Journal of Biopesticides, 4 (1): 61 - 64 (2011)

Assessment of two natural toxin microcystin and nodularin for the control of *Anopheles multicolor* (Diptera: Anophelidae)

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ABSTRACT

Laboratory experiments were conducted to determine the efficacy of two cyanobacterial toxins (microcystin and nodularin) against the different larval instars, pupal and adult stages of *Anopheles multicolor*. Experiments were carried out in plastic cups, and the two toxins were tested at four concentrations, where the LC_{10}, LC_{25}, LC_{50} and LC_{90} for the first instar larvae under laboratory conditions were 2.95, 3.80, 4.95 and 7.45 µg /ml and 4.37, 6.40, 8.85 and 14.39 µg/ml for microsystin and nodularin respectively. While the LC_{50} for the first, second, third and fourth larval instars were 6.70, 8.92 and 9.70 µg/ml for microsystin and nodularin, respectively. The most sensitive instar was the 1st and the most resistance instar was the fourth larval instar. The delayed effects of sublethal concentrations (LC_{10}, LC_{25} and LC_{50}) of the two tested toxins on some biological activities were also studied and discussed when treating the fourth larval instar.

Key words: Microcystin, nodularin, *Anopheles multicolor*, mosquito, toxicity, vector

INTRODUCTION

Mosquitoes are of great economic impact because their bites are annoying and cause skin allergies and they are vectors for a number of diseases, such as malaria yellow fever, dengue filariasis and certain types of encephalitis such as west Nile fever. *Anopheline mosquitoes* were the major vectors of malaria recorded in Oases and other desert regions (Gad *et al*., 1984; Ali *et al.*, 2011). Most of the mosquito control programmers target the larval stage in their breeding sites with larvicides because adulticides may only reduce the adult population temporarily (Knio *et al*., 2008).

Cyanobacteria produce a wide array of bioactive secondary metabolites, some which are toxic. Those toxic to mammals include the microcystins, cylindrospermopsins, saxitoxins, nodularins, anatoxin-a, homoanatoxin-a, and anatoxin-a(s). The toxic of cyanobacteria still constitute a major source of natural products toxins (biotxins) found in the surface supplies of freshwater. Approximately 40 species of cyaobacteria are benign implicated in toxic bloom (Skulberg *et al*., 1993). *Microcystis aerginosa* and *Nodularia harvenya* are two species of cyaobacteria that have been detected in freshwater ponds and lakes all over the world and are of the most important toxic members containing essential oils (Carmichael 1986 and Winne *et al*., 1988). Also their blooms produce a cyclic peptide toxic known to be toxic fish, birds and invertebrates (Penalozax *et al*., 1990; Williams *et al*., 1997; Campo *et al.*, 2010), theses toxins are termed microcystin and nodularin. Microcystins (MCs) are a group of at least 80 variants based on a cyclic heptapeptide structure. Nodularins are hepatotoxic cyclic peptides of similar structure to the microcystins except that they are composed of 5 amino acids rather than 7. There have been several studies on the toxicity of microcystin and nodularin against vertebrates and aquatic invertebrates (Winne *et al*., 1988; Nasser, 2000; Anna Lankoff and Adam Kolataj, 2001; Deng *et al.*, 2009; da Silva *et al*., 2010) but there are little comprehensive investigations on the toxicity of these toxins against insects (El-Blok and Moawad, 1996; Abou-El-Ela, 2006). Impact of spinatoran and vertemic on this pest has also available previously (El-Kady *et al.*, 2008). Microbial insecticides are being considered as alternative to chemical insecticides because of their selective toxicity and ready decomposability in the ecosystem. The aim of the present study is to evaluate the efficiency of micro cystin and nodularin against the different larval instars of *Anopheles multicolor*. Also the investigation extended to latent effect of sublethal concentrations of the tested toxins on some biological activities of *A. multicolor*.

MATERIALS AND METHODS

Toxins used

The two toxins, microcystin and nodularin used in this study were extracted and identified from cyanobacterial algae (*Microcystis aeruginasa* PCC 7806 and *Nodularia harvenya* according to the method of Abou-El-Ela (2006).

Laboratory bioassay

The original strain of *A. multicolor* was obtained from wild larvae collected from Fayoum Governorate, Egypt. They were

reared in our laboratory, Faculty of Sciences, Fayoum University from two different generations at room temperature 21.7 ± 2° C and 70-80 ± 5 % RH. Pure population of the first, second, third and fourth instar larvae of *A. multicolor* was used in this study.

Five concentrations of both toxins were tested to cause mortality ranging from 20 to 90%. For each treatment 20 larvae were tested with four groups. All individuals were maintained in sterilized transparent plastic cups containing 150 ml tap water with 2 grams of dry yeast. In each cup different concentration of microcystin or nodularin were used. Each experiment was replicated thrice. The experimental arena was kept at the laboratory conditions for 24 hr. Mortality was recorded 48 hrs. after exposure and the mortality corrected according to Abbott's formula (Abbott, 1925). The larvae were classified as 'dead' if they did not move when gently touched with the point of tooth pick. In addition to the treated larvae, another group of larvae were used without using any toxin as control. Corrected mortality was subjected to Finney (1971) formula to find out the sub-lethal concentrations (LC_{10}, LC_{25}, LC_{50} and LC_{90}).

Latent effect

The latent effect of sub-lethal concentrations (LC_{10} and LC_{25}) of tested toxins on larvae and pupal duration as well as on the emergence, fecundity and fertility of adult progeny were determined.

Statistical analysis

In all cases a minimum of three independent experiments were conducted and each sample was triplicated. Data presented were means ± standard deviations. Data were analyzed with two way analysis of variance, and pair wise multiple comparison procedure (Student Newman Keuls method) were used to compare insecticides at $p<0.05$.

RESULTS AND DISCUSSION

Toxic effect

Data presented in Table 1 clearly indicates that the selected toxins had toxic effect against the different larval instars of *A. multicolor*. The response of the first, second, third and fourth larval instars against the tested biopesticides appear to be concentration dependent one. Data showed also that the susceptibility of first instar larvae of *A. multicolor* to fresh water toxins after 48 hrs. of exposure was higher than the second, the third and the fourth instar larvae at all tested toxin concentrations. The LC_{10} in descending order of toxicity were 2.95 µg /ml (first instar larvae), 3.78 µg /ml (second instar larvae), 4.15 µg /ml (third instar larvae) and 4.96 µg /ml (fourth instar larvae) for microcystin. While the LC_{10} of nodularin toxin against the first, second, thirds, and fourth instar larvae were 3.31, 6.45, 7.95 and 8.75 µg /ml respectively, the respective values of LC_{50} for microcystin were 4.95, 6.70, 8.92 and 9.70 µg /ml, while the LC_{50} values of nodularin were 8.85, 12.25, 15.90 and 18.20 µg /ml respectively. The toxicity of microcystin and nodularin were due to the presence of polypepide chain endotoxin produced as part of the cyanobacteria which also cause contact irritation (Carmichael, 1986; Codd and Bell, 1985; Nassar, 2000; Adel and Abou-El-Ela, 2006).

The sensitivity of different larvae instars of *A multicolor* to nodularin toxin was less than microcystin. Among the lareve stage the most tolerant instar was the fourth instar larval which showed highly lower lethality. It is clear that from Table 1 microcystin was more toxic against*A multicolor* larvae than that of nodularin. This may be due to the variation in the chemical structure of two toxins as observed by Adel and Abou-El-Ela (2006).

Table 1. Toxic effect of fresh water toxin microcysatin and nodularin on the different larval instar of *Anopheles multicolor.*

Toxin	LC concentrations	Larval Stage (Fiducial limits at 95%)			
		1st intar	2nd instar	3rd instar	4th instar
Microcysatin	LC_{10}	2.95(1.6 - 4.9)	3.78 (2.4 - 6.8)	4.15 (2.75 - 6.70)	4.96 (2.90 - 7.10)
	LC_{25}	3.80 (2.55 - 5.90)	5.20 (3.65 - 7.80)	6.40 (3.90 - 8.76)	7.97 (4.50 - 10.68)
	LC_{50}	4.95 (2.80 - 670)	6.70 (4.65 - 730)	8.92 (5.91 - 1145)	9.70 (6.45 - 12.7)
	LC_{90}	7.45 (5.56 - 9.70)	10.95 (7.40 - 13.30)	13.40 (9.25 - 17.43)	17.80 (12.65 - 21.86)
Nodularin	LC_{10}	4.37 (2.95 - 560)	6.45 (4.80 - 7.95)	7.95 (5.22 - 9.32)	8.75 (5.85 - 10.79)
	LC_{25}	6.40 (4.75 - 822)	9.35 (6.29 - 11.78)	12.10 (9.35 - 14.70)	14.32 (11.86 - 17.62)
	LC_{50}	8.85 (5.56 - 979)	12.25 (9.46 - 14.95)	15.95 (11.85 - 18.76)	187.20 (14.15 - 21.39)
	LC_{90}	14.39 (11.90 - 17.2)	19.78 (16.20 - 21.80)	25.90 (20.70 - 20.73)	33.67 (29.67 - 38.85)

Value in parentheses indicates the range

Table 2. Effect of microcysatin and nodularin on some biological activities of *Anopheles multicolor*

Toxin	LC concentrations	Larval duration(days)	Pupal duration (hrs)	Stage	Adult stage (in %)		
					Fecundity	Hatching	Sterility (in %)
Microcysatin	LC_{10}	7.55±0.21	52.39±0.76	77.95 ±1.21	80.50±0.98	74.80±0.39	25.20±0.85
	LC_{25}	8.95±0.85	53.45±0.97	70.52 ±0.78	78.65 ±0.39	70.60±1.25	29.40 ±0.58
	LC_{50}	9.55±1.022	53.85 ±0.48	63.75 ±1.08	74.90±1.03	65.38±0.76	34.62±0.83
	Control	7.32±0.20	48.50± 0.17	96.80±0.35	94 ±0.22	92.70±0.25	7.30± 0.43
	LSD value	2.78	3.90	9.50	5.50	7.65	-
Nodularin	LC_{10}	8.35 ± 0.55	44.63 ± 1.34	80.78 ± 1.05	83.60 ± 0.48	76.30 ± 0.50	23.70 ± 0.25
	LC_{25}	8.79 ± 67	46.63 ± 1.34	73.45 ±0.90	80.50 ± 0.79	72.80 ± 0.95	27.20 ± 0.54
	LC_{50}	9.58 ± 0.98	47.62 ± 0.93	68.95 ± 1.05	77.68 ± 0.95	68.38 ± 1.05	31.65 ± 0.75
	Control	7.24 ± 0.36	42.95 ± 0.93	94.75 ± 0.22	92.70 ± 0.25	93.78 ± 0.80	6.22 ± 1.02
	LSD value	3.10	4.20	10.60	5.70	8.50	-

The delayed effect

The latent effect of some sub-lethal concentrations of LC_{10}, LC_{25}, LC_{50} and LC_{90} of the tested toxins on larval and pupal duration as well as on the emergence fecundity and fertility of the adult progeny were determined. Data presented in table 2 show that the two tested toxins increased the larval duration of *A. multicolor* after treatment with sub lethal concentrations of microcystin and nodularin.

The percentages of developed pupae from treated larvae were lower than that of the untreated ones. Also the pupal duration significantly increased at $p<0.05$ with increasing the cyanobacterial concentrations. Microcystin toxins was more active than nodularin. The increase of larval and pupal mortalities of their durations may be related to the effect of these toxins on the insect tissues. Our results are in agreement with the findings of Rashed and El-Ayouty (1992); Rao *et al*. (1995) and Adel and Abou-El-Ela (2006). Results in table 2 showed that the fecundity and fertility of *A multicolor* adults emerging from treated larvae with the two toxins reduction in negatively correlated with the concentration level of the toxin.

The reduction in fecundity and fertility of *A. multicolor* may be due to the effect of these toxins on the ovary development including tissues and biochemical level. Also it may be related to the disturbance in insect testes. These results coincide with the finding of Sanker *et al*. (1995) who tested the petroleum ether extract (PEX) of alga *Chara zeylanica* against *Dysedercus koenigii* which showed that some fractions of (PEX) were active on eggs and induced maximum sterility and reduced the average fecundity. Finally the cyanobacterial toxins of *Microcystin aeruginosa* and *Nodularia harvenya* are effective in suppressing populations of *A multicolor* larvae either directly through their acute toxicity to the larvae or indirectly through their delayed effect on the survivors resulting from larval treatment. It may be concluded that the two toxins of blue-green algae (cyanobacterial microcystin and nodularin) have acute effects on the different stage of *A multicolor* and can be considered in the integrated program of mosquito control in Egypt.

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Received: April 5, 2011 Revised: May 20, 2011 Accepted: May 22, 2011