among them (df = 3; f = 283.87; $p < 0.05$; LSD = 0.300) (Table 2). There was no significant correlation between DT_{50} and DT_{90} when mosquito eggs were exposed to pyriproxyfen, azadirachtin and diflubenzuron (r = −0.23 to 0.35; $p > 0.05$).

Table 2
Estimation of desiccation time of eggs (0–2 h old) of four mosquito species using probit analysis.

Species	DT50 (h)	DT90 (h)	Slope	Intercept	χ^2	df	p
Aedes albopictus	3.93a (3.85–4.01)[a]	4.84a (4.71–5.02)[a]	14.15	−8.42	225.31	37	0.0001
Aedes aegypti	4.85b (4.73–4.96)	5.72b (5.52–5.99)	17.91	−12.28	587.65	40	0.0001
Aedes atropalpus	5.11c (5.01–5.21)	5.96b (5.81–6.19)	19.16	−13.58	355.84	37	0.0001
Culex pipiens	1.72d (1.61–1.81)	3.29c (3.03–3.66)	4.52	−1.06	375.96	51	0.0001

DT_{50}, median desiccation time; DT_{90}, desiccation time for 90% eggs. Values denoted with same small letter in column are not significantly different with One-way ANOVA ($p < 0.05$) using Fisher's least significant difference (LSD) method (DT_{50}: df = 3, f = 283.87, p = 0.0001, LSD = 0.300; DT_{90}: df = 3, f = 117.41, p = 0.0001, LSD = 0.359).

[a] Lower–upper fiducial limits were shown in parenthesis for respective DT_{50} and DT_{90}.

4. Discussion

In the present study we revealed the effects of three insect growth regulators, pyriproxyfen, azadirachtin, and diflubenzuron on the egg hatch inhibition and other developmental parameters in four species of mosquitoes. We found, differences in egg hatching and egg mortality of the four species of mosquitoes which can be attributed to different modes of action, types and concentrations of pyriproxyfen, azadirachtin, and diflubenzuron. Previously, Hoffmann and Lagueux (1985) have reported a role of ecdysteroid and juvenile hormone in the embryonic development of various insects. Insect growth regulators disrupt hormonal balance due to change in hormonal titer affecting normal embryonic development. Our results confirm abnormal development of mosquito embryo when exposed to pyriproxyfen, azadirachtin and diflubenzuron. Juvenile hormone analog (pyriproxyfen) and ecdysone agonist (azadirachtin), arrest embryonic development at various stages; whereas, chitin synthesis inhibitor (diflubenzuron), affects chitinization of embryonic cuticle, thus making it unviable. Abnormal egg hatching through the egg side wall has been reported in *Cx. quinquefasciatus* eggs, which were exposed to chitin synthesis inhibitors (Miura et al., 1976; Miura and Takahashi, 1979). We observed morphological and physical changes in mosquito eggs exposed to diflubenzuron at higher concentrations suggest that chitinization and hardening processes of the egg shell and embryonic chitinous structures were compromised resulting in hatch from the side wall of egg instead of operculum. As a result of abnormal hatching in *Cx. pipiens*, the egg raft defragmented into individual eggs causing egg mortality, probably because of their submersion in water. Normally, egg hatching is accomplished by the pressure generated from egg burster present on the head of the larva that causes the shell to crack along the normal line of dehiscence at the anterior end of the egg (Clements, 1992). Chitinous egg burster development affected in diflubenzuron treated embryos and probably, pharate larvae use body pressure for the hatching which disrupts the egg shell from weaker places. As far as changes in the egg structure and increase/decrease in egg mortality/egg inhibition is concerned our findings suggest that freshly laid eggs are more vulnerable to insect growth regulators. In contrast, pyriproxyfen and azadirachtin substantiated their role in altering hormonal actions during embryonic development of the *Aedes* and *Culex* eggs rather than altering egg hatching process.

Vasuki (1990), Ali et al. (1995), Su and Mulla (1998) and Suman et al. (2010) have suggested that larvicidal and ovicidal efficacies are governed by the type and concentration of different classes of insect growth regulators. We found a similar phenomenon with pyriproxyfen, azadirachtin and diflubenzuron. In the present study, *Ae. albopictus* was most susceptible to all the three insect growth regulators followed by *Ae. aegypti*, *Cx. pipiens* and *Ae. atropalpus*. Our results are in conformity with Vasuki (1990) and Su and Mulla (1998) who reported diflubenzuron, pyriproxyfen and azadirachtin to be less toxic to *Cx. quinquefasciatus* eggs at a WHO recommended concentrations (WHO, 2006) for larviciding. In order to manage mosquito populations at egg stages, the eggs need to be exposed at higher concentrations of insect growth regulators for shorter rather than long durations. Eggs exposed for longer durations at low concentrations will defeat the idea of mosquito control as eggs would hatch unaffected since there is always a short duration between when the eggs are laid (susceptible stage) and embryonic development (resistant stage) begins. Freshly laid eggs are more vulnerable to the toxicity of insect growth regulators than embryonated eggs. Similar observations were made by Miura et al. (1976) and Vasuki (1990). Miura et al. (1976) suggested that freshly laid and 2–14 h old eggs were more vulnerable to diflubenzuron exposure. While, Vasuki (1990) demonstrated species specific variation for ovicidal action of chitin synthesis inhibitor and juvenile hormone analog against eggs of *Anopheles stephensi* (Liston), *Cx. quinquefasciatus* and *Ae. aegypti*. These differences may be attributed to the inability of insect growth regulators to disrupt hormone actions during egg development and the loss of shell permeability due to endochorion tanning and wax layer formation (Clements, 1992).

Interspecific variations in *Aedes* eggs from different habitats have shown differences in desiccation survival-time (Sota and Mogi, 1992). We found that *Ae. atropalpus* eggs were more tolerant to desiccation than the eggs of *Cx. pipiens*. Such disparity seems to be due to their type of habitats. *Aedes atropalpus* prefer moist places to lays their eggs where there is a significant desiccation pressure, whereas *Cx. pipiens* deposits egg raft in larger water habitats where there is minimum chances of dehydration. *Aedes aegypti* and *Ae. albopictus* survive in containers since they developed tolerance to desiccation up to some extent. Although, we could not find any relationship between water permeability of eggs and ovicidal activity of insect growth regulators based on egg desiccation time but assuming this is one of the reasons for differential ovicidal efficacy of insect growth regulators. Further studies are needed to look into molecular evolution of egg shell proteins and physiological basis of shell permeability.

Mode of action of the three classes of insect growth regulators used during the present study was somewhat species specific. Pyriproxyfen was highly effective against *Ae. albopictus* and *Ae. aegypti* whereas diflubenzuron was found to be most toxic to *Cx. pipiens* eggs at the highest concentration. In contrast, none of the insect growth regulator affected egg viability and hatching of *Ae. atropalpus*. Such variations associated with the egg structure, egg morphology and physiological adaptation of eggs to a particular habitat bear more significance in the light of the observations made by Linley and Craig (1994), Suman et al. (2008, 2011) and Jagadeeshan and Singh (2007). They concluded that variations in a habitat bring changes to egg structures that may result into decreased ovicidal efficacy.

We conclude that the three classes of insect growth regulators, pyriproxyfen, azadirachtin and diflubenzuron possess ovicidal properties but their efficacies depend on the mode of action, type and concentrations and mosquito species. Egg hatching inhibition was dose dependent, and freshly laid eggs are more susceptible than the embryonated eggs, but, with no correlation between water permeability of the egg shell and ovicidal efficacy of the insect growth regulators. Overall, insect growth regulators showed potential to be an ovicidal agent and this study suggests further investigations to improve the ability of formulations to penetrate the egg membranes particularly embryonated eggs and to elucidate species specific action of these compounds which can be helpful in: (1) treatment of larval habitats at lower doses will result in oviciding that will delay in population build up, (2) treatment of key habitats, *i.e.*, used tires, will reduce the spreading or introduction of mosquitoes in uninfested areas, and (3) seasonal transmission of vectors, particularly container mosquitoes, *Ae. albopictus* and *Ae. aegypti* passed unfavorable season in egg stages, will be curtailed.

Acknowledgements

We thank Don Hanson for providing samples of azadirachtin and diflubenzuron insect growth regulators. We thank Linda McCuiston for her help in maintaining mosquito colonies. We also thank MGK® (Minneapolis, MN, USA) for financial support.

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