RESIDUAL TOXICITY OF SLOW-RELEASED FORMULATIONS (SRFs) OF TEKNAR AND SPINOSAD AGAINST *CULEX PIPIENS* MOSQUITOES.

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ABSTRACT

Sustained-release formulation of *Bacillus thuringiensis* var. *israelensis* (*B.t.i.*), Spinosad and their mixture were evaluated against mosquito larvae of *Culex pipiens* in tap and pond water. Teknar, Spinosad and their mixture provided continuous effective control with 100% larval mortality for 21, 42 and 49 days post-treatment, respectively in tap water, while in pond water these values were 21, 21 and 28 days post-treatment for the same treatments, respectively. The residual toxicity of these treatments in tap water was higher than in pond water. The residual toxicity of Teknar and Spinosad mixture was higher than the residual toxicity of Teknar or Spinosad alone. At the same time, the residual toxicity of Spinosad was higher than Teknar. The average of pupation occurred in Teknar treatment was higher than Spinosad in both water types tested, while the average of adult inhibition in Teknar was lower than in Spinosad in tap and pond water. Generally, results for the three treatments were higher in tap water than in pond water.

INTRODUCTION

Mosquitoes are the most important vectors of certain human diseases including malaria, encephalitis, filariasis and yellow fever. The members of *Culex pipiens* complex are the most widely distributed species in the world (Hoogstraal *et al.* 1977). In Egypt, the common house mosquito *C. pipiens* has been recorded from all governorates without exception (Wassif 1969 and Farghal 1974) causing severe morbidity to man and animals. It is the main vector of Bancroftian filariasis (Sabry 1991 and Harb *et al.* 1993). It is also the vector of Rift valley fever in Egypt (Hoogstraal *et al.* 1979) and other viral diseases (Darwish and Hoogstraal 1981).

The prolonged use of synthetic insecticide for mosquito control has been accompanied by harmful effects on human health and the environment (Attaran *et al.*, 2000 and Walker 2000). Also, the widespread use of synthetic insecticides in mosquito control have resulted in the development of resistant strains and caused the death of non-target organisms. The appearance of such problems has been companied by growing interest to find out other alternative control methods with a new mode of action that can effectively control mosquito larvae with minimal damage to the environment. Among these alternatives are the bioinsecticides such as Spinosad and *Bacillus thurengiensis israelensis*.

Spinosad (mixture of Spinosyn A and D), a naturally occurring product of the bacterium *Saccharopolyspora spinosa* fermentation, is a highly effective bioinsecticide against a wide range of agriculturally important insect pests, and this agent has an excellent environmental and mammalian toxicological profile (Romi *et al.* 2006).

Bacillus thurengiensis israelensis acts on insect larvae after ingestion of the insecticidal crystalline protein (ICP) spore complex. In the midgut, the ICP-spores are dissociated to their protoxins and activated by gut proteases, including the arrest of feeding and leading to larval death (Aronson *et al.* 2001). Different authors had been pointed to the effectiveness of *B.t.i.* against several mosquito species and to its safety to the environment (Flexner *et al.* 1986, Gharib and Hilsenhoff 1988, Lacey *et al.* 1984, Yap *et al.* 2002, Lima *et al.* 2005).

In order to reduce and delay the development of resistant populations, insect control by chemical insecticides should be accomplished with fewer applications at far lower doses. This aim might be realized, for example, by combining the insect control agents with each other. This trend had been used before by many researchers. Zahran (2008) used Spinosad mixed with some plant oil extracts against *C. pipiens* larvae. Also the mixture of Spinosad and pyriproxyfen against *Aedes aegypti* were tested (Darriet and Corbel 2006).

The aim of this investigation was to evaluate the residual toxicity of Sustained-release formulation of *B.t.i.* and Granule formulation of Spinosad against *C. pipiens* larvae. The residual toxicity of both *B.t.i.* and Spinosad mixture against *C. pipiens* was also evaluated.

MATERIALS AND METHODS

Mosquito larvae used: A field strain of *C. pipiens* larvae was collected from a water pond in Abees area, Alexandria Governorate, Egypt, in August 2001. The obtained larvae were reared foe 10 generations under laboratory conditions of 25±1°C and 70±5% R.H. The 2nd instar larvae were used in this study.

Bioinsecticides tested: *Bacillus thuringiensis israelensis* used as Teknar G 1.7% (200 ITU/mg), obtained from CERTIS Company, USA. Spinosad used as Spinosad G 0.5% provided by Dow Agrosciences.

Experiments: Susceptibility tests were carried out in large galvanized pools (140 x 40 x 30 cm) containing approximately 40 liters of water. The experiments were carried out in two types of water (tap water and pond water). The pond water was taken from a known mosquito breeding site in Abees area, Alexandria. The two biocides were used according to the recommended dosages for field trials in both tap and pond water (0.05 gm/L for Teknar G, 0.075 gm/L for spinosad G). The mixture of Teknar G and spinosad G at their half recommended dosages were also tested. Each pool received 100 second instar larvae of *C. pipiens* held in five white plastic pots (10cm long; 13cm diam.). Each pot had four openings (2.5 - 5cm diam.) covered by muslin cloth to allow water ventilation. The larvae were provided with food during the experiments. Water was slowly added to the pools daily to compensate evaporation. All trials and controls were replicated three times. Larval mortalities were recorded daily until all larvae either died or pupated (one week for each test). The live pupae were transferred to untreated water in clean glass beakers for emergence. When complete larval mortality or pupation occurred (after 7 days), a new 100 live 2nd instar larvae were added to the test pools. Each new added larvae were carried out as a new test. This procedure was continued sequentially until the effectiveness of each formulation reached a low level of toxicity (less than 50% inhibition of adult emergence).

Data analysis: Data were analyzed by one-way ANOVA, Newman Keuls multiple comparison test to determine differences between the efficiency of Teknar G, Spinosad G and the mixture of Teknar G and Spinosad G.

RESULT AND DISCUSSION

Toxic viability of Teknar, Spinosad and their mixture in pond and tap water:

Biolarvicides, based on mosquitocidal toxins of certain strains of *B. sphaericus* and *B.t.i.* are highly effective against mosquito larvae at very low doses and safe to other non-target organisms (Mittal 2003). Spinosad, a naturally occurring product from the fermentation of the bacterium *S. spinosa*, proved to be highly effective as bioinsecticide against different mosquito species (Romi *et al.* 2006, Darriet *et al.* 2005, Bond *et al.* 2004, and Cetin *et al.* 2005). The combining between the two aforementioned bioinsecticides can leads to the increase of efficiency.

Residual toxicity of Teknar, Spinosad and their mixture (at the half concentrations for each) against *C. pipiens* 2^{nd} instar larvae in pond water showed that the residual toxicity of Teknar and Spinosad mixture was significantly higher than the residual toxicity of Teknar alone or Spinosad alone (Fig.1). The toxicity of the Teknar and Spinosad mixture reached 95%, 70% and 34% mortality after 35, 49 and 70 days, respectively, from the beginning of the test. Spinosad alone caused 91%, 50% and 7% mortality 35, 49 and 70 days after the beginning of the test, respectively. Teknar alone caused 74, 22 and 3% mortality 35, 49 and 70 days after the beginning of the test.

Toxic viability of Teknar, Spinosad and their mixture in tap water is shown in (Fig.2). Teknar and Spinosad mixture achieved 100%, 95% and 85% mortality after 49, 56 and 70 days from the beginning of the test, respectively. Spinosad alone caused 94%, 88% and 40% mortality 49, 56 and 70 days after the beginning of the test, respectively. Teknar alone caused 87%, 64% and 8% mortality 49, 56 and 70 days after the beginning of the test. From these data it is obvious that the Teknar and spinosad mixture was significantly more toxic than Teknar alone or Spinosad alone in tap water.

Results provided in this investigation were in accordance with many authors. Zahran (2008) evaluated the mixture of Spinosad with different plant oil extracts against *C. pipiens* larvae and concluded that the mixture was more toxic than the Spinosad alone or plant oil extracts alone. On the other hand, Darriet and Corbel (2006) mentioned that the mixing between pyriproxyfen and Spinosad allows a reduction in both pyriproxyfen and Spinosad amounts by 5 and 9 fold to kill almost 100% mosquitoes.

Comparison between the efficiency of Teknar, Spinosad and their mixture in tap and pond water:

Figures (3, 4 and 5) showed that the residual toxicity of Teknar and Spinosad in tap water was higher than in pond water. The viability of Teknar in tap water was decreased from 97%, 87% and reached to 8% mortality after 28, 49 and 70 days from the beginning of the test, respectively, while the efficiency of Teknar in pond water was 94%, 22% and 3% mortality after 28, 49 and 70 days from the beginning of

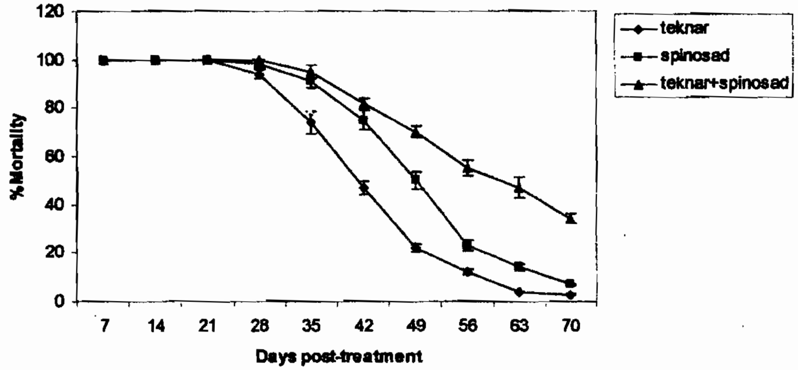

Fig. (1): Residual toxicity of Teknar, Spinosad and their mixture in pond water. Error bars represent standard deviation of three replications.

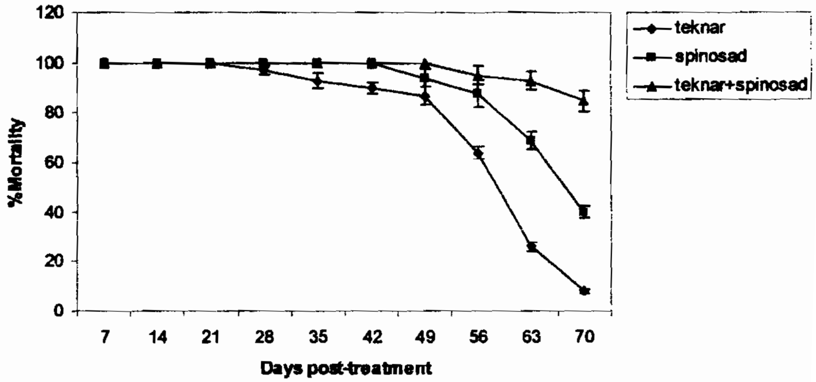

Fig. (2): Residual toxicity of Teknar, Spinosad and their mixture in tap water. Error bars represent standard deviation of three replications.

the test, respectively (Figure 3). Spinosad in tap water achieved 100%, 88% and 40% mortality after 42, 56 and 70 days from the beginning of the test, respectively, while Spinosad in pond water achieved 75%, 23% and 7% mortality after 42, 56 and 70 days from the beginning of the test, respectively (Figure 4). Also, the residual toxicity of Teknar and Spinosad mixture in tap water was higher than in pond water. The efficiency of the mixture in tap water decreased from 100%, 95% and reached to 85% mortality after 49, 56 and 70 days from the beginning of the test, respectively, while the efficiency of the mixture in pond water decreased from 70%, 55% and reached to 34% mortality after 49, 56 and 70 days from the beginning of the test, respectively (Fig.5).

The possible explanation of these results is, there are competing reactions occurred in the pond water where the toxicant was adsorbed to organic matter or suspended soil constituents and accordingly led to reduce its effectiveness against mosquito larvae (Stockman *et al.* 1970, Ramoska *et al.* 1982, Saleh *et al.* 2003, El-Banoby, 2005). They pointed out that the presence of soil constituents in pond water was associated with lower larval mortalities and tend to reduce the durations of effective control against mosquito larvae. However, consideration must be taken to avoid the influence of this factor. Several other factors can also influence the duration of larvicidal activity of SRFs when applied against mosquito larvae under field conditions such as target species, vegetative cover, sunlight, water turbidity and method of application (Lacey and Lacey, 1990). Modifications in formulation components and increase the dosage levels of active ingredient may necessary for future widespread use of such formulations.

Comparison between averages of larval %mortality, %pupation and % inhibition of adult formation after exposure to Teknar, Spinosad and their mixture in tap and pond water.

Table (1) showed that Teknar, Spinosad and their mixture caused 76.5, 89.1 and 97.3% average of larval mortality in tap water, respectively. The average of pupation caused by Teknar, Spinosad and their mixture in tap water was 23.5, 10.9 and 2.7%, respectively. On the other hand, the average inhibition of adult formation caused by Teknar, Spinosad and their mixture in tap water was 40.0% 53.5% and 55.7%, respectively.

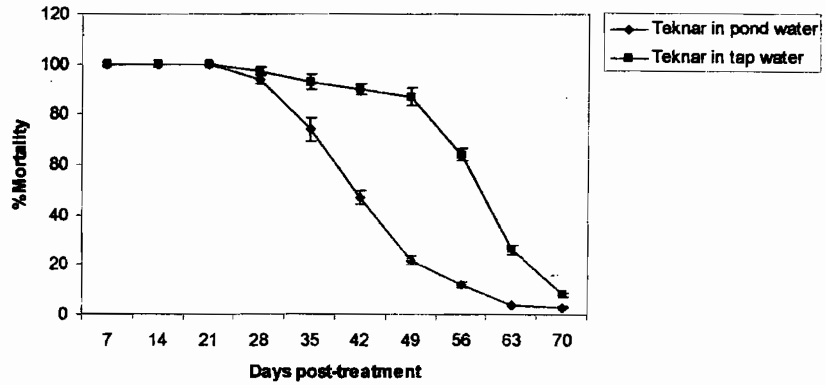

Fig. (3): Residual toxicity of Teknar in tap and pond water. Error bars represent standard deviation of three replications.

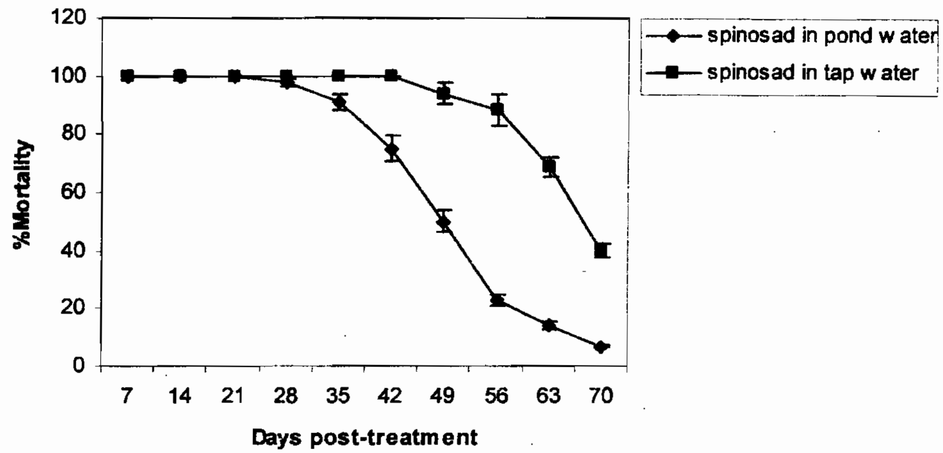

Fig. (4): Residual toxicity of Spinosad in tap and pond water. Error bars represent standard deviation of three replications.

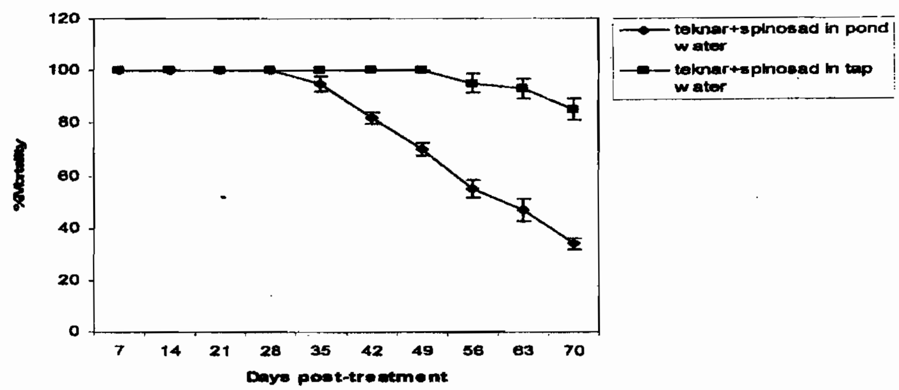

Fig. (5): Residual toxicity of Teknar and Spinosad mixture in tap and pond water. Error bars represent standard deviation of three replications. Error bars represent standard deviation of four replications.

Table (2) showed that the average of larval mortality caused by Teknar, Spinosad and their mixture in pond water was 55.6, 66.8 and 78.3%, respectively. The average of pupation was 44.4, 33.2 and 21.7% caused by Teknar, spinosad and their mixture, respectively. Teknar, Spinosad and their mixture caused average inhibition of adult formation 32.8, 39.2 and 44.3% in pond water, respectively. From these data, it is obvious that the mixture of Teknar and Spinosad achieved the highest efficiency in mosquito control both in pond and tap water.

Table (1): Average of larval mortality, %pupation and % inhibition of adult formation after exposure to Teknar , Spinosad and their mixture in tap water:

Treatment	Average of larval mortality at the end of test.	Average of pupation.	Average of inhibition of adult formation
Teknar	55.6 c ± 3.0	44.4 a ± 3.4	32.8 c ± 2.0
Spinosad	66.8 b ± 4.9	33.2 b ± 2.8	39.2 b ± 1.7
Teknar + Spinosad	78.3 a ± 3.1	21.7 c ± 1.8	44.3 a ± 3.0

Within the same column, numbers followed by the same letters are not significantly different according to SNK test.

Table (2): Average of larval mortality, %pupation and % inhibition of adult formation after exposure to Teknar , Spinosad and their mixture in pond water:

Treatment	Average of larval mortality at the end of test.	Average of pupation.	Average of inhibition of adult formation
Teknar	76.5 c ± 3.7	23.5 a ± 1.9	40.0 b ± 2.2
Spinosad	89.1 b ± 3.1	10.9 b ± 1.1	53.5 a ± 3.4
Teknar + Spinosad	97.3 a ± 1.2	2.7 c ± 0.05	55.7 a ± 2.9

Within the same column, numbers followed by the same letters are not significantly different according to SNK test.

In general, the present results suggest that long-term effective control of *C. pipiens* can be achieved economically with a single application of the tested formulations especially the mixture between Teknar and Spinosad in mosquito breeding sites. In other words, one treatment with any of these SRFs per season is a cost-effective control strategy compared with 2-3 treatments using other conventional chemical mosquitocides (McCarry, 1996). However, such sustained-release formulations may be particularly useful, especially as a mixture, for application in any location near the household where water collects and remains for long periods (i.e: ditches, pond, irrigated pastures, unused swimming pools and artificial containers).

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الملخص العربي

السمية المتبقية للتجهيزات بطيئة الإنطلاق للتكنار والإسبينوساد ضد بعوض الكيولكس بيبينز.

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تم تقييم التجهيزات المتحكم فى إطلاقها للمادة الفعالة للـ *B.t.i.*، الإسبينوساد ومخلوطهما ضد يرقات بعوضة الكيولكس بيبينز فى مياه البرك. أظهر التكنار والإسبينوساد ومخلوطهما مكافحة فعالة ومستمرة تصل إلى ١٠٠% موت ليرقات البعوض حتى ٢١، ٤٢، ٤٩ يوم بعد المعاملة على الترتيب فى مياه الصنبور، بينما فى مياه البرك كانت هذه القيم هى ٢١،٢١،٢٨ يوم بعد المعاملة على الترتيب بنفس هذه المركبات. السمية المتبقية لهذه المعاملات فى مياه الصنبور كانت أعلى من مثيلتها فى مياه البرك. السمية المتبقية لمخلوط التكنار والإسبينوساد كانت أعلى من السمية المتبقية للتكنار بمفرده أو الإسبينوساد بمفرده. أيضا السمية المتبقية للإسبينوساد كانت أعلى من السمية المتبقية للتكنار. متوسط التعذير عند المعاملة بالتكنار كان أعلى منه فى حالة المعاملة بالإسبينوساد وفى كلا من مياه الصنبور ومياه البرك، بينما متوسط تثبيط خروج الحشرات الكاملة فى حالة المعاملة بالتكنار كان أقل منه فى حالة المعاملة بالآسبينوساد فى كلا من مياه الصنبور ومياه البرك. وعامة كانت النتائج المتحصل عليها من الثلاث معاملات فى مياه الشرب أعلى عنها فى مياه البرك.