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Bioactivity of three Cyanobacterial blooms against *Culex pipens molestus* (Diptera: Culiciday).

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Abstract
The cyanobacteria (blue - green algae) were isolated and screened from the aquatic habitates of the *Culex pipiens molestus*. The algae; *Nostoc commune Osallatoria tenuis* and *Lyngbya alementaria* have activity on the immature stages with the concentrations 0.25, 0.05, 0.75 and 1.0µg/ml of *N. commune*. The suspension bloom of *O. tenius* induced malformations began with 23.3 and 11.7 present at 0.25µg/ml until 83.3 and 93.3% for larvae and pupae, at the concentrations 0.25 and 1.0µ/g/ml respectively. The cyanobacterium *L. alementaria* specific with the life cycle extension of larva and pupa starts with 0.5µg/ml(18.2 and 3.0 days) and proportionally exceeded to 30.5 and 4.7days at 1.0 µg/ml in comparison with 13.3 and 1.8 days for control.

The adult emergence decreased to 50 and 6-7% at the concentration 1.0 for *L. alementaria* and *O. tenuis*.

Keywords: *Culex pipens*, cyanobacteria, mortality, malformation, growth disruption, emergence failure.

Introduction

Mosquitoes are vectors of many vertebrate blood parasites and nuisance for man and domestic animals. Mosquitoes are controlled either as larvae or as adults. Adults control is the mainly method in developing countries, while larvae control are practical in urban and periurban communities (Mulla, 1999).

Mosquito larvae are filtrate-feeding on planktons and fragmented organic matter in the water body column cyanobacteria represent the largest part the diet of mosquito larvae (Thiery et al, 1991; Vazquez-Martinez et al., 2002). It was early found that the blooms of certain blue – green algae were associated with the absence of mosquito larvae from rice fields in California (purdy, 1924). Currently, the algae as *anabaena* spp. kill the mosquito larvae because they are indigestible (Marten, 1986), also, cyanobacteria can produce secondary metabolites named cyanotoxins with effect on aquatic herbivorous zooplanktons, secondary consumer and vertebrates (Murphy and Stevens, 1995; Ferrao-Filbo and kozlowsky-Suzuki, 2011). These toxins may offer possibilities for dealing larvicides and to be useful for Mosquito control (Marten, 2007).

Mosquito larvae were killed by species of blue-green algae of genera: *Anabaena Cylindrosprmum, Eucapsis, lyngbya, Microcysis, Nodularia, Nostoc,* three day old females nightly fed pigeon blood. The egg rafts were oviposited in plastic containers filled with dechlorinated tap water. The rafts transferred to 35×20×10 cm melamin containers at 27±2°c and 70-80% relative humidity under a photoperiod cycle of 16 h light /8 h dark. The hatched larvae daily fed with 1.0 gm rabbit diet.

Mosquito larvicidal assay commonly use second instar (Elango et al.; Mosleh and Abou-EL-Ela, 2011). For exhibit the sub-lethal effect on development immature stages the 1st instorwas chosen.

Cyanobacteria blooms

The algal samples were collected from shallow ponds. Of these, seven cyanobacteria species cultivated in the lab. for biomass blooms, under 20±°c and 70-80 relative humidity. The cyanobacterial isolates have been screened to evaluate their mosquito larvicidal activity. especially; morlality, malformations and developmental disruption. The blue-green algae: *Lyngbya alementaria, Nostoc commune* and *Oscllatoria tenuis* were selected on the bases of immatures mortality abnormal growth and development, with promised alternative mosquito control.

Treatment and suspensions

Bioassays

Four concentrations with triplicate of the applied 1-hr-setteled wet bloom blue-green algae were added to 900 ml of dechloronated top water and completed with hatch water to 1 liter. For algal suspentions, the mixtures were homogenized by stirring for 5 minuits, then transformed into plastic containers

Oscillatoria , *phormidium* and *spirulina* (Burja et al., 2001; Kelseolou and Bouwer, 2012; Marten, 2007). Nasser et al., (1999) found that the crude extracts from cyanobacteria had larvicidal activity with microgram per larva levels.

The Lc_{90} cyanobacterial Toxins of *Microcustis aeryinosa* and *Nodularia harvenya* 7.45 and 14.39 µg/ml for 1st instar of *Anopheles multicolor* (Mosleh and Abu-El-Ela, 2011).

Besides the mortality the body size of the treated mosquito larvae with the algae enlarged or reduced(Ahmad et al., 2004). Also, sub-lethal concentrations delayed mosquito larvae development and pupation (Ahmad et al., 2004; Becher and Juttner, 2006), the micro cystin toxins damage to the midgut cells of *aedes aegypti* (Sorrio et al., 1994). Also, dry biomass of *Nostoc musorum* prolonged pupation and malformed immature stages of *Agrot isipsilon* (Abdel-Rahim and Mohamed 2013).

The objective of the present study was being as the first trait to evaluate the role of cyanobacterial blooms suspension in control mosquito immature stages in Iraq, through application with the named blue–green algae, *Lyngba alementaria, Nostoc commune* and *Oscllaloria tenuis*.

Materials and Methods

Insects

The mosquito, *Culex pipiens molestus* colony were established from immature stages collected from temporary ponds after rainfall, at the insectary of Biology building, College of Pure Science, University of Mosul. The adults on a cotton pad soaked in 15% honey solution. To ontain egg rafts, the

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2000), and later, several species and strains inhibit mosquito larvae development (Rao, et al., 1999). Which behaved as insect growth regulators (Ahmed et al., 2004; Becher and Jullent).

A few studies applied cyanobacteria blooms as direct treatment with low cost. For this purpose; we chose three blue-green algae as more significant effect on mortality, malformation, development extension and emergence.

Habitate clearance

Table 1 show that daily mortality as application with *Nostoc commune* not homogenous distribution through the testing week time, peak of mortality happens at fourth day of treatment, and 85% of the mortalily occurs from third to fifth day at the concentrations 0.75 and 1.0 µg/ml. The mosquito larvae sensitive to *L. alementaria* with Lc_{50} 0.52 µg/ml and accumulative mortality reached 95.1% at 1.0 µg/ml.

The larvicidal effect of the blooms was not directly appeared after ingestion and the larvae moribund and dead clearly notable in third day of the application as the bloom digested and the cyanotoxins interfered with the metabolic pathways.

and adding twenty neuly hatched or 2nd instar larvae in each container as well as negative control.

Immature evaluation

Treated 2nd instar larvae with 0.25, 0.5, 0.75 and 1.0 µg/ml of the applied blue-green algae; *L. alementaria, N. commune* and *tenuis* were daily observed for 7 days, the dead and moribund larvae removed for mortality account. Also sub lethal effect on development larvae and pupa as malformations and adult emergence. Digital comera based macro-photographic technique for dedection the morphological growth disruption.

Life cycle disturbance

For assessment the effect of the body water treatment with the blue-green algae on the developmental time of the immature stages, twenty newly hatched larvae transformed into 0.25, 0.5, 0.75 and 1.0 µg/ml of suspension algal and negative control for triplicates. The metamorphosing condition and adult emergence were continually had been seen every day with binocular microscope or naked eye.

Results and Discussion:

The first studies were focused on larvicidal activity of cyanobacteria (Kiviranta et al., 1993; Harada et al.,

Table 1 : Weekly Clearanse from *Culex pipiens molestus* larvae treated with *Nostoc commune* bloom*

Day no.	Mortality at the bloom concentration (µg/ml)			
	1.0	**0.75**	**0.5**	**1.0**
First	0.0**i**	0.0**i**	0.0**i**	0.0**i**
Second	6.7 **ef**	5.0 **f**	3.3 **f**	0.0**i**
Third	11.7**e**	8.3**e**	6.7**ef**	1.7**h**
Fourth	46.9**a**	40.0**ab**	10.0**e**	6.7**ef**
Fifth	21.7**ed**	16.7**d**	10.0**e**	5.0**f**
Sixth	8.3**e**	6.7**ef**	3.3**f**	1.7**h**

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Seventh	0.0**i**	0.0**i**	0.0**i**	0.0**i**
Accumulative mortality	95.1	76.7	45.0	15.1

*Numbers Followed by the same letters are not significantly (P≤0.05).

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mosquito control (Alouani et al., 2009; Mekhlif, 2007). This delation of metamorphosis may be attributed to cellular disturbance of the larval stage, and active ingredient interference with tissue differentiation of the pupal stage.

The purified compounds from cyanobacteria can act as mosquites larvicides (kiviranta et al., 1993; Harada et al., 2000) but crude extracts or algal bloom more effective due to synergism between the metabolic ingredients (Skulberg et al., 1993).

Effect on life cycle

It was found suspension bloom of *Lyngbya alementaria* more significant in extension of larvae and pupae development and emergence failure. The significant extension began with 0.5 µg/ml and folded at 0.75 µg/ml. and few larvae met morphed to adults with 30.5 and 4.7 days for larval and pupal stages while 13.3 and 1.8 days for control (table 2). It seems the allelopatic strategy of *L. alementaria* and higher plant not deference through insect development extension and emergence failure in

Table 2: Effect of *Lyngbya alementaria* bloom on the development of *Culex pipiens molstus* (days)*

Concenration (µg /ml)	Immature Stage		Emergence
	Larva	Pupa	
0.0	13.3±0.6**d**	1.8±0.1**cd**	100
0.25	13.2±0.6**d**	1.7±02**cd**	83.5
0.5	18.2±0.9**c**	3.0±0.2**b**	565
0.75	25.7±0.6**b**	4.7±0.3**a**	53.6
1.0	305±1.1**a**	4.7±0.5**a**	50

*means within in column followed by the same letters are not significantly different (P ≤ 0.05).

Immature malformation

The malformations were represented by elephantoids, unsclerotized integument and asymmetrical body region sizes (Fig.1). Also, these abnormal growth of the mosquito immature can be induced with plant extracts (Ahmed et al., 2004; Khater and shalaby, 2008; Bream, et al., 2010). The deformities were increased with *O. tenuis* concentran, and drastic adult emergence from 66.7 to 6.7%

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during metamorphosis of the pupal stage (Hafez, 2000).

between the concentrations 0.25-1.0µg/ml(Fig 2).

The emergence inhibition may be attributed to certain inhibiting activity

Fig 1: Elephantoid and unsclerotized integument: **A-** Larvae and **B-** Pupae of *Culex pipiens molestus* treated with bloom suspension of *Oscllatoria tenuis.* **C-** control

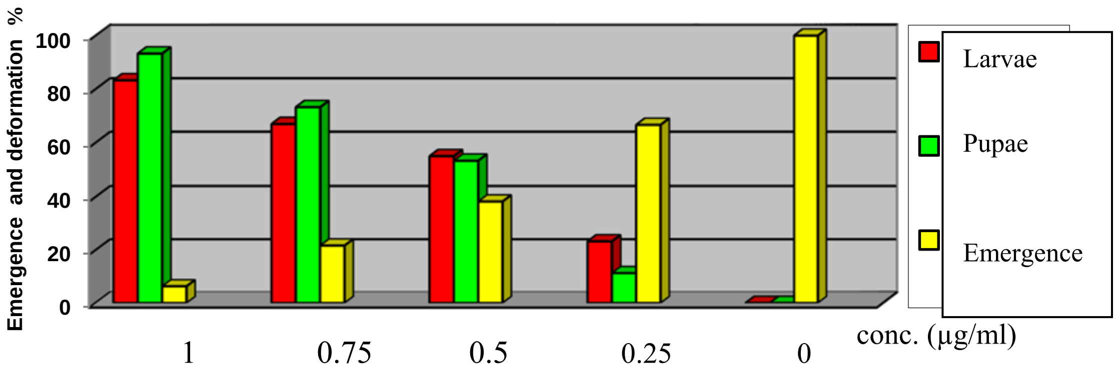

Fig 2: Malformations of the immature stages of mosquito, *culex pipiens molestus* and emergence percentage in presence of blue - green alga, *Oscillatoria tenuis* bloom suspension.

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Conclusion and Recommendation

Interest in Bioinsecticidal resources derives from the need of alternatives to synthetic insecticides, those alternatives well known in the last decades which characterized as; friendly to eco-biotic system, more specific and save for non-target organisms. Cyanobacteria are recognized as a enormous source for future insecticides (Becher et al., 2007). Most of the studies used cyanobacteria extracts, in the present study, all the applied blue-green algae and considerable number about 26% (berry et al., 2008) inhibit with developing mosquito larvae, they habitat mosquito freshwater system with high bloom growth. It was noticed that most of the malformations included with elephantoid ones, this will increase the biomass of the trophic chain. The Integrated Mosquito Management (IMM) which applied more than method in mosquito control, so, odonata and hemipteran predators can be used in IMM. The sub-lethal concentration eliminate the fitness through predation of handicapped immature stages and emergence failure. On the other hand, extension of the life cycle (table 2) reduces number of generations to less than half annual mosquito offsprings.

We are suggested to investigate more studies about the effect of the present cyanobacteria on other mosquito species, non-target aquatic invertebrates, supernumerary growth as firstly induced with blue-green algae and bioaccumulation in food-web.

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