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Efficacy of a Microbial Bioinsecticide and Three Insecticidal Compounds against the Mosquito *Culex pipiens* from Riyadh, Saudi Arabia

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ABSTRACT

Efficacy of four insecticidal commercial formulations was tested against the mosquito *Culex pipiens*, a major vector of diseases in Saudi Arabia and the world, such as filariasis and many arboviral diseases. The tested insecticides are the major bacterial bioinsecticide against mosquitoes, *Bacillus thuringiensis israelensis* (*Bti*), Bacilod®5000ITU and three chemical insecticides, the insect growth regulator (IGR) pyriproxfen, Sumilarv®0.5G, the organophosphate Sumithion NB® and the pyrethroid Pesguard®FG81. Bioassays were carried out on mosquito larvae collected from different localities in Riyadh. The F1 adults of these larvae were also used. The efficacy of each tested insecticide was expressed as lethal concentration (LC_{50}) values that kill half of the population tested. Concentrations of each insecticide recommended by the manufacturer for field application as shown on the commercial formulations were used and tested in this study as such. For Bacilod, two LC_{50} values were reported against larvae, 2645 and 1568 IU (International Unit) at 24hr and 48hr post treatment, respectively. The highest concentration, 6150 IU caused 96 and 100% larval mortality at 24hr and 48hr post treatment, respectively. For Sumilarv, the LC_{50} against larvae is 22.7 kg/ha at 7days post treatment, as the effect of IGRs increases with time after the first day of application. The highest concentration, 50kg/ha, caused 80% larval mortality at 7 days post treatment. For Sumithion, the LC_{50} against larvae was 6.8% at 24hr post treatment, while the highest mortality was 92% produced by the concentration 27.5%. Pesguard was tested against the adult stage, with LC_{50} of 0.004% at 24hr post treatment, while the highest concentration used, 0.02% caused ~97% mortality. The results indicate that these insecticides exhibited high efficacy against Cx. *pipiens* mosquitoes, *i.e.*, this mosquito species is highly susceptible to these insecticides. Therefore, these insecticides are effective tools for the control of *C. pipiens* and probably other mosquito species in the region. For the highest efficacy, these insecticides can be applied as a component of an integrated control programme according to the target mosquito stage and insecticidal mode of action. For example, the biological agent *Bti* and the IGR pyriproxfen can be used for the control of aquatic stages of the mosquito. In addition, a third component, as Pesguard may be included to target the adult stages that survived the larval control operations.

Key Words: *Culex pipiens*, *Bti*, Efficacy, Insecticides, Integrated control, Saudi Arabia.

INTRODUCTION

Mosquitoes are major insect vectors of various diseases of global public health and economic importance. Mosquitoes, such as *Anopheles, Culex*, and *Aedes* are vectors of many parasitic and viral diseases, such as malaria, filariasis, yellow fever and dengue. These diseases infect millions of people worldwide with huge economic costs, due to lost human power, disease control and treatment (Rathor, 2000, El-Setouhy and Ramzy, 2003 and WHO, 2003a). Control of many of these diseases depends largely on mosquito vector control using insecticides, alone or in an integrated control program, in combination with drugs, vaccines or environmental management. Many vector control campaigns are hampered and even broken-down due partly to the development of vector resistance to almost all insecticides used. The rate of resistance development and spread among vector populations has been increasing due to the use of a relatively small number of insecticides both in the control of disease vectors and agricultural pests. Insecticide resistance is therefore, a serious problem that renders disease control efforts ineffective with unpredicted public health consequences and resurgence of disease epidemics (Collins *et al.*, 2000, Hemingway *et al.*, 2002, Krzywinsky and Beasansky, 2003and WHO, 2003a).

In Saudi Arabia, *Culex pipiens* complex mosquitoes are vectors or suspected vectors of many diseases. For example, they are vectors of the Rift Valley fever virus, where a recent outbreak has occurred (CDC, 2000 and Shoemaker *et al.*, 2002). This in addition to the Japanese encephalitis virus and West Nile fever virus, which are serious diseases in other parts of the Eastern Mediterranean Region and Southeast Asia. However, the epidemiology of these diseases is largely not fully understood and the risk of transmission is possible. There are many factors that increase the risk of disease transmission and epidemics, such as global warming and seasonal fluctuations, changing land use, re-use of sewage water in agriculture

and increasing population movements either for work or for religious rituals. All these factors will have significant impact on the dynamics of mosquito vector populations.

Vector control strategies rely largely on chemical insecticides and bacterial biopesticides. However, each method has its own advantages and limitations, in terms of efficacy, specificity, effect on non-target organisms and environmental impact. Major obstacles in implementation of effective control programs is the lack of reliable diagnostic tools for disease agents, accurate identification of the vector species and the spread of insecticide resistance in many vectors. Therefore, it is becoming essential for effective control of a vector species, to implement an integrated vector management program. This program will employ more than one strategy, and in addition, it may affect another vector species, as a by-product of control operations.

For an effective and sustainable control campaign, the susceptibility of vector species to different types of insecticides in use, should be studied and monitored regularly. This provides invaluable information on the efficacy of the tested compounds and will provide an early warning on the development of resistance. These studies are of very important practical and epidemiological consequences for disease vector control programs and decision-making.

Therefore, the objective of the present study was the evaluation of the susceptibility status of the mosquito *Cx. pipiens* to commercial formulations of four insecticides including one bacterial biopesticide and three chemical insecticides, which are widely used for the control of many vector species in Saudi Arabia and various regions in the world.

MATERIALS AND METHODS

1. Insects

Larvae of *Cx. pipiens* mosquito were collected from different localities in Riyadh, Saudi Arabia, using the dipping technique as described (WHO, 2003a). The collected larvae were transferred to the laboratory for species identification using the key of Harbach (1985). To obtain the adult stage, larvae were reared in the laboratory and first generation adults were used in the experiments.

2. Insecticides

Four compounds belong to different classes of pesticides, the biopesticide *Bacillus thuringiensis israelensis* (*Bti*), Bacilod 5000ITU, Sumilarv 0.5G (pyriproxfen), Sumithion NB (fenitrothion + d-tetramethrin) and pesguard FG81 (d-tetramethrin + cyphenothrin). The four compounds used are the commercial formulations obtained from Sumitomo Chemicals Company. All are larvicides, except pesguard is an adulticide. To carry out the bioassay experiments, concentrations equivalent to the doses recommended for field application of each insecticide were made and used as described below.

2.1. *Bacillus thuringiensis israelensis* (*Bti*) (Bacilod® 5000 IU)

This is a bacterial biopesticide contains the active ingredient of the dry soluble toxin of the naturally-occurring spore-forming *Bacillus thuringiensis* var. *israelensis* (Bti), Serotype H–14, strain 164 with potency of 5000 ITU*/mg (ITU= International Toxic Unit). When sprayed upon the surface of larval aquatic breeding sites, this larvicide becomes part of the food eaten by larvae in the water. The larvae ingest the bacterial spores and crystalline toxins. Only when activated by the larvae specific gut pH and enzymes, the crystals become toxic. Due to the action of toxins on midgut epithelium, infected larvae lose their appetite, stop feeding and therefore, die before they can pupate and reach adulthood.

For the bioassay, five concentrations were used, 381.2, 762.5, 1525, 3050 and 6150 ITU, of which the 6150 ITU is the field recommended dose.

2.2. Pyriproxfen (Sumilarv® 0.5G)

This is an insect growth regulator (IGR), which targets the hormones involved in insect development. The recommended field dose is 50kg/ha used as granular formulation of 0.5% in water (w/v). To establish the bioassay baseline susceptibility data, five concentrations in acetone were made equivalent to 10, 20, 30, 40 and 50kg/ha.

2.3. Fenitrothion (Sumithion®NB 25/2.5 EC)

This insecticide belongs to the organophosphate group of insecticides. The commercial formulation is fenitrothion 25% emulsifiable concentrate (EC), contains 2.5% of the pyrethroid tetramethrin (Neo-pynamin). To establish the bioassay baseline susceptibility data, five concentrations in acetone were made equivalent to

1.7, 3.7, 6.9, 13.8 and 27.5% w/v.

2.4. Pesguard®FG 81

A pyrethroid containing two insecticides, 2% w/v Neo-pynamin Forte (d-tetramethrin) and 6% w/v Gokilat (cyphenothrin). The formulation used in the bioassays is the emulsfiable concentrate in five concentrations, 0.00125, 0.0025, 0.005, 0.010 and 0.020% w/v; the latter concentration, 0.020%, is the recommended for field use.

3. Bioassay Procedure

The susceptibility assays on both mosquito larvae and adults were carried out according to the standard guidelines of the WHO bioassay methods (WHO 1981 and 1997).

Third instar larvae were exposed to the insecticide in 250-ml paper cups, at 25 larvae per concentration. Tests were repeated 2-3 times when were applicable and the number of insects was sufficient. For the IGR Sumilarv, mortality percentage was calculated daily from 3 to 7 days post treatment (dpt). For the other insecticides, mortality percentage was calculated 24hr post treatment (hpt).

For adult bioassays, mosquitoes were exposed in the WHO exposure tubes lined with papers-impregnated with the required insecticide concentration. All assays were conducted in the laboratory at a temperature of 25±2°C and 75±10% RH in a light: dark cycle of 12:12h. The test larvae were not fed during the 24-hr exposure period. Bioassay data were analyzed using log-probit analysis software program. Lethal concentrations that kill 50% (LC_{50}) and 90% (LC_{90}) for each insecticide, the slope and Chi^2 values were determined from the resultant concentration-mortality regression line.

RESULTS AND DISCUSSION

1. Susceptibility to *Bti* (Bacilod® 5000 IU)

The efficacy of the bacterial biopesticide *Bti* against *Cx. pipiens* larvae was evaluated at two time points, 24 and 48 hpt (Table 1). At 24 hpt, the highest mortality recorded was 96% in larvae exposed to the highest concentration used, 6150 ITU. The second effective concentration was 3050 ITU, which caused 76% mortality. At 48 hpt, the mortality rate increased to 100 and 96% at the same two concentrations mentioned above, respectively. In addition, at 48 hpt, high mortality of 84% was recorded at the third concentration used, 1525 ITU. These results clearly indicate the strong efficacy of this biopesticide against this species of mosquitoes, and show the possibility that with concentration of the biopesticide lower than that commercially-recommended for field application (6150 ITU) and longer period of exposure could cause high mortality in the targeted mosquito population such as *Cx. pipiens*.

Zahiri and Mulla (2006), reported the effect of exposure to *Bti* water dispersible granules (WDG) on oviposition and hatching of a laboratory strain of the mosquito *Cx. quinquefasciatus*. The study showed that exposure of mosquitoes to *Bti* WDG resulted in a reduction in the number of eggs in rafts and the shape of the raft was irregular. The effect of exposure caused up to 88% reduction in egg hatching rate, with only 30% survival rate of hatched larvae. This accumulative effect of treatment with *Bti* could have greater control efficacy against the target mosquito vector. Significant reduction in oviposition of *Cx. quinquefasciatus* exposed to aqueous suspensions of *Bti* and *Bacillus sphaericus* WDG was also reported. In addition, the effect of exposure to the two bacterial agents was extended to the adult stages; the mortality rate in male and females was increased on landing and imbibing on the treated waters. This extended effect will significantly decrease the number of gravid females coming to the water for oviposition or drinking, with the subsequent reduction in transmission of pathogens and increased control outcomes (Zahiri and Mulla, 2005). High susceptibility levels to *Bti* H-14 of many laboratory-reared and field-collected strains of *Ae. aegypti* and *Ae. albopictus* were reported in a dengue-endemic area in Malaysia. The LC_{95} was 20 times less than that the 6000 ITU recommended internationally. The laboratory-reared strains were more susceptible than the field-collected ones. Even after >6 months storage (up to 24 months), the *Bti* formulation was still highly effective against the mosquitoes (Lee and Zairi, 2006**a**). In the laboratory and under semi-field conditions, *Bti* showed high efficacy against *Ae. aegypti* and *Ae albopictus* from Thailand, with lasted efficacy of control of >80% mortality up to 11 weeks post-treatment. The efficacy of treatment increased with increasing the exposure period (Lee and Zairi, 2005, Lee and Zairi 2006b and Fansiri *et al.*, 2006). In contrast to these high levels of susceptibility, Paul *et al.*, (2005), reported high level of resistance of *Cx. pipiens* collected from New York to *Bti*, while the mosquito was still susceptible to a second bacterial biopesticide, *B. sphaericus*. Su and Mulla

Table (1): Efficacy of the biopesticide *Bacillus thuringiensis israelensis* (*Bti*) against *Culex pipiens* 3rd instar larvae.

Concentration (ITU)*	Mortality (%) hours post-treatment (hpt)	
	24 hpt	48 hpt
381.2	8	24
762.5	24	64
1525	52	84
3050	76	96
6150	96	100
Control	0	4
LC_{50}	2.645	1.568
LC_{90}	5.288	3.393
Ch^{i2}	3.94	0.31
Slope	1.712	1.820

Table (2): Efficacy of Sumilarv 0.5G against *Culex pipiens* 3rd instar larvae.

Kg/Ha*	Mortality (%)-days post-treatment				
	3rd day	4th day	5th day	6th day	7th day
10	1.33	5.33	6.66	6.66	21.33
20	4.0	12.0	30.66	30.66	52
30	5.33	20.0	36.0	44.0	58.66
40	6.66	21.33	40.0	48	61
50	10.66	37.33	42.66	52	80
Control	0	1.33	2.66	2.66	4.0
LC_{50}	503.8	90.4	55.1	41.2	22.7
LC_{90}	4934.7	481.9	328.4	172.6	97.6
Chi^2	0.27	1.97	4.63	3.54	4.37
Slope	5.874	3.664	3.997	3.040	3.096

* International Toxic Unit

(2004), reported high level of resistance of laboratory strain of field collected *Cx. quinquefasciatus* mosquitoes to *B. sphaericus*, where control operations failed after 4 months post treatment with WDG formulation named VectoLex of the bacteria. Interestingly, the same mosquito was susceptible to *Bti*, and it decreased the level of resistance to *B. sphaericus* when combined together, an indication of the synergistic effect of *Bti* (Zahiri and Mulla, 2003).

Molecular genetics approaches to increase the efficacy of *Bti* have been followed through combining the binary toxins proteins Cry proteins of *Bti* with the BsB protein of *Bs*. A *Bti/Bs* recombinant toxin protein synthesized in *Bti* strains showed high potency against *Cx. quinquefasciatus* larvae, an important vector of West Nile Virus and *Cx. tarsalis*. This approach increased the efficacy of *Bti* and *Bs*, reduced resistance of *Cx. quinquefasciatus* to *Bs*, and will therefore; reduce the high cost of repeated applications (Park *et al.*, 2005).

Another report (Gayathri *et al.*, 2004) showed high efficacy in mosquito control with rotational application of *Bti* or *Bti/Bs* mixture followed by the pyrethroid deltamethrin EC formulation against *Cx. quinquefasciatus*, the filaria mosquito vector in India.

2. Sumilarv 0.5® (Pyriproxfen)

The efficacy of the IGR sumilarv on *Cx. pipiens* larvae was studied over a period of 7 days, where mortality was recorded daily starting from 3 dpt. The mortality percentages recorded (Table 2); show that there is an increased effect on larvae with increased exposure period at one concentration, and with increased concentration at a given time point. The highest mortality (80%) was recorded at concentration equivalent to 50kg/ka (the recommended concentration for field use) at 7dpt. The effect of the insecticide greatly varied with increased exposure period. For example, exposure of larvae to the concentration equivalent to 20kg/ka caused mortality of 52% at 7dpt equal to the mortality caused by 50kg/ka (52%) at 6dpt (Table 1). This means that increased exposure time of 1 day (14%) might reduce insecticide concentration used by 250%, with an overall reduction in the amount of insecticde applied and costs and might reduce the development of resistance to this insecticide. The lethal values LC_{50} and LC_{90} were 22.7 and 97.6kg/ha, respectively at 7dpt. These results showed that *Cx. pipiens* larvae were highly susceptible to Sumilarv field applied concentration of the commercial formulation recommended by the manufacturer.

Due to the latent effects of IGRs on mosquito hormones involved in the regulation of developmental processes, this rate of mortality could rise with time. The exposure to growth regulators inhibitors delayed the developmental transition (duration) of each stage, i.e., larva-pupa transition. A by-product of this delay in the development of mosquito immature stages (e.g., the lifespan), would increase the exposure time to the natural predators and parasites, which results in increased mortality rates. The residual effect of IGRs such as pyriproxfen was highly effective and could remain for several weeks with complete inhibition of adult emergence of tested mosquitoes such as *Culex, Anopheles* and *Aedes* (Ali *et al.*, 1999 and Nayar *et al.*, 2002). Therefore, the WHO recommended the use of pyriproxfen for the control of many mosquito vector species, but with specified rates of application according to the mosquito habitat (Nayar *et al.*, 2002). In

combination with other types of insecticides such as the bacterial formulation Spinosad® and *Bti*, the efficacy of pyriproxfen and the other insecticide could increase significantly, as was reported on *Aedes* mosquitoes (Lee *et al.*, 2005). These synergistic combinations are particularly very helpful in situations where resistance to insecticides is developing (Darriet and Corbel, 2006).

In Mexico, a field trial employed curtains impregnated with the pyrethroid lambdacyhalothrin and treated the covers of water containers with pyriproxfen chips. This strategy significantly reduced the population of the mosquito *Ae. aegypti* based on entomological indices over a period of 12 months. This result showed that combination of two different methods could have great synergistic effect in reduction of vector population and transmission of dengue disease (Kroeger *et al.*, 2006). If adult mosquitoes exposed to surfaces treated with pyriproxfen; they will pick the insecticide particles through tarsal contact exposure and horizontally transfer them to larval habitat (microenvironments), where they oviposit (Dell-Chrism and Apperson, 2003). In laboratory experiments, despite exposure to IGR-treated paper surfaces did not affect the fecundity of mosquitoes; there was a 30% reduction in hatching rate in the first and second gonotrophic cycles (Dell-Chrism and Apperson, 2003). This could be a very effective mosquito management technique, taking into consideration biology of the target mosquito, such as oviposition behavior and potential interactions between different mosquito species in oviposition sites. Another important aspect of pyriproxfen and other IGRs is their safe effect on non-target beneficial predators such as the larvivorous crimson-spotted rainbow fish *Melanotaenia duboulayi* in Australia. Laboratory studies showed that there is no acute toxic effect of the IGRs pyriproxfen and s-methoprene on this fish, as indicated by normal living of the young fishes and the critical swimming speed (Ucrit) of insecticide-treated adult fishes (Brown *et al.*, 2002 and Hurst *et al.*, 2007). These studies showed the beneficial inclusion of these insecticides as elements of an integrated pest management program, due to their insecticidal activities and environmental safety towards the non-target organisms co-inhabitant of mosquito larvae

3. Sumithion-NB® EC

The mortality values caused by Sumithion-NB® EC against *Cx. pipiens* larvae are shown in (Table 3). Highest mortality was 92% on exposure to the concentration 27.5 kg/ha, the concentration equivalent to that recommended by the manufacturer for field control operations, followed by the concentration 13.8 kg/ha (76%). The LC_{50} and LC_{90} as deduced from the susceptibility regression line were 6.84 and 26.90 kg/ha, respectively. These results clearly showed that the insecticide was highly effective against mosquito larvae.

Few studies have been reported that investigated the susceptibility of mosquitoes to the organophosphate fenitrothion, the major component of this Sumithion NB® EC formulation. In a recent study in Japan (Kasai *et al.*, 2007), various colonies of *Cx. pipiens* and *Cx. quinquefascaitus* were susceptible to fenitrothion. High levels of susceptibility to fenitrothion were also reported in *Cx. annulirostris* and *Ae. aegypti* (Essam *et al.*, 2006). In contrast to the above studies, high level of resistance to fenitrothion was reported in *Cx. quinquefascaitus* in Thailand, while it was still highly susceptible to another organophosphate, malathion (Sunaiyana *et al.*, 2006). This resistance was suggested to be due to the use of fenitrothion in the control of *Cx. quinquefascaitus*, the main dengue vector in the area. At the same study (Sunaiyana *et al.*, 2006), *Ae. aegypti* was completely susceptible to fenitrothion, despite it was resistant to other pyrethroids tested. In another study, high levels of susceptibility to fenitrothion were recorded in *Ae. aegypti* and *Ae. albopictus* from Thailand. In contrast in other localities, these two mosquitoes were resistant to the insecticide (Jirakanjanakit *et al.*, 2007). These studies clearly show the various responses to the same insecticide of the same mosquito species from different localities or different genera. It also showed the variable response of one mosquito vector to members of one insecticidal group, which probably might result from different biochemical mechanisms and exposure to the insecticide.

4. Pesguard FG81

For the adulticide, Pesguard FG81, similar to Sumilarv and Sumithion, the highest mortality percentage, 96.6% was caused by the concentration 0.020%, which equivalent to the rate applied in the field. The concentration 0.010% caused high mortality of 77.7%, which indicates high susceptibility of adult mosquitoes

Table (3): Efficacy of Sumithion NB 2.5/25 EC against *Culex pipiens* 3rd instar larvae.

Concentration	1.7	3.7	6.9	13.8	27.5	Control	LC_{50}	LC_{90}	Chi^2	Slope
Mortality (%)	12	28	44	76	92	0	6.841	26.894	0.67	2.893

Table (4): Efficacy of Pesguad FG81 against *Culex pipiens* first generation adult mosquitoes reared from field collected-larvae.

Concentration	0.00125%	0.0025%	0.005%	0.010%	0.020%	Control	LC_{50}	LC_{90}	Chi^2	Slope
Mortality (%)	12	32	55	77.7	96.6	3.3	0.004%	0.016%	0.69	2.752

to this insecticide. The LC_{50} and LC_{90} were 0.004% and 0.016%, respectively. The results of the bioassay are summarized in table 4.

No studies have been carried out to test the susceptibility of mosquitoes to Pesguard FG 81 (2% d-tetramethrin, 6% cyphenothrin) formulation tested in this study. However, other formulations were tested and all proved highly effective against various mosquito species. For example, Pesguard FG 161 (4% d-tetramethrin, 12% cyphenothrin) was highly effective as larvicide and adulticide against four species of mosquitoes including *Cx. quinquefasciatus, Ae. aegypti, Ae. albopictus* and *Anopheles sinensis* from Malaysia (Yap *et al.*, 2001). Other formulations such as Pesguard 102 (0.5% d-allethrin, 0.5% phenothrin) ultra low volume together with the bacterial Vectobac 12AS (1200 ITU/mg water based suspension of *Bti*) was also proved highly effective against larvae and adults of *Cx. quinquefasciatus* and *Aedes* from Malaysia (Yap *et al.*, 1997), indicating their high synergistic effect to control different species of mosquitoes.

The present study shows that the mosquito *Cx. pipiens* collected from Riyadh is highly susceptible to the four types of insecticides tested. This level of susceptibility should be monitored regularly in the field using both bioassays and biochemical molecular assays when applicable. This is in order to spot any change in susceptibility, which could be an early indication of the development of resistance to one or more of the insecticides in use. Experiments, in which the mechanisms of resistance to these insecticides have to be conducted on laboratory strains of the target mosquito that are selected for resistance. These studies will reveal the potential biochemical and molecular mechanism of resistance to a given insecticide and the pattern of resistance spread in the population. Such studies will help monitor the situation in the wild population and will guide decision-making regarding control operations in practice. In addition with wider understanding of vector biology and species composition and mode of action of various control strategies, an integrated vector control operations that target one or more vector species sharing similar biological and ecological aspects, will be highly effective and sustainable. Such strategic planning will significantly reduce the potential and implications of development of resistance, reduce undesirable effects on the non-target organisms and environment and will increase the outcomes of disease prevention, control and treatment efforts.

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الملخص العربي

فاعلية مبيدات حيوية وكيميائية على بعوض *Culex pipiens* من الرياض، المملكة العربية السعودية

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تم اختبار تاثير أربعة مبيدات حشرية على البعوض المنزلي *Culex pipiens*، الناقل لعديد من الأمراض الهامة فى السعودية والعالم، مثل مرض الفيلاريا والعديد من الأمراض الفيروسية المنقولة بالمفصليات. وتشمل المبيدات المختبرة المبيد الحيوي الرئيسي ضد البعوض وهو بكتيريا *Bacillus thuringiensis israelensis* (*Bti*), Bacilod®5000ITU مقارنة بثلاث مبيدات كيميائية وهى: منظم النمو pyriproxfen, Sumilarv®0.5G والمبيد الفوسفوعضوى Sumithion NB® والمبيد البيريثرويدى Pesguard®FG81. وقد أجريت الاختبارات الحيوية على يرقات للبعوض والمجمعة من الرياض، والطور اليافع الذى تم تربيته من اليرقات المجمعة. وتم قياس تاثير المبيدات المستخدمة باستخدام تركيزات تكافئ القيم الموصى بها على المستحضر التجاري من قبل الشركة المنتجة لها فى التطبيق الحقلي فى المكافحة وبتحديد التركيز الذي يقتل نصف الحشرات المستخدمة فى التجربة أو LC_{50}. بالنسبة للمبيد البكتيري، تم رصد قيمتين: 2645 و 1568 وحدة دولية ضد يرقات البعوض بعد ٢٤ و ٤٨ ساعة من التعريض للمبيد على التوالي. وبالنسبة لمنظم النمو بايريبروكسفين، فان القيمة المسجلة هى ٢٢كجم/هكتار بعد ٧ أيام من التعريض ضد اليرقات، حيث ان تأثير منظمات النمو يبدأ ويزيد بعد فترة من التعريض. وقد سجل مبيد Sumithion NB® ٦,٨% ضد اليرقات بعد ٢٤ ساعة من التعريض له. المبيد الوحيد الذى تم اختبره ضد الطور اليافع، سجل ٠,٠٠٤% بعد ٢٤ ساعة من التعريض. وتشير هذه النتائج المتحصل عليها أن البعوض المختبر مازال حساسا بدرجة عالية لها و ليس هناك اية بوادر تدل على انخفاض فى هذه الحساسية أو بمعنى اخر بدء ظهور مقاومة لاى من هذه المركبات والتى تستخدم فى مكافحة أطوار البعوض المختلفة. ولهذا تعتبر هذه المبيدات طرق فعالة فى مكافحة البعوض ويمكن ان تطبق فى برنامج مكافحة متكامل حسب طور البعوض المستهدف والية تأثير المبيد. على سبيل المثال استخدام مبيد بكتيري ومنظم النمو ضد اليرقات وبالإضافة إلى مبيد Pesguard®FG81 لمكافحة الاطوار اليافعة والتى نجت من عمليات مكافحة اليرقات.