

Evaluation of Two Insect Growth Regulators (IGRS) As a Feed- Through Treatments against Adults of *Culex pipiens* L. mosquito

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ABSTRACT

This study was carried out to evaluate the biological effects of two IGRs (Match[®] and Baycidal[®]) on *Culex pipiens* mosquitoes when fed the adults on sugar solution mixed with 0.1, 0.5 and 1 ppm of each compound. Some delayed effects of these treatments were also determined. The results indicated that the use of each of the tested IGRs caused a reduction in the blood-feeding activity of mosquito female when compared with the controls. The reductions were 18.2, 22.7 and 31.8% by using Match and 25, 27.3 and 36.3% by using Baycidal[®], respectively, at the tested concentrations. The reduction in biting activity of mosquito females may lead to a decrease in the number of fully engorged females and accordingly affect their reproductive capacity of mosquitoes. The use of Match[®] and Baycidal[®] at the tested concentrations caused a marked prolongation in the time needed for blood meal digestion by *C. pipiens* females. The prolongation percentages were 44.4, 48.8 and 55.5% by using Match and 51.1, 55.5 and 62.2% by using Baycidal[®], respectively. Also, treatments caused a reduction in the reproductive capacity. The maximum decreasing in egg production was 30.3% at concentration of 1 ppm of Match[®]. The result showed that the treatment by Baycidal[®] at 0.1, 0.5 and 1ppm led to a remarkable reduction in the egg production by 61.9, 66.1 and 69.2%, respectively and also the hatchability was reduced in the treated females. Subsequent study was carried out to investigate the delayed effect of IGRs Match[®] and Baycidal[®] on the hatched larvae. The result showed that the larval development was gradually inhibited by 17.8, 49.8 and 92.7% by using Match[®] at 0.1, 0.5 and 1 ppm, respectively. On the other hand the larval development was completely inhibited after the treatment of adults fed by 0.5 and 1 ppm Baycidal[®]. The larvae developed till the 2nd instar but completely failed in molting to the 3rd instar. Subsequent study was conducted on the effect of Match[®] and Baycidal[®] on the longevity of the survived mosquito adults that, the records showed that no significant differences in the mean of adult longevity between treatments and control.

INTRODUCTION

Female mosquitoes are transmitters for many infectious diseases thus, their control is urgently needed. The harmful effects of conventional insecticides beside the development of resistant strains call the mosquito control specialists for searching about an effective and safe control agents. Between these agents, insect growth regulators (IGRs) which have high levels of activity and

efficacy against various species of mosquitoes in a variety of habitats (Mulla *et al.*, 1989), since IGRs could alter adult emergence, reproduction and ecdysteroid production in surviving females (Fournet *et al.*, 1995). IGRs are suitable candidate larvicides for mosquito control due to their greater margin of safety for non-target biota (Mulla *et al.*, 1986). On the basis of these attributes, IGRs are likely to provide additional tools for mosquito control, supplementing microbial, pyrethroids and organophosphorus larvicides (Mulla *et al.*, 1989).

More attention has been directed to the use of IGRs as a feed-through treatment for controlling different species of dipterous flies (Huybrechts and DeLoof, 1981; Pochon and Casida, 1983; Friedel and McDonell, 1985). In this concern, the present study was conducted to evaluate the biological effects of two IGRs (Match[®] and Baycidal[®]) on *Culex pipiens* mosquitoes as a feed-through treatment against the adults. The possible effects on feeding activity, time of blood meal digestion and reproductive potential of mosquitoes were investigated. The delayed effects of the above IGRs on subsequent larval development and longevity of mosquito adult survivors were also considered.

MATERIALS AND METHODS

Compounds used:

Triflumuron (TFM) (Baycidal[®] 48% EC); benzoylphenylurea, 1- (2- chlorobenzoyl) -3- (4-trifluoromethoxyphenyl) urea, was supplied by Bayer AG (Germany). Lufenuron (LUF) (Match[®] 5%EC); benzoylurea, (RS) -1- [2,5- dichloro-4- (1,1,2,3,3,3-hexafluoropropoxy) phenyl] -3- (2,6-difluorobenzoyl) urea was supplied by Syngenta.

Insects:

A *Culex pipiens* L. (Diptera: Culicidae) colony maintained in the laboratory of Medical and Veterinary Insects, Department of Applied Entomology Faculty of Agriculture, Alexandria University, for more than 10 years was used. Mosquitoes were held at 27±1 °C, 70±5% RH, and a photo regime of 14:10 hr (light:dark) Adults were provided with a 10% sucrose solution as food source. A pigeon was introduced twice a week to the adults for blood feeding. Larvae were reared in dechlorinated water under the same and conditions and were fed daily with baby fish food.

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Experimental tests:

Match[®] or Baycidal[®] was included in 10% sugar solution at concentrations of 0.1, 0.5 and 1.0 ppm. For each concentration, the newly emerged mosquito adults from batches of 200 two days old pupae were confined in cages provided with a source of water. They were allowed to feed on treated sugar solutions soaked on cotton pads in small vials for 48 hrs. Untreated sugar solutions were served as controls. Each concentration was replicated four times. Four days post-treatment, mosquito females a pigeon was subjected to mosquito females as a source of the blood meal. The percentage of biting (the number of blood-fed females/total number of tested females x 100) was estimated after 2 hrs. Then, each engorged female was kept with a male in a small plastic cup (10cm in diameter and 15cm in height), half-filled with tap water and covered with muslin cloth. The reproductive potential of mosquito females (based on egg production and hatchability of eggs) and the time of blood digestion (the period between feeding and oviposition) were recorded for the 1st gonotrophic cycle.

The effect of the tested IGRs on the progeny resulting from the treated females was determined by monitoring larval development from hatching to adult emergence. Four hundred first instar larvae from each concentration were randomly selected and placed into white enamel bowls (4 replicates, 100 larvae each) containing fresh tap water and given the usual larval food. Data on larval mortality, pupation and adult emergence were recorded daily, and compared with control trials. Adult longevities were also estimated for the mosquito survivors. All data were evaluated by a general linear model procedure comparing least square means using a SAS computer program.

RESULTS AND DISCUSSION

Effects of the Match[®] and Baycidal[®] on some behavioral and biological aspects of *C. pipiens* when fed

Table1. The effect of Match and Baycidal as a feed-through treatment on the blood-feeding activity in *C. pipiens* mosquitoes

IGRs	Match			Baycidal			Control
	Conc.(ppm)	0.1	0.5	1.0	0.1	0.5	
No.of engorged females*	36	34	30	33	32	28	44
Feeding activity (FA) %	72	68	60	66	64	56	88
Reduction (R)%	18.2	22.7	31.8	25	27.3	36.3	-

* 50 mosquito females were used. - No data

$$F.A. = \frac{\text{No. of engorged females}}{\text{Total no. of females used}} \times 100.$$

$$R = 1 - \left(\frac{\text{F.A. in treated females}}{\text{F.A. in control}} \right) \times 100.$$

as adult mosquitoes via the sugar solution have been evaluated. The results showed that the use of Match[®] and Baycidal[®] as feed-through trials against *C. pipiens* at concentrations of 0.1, 0.5 and 1 ppm for each compound caused a reduction in the blood-feeding activity of mosquito females (Table 1). At the tested concentrations (0.1, 0.5 and 1 ppm), the feeding activity of mosquito females was reduced by about 18.2, 22.7 and 31.8% in the case of Match[®] treatments and 25, 27.3 and 36.3% in the case of Baycidal[®], respectively, compared with the control. These results are in accordance with Saleh and Wright (1990). They conducted similar trials using cyromazine (0.5 – 25 ppm) as a feed through treatment against *C. pipiens* and *Ae. egypticus*. Also, feeding activity was significantly reduced in *An. dirus* by about 10% and 25% after exposure to 0.01 and 0.1 ppb of the IGRs methoprene (Sithiprasasna *et al.* 1996).

Generally, the initiation of biting behavior is mediated by juvenile hormone in *C. pipiens* (Meola and Petralia, 1980). Therefore, compounds with anti-juvenile hormone activity would presumably be highly effective in preventing blood feeding by blocking juvenile hormone release or its action in adult female mosquitoes. However, the reduction in biting activity of mosquito females may lead to a decrease in the number of fully engorged females and accordingly affect the reproductive capacity of mosquitoes.

Results in Table (2) showed that the times of blood digestion (2nd phase of gonotrophic cycle) in mosquito females of *C. pipiens* treated by the tested concentrations of Match[®] were 6.5, 6.7 and 7.0 days, respectively. These times were 6.8, 7.0 and 7.3 days when mosquito females were treated by the tested concentrations of Baycidal[®], respectively. In the control, this time was 4.5 days. Therefore, treatment with Match[®] and Baycidal[®] as a feed-through treatment caused a significant

prolongation in the time needed for blood meal digestion by about 44.4, 48.8 and 55.5% in case of Match[®] and 51.1, 55.5 and 62.2% in the case of Baycidal[®], respectively. These results are in parallel with other investigations using six JH-like activity compounds (Kelada *et al.*, 1980) and *Bacillus thuringiensis* H-14 (Saleh *et al.* 1990; Saleh and Wright, 1990) against *C. pipiens* mosquitoes. They stated that a marked prolongation in the time needed for blood meal digestion ingested by mosquito females. However, such a study is useful for the estimation of the frequency of mosquito contact with the host. For instance, if the gonotrophic cycle is 4 or 5 days it means that the mosquito will feed on average every 4 or 5 days; this has an important effect on the transmission of the disease. Therefore, determination of the time of gonotrophic cycle is one of the necessary parameters for the calculation of vectorial capacity (WHO, 1975).

The effect of Match[®] and Baycidal[®] on the reproductive potential of *C. pipiens* when fed to adult mosquitoes in the sugar solution is summarized in Table (3). Results showed that Match[®] treatments at concentrations of 0.1 and 0.5 ppm had no effect on egg production by mosquito females. A maximum reduction in fecundity (30.3%) was observed in mosquito females received sugar solution containing 1 ppm of Match[®]. On the other hand, results indicated that Baycidal[®] treatments at concentrations of 0.1, 0.5 and 1 ppm led to a marked reduction in egg-laying capacity of *C. pipiens* mosquitoes. The mean numbers of eggs were 52.1, 46.4 and 42.1/ female, while, it was 136.9/ female in control (Table 3). Therefore, the reduction percentages in the fecundity of female were 61.9%, 66.1% and 69.2% at the tested concentrations, respectively.

Data on the hatchability of eggs are shown in Table (3). The percentages of egg hatching were 92.3, 86.3, and 85.3% in mosquito adults that fed Match[®] at concentrations of 0.1, 0.5 and 1.0 ppm in the sugar solution. Statistical analysis showed that the difference

in the hatchability of eggs between the treatment groups and the control (89.7%) was insignificant. On the other hand, the records showed that the percentages of egg hatching were 55.5, 46.9 and 47.9% in mosquito adults that fed Baycidal[®] at doses of 0.1, 0.5 and 1.0 ppm, respectively, when compared with the control. This means that Baycidal[®] as a feed-through treatment at the tested concentrations caused 34.2, 42.8 and 41.8% reduction in the hatchability of eggs.

It has been reported in the literature that egg hatching is strongly inhibited, in *Drosophila melanogaster* females after feeding on a diet containing lufenuron, the percentage of hatching of eggs laid by lufenuron-treated females was insignificant compared to that of untreated females. (Wilson and Cryan, 1997). Similar results were observed following exposure of *Tribolium castaneum* to novaluron (Kostyukovsky and Trostanetsky, 2006).

Our results are in accordance with Saleh and Wright (1990). They used cyromazine against *C. pipiens* and *Ae. egypticus* and they mentioned that cyromazine treatments led to a reduction in the egg-laying capacity. Most studies described tests of Baycidal[®] against different species of dipterous flies. Friedel and McDonell (1985) mentioned that the egg production was decreased and the hatching of eggs remained unaffected in the sheep blow fly *Lucilia cuprina* fed on cyromazine (1 – 20 ppm) in the drinking water. A concentration of up to 100 ppm cyromazine in the drinking water did not affect egg-laying capacity in *Musca domestica* (Pochon and Casida, 1983). Generally, it has been suggested that treatment with the test IGRs as feed-through trials may affect the ovaries (e.g., the germarium) or vitellogenesis and consequently the fecundity of mosquito females (Huybrechts and DeLoof, 1981). However, the present study suggests that the present IGRs particularly Baycidal[®] will also prove to have a significant effect on the reproductive potential of other dipteran species that

Table 2. The effect of Match and Baycidal as a feed-through treatment on the time of blood digestion (2nd phase of gonotrophic cycle) in *C. pipiens* mosquitoes

	IGRs			Baycidal			Control	
	Conc. (ppm)	0.1	0.5	1.0	0.1	0.5		1.0
	Range	4-8	4-11	4-11	4-10	4-10	5-11	4-6
Digestion time (in days)	Average* ± S.E.	6.5 ^b ± 0.28	6.7 ^b ± 0.36	7.0 ^b ± 0.44	6.8 ^b ± 0.34	7.0 ^b ± 0.45	7.3 ^b ± 0.15	4.5 ^a ± 0.19
	Increase in time of blood digestion (%)	44.4	48.8	55.5	51.1	55.5	62.2	-

* 20 engorged mosquito females.

- No data

Numbers with the same letter are not significantly different at 0.05 level

Table 3. The effect of Match and Baycidal as a feed-through treatment on the reproductive potential of *C. pipiens* mosquitoes

IGRs	Match			Baycidal			Control
Conc. (ppm)	0.1	0.5	1.0	0.1	0.5	1.0	-
Total No. of eggs	2597	2467	1939	1055	945	876	2702
Mean No. of egg female	128.4 ^a ± 1.10	121.6 ^a ± 1.7	95.3 ^b ± 1.1	52.1 ^c ± 2.12	46.4 ^c ± 1.6	42.1 ^c ± 0.41	136.9 ^a ± 2.15
(%) fecundity reduction	6.2	11.2	30.3	61.9	66.1	69.2	-
Total No. of hatched eggs	2396	2129	1655	586	443	420	2425
Hatchability (%)	92.3	86.3	85.3	55.5	46.9	47.9	89.7
Effect on hatchability (%)	+2.6	-3.4	-4.4	-34.2	-42.8	-41.8	-

* 20 engorged females were used.

-- No data

Numbers with the same letter are not significantly different at 0.05 level

require a blood meal for the initiation and completion of vitellogenesis. Mansur *et al.* (2010) explained the reduction of number of chorionated oocytes, the reduction of eggs laid and the reduction in the viability of the eggs of *Rhodnius prolixus* after exposure to lufenuron (Match[®]) as a consequence of osorption following inhibition of chitin synthesis and/or cuticle assembly.

Subsequent studies were also conducted to investigate the possible delayed effect of Match[®] and Baycidal[®] on the development of *C. pipiens* larvae when fed to adult mosquitoes (Table 4). Although Match[®] had no apparent effect on egg hatch (Table 3), subsequent larval development was inhibited progressively as the concentrations of Match[®] increased. At concentrations of 0.1, 0.5 and 1.0 ppm, most larvae that formed apparently normal pupae often died before the adult emergence and accordingly, 17.8, 49.2 and 92.7% inhibition of adult formation was occurred. However, this decline in the survival rates may indicate that some

of delayed fetal effects of Match[®] occurred during the development from larvae to pupae, and accordingly, the resulting pupae and emerging mosquito adults were affected. On the other hand, the results indicated that the larval development of *C. pipiens* was completely inhibited when adult mosquitoes fed Baycidal[®] at the dosage of 0.5 and 1.0 ppm (Table 4). Results showed that most hatched larvae were fed and developed normally for the duration of the 2nd instar, but failed to molt completely to the 3rd larval instar. However, it can be suggested that the difference in the trend of inhibitory effect of Match[®] and Baycidal[®] on the subsequent larval development of *C. pipiens* may be due to a special mechanism of the tested compounds. The obtained findings are in agreement with a previous study (Kawada *et al.*, 2003) that discussed the larvicidal efficacy of IGR Match[®] on anopheline mosquito (*A. sergenti*, *gambiae* and *albimanus*).

Table (4) shows the delayed effect of Match[®] and Baycidal[®] as a feed-through treatment on the longevity

Table 4. The effect of Match and Baycidal as a feed-through treatment on subsequent larval development and the longevity of adult survivors of *C. pipiens* mosquitoes

IGR (ppm)	No. of Dead-larvae ^a	No. of pupae produced	Adult emerge			Adult longevity ^c (in days) ± S.E.	
			Total	Inhibition ^b (%)		Males	Females
				Obs.	Cor.		
Match							
0.1	50	350	301	24.7	17.8	17.99 ± 0.78	28.01 ± 0.90
0.5	147	253	187	53.2	49.2	15.3 ± 0.87	31.0 ± 0.76
1.0	192	208	18	95.5	92.7	18.3 ± 0.82	23.9 ± 0.89
Baycidal							
0.1	246	154	86	78.5	76.7	18.9 ± 0.81	26.7 ± 0.94
0.5	365	35	0.0	100	100	-	-
1.0	400	0.0	0.0	100	100	-	-
Control	21	378	368	8		7.4 ± 0.83	27.5 ± 0.90

^a 4 replicates, 100 larvae each.^c 20 males or females were used.^b Corrected by Abbott's formula.

- No data.

of mosquito adults that survived from the above hatched larvae. In general, the records showed that mosquito females of both treated and untreated groups live much longer than the males.

Finally, it can be concluded that mosquito females that fed on (in water) treated surfaces with Match® or Baycidal® are likely not only lay fewer eggs, but also lay eggs that will ultimately produce fewer (or no) adults, even if the mosquito larvae do not come in contact with these compounds.

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الملخص العربي

تقييم نوعين من منظمات نمو الحشرات "كمعاملات من خلال التغذية" ضد الحشرات الكاملة لبعوض الكيولكس بيبينز

حسام الدين مجدي زهران

هذه المركبات في المحلول السكري. لوحظ أقصى إنخفاض في إنتاج البيض بمقدار ٣٠,٣% في إناث البعوض المغذاه على محلول سكري بجوى ١,٠ جزء في المليون من مركب الـ Match. من ناحية أخرى أوضحت النتائج أن المعاملة بمركب الـ Baycidal عند التركيزات ١,٠, ٠,٥, ٠,١ جزء في المليون قد أدت إلى إنخفاض واضح في قدرة الإناث على وضع البيض بمقدار ٦١,٩, ٦٦,١ و ٦٩,٢% على الترتيب وذلك عند المقارنة بالكنترول. كما إنخفضت أيضاً نسبة قفس البيض الناتج عن تلك الحشرات المعاملة.

أجريت أيضاً دراسات لاحقة لبحث التأثير المتأخر لمنظمات نمو الحشرات: الـ Match والـ Baycidal على اليرقات الفاقسة. أوضحت النتائج أن نمو وتطور اليرقات حتى ظهور الحشرات الكاملة قد تم تثبيطه تدريجياً بمقدار ١٧,٨, ٤٩,٢ و ٩٢,٧% عند استخدام الـ Match بالتركيزات ١,٠, ٠,٥, ٠,١ جزء في المليون على الترتيب. من ناحية أخرى أكدت النتائج أن نمو اليرقات وتطورها حتى ظهور الحشرات الكاملة قد تم تثبيطه كلياً عندما تغذت الحشرات الكاملة للبعوض على التركيزات ١,٠, ٠,٥ جزء في المليون من الـ Baycidal حيث أظهرت النتائج أن معظم اليرقات الفاقسة قد تغذت وتطورت طبيعياً حتى العمر اليرقي الثاني ولكنها فشلت في الإنسلاخ كلياً إلى العمر اليرقي الثالث.

كما تم دراسة تأثير الـ Match والـ Baycidal على طول عمر الحشرات الكاملة (ذكور و إناث) الناتجة من معاملة اليرقات بهذه المركبات وأظهرت النتائج عدم وجود فرق معنوي بين طول عمر الحشرات الكاملة الناتجة من اليرقات المعاملة والكنترول.

أجريت هذه الدراسة لتقييم التأثير الحيوي لنوعين من منظمات نمو الحشرات هما الـ Match و Baycidal كمعاملات من خلال التغذية ضد الحشرات الكاملة لبعوض *C. pipiens*. باستخدام التركيزات ١,٠, ٠,٥, ٠,١ جزء في المليون لكل مركب. أوضحت النتائج أن استخدام الـ Match و الـ Baycidal كمعاملات من خلال التغذية ضد الحشرات الكاملة لبعوض الـ *C. pipiens* أدى إلى إنخفاض في نشاط التغذية على الدم عند إناث البعوض بمقدار ١٨,٢, ٢٢,٧ و ٣١,٨% (في حالة المعاملة بالـ Match) وبمقدار ٢٥, ٢٧,٣ و ٣٦,٣% (في حالة المعاملة بالـ Baycidal) على الترتيب وذلك عند المقارنة بالكنترول. هذا الإنخفاض في عدد الإناث المتغذية على الدم يؤثر بالتالي على الكفاءة التناسلية للبعوض.

أظهرت النتائج أن استخدام الـ Match والـ Baycidal كمعاملات من خلال التغذية بالتركيزات المستخدمة قد سبب إطالة ملحوظة في الوقت اللازم لهضم وجبة الدم المبتلعة بواسطة إناث بعوض *C. pipiens* بمقدار ٤٤,٤, ٤٨,٨ و ٥٥,٥% (في حالة الـ Match) وبمقدار ٥١,١, ٥٥,٥ و ٦٢,٢% (في حالة الـ Baycidal) على الترتيب وذلك عند المقارنة بالكنترول. تفيد هذه الدراسة في تقدير مدى تكرار تلامس البعوض مع العائل، وحساب مقدرته كناقل للمسببات المرضية.

تم دراسة تأثير منظمات نمو الحشرات الـ Match والـ Baycidal على القدرة التناسلية لبعوض *C. pipiens* المتغذى على