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Synergism and antagonism of Baygon with some additives against Baygon–resistant strain of *Culex pipiens* larvae

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ABSTRACT

Larvae of the mosquito *Culex pipiens* were subjected to continuous laboratory selection with Baygon for 15 successive generations. This resistant strain was tested with some additives, piperonyl–butoxide, sesame oil and clove oil, to investigate their synergistic or antagonistic effect. The use of sesame oil and piperonyl-butoxide considerably enhanced the toxicity of Baygon. Clove oil also potentiated Baygon but to a lower extent than sesame oil and piperonyl-butoxide. The activity of each synergist was found to be concentration dependent. Results showed the possibility of using the piperonyl-butoxide and sesame oil as a synergist against a Baygon–resistant strain of *C. pipiens*.

KEYWORDS: mosquitoes, insecticide, clove oil, sesame oil, resistant

INTRODUCTION

For many years synergists have been used extensively in combination with insecticides for controlling mosquitoes and other insects. For example, Plapp *et al*. (1963) achieved a 96-fold synergistic effect with DEF in a strain of *Culex tarsalis* exhibiting specific malathion resistance. There is a remarkable synergism of carbamate by methylene ether, 2,3-naphthalenediol and piperonyl–butoxide (Georghiou *et al*. 1966). Apperson & Georghiou (1975) tested *in vivo* a multiresistant strain of *C. tarsalis*, and showed clearly the synergism of organophosphorous compounds by DEF and antagonism by piperonyl-butoxide. Georghiou *et al*. (1975) found that the resistance of a field strain of *C. pipiens quinquefasciatus* to chlorpyrifos and malathion was overcome by a DEF synergist but not by piperonyl-butoxide. However, Priester & Georghiou (1978) observed a limited effect of piperonyl-butoxide and DEF when used against a permethrin-resistant strain of *C. pipiens quinquefasciatus*. Losada *et al.* (1991) tested the effect of the synergists s,s,s-tributyl, phosphorotrithioate and piperonyl-butoxide on resistant strain of *C. quinquefasciatus* to malathion, temephos and propoxur. They found that in the presence of s,s,s-tributyl, phosphorotrithioate suppressed the resistance to malathion, while piperonyl-butoxide had no effect. Gonzalez *et al.* (1996) studied the change in resistance to different insecticides in a *C. quinquefasciatus* strain subjected in the laboratory to doses of pyrethroid lambda-cyhalothrin that would cause a larval mortality of 90%. The strain became 144.5× more resistant to this insecticide compared to the original level, and a resistant strain emerged (287×). There was an increase of the levels of resistance to methyl-pyrimifos (2.4×), propoxur (6×), DDT (5,2×), clorpirifos (22×), cypermethrin (67.5×), and deltamethrin (20.2×). The authors detected synergism between DEF and PB with lambda-cyhalothrin. In addition, Tadas *et al*. (1994) found after field trials that the efficacy of fenvalerate insecticide against some cotton pests increased by adding sesame oil.

According to the World Health Organization (WHO), however, the resistance of insect pests to insecticides constitutes a serious threat to their successful control. Resistance has

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been reported not only by the new synthetic insecticides but also to insect growth regulator, chemo-sterilants and even natural control agents (Sawicki 1979).

The present study aims to investigate the activity of three additives, piperonyl-butoxide, sesame oil and clove oil, to be used in combination with the insecticide Baygon against a resistant strain of *C. pipiens* larvae.

MATERIALS AND METHODS

C. pipiens larvae were obtained from Miet El-Attar village, Qualyubia Governorate. The larvae were reared and maintained at the Entomology Department, Faculty of Science, Zagazig University, Benha branch. To raise their susceptibility, the field-collected larvae were reared without being exposed to any insecticides, and are referred to as the "normal" strain. This normal strain formed the basic stock from which a strain resistant to Baygon was created. This "resistant" strain was achieved by treating the third instar larvae of the normal strain with an insecticide/water mixture at each generation. Large numbers were employed and the selection pressure was always sufficient to cause 75% mortality or more. According to the response of the treated larvae to selection, a higher concentration of the toxicant was sometimes used in subsequent generations.

Further selection was carried out for many generations until a high level of resistance was achieved, maintained for three generations after relaxation of selection with the insecticide (evidence of stability of resistance). The resistant strain was compared with the normal strain to determine the resistance level or the resistance ratio by the following equation:

$$R.R. = \frac{LC_{50}\text{ of the resistant strain}}{LC_{50}\text{ of the normal strain}}$$

C. pipiens of both strains were kept at room temperature 25 ± 2°C and 80 ± 5% R.H. The field-collected larvae of *C. pipiens* were transferred to white enamel pans (35 cm. diameter 9 cm. depth) containing tap water where they were kept until emergence. Larval food (tetramin tropical fish food-tetra, W. Germany) was supplied once daily. These pans were covered with muslin for protection against foreign insects. Pupae were collected daily from the breeding pans by means of a wire mesh or a plastic dropper. They were transferred to small plastic cups half-filled with distilled water and then introduced into cages for adult emergence. The breeding cages were made of wooden frames (30×30×30 cms.) with the bottom covered by plywood, and sides screened with a fine mesh. The front side of the cage had a long sleeve, closed by a rubber band when the cage was not in use. Emerged adults were provided with 10% sucrose solution soaked in a piece of cotton pad, renewed daily. Adult females were allowed blood meals from a pigeon at intervals for egg production. The breeding cages were provided with plastic cups (15 ml) half-filled with distilled water for egg laying. The egg rafts were gently collected and distributed in enamel pans half-filled with water until hatching.

The standard method of the WHO (1981) for measuring the susceptibility level (i.e. the resistance) of larvae was used. Tests were carried out on the early and the late third instar larvae, in plastic cups (500ml capacity) each containing 249ml distilled water. 1ml of the desired concentration of insecticide or insecticide/additive mixture was applied under the water surface with a pipette and mixed well. A batch of 20-25 healthy and active larvae was transferred to the cup 30 minutes after preparing the insecticide/additive mixture. Each test was replicated 5 times.

Larvae from the resistant strain were used for the experiment. The tested larvae were kept at the same temperature and relative humidity as was used for rearing. They were left in the insecticide or insecticide/additive mixture for 24 hrs, after which mortality was recorded.

A larva was considered dead when it appeared unable to move. Control experiments were prepared and tested in a similar way but without applying insecticide. Mortality percentages were corrected by Abbott's formula (Abbott 1925) if the mortality in the control exceeded 10%. Mortality percentages were plotted against the log of the concentration used, and the median lethal concentrations were determined graphically. The slope function of the mortality lines was taken as a criterion of the degree of homogeneity of the population in its response to the toxicant (Hoskins & Gordon 1956). The chemical insecticide used was the carbamate Baygon (2-isopropoxyphenyl methyl carbamate, technical 97%). The additives used are:-

1. Piperonyl-butoxide : (5-2-(2-butoxyethoxy) ethyl methyl-6-propyl 1-3 benzodioxole.
2. Sesame oil (Sesamex) : 2-(3,4- methylenedioxy phenoxy)-3,6,9-trioxaundecane.
3. Clove oil: the chief constituent of the oil (morthan, 85%) is eugenol (4-allyl-2-methoxyphenol), terpene (caryophyllene), acetyleugenol, methyl urfural, dimethylfurfural and methyl salicylate.

The different concentrations of insecticide and additives were prepared from the stock solution by dissolving a known amount of each in distilled water. Ethyl alcohol was used as a solvent for all the chemicals tested. Concentrations were expressed in parts per million (ppm) or in percentages. The synergistic factor was calculated by using the following formula:

$$S.F. = \frac{LC_{50}\text{ of the insecticide alone}}{LC_{50}\text{ of the insecticide/additive mixture}}$$

where values of S.F.>1 indicate synergism and <1 indicate antagonism.

In order to find the synergistic action of a mixture, the concentration of the synergist should have little or no toxicity when applied alone. Hence, the three additives were first tested alone against the larvae, before their potentiating effects on Baygon were investigated. 0.01%, 4% and 1% concentrations of the additives piperonyl-butoxide, sesame oil and clove oil respectively were used as a maximal synergistic concentration for testing, both alone and in conjunction with different concentrations of Baygon insecticide. Statistical analysis was performed using t-tests comparing the treatments with and without additives.

RESULTS

The additives alone showed no appreciable toxicity even at the highest concentration used. For the insecticide/additive mixtures, the results are presented in tables 1-4. Table 1 indicates that the additions of 0.001, 0.005 & 0.01 % of piperonyl-butoxide to each concentration of Baygon caused a progressive decrease in LC_{50} values, i.e. increased larval mortality.

Table 2 shows the synergistic effects of sesame oil when used in combination with Baygon insecticide against the Baygon-resistant *C. pipiens* larvae. There is a significant increase in larval mortality due to the combined effect of Baygon with sesame oil. The addition of increasing concentrations of sesame oil to Baygon greatly decreased the LC_{50} values, and hence increased Baygon's efficacy up to 10 times. The activity was clearly concentration dependent. Combined effects of clove oil with Baygon insecticide are shown in table 3. The LC_{50} values for Baygon declined with increasing concentrations of clove oil, although the cotoxicity coefficients indicated only a slight increase in the toxicity of Baygon. Table 4, showing the cotoxicity coefficient, indicates clearly the effectiveness of the piperonyl-butoxide and sesame oil as synergists on the toxicity of Baygon. In each case the activity of the synergist was concentration dependent. Statistical analysis revealed, moreover, that these observed increases in larval mortality for the insecticide/additive mixtures over that caused by the insecticide alone were highly significant at the highest additive concentrations ($p<0.001$), significant at the middle concentrations ($p<0.05$) but not significant at the lowest concentrations.

Table (1): Susceptibility of Baygon-resistant strain of *C. pipiens* larvae to Baygon and its combination with different concentrations of piperonyl-butoxide.
R. R. =Resistance ratio.
S. F. = Synergistic factor.
P.b.= Piperonyl-butoxide

Baygon Conc.	Piperonyl-butoxide concentration (%)			
(ppm)	0.0	0.001	0.005	0.01
5	--	--	5.0± 0.33	18.3±0.67
6	--	10.0±0.58	31.7±0.88	35.0±0.58
10	--	26.7±0.88	50.0±1.16	60.0±1.16
20	0.0	40.5±0.56	65± 1.50	80.0±1.53
30	5.3 ± 0.8	70.0± 1.16	88.3±1.45	95.0±1.0
50	25.0± 162	90.0± 0.67	--	--
60	46.7±0.88	--	--	--
80	72.7 ± 1.73	--	--	--
$LC_{50\ (ppm)}$	61.75	17.78	10.07	9.16
Slope function	1.49	2.31	2.65	2.21
R.R	308.7	88.9	50.35	45.8
S.F.	0.0	3.47	6.13	6.74

Table 2: Susceptibility of Baygon-resistant strain of *C. pipiens* larvae to Baygon and its combination with different concentrations of sesame oil.

Baygon Conc.	Sesame oil concentration (%)			
(ppm)	0.0	1	2	4
5	--	20.0±0.58	31.7±1.33	36.7±0.33
10	--	46.7±0.33	66.7±1.20	75.0±1.16
20	0.0	60.0±1.0	82.0±1.0	88.3±0.88
30	5.3 ± 0.8	76.7± 0.88	90.0±0.58	96.7±0.68
50	25.0± 162	90.0± 0.67	--	--
60	46.7±0.88	--	--	--
80	72.7 ± 1.73	--	--	--
$LC_{50\ (ppm)}$	61.75	13.04	7.78	6.20
Slope function	1.49	3.52	2.93	2.48
R.R.	308.7	65.2	38.9	31.0
S.F.	0.0	4.47	7.94	9.96

Table 3: Susceptibility of Baygon-resistant strain of *C. pipiens* larvae to Baygon and its combination with different concentrations of clove oil.

Baygon Conc.	Clove oil concentration (%)			
(ppm)	0.0	0.25	0.5	1
10	--	--	16.7± 0.33	28.3±0.68
20	0.0	8.3±0.88	40.0±1.0	46.7±0.88
30	5.3±0.88	13.7±1.0	56.7±0.88	71.7±1.4
40	24.0±0.33	31.7 ±133	78.3±1.16	80.0±1.0
60	46.7±0.88	68.3±1.67	--	--
80	72.7 ± 1.73	--	--	--
$LC_{50\ (ppm)}$	61.75	51.26	23.70	18.84
Slope function	1.49	1.81	2.25	2.60
R.R	308.7	256.3	118.5	94.2
S.F.	0.0	1.20	2.61	3.28

Table 4: Cotoxicity coefficient of different combinations of Baygon insecticide and additives tested on Baygon-resistant strain of *C. pipiens* larvae under laboratory conditions.

Additive used	Concentration (%)	Cotoxicity coefficient
Piperonyl-butoxide	0.001	3.47
	0.005	6.13
	0.01	6.74
Sesame oil	1	4.74
	2	7.94
	4	9.96
Clove oil	0.25	1.20
	0.5	2.61
	1.0	3.28

DISCUSSION

Tested alone, none of the three additives showed appreciable toxicity to Baygon resistant *C. pipiens* larvae, even at the highest concentration used. However, when mixed with Baygon, all of the tested concentrations of additives increased its toxicity over that of merely the insecticide alone. The synergistic effectiveness of the three additives to Baygon was very clear. That insects resistant to an insecticide remain susceptible to an insecticide/synergist mixture is practically very important.

Insecticide synergists are generally believed to inhibit the detoxication process. For example, synergists appear to prevent the detoxication of pyrethrins in insects (Metcalf 1955); this may come about through the inhibition by the synergists of naturally detoxifying oxidation reactions (Sun & Johnson 1960).

That piperonyl-butoxide and sesame oil both potentiate Baygon suggests that the mixed function oxidase system of microsomes (MFO) is the major defence mechanism responsible for Baygon-resistance in *C. pipiens* larvae. It is well-known that the methylenedioxyphenyl compounds (piperonyl-butoxide and sesame oil) serve as alternative substrates for microsomal oxidases, and thereby reduce the rate of pesticide metabolism (Casida *et al.* 1966).

The results presented here agree with most existing literature on the synergists in question. Georghiou *et al*. (1966) investigated the synergistic activity of piperonyl-butoxide against a highly Baygon–resistant strain of *C. pipiens* adults and found that piperonyl-butoxide considerably enhanced the toxicity of Baygon. However, Georghiou & Metcalf (1961) investigated the synergistic action of carbamate piperonyl-butoxide combinations on a laboratory strain of *C. pipiens* larvae and adults and found only limited potentiation. This apparently weak synergism was thought to be due to absence of strong mechanism for carbamate degradation in the strain studied. Yasutomi & Takahashi (1989) studied the synergistic effects of piperonyl-butoxide to permethrin and cypermethrin on *C. tritaeniorhynchus* and found that their LC_{50}s decreased by 1/3 and 1/23 respectively. Atkinson *et al.* (1991) studied a field–collected strain of *Blattella germanica* highly resistant to cyfluthrin, cyhalothrin, cyermethrin, fenvalerate, esfenvalerate, fluvalinate, permethrin, resmethrin, sumithrin [the (1 R)-cis-trans-isomer of phenothrin] and tralomethrin (resistance determined by topical applications and comparison with a known susceptible strain); resistance ratios ranged from 29 to 337. They tested both the metabolic inhibitors s,s,s-tributyle phophorotrithioate (DEF) and piperonyl-butoxide for synergism in conjunction with cypermethrin and permethrin: their results showed that the application of synergists partially eliminate the resistance. Rajasekhar *et al*. (1996) tested the efficacy of sesame oil as a synergist in combination with endosulfan and cypermethrin for the control of *Heliocoverpa armigera,* finding that sesame oil (0.5%) enhanced the action of endosulfan and cypermethrin.

It may be concluded that piperonyl-butoxide, sesame oil and clove oil differ in their intensity of synergism with Baygon insecticide, sesame oil and piperonyl-butoxide being more active synergists to this insecticide than clove oil. Their synergistic efficiency thus confirmed, piperonyl-butoxide and sesame oil may be recommended for use in pest-control strategies to increase pesticide efficiency and reduce working costs.

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الملخص العربى

التأثير المثبط أو المنشط لبعض المنشطات على سلالة يرقة *الكيولكس ببينز* المقاومة للبايجون

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تم عمل هذه الدراسة على سلالة يرقة *كيولكس ببينز* التى تم تجميعها من منطقة ميت العطار بالقليوبيـة وتـم تربيـة سلالتين أحدهما لم يتم تعريضها لأى مبيد والأخرى تم تعريض أجيالها المختلفة لمبيد البايجون حتى تم الحصول علـى سلالة عالية المقاومة وعلى هذه السلالة المقاومة لمبيد البايجون تم دراسة تأثير كلا من البيبرونايل بيوتكسـيد وزيـت السمسم وزيت القرنفل كمنشطات لمبيد البايجون وأوضحت النتائج أن زيت السمسم ، والبيبرونايل بيوتكسيد كمنشـطات تؤدى إلى زيادة سمية مبيد البايجون ويتضح ذلك من خطوط السمية والانحدار الاماتى ومعاملات السمية . وأيضا زيت القرنفل زاد من جهد مبيد البايجون ولكن بدرجة أقل من منشط زيت السمسم والبيبرونايل بيوتكسيد كما لوحظ أن نشاط هذه المنشطات يعتمد على التركيز حيث لوحظ تغييرا معنويا عاليا عند أعلى تركيز من كل منشـط ولا توجـد زيـادة معنوية عند أقل تركيز فى كلا منهما وبناء على النتائج المتحصل عليها يمكن التوصية باستخدام كلا مـن البيبرونايـل بيوتكسيد وزيت السمسم كمنشطات لمبيد البايجون على يرقات *الكيولكس ببينز* المقاومة لهذا المبيد .