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Effect of insect growth regulators on hatching of eggs of three vector mosquito species

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Abstract. Hatchability of eggs of *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* was studied by exposing freshly laid (0–1 h) and older (12–18 h) eggs to varying concentrations (0·0001–1·0 mg/l) of 6 insect growth regulators viz. OMS 3019, OMS 3007, OMS 2015, OMS 3031, OMS 3013 and OMS 3009. In all the 3 species, dosage dependent response was observed. The response was also dependent on the age of eggs. Among the 6 IGR compounds OMS 3031 was found highly active resulting in maximum reduction in hatching of freshly laid eggs of *Anopheles stephensi* (99·95%) followed by *Aedes aegypti* (89·9%) whereas 50% inhibition in hatching occurred in older eggs of *Culex quinquefasciatus* exposed to OMS 3009 at the same dosage. Dose, age and dose and age dependent hatching was evident in eggs of the 3 test species exposed to insect growth regulators OMS 3019, OMS 3013 and OMS 2015. Higher proportion of unhatched eggs with varying abnormalities was noticed in test species. Percentage mortality observed was higher in first instar larvae hatched from treated eggs reared in untreated water. Therefore, insect growth regulators have great potency in suppressing the population by affecting hatching of mosquito eggs in addition to inhibition of adult emergence, thus providing a useful tool for integrated vector management.

Keywords. Insect growth regulators; egg hatchability; post-treatment effects; *Culex quinquefasciatus*; *Aedes aegypti*; *Anopheles stephensi*.

1. Introduction

Insect growth regulators (IGRs) have received a great deal of attention in the last two decades as promising insect control agents (Retnakaran *et al* 1985) because of their unique mode of action of disrupting the metamorphic development, higher selectivity and less persistence in the environment (Post and Vincent 1973; Mulder and Gijswijt 1973). In addition, many new IGRs currently under development have low mammalian toxicity and are potentially compatible with natural enemies and safer to economically important insect species (Staal 1975). Such attributes are desirable when dealing with problems of vector resurgence, secondary vector outbreaks and insecticide resistance. Thus, IGRs can be effectively used in integrated vector management (IVM) programme designed to decelerate or prevent the development of resistance (Sparks and Hammock 1982).

IGRs, not only interfere with metamorphosis or chitin deposition during moulting (Mulder and Gijswijt 1973; Staal 1975; Sparks and Hammock 1982; Estrada and Mulla 1986), but also affect the hatching of eggs of medically and agriculturally important insects (Chokalingam and Noorjahan 1984; Jordan *et al* 1979; Saxena and Girish Mathur 1981; Ascher and Nemny 1974; Moore and Taft 1975). However, very little information is available on the effect of IGRs on hatching of mosquito eggs.

The present study is undertaken to determine the effect of 6 IGRs on hatching of

eggs of 3 major vector mosquitoes, *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*.

2. Materials and methods

Six new IGRs (4 chitin synthesis inhibitors and two juvenoids) were used for this study. Four chitin synthesis inhibitors are OMS 2015 (Triflumuron, 1·04% gr), OMS 3009 (CME 13406, 15% water based suspension concentrate), OMS 3031 (XRD-473, 5% ec) and OMS 3013 (IK 17899, 5% ec) and two juvenoids are OMS 3019 (S31183, 5% wp) and OMS 3007 (S21149, 5% wp). For each compound 1% stock solution was prepared and serial dilutions were made thereafter. The test species of mosquitoes *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* were obtained from cyclic colonies maintained in Vector Control Research Centre.

Newly emerged mosquitoes were held in 30 × 30 cm netted cage provided with raisin and 10% glucose solution. They were offered blood meal from a chicken or rabbit as the case may be, on the third day after emergence. Oviposition cups were provided after 3 days. In this way 12–18 h old eggs were obtained. A single egg raft of *C. quinquefasciatus* and 100 eggs of *Ae. aegypti* and *An. stephensi* were exposed to IGR treated waters with concentrations ranging from 0·0001–1·0 mg/l. In another set of experiments individual gravid females were allowed to oviposit in small cups (50 ml) gauzed with nylon net. Thus, freshly laid eggs were exposed to IGR treated water within 1 h. Four replicates for each concentration and appropriate controls were maintained. Hatchability observations were made after 36–48 h of incubation period and abnormalities in hatched and unhatched eggs were recorded. The larvae hatched from treated (1·0 mg/l) eggs were reared in treated and untreated water for the observation on post embryonic development.

Analysis of variance was performed using the ANOVA procedures (Sokal and Rohlf 1981) depending on whether the experiment involved a balanced or unbalanced design. The data from the egg hatching studies, expressed as percentages, were transformed to arcsin angular transformation to normalize the distribution before testing by ANOVA. This procedure was used because the comparison wise error rate was considered to be more important than the experiment-wise error rate. This allows all possible comparisons between all experimental groups.

3. Results and discussion

Figure 1 summarizes the level of inhibition in hatching of eggs (12–18 and 0–1 h old) of *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* exposed to 6 IGRs mentioned above at series of concentrations. On screening the effect of these IGRs it is evident that the hatching of treated eggs at dosages ranging from 0·0001–1·0 mg/l is greatly reduced. On the basis of percentage inhibition in hatching of eggs (0–1 and 12–18 h old) of the three test species at the maximum dosage, the compounds can be compared in the order of their efficacy. OMS 3031 was found to have significantly highest efficacy causing maximum suppression in hatching in (0–1 and 12–18 h old) eggs of *Ae. aegypti*, 12–18 h old eggs of *C. quinquefasciatus* and 0–1 h old eggs of *An. stephensi* while OMS 3009 was more active against 0–1 h old *C. quinquefasciatus* and 12–18 h old *An. stephensi*.

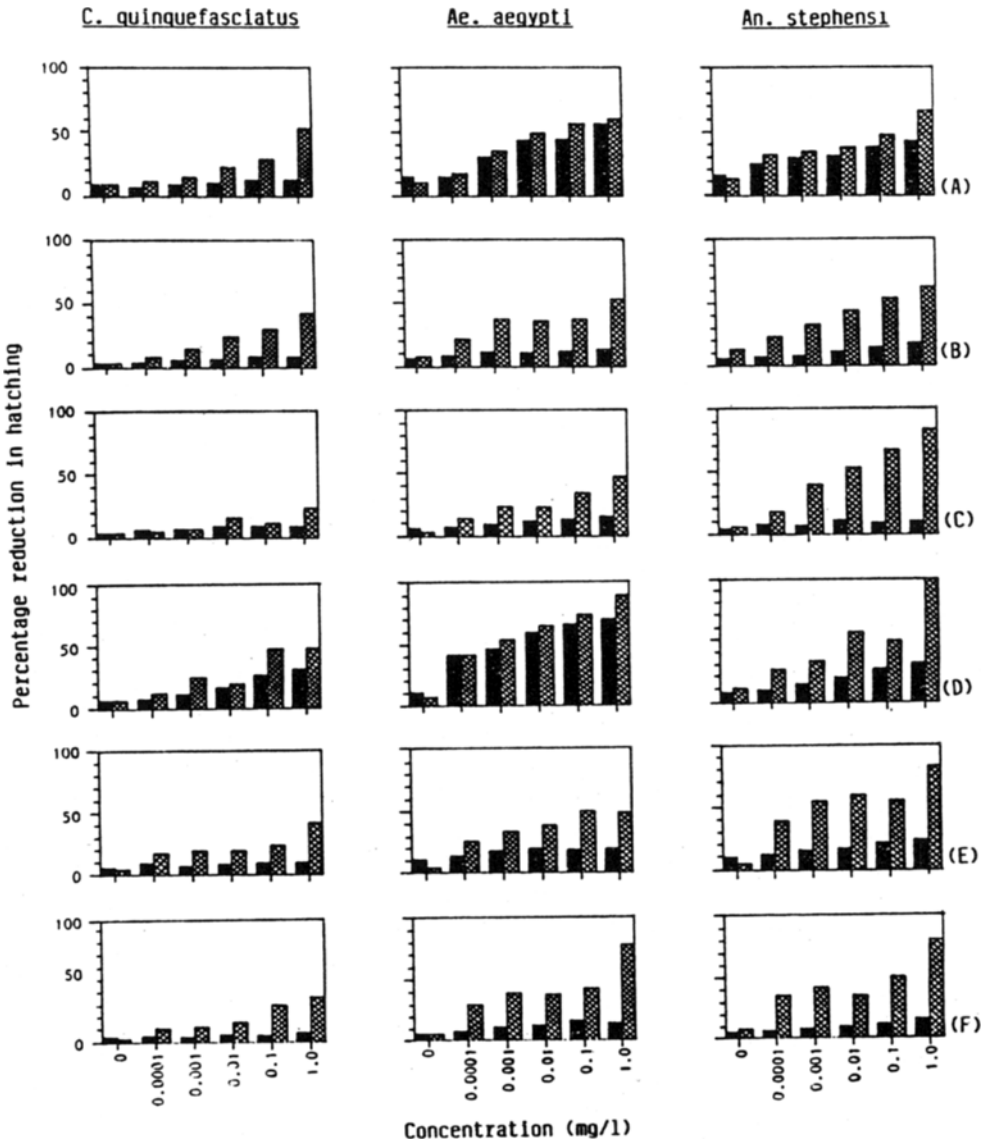

Figure 1. Percentage reduction in hatching of 12–18 h old (■) and 0–1 h old (□) eggs of *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* exposed to 6 IGRs. (A), OMS 3009; (B), OMS 3007; (C), OMS 2015; (D), OMS 3031; (E), OMS 3013; (F), OMS 3019.

The results show that the reduction in hatching of mosquito eggs is significantly related to dosage ($P < 0{\cdot}05$) in all cases. However, significant age dependence could only be demonstrated in the case of *C. quinquefasciatus* (for all the IGRs). Dose and age dependence could be observed in all the 3 species tested only for 3 IGRs (OMS 3019, OMS 3013 and OMS 2015).

The overall response in the eggs of all test species varied at different concentrations of treated waters showing more unhatched eggs with various abnormalities at increasing concentrations. When the eggs of 3 species were exposed

to 6 IGRs younger embryos-preblastoderm (Idris 1960) were found to be more sensitive than older ones suggesting the influence of the age of the embryos at the time of treatment on the activity of the candidate IGR compounds at increasing dosages. Similar inverse relationship between the age of the egg and the susceptibility to chitin synthesis inhibitor, diflubenzuron has been observed in Egyptian cotton leaf worm (Ascher and Nemny 1974), Boll weevil (Moore and Taft 1975), Simulium (Lacey and Mulla 1978) and Southern house mosquito (Miura *et al* 1976).

Percentage reduction in hatching is found to be higher in eggs exposed to chitin synthesis inhibitors than eggs exposed to juvenoids irrespective of the species and age of the eggs.

Typical symptoms of toxicity and developmental abnormalities induced by IGRs on exposure of mosquito eggs (both younger and older) have been observed and are as follows.

Unhatched eggs contained fully developed embryos which failed to hatch and apparently died just before hatching. Segmentation, eye spots, eggspine and setae were visible through the eggshell of *C. quinquefasciatus*.

In some cases larvae eclosed from a longitudinal line of weakness at the mesal dorsum of eggshell which is different from normal hatch where a portion of the anterior end of the egg shell is forced open transversely at a line of dehiscence, forming an egg cap which is not completely detached but hinged to the remaining egg shell in the case of *C. quinquefasciatus* and *An. stephensi*. Partial side hatch resulted in the death of the larva during ecdysis. Larvae were found dead with (i) head capsule free, but caudal end still caught in the egg shell, (ii) caudal end free but head capsule inside the egg shell and (iii) thorax and abdomen free but head capsule and caudal end in egg shell. IGR treatment may also have caused slower embryonic development as reported earlier in *C. pipiens quinquefasciatus* (Miura *et al* 1976).

These compounds may affect the chitin deposition in cuticle thus the rigidity of the cuticle is of lower degree and it fails to resist the muscular traction during hatching thereby resulting in the death of the larvae within the eggs as observed in *Spodoptera littoralis* treated with diflubenzuron (Ascher and Nemny 1974). It is also possible that these compounds interrupt the development shortly before hatching making the heads of the larvae to be seen outside the egg shell as suggested by Saxena and Girish Mathur (1981).

The first instar larvae hatched from eggs treated with OMS 3031 and OMS 3009 showed 100% mortality in the case of *C. quinquefasciatus* followed by 94–96% in *Ae. aegypti* and 72–98% in *An. stephensi*. Whereas, 54–90% mortality was observed in first instar hatched from the eggs treated with the other compounds (OMS 3007, OMS 3019, OMS 2015 and OMS 3013) in the 3 species tested (tables 1–3). High mortality recorded in first instar larvae hatched from IGR treated eggs (1 mg/l) reared in untreated water may be due to the transfer of the compound from egg to larva as reported earlier (Saxena and Girish Mathur 1981). Complete inhibition of adult emergence resulted in the larvae of the 3 test species hatched from the eggs treated with OMS 3031, OMS 3009 and OMS 3013. Only 6–14%, 4–41% and 8–10% adult emergence was recorded in *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* respectively as an indication of post treatment effect on the larvae hatched from eggs treated with 3 IGRs (OMS 3007, OMS 2015 and OMS 3019). However,

Table 1. Post-treatment effects of IGRs on the larvae of *C. quinquefasciatus* hatched from treated eggs.

Name/code of the IGR	Larval mortality (%) I	II	III	IV	Pupation (%)	Pupal mortality (%)	Adult emerged (%)
OMS 3019	62(3·6)	22(2·2)	10(1·0)	—	6(1·0)	—	6(1·0)
OMS 3007	60(5·1)	10(2·2)	8(2·8)	2(1·0)	20(4·4)	6(1·0)	14(3·6)
OMS 2015	76(1·4)	2(1·0)	—	—	22(1·0)	10(2·2)	12(2·8)
OMS 3031	100(0·0)	—	—	—	—	—	—
OMS 3013	79(3·0)	19(2·6)	2(1·0)	—	—	—	—
OMS 3009	100(0·0)	—	—	—	—	—	—
Control	6(1·0)	2(1·0)	—	2(1·0)	90(1·0)	—	84(2·0)

Values are mean ± SE.

Table 2. Post-treatment effects of IGRs on the larvae of *Ae. aegypti* hatched from treated eggs.

Name/code of the IGR	Larval mortality (%) I	II	III	IV	Pupation (%)	Pupal mortality (%)	Adult emerged (%)
OMS 3019	4(3·2)	18(2·2)	14(2·2)	—	4(1·4)	—	4(1·4)
OMS 3007	54(5·4)	3(0·9)	—	—	43(5·1)	2(1·0)	41(4·3)
OMS 2015	60(4·5)	6(1·0)	4(1·4)	—	30(2·2)	2(1·0)	28(3·1)
OMS 3031	94(2·2)	6(2·2)	—	—	—	—	—
OMS 3013	90(2·2)	8(2·8)	2(1·0)	—	—	—	—
OMS 3009	96(1·4)	4(1·4)	—	—	—	—	—
Control	2(1·0)	4(1·4)	10(4·1)	2(1·0)	82(5·0)	2(1·0)	80(4·2)

Values are mean ± SE.

Table 3. Post-treatment effects of IGRs on the larvae of *An. stephensi* hatched from treated eggs.

Name/code of the IGR	Larval mortality (%) I	II	III	IV	Pupation (%)	Pupal mortality (%)	Adult emerged (%)
OMS 3019	58(3·6)	6(2·2)	12(4·5)	4(1·4)	20(4·4)	10(3·0)	10(2·2)
OMS 3007	70(2·2)	1(0·9)	12(1·4)	6(2·2)	10(2·2)	2(1·0)	8(1·4)
OMS 2015	60(4·2)	—	12(4·5)	6(2·2)	22(2·2)	12(0·0)	10(2·2)
OMS 3031	98(1·0)	2(1·0)	—	—	—	—	—
OMS 3013	64(5·8)	12(2·4)	24(2·8)	—	—	—	—
OMS 3009	72(4·5)	28(3·2)	—	—	—	—	—
Control	3(1·7)	2(1·0)	6(1·0)	—	88(2·4)	1(0·8)	87(2·1)

Values are mean ± SE.

large proportion of the larvae hatched from abnormal hatching or normal hatching died during next ecdysis and all the larvae were dead within 48 h in treated waters.

Besides being potent chitin synthesis inhibitors and disruptors of metamorphic development causing higher mortality in immatures, IGRs are also found to greatly affect hatching of mosquito eggs soon after oviposition or just before hatching.

Therefore, IGRs true potentiality is much higher than the anticipated inhibition in adult emergence as often indicated by EI_{50} values. Hence, the significant role of IGRs in preventing the hatching of mosquito eggs may be included in the evaluation as their promise in integrated vector control is obvious.

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