

## JOINT ACTION OF INSECTICIDE MIXTURES ON RESISTANT STRAIN OF *Culex pipiens* LARVAE

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### ABSTRACT

The joint action of baygon insecticide with sumithion, diazinon, dursban, sumicidin and permethrin insecticides was studied against *Culex pipiens* resistant strain larvae. This strain was subjected to continuous laboratory selection with baygon for 15 successive generations. The results showed that the baygon produced synergistic effects with permethrin and diazinon. An additive effect was observed with sumicidin and sumithion; while an antagonism effect was only produced with dursban. The joint action of baygon with the insect growth regulator produced a synergistic effect with IKI after 24 hour of exposure period. However an additive effects were produced with IKI and bay sir after 48 &72 hours and 24, 48 &72 hours of treatments respectively. The obtained results showed the possibility of using the baygon insecticide in combinations with permethrin, diazinon, sumicidin, sumithion and with the insect growth regulator (IKI and bay sir ) for controlling *C. pipiens* larvae.

### INTRODUCTION

The use of the insecticide mixture in insect control has got advantage methods than one insecticide alone as reported by many authors. Gordon and El-defrawi (1960), suggested that the interference of one insecticide with the detoxifying enzymes of the other was the mechanism responsible for analogsynergism of carbamates in insects. Dubois (1961), observed cases of antagonism or strictly additive effect of paired combinations of organophosphorous insecticides, he also reviewed the possible mechanisms that had been suggested

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to be responsible for the potentiation of the toxicity of organophosphorous compounds in mammals and insects. Mansour, *et al.* (1966) reported that the potentiator insecticide had an affinity to react faster with the detoxifying enzyme-complex. It will block the detoxifying enzyme, thus allowing the other weak insecticide, owing to its susceptibility to detoxication to exert its toxic action. The strong insecticide could then be considered as potentiator and the weaker as potentiabile. Antagonism could be produced if an insecticide interfered with the activation of the other by retarding it. So maximal effects were not arrived at simultaneously, and the detoxifying enzymes would have more chance of acting on the less toxic parent compound. El-Sebae, *et al.* (1964) emphasized the phenomenon of synergizing carbamate insecticides by mixing them with organothiocyanates. They stated that the synergism was due to blocking certain enzymatic sites responsible for multifunction oxidation, which was the major step in metabolism and detoxification of carbamates. Busvine (1970) pointed out that the insecticidal combinations had permitted continued use of the insecticide for which insects had developed resistance. This could be theoretically preventing the emergence of resistant strain. Yoshihiko (1971), made a comparative evaluation of tetramethrin / resmethrin mixtures and natural pyrethrins synergized with piperonyl-butoxide. He found that the efficacy of a suitable formulation of mixture of tetramethrin (knockdown) and resmethrin (killing agent) surpassed than that of natural pyrethrin synergized with piperonyl-butoxide. Lyarskii *et al.* (1983) found that the combination of permethrin with neopynamine displayed synergism. The most active combination contained permethrin and neopynamine was in the ratio of 9:1. This combination surpassed the insecticidal activity of the most effective combination of permethrin with neopynamine. Also Warui (1992) found that the combinations of pyrethrins and bioallethrin insecticides at high doses produced greater levels of knockdown than the individual constituents in mats for mosquito control.

In this study, the joint actions of baygon with some insecticides as well as

with two insect growth regulators were studied against baygon-resistant strain of the *Culex pipiens* larvae.

## **MATERIALS AND METHODS**

### **Chemicals used**

The chemical insecticides used were the carbamate, baygon (technical 97%), the organophosphates, sumithion (technical 96.5%), diazinon (technical 92%) and dursban (technical 56.4%Ec), the synthetic pyrethroids, permethrin (technical 92.6%) and sumicidin (technical 95.5%) and the insect growth regulators, bay sir 8514 (Ec. 6.5%) and IKI (Ec. 5%)

The different concentrations of the insecticides or the insect growth regulators were prepared from the stock solution by dissolving a known amount of each of them in distilled water. Usually ethyl alcohol was used as a solvent for all the chemicals tested except the insect growth regulators, which they were dissolved in acetone. Concentrations were expressed in parts per million (ppm); in order to calculate the LC's.

### **Test insect**

The *C. pipiens* larvae were obtained from Miet El-Attar village, Qualyubia Governorate. The larvae were successfully reared and maintained at the Entomology Department, Faculty of Science, Zagazig University, Benha branch.

The field-collected larvae were reared without being exposed to any insecticides or the insect growth regulators to raise their susceptibility and it was referred as the original normal susceptible strain. This strain is also considered as the base line of the selected pressured resistant strain with which the results of selections were compared. Assessment of toxicity was based on the mortality of the tested larvae. The early third or the late third larval instars of *C. pipiens* were exposed for 24 hours to different concentrations of baygon and/or the tested insecticide mixture /or the baygon with the insect growth regulator mixtures in distilled water. Another groups of larvae were left without any treatments, or treated with baygon alone or the other tested toxicants alone and served as control. Four

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replicates of the desired concentrations of each compound were prepared, in 250 ml glass beaker. Each beaker received 25 larvae of the early third or the late third larval instars according to the experiment carried out. The percentages of mortality were plotted against the tested concentrations and the LC<sub>50</sub> and the LC<sub>95</sub> values were determined graphically. Mortality percentages were corrected by Abbott's formula (1925), if the mortality in control exceeds 10%.

**Joint action of the insecticides mixtures against *C. pipiens* larvae.**

Pairs of insecticides (baygon with the other insecticides) were mixed in equal volumes at concentrations equivalent to half of the LC<sub>50</sub> values. The expected mortalities of the mixtures were calculated by the summation of the expected mortalities of the toxicant used in the mixture.

The equation of Sun and Johnson (1960) was used as follows:

$$\text{Co toxicity Factor} = 100 \times \frac{\text{Observed \% mortality} - \text{Expected \% mortality}}{\text{Expected \% mortality}}$$

This factor differentiates the results into three categories as follows:

A positive factor of 20 or more is considered as indicating potentiation.

A negative factor of -20 or more indicating antagonism.

The intermediate values between -20 and +20 indicating additive effect.

It must be noted that, all these experiments were conducted at room temperature (25±2 °C).

**The statistical analysis**

The statistical analysis was performed using t-test.

**RESULTS**

Results of the present experiments are shown in the tables (1, 2, 3, 4 and 5) and graphically illustrated in figure (1). The obtained data in table (1) indicated that the insecticides tested appear highly effective against normal strain of *C. pipiens*. Their activities range between susceptible to vigour tolerance. On the basis of their LC<sub>50</sub> values, the efficiency of the tested chemical insecticide could be arranged in the following order: dursban > permethrin > sumicidin > sumithion > baygon >

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diazinon. Further support of the previous results could be obtained from the results of superior potency, which depends mainly on the insecticide activity ratios or comparative insecticidal activities. The calculated results of potency are tabulated in table (2). It is apparent that the potencies of the insecticides tested showed a superior efficiency of dursban on this normal strain of *C. pipiens* larvae followed by permethrin, sumicidin, sumithion, baygon and diazinon, respectively. The results indicated a negligible development of resistance to any of the tested insecticides or the appearance of heterogeneous response of the individuals to the action of the tested insecticides.

The early third instar larvae were tested with two insect growth regulators IKI and bay sir for their susceptibility. Mortality resulted after 24, 48 and 72 hours post-treatments are presented in table (3). The obtained data showed that the two insect growth regulators have different levels of activities against the larvae. The susceptibility of the treated larvae was increased with the increase of exposure time. For the IKI the  $LC_{50}$  values after 24, 48 and 72 hrs were 1.30, 0.30 and 0.13 ppm, respectively and the slope functions were of low values of 1.85, 2.92 and 3.38 indicating high degree of homogeneity of the tested population. Complete kill was achieved at the concentration 2.5 ppm, after 48 hrs of treatment.

For bay sir the  $LC_{50}$  values were 1.79, 0.26 and 0.19 ppm, and the slope functions are also of relatively low values of 3.16, 3.36 and 3.63. Complete larval mortality occurred at the concentration 3.25 ppm after 72 hrs post-treatment.

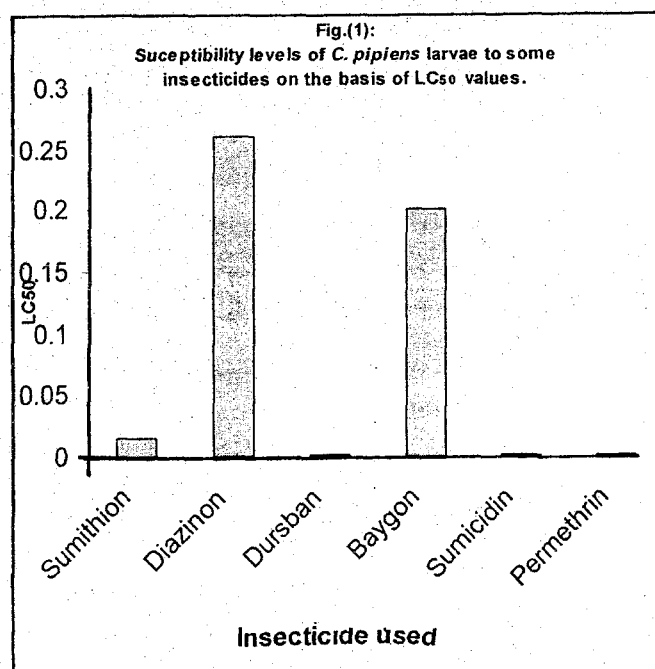
The joint action of various insecticides mixtures was tested by mixing the insecticides and /or with the insect growth regulators in proportion to the concentrations equivalent to half of the  $LC_{50}$  values. The potencies of baygon and the other insecticides mixtures on the resistant strain larvae are demonstrated in table (4). The results revealed that baygon produced synergistic effects (potentiation) with permethrin and diazinon. An additive effect was observed with sumicidin and sumithion. Antagonistic effect was only produced with dursban. The joint action of baygon insecticide with the insect growth regulators is shown in table

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(5). Baygon produced synergistic effect with IKI after 24 hour of exposure period. However, additive effects were produced with IKI and bay sir after 48 and 72 hrs and 24, 48 and 72 hrs of treatment, respectively.

Table (1): Susceptibility of the early 3<sup>rd</sup> instar of *C. pipiens* larvae (normal strain) to certain chemical insecticides under laboratory conditions, after 24 hours of exposure.

Insecticide used	LC <sub>50</sub>	LC <sub>95</sub>	Slope function
Dursban	0.0016	0.0056	2.40
Permethrin	0.0018	0.0071	2.11
Sumicidin	0.0020	0.0400	6.33
Sumithion	0.0150	0.0700	2.59
Baygon	0.2000	1.9900	4.33
Diazinon	0.2600	0.9600	2.16



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**Table (2): Comparative insecticidal activity of certain insecticides against *C. pipiens* larvae after 24hours of exposure.**

Relative potency based on

Insecticide used	LC <sub>50</sub> (ppm)	Sumithion	Diazinon	Dursban	Baygon	Sumicidin	Permethrin
Sumithion	0.0150	01.00	0.058	009.372	0.075	007.5	008.33
Diazinon	0.2600	17.33	1.000	162.500	1.300	130.0	144.40
Dursban	0.0016	00.11	0.006	001.000	0.008	000.8	000.88
Baygon	0.2000	13.33	0.769	125.000	1.000	100.0	111.11
Sumicidin	0.0020	00.13	0.008	001.250	0.010	001.0	001.11
Permethrin	0.0018	00.12	0.007	001.125	0.009	000.9	001.00

**Table (3): Biological activity of the Insect growth regulators IKI and bay sir against the early 3<sup>rd</sup> instar of *C. pipiens* larvae (normal strain), at different time of exposure (24,48&72 hours).**

Insect growth regulator (ppm)	Exposure time per (h.)	LC <sub>50</sub> (ppm)	LC <sub>95</sub> (ppm)	Slope function
IKI	24	1.30	3.70	1.85
	48	0.30	1.59	2.92
	72	0.13	1.12	3.38
Bay sir	24	1.79	9.20	3.16
	48	0.26	1.87	3.36
	72	0.19	1.22	3.63

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Table (4): The joint action of baygon with various insecticides against baygon –resistant strain of *C. pipiens* larvae, after 24 hours of exposure.

Insecticide mixture	Concentrations (ppm)	Mortality percent		Cotoxicity factor
		Expected	Observed	
Baygon (alone)	61.5	50	49.3	—
B. +Sumithion	61.5+0.045	50	48.0	-4.0
B. +Diazinon	61.5+0.38	50	62.7	+25.4
B. +Dursban	61.5+0.0072	50	38.7	-22.6
B. +Sumicidin	61.5+0.0034	50	57.3	+14.6
B.+ Permethrin	61.5+0.0022	50	63.3	+30.7

Table (5): The joint action effect of baygon insecticide with the insect growth regulators both IKI and bay sir against baygon –resistant *C. pipiens* larvae when used in mixture for different time of exposure.

Baygon +Insect growth regulator (mixture)	Concentrations Used B + IGR (ppm)	Exposure Time (hour)	Mortality percent		Co toxicity factor
			Expected	Observed	
			Baygon	61.5 + 1.93	
+IKI		48	78.7	94.0	+19.44
		72	96.3	100	+3.84
Baygon +Bay sir	61.5 + 1.80	24	50	57.3	+14.6
		48	77.3	81.3	+5.17
		72	84.0	89.3	+6.31



## DISCUSSION

It is well known that the insecticide mixtures are used satisfactorily in practice as a tool to combat resistance. Certain aspects should be fulfilled in this respect:

- 1- The components in the mixture should synergies each other.
- 2- The concentrations of the insecticides in the mixture must be used at very low concentrations.
- 3- The components of the mixture should have similar decaying rates to reduce their hazards.
- 4- Preferably the two insecticides in the mixture should have different modes of action.

The obtained results in the present study can meet well with the above-mentioned points. The mixtures of baygon with various insecticides and insects growth regulator substances were performed on compounds, which have not developed cross-resistance to baygon. Low concentrations of the selective compounds, which have different modes of action, were used on the resistant larvae. The obtained results revealed that baygon insecticide produced synergistic actions with diazinon, sumicidin, permethrin and sumithion as well as with the insect growth regulator the IKI and bay sir. Antagonistic effect was only found with dursban insecticide. This was found to be explained by Dubois *et al.* (1961), whom stated that if the two insecticides are applied on an insect, one might interfere with the other's activation or with the detoxification reaction or with both. Antagonism results when interference with the activation mechanism occurs, while potentiation results when interference with detoxification takes place. If both reactions encounter interference, antagonism, potentiation or additive effects could result. They are depending on the degree of interference with different reactions.

The obtained larvicidal action of the IKI and bay sir is in agreement with that of Mckague and Pridmore (1978) who concluded that the ingestion of Juvenile hormone or Insect growth regulator by larvae of the rain bows trout disrupt the

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normal process of cuticle deposition. They also stated that the higher doses greatly reduced emergence of adults. Also this result is in accordance with the findings of other workers Jakob, (1972), Mulla *et al.* (1975) and Bakr *et al.* (1997).

High doses is needed to achieve the best control levels, however, the joint action improve such situation.

In summary, the obtained results confirmed the insecticidal efficiency of the combined effect of the baygon insecticide with diazinon, sumicidin, permethrin and sumithion insecticides, as well as, with the insect growth regulator both the IKI and bay sir to be used against *Culex pipiens* larvae baygon resistant strain, thus it may be recommended the usage of the tested toxicants in control management strategy to enhance the activity.

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## التأثير المشترك لخلط المبيدات على يرقات سلالة مقاومة لبعوضة الكيولكس ببيبنز

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تم قياس حساسية يرقة الكيولكس ببيبنز لبعض المبيدات من مجموعات مختلفة مثل المبيدات الفوسفورية والكراميت والبيروثرويد من المبيدات التالى ذكرها وأمكن ترتيب كفاءة المبيدات التى تم اختبارها حسب قيمة التركيز القاتل لنسبة ٥٠٪ من اليرقات LC<sub>50</sub> كالتالى:

دورسيان \* بيرميثرين \* سوميدسيدين \* سوميثيون \* بايجون \* ديازينون ، حيث كانت تركيزاتها القاتلة لنسبة ٥٠٪ من اليرقات كالتالى:

٠,٠٠١٦ ، ٠,٠٠١٨ ، ٠,٠٠٠٢ ، ٠,٠١٥ ، ٠,٢٠ ، ٠,٢٦ ، جزء فى المليون .

وتم قياس أيضا حساسية اليرقات بالنسبة لمنظمات النمو لكل من أى كه أى (IKI) وبأى سي ر (Bay Sir) التى اتضح أنها متكافئة تقريبا على الرغم من أن تأثير أى كه أى كان أعلى قليلا من مركب باى سير وذلك بمقارنة التركيز القاتل لنسبة ٥٠٪ من اليرقات . وكانت نسبة التركيز لـ أى كه أى هى ١,٣ ، ٠,٣ ، ٠,١٣ ، جزء فى المليون وذلك لمدة ٢٤ ، ٤٨ ، ٧٢ ساعة من التعريض على التوالى بينما كانت ١,٧٩ ، ٠,٢٦ ، ٠,١٩ ، جزء فى المليون بالنسبة لـ باى سير (Bay Sir).

وتم تعريض يرقات الكيولكس ببيبنز لأجيال مختلفة لمبيد البايجون حتى تم الحصول على سلالة عالية المقاومة وعلى هذه السلالة المقاومة لمبيد البايجون تم دراسة تأثير خلط مبيد البايجون مع بعض المبيدات المختلفة ومنظمات النمو على هذه السلالة المقاومة . لوحظ ظهور التأثير المنشط للبايجون على السلالة المقاومة عند خلطه بالمبيدات الأخرى حتى أوضحت النتائج التأثير التنازلى الآتى:

(بايجون + بيرميثرين) < (بايجون + ديازينون) < (بايجون + سوميسيدين) < (بايجون + سوميثيون) بينما ظهر التأثير المثبت فقط مع مبيد الدوسبان.

كما أوضحت الدراسة التأثير المنشط لخلط البايجون مع منظمات النمو لكل من أى كه أى (IKI) والبأى سي ر (Bay Sir) على يرقات الكيولكس ببيبنز المقاومة لمبيد البايجون .