Potential studies of non-conventional chemicals against the housefly larvae *Musca domestica* L.

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Abstract: Three insect growth regulators (IGRs) triflumuron, cyromazine and pyriproxyfen as well as the plant extract neem oil were evaluated against 2nd instar larvae of *Musca domestica* by feeding and dipping bioassay methods. In both assays, cyromazine proved to be the most effective compound against housefly larvae, followed by triflumuron and pyriproxyfen, while the plant extract neem oil was the least effective one. According to IC_{50} values (concentration which to inhibit the emergence of 50% of adults), the results indicated that larval treatments with the test compounds using feeding method (0.6, 0.35, 0.66 and 43 ppm, respectively) were more effective for larvicidal activity than dipping assay (0.8,0.46, 0.9 and 60 ppm, respectively). Different levels of potentiation reflected by the inhibition of adult emergence were also obtained when the test IGRs were applied jointly with the plant extract neem oil against housefly larvae.

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1. Introduction

The common housefly, *Musca domestica* L. (Diptera: Muscidae) is a major insect pest, particularly in tropical countries. It is an important mechanical vector of several bacterial and pathogenic organisms of humans and animals (Greenberg, 1973; Pandian and Asumtha, 2001; Sehgel *et al*., 2002).

Drawbacks associated with wide spread use of conventional insecticides for controlling housefly populations have not resulted in the development of insect resistance to different insecticides, but have also caused environmental pollution and toxic side effects to human and non-target organisms. Therefore, more attention has been recently paid to the use of non-conventional insecticides such as insect growth regulators (IGRs) and plant extracts for controling housefly in different parts of the world (Kaufman *et al*., 2001; Crespo *et al*., 2002; Kristensen *and* Jespersen, 2003; Bisselleua *et al*., 2008; Begum *et al*., 2013).

The present study was planned in part to evaluate the biological activity of three IGRs triflumuron, cyromazine and pyriproxyfen as well as, the plant extract neem oil against larvae of *M. domestica*. The possible joint action of the test IGRs with the neem extract against housefly larvae was also conducted.

2. Materials and Methods

House fly strain

Tests were performed on a field strain of *M. domestic* collected by using sweep nets from sheep market, Jeddah, KSA. The collected flies were transported in muslin cages (40 x 40 x 40 Cm) to the laboratory and had been maintained under controlled conditions of 27± 1 ^{o}C and 65+ 5 % R.H with a 12:12 (L:D) photoperiod. Adult flies were fed on dry milk powder, sugar and water while larvae were reared on a medium of yeast, dry milk powder, wheat bran and water.

Tested Compounds

1. Three IGRs: triflumuron 25 %, Bayer Env. Sc. SAS; pyriproxyfen 0.5%, sumitomo Chem Co. and cyromazine 5 %, Ciba – Giege Ltd.

Aprimary aqueous suspention of each tested compound was prepared at 100 ppm in 100 ml of distilled water, and serial dilutions were prepared in distilled water for testing.

2. The plant extract neem oil (*Azadirachta indica*), kindly supplied by Dr. M.A. Khan, Dept. of Zoology, Saifia science college Bhopla, India. The stock solution of the plant extract was prepared by adding 1 ml of it to 99 ml of distilled water containing 0.5% triton X-100 as an emulsifier to ensure complete solubility of the extract in water. Series of concentrations were prepared in distilled water.

Larval bioassay methods

Two bioassay methods were conducted in this study

Feeding method:

The feeding method was applied according to the method of Vazirianzadeh *et al*. (2007) with some modifications. Tests were performed in groups of glass beakers (400 ml capacity) containing 50 gm of larval

rearing medium treated with different concentrations of the test compound. Untreaed larval media were served as controls. Five replicates of 20 second instar larvae for each concentration and so for the control trials were conducted. Each beaker was covered with muslin cloth and held at 27 ±1 °C and 65+5% R.H with a 12:12 (L:D) photoperiod for two weeks.

The mortality of larvae and the number of formed pupae and adults emerging were recorded. Flies were scored as survived if they were able to emerge successfully from the puparium.

Dipping Method:

The dipping method was applied following the method of Sukontason *et al*. (2004) with some modifications. larval treatments were carried out by exposing the 2nd instar larvae to various concentrations of the test IGRs. Five replicates of 20 larvae each per concentration and so for the control trials were set up. Larvae were gently dipped into compound solutions for 30 sec with a dip net. The larvae of controls were dipped in tap water. After the larvae had been dipped, they were transferred to the rearing glass beakers containing larval medium. The number of emerged flies was counted. All test trials were carried out under the same above controlled conditions.

Joint action tests

Values of IC_{25} and IC_{40} (concentrations which to inhibit the emergence of 25 and 40 % of fly adults, respectively) were obtained from the toxicity line of the plant extract neem oil. The concentrations corresponding to these values were prepared. Paired mixtures were applied at the above sublethal concentrations of the plant extract with the IC_{25} values of the test IGRs.

Five replicates of 20 larvae for each mixture were tested by using the feeding bioassay method. The efficacy of the test mixtures was calculated as the percentage of larvae that did not develop into successfully emerged adults or the inhibition of emergence. The joint action of different mixtures was expressed as the co-effective factor (C.F.) according the equation given by Mansour *et al*. (1966) as follows:

$$C.F. = \frac{X - Y}{Y} \times 100$$

X= Observedinhibition

Y = Expected inhibition of adult emergence

This factor was used to differentiate results into three categories. A positive factor of 20 or more is considered potentiation; a negative factor of 20 or more means antagonism and intermediate values between -20 and + 20 indicate only additive effects.

Statistical analysis

The percentage of inhibition of adult emergence was corrected for control mortalities using Abbott's formula (Abbott, 1925). The inhibition– concentration – probability lines (IC-p lines) were drawn for each compound and the median inhibitory concentration of adult emergence (IC50) was calculated thereby following the method of Litchfield and Wilcoxon (1949).

3. Results and Discussion

Table 1 shows toxicity of the IGRs triflumuron, cyromazine and pyriproxyfen as well as the plant extract neem oil against housefly larvae *M. domestic4*about 1.7, 1.9 and 123 times, respectively.

By using dipping method, the effective concentrations of triflumuron (0.3- 2 ppm), cyromazine (0.2 - 1.2 pmm), pyriproxyfen (0.4 - 2ppm) and neem extract (20-150ppm) against 2nd larval instars caused 14.9 - 87.2%, 19.1 -89.4%, 17.2 - 86% and 15 - 87% inhibition of adult emergence, respectively. According to IC_{50} values, cyromazine (0.46ppm) proved to be the most effective of the tested IGRs followed by trifulumuron (0.8 ppm) and pyriproxyfen (0.9 ppm), while the plant extract neem oil (60 ppm) was the least effective one. In other words, results thus indicated that trifulmuron is 1.7, 2 and 130 times as effective as the above compounds, respectively.

Generally, the records indicated that larval bioassay treatments by using feeding method were more effective for larvicidal activity than dipping one. Such a fact was highly pronounced on the basis of IC_{50} values obtained for the tested compounds against housefly larvae treated by feeding assay as compared to the dipping method (Cetin *et al*., 2006). However, it can be concluded that the response of larvae to the test compounds depends entirely on the differential mode of action of these compounds and its effective concentrations. Studies in this respect were carried out by other investigators using many different IGRs (Vazirianzadeh *et al*., 2007; Sulaiman *et al*., 2008; Msangi *et al*., 2011) and plant extracts (Khan *et al*., 1991; Sukontason *et al*., 2004; Ghoneim *et al*., 2007; Dad *et al*., 2011; Islam and Aktar, 2013) against larvae of *M. domestica*

Table 2 shows the percentage expected and observed inhibition of adult emergence, co-effective factor (C.F) and the type of effects resulted from the combinations of the plant extract neem oil with the IGRs triflumuron, cyromazine and pyriproxyfen. The combinations were applied at the IC_{25} and IC_{40} values of neem oil (26 and 35 ppm) and the IC_{25} level of triflumuron (0.42 ppm), cyromazine (0.22 ppm) and pyriproxyfen (0.44 ppm). In general, values of C.F. indicated that all combinations of the plant extract with the test IGRs produced different levels of potentiation reflected by the inhibition of adult formation. The

mixture of neem oil at IC_{25} + triflumuron at IC_{25} showed the highest C.F (+88), while the mixture of neem oil at IC_{40} + pyriproxyfen at IC_{25} gave the lowest C.F. (+26.1). Variations in the levels of potentiation among the test mixtures may reflect the differences in their mode of action and the tested IC values (Saleh and Wright, 1989). Similar findings have been reported by Mansour *et al*. (2012) who found that the combinations of 11 botanical extracts with 4 insecticides against the housefly *M. domestica* were resulted in potentiating mixtures of co-toxicity factors exceeding 90.0. However, long term follow–up studies are needed to determine how the environmental conditions affect the larvicidal effectiveness of such non-conventional insecticides when applied jointly for field control measures.

Table 1: Toxicity of the IGRs triflumuron, cyromazine and pyriproxyfen as well as the plant extract neem oil against housefly larvae *M. domestica* by using feeding and dipping bioassay methods.

Compound	Effective Concentrations (ppm)	Larval mortality (%)	Adult emergence Total Inhibition** (%)		IC_{50} (ppm)	Slope
Feeding Method						
Triflumuron	0.3 - 1.5	7 - 34	78 - 4	17 - 95.7	0.6	4.3
Cyromazine	0.1 - 0.9	10 - 44	83 - 8	9.8 - 91.3	0.35	3.1
Pyriproxyfen	0.3 - 1.8	6 - 31	80 - 5	14 - 94.6	0.66	3.9
Neem oil	15 - 150	5 - 25	82 - 3	12.8 - 96.8	43	3.3
Control		3 - 4	94 - 92	6 - 8		
Dipping method						
Triflumuron	0.3 - 2	6 - 24	80 - 12	14.9 - 87.2	0.8	2.8
Cyromazine	0.2 - 1.2	4 - 37	76 - 10	19.1 - 89.4	0.46	3.9
Pyriproxyfen	0.4 - 2	6 - 36	77 - 13	17.2 - 86	0.9	2.9
Neem oil	20 - 150	4 - 22	85 - 13	15 - 87	60	2.2
Control		2 - 5	96 - 93	4 - 7		

* 5 replicates, 20 second instar larvae; ** Corrected for control mortalities (Abbott, 1925).

Table 2 : The joint action of the plant extract neem oil with IGRs triflumuron, cyromazine and pyriproxyfen against 2nd instar larvae of *M. domestica* by using feeding method.

Mixures and IC levels	Concentrations used (ppm)	Inhibition of Adult emergence (%) Expected	Observed	C.F*	Effect
Neem oil + Triflumuron					
IC_{25}+ IC_{25}	26 + 0.42	50	94	+88	(XX)
IC_{40} + IC_{25}	35 + 0.42	65	85	+30.7	(XX)
Neem oil + cyromazine					
IC_{25}+ IC_{25}	26 + 0.22	50	90	+80	(XX)
IC_{40} + IC_{25}	35 + 0.22	65	93	+43	(XX)
Neem oil + pyriproxyfen					
IC_{25}+ IC_{25}	26 + 0.44	50	79	+58	(XX)
IC_{40} + IC_{25}	35 + 0.44	65	82	+26.1	(XX)

* Coeffective factor (Mansour et al., 1966) (xx) potentiation

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