EFFICACY OF ENTOMOPATHOGENS AGAINST MOSQUITOES *ANOPEHELES MACULIPENNIS* AND *CULEX PIPIENS MOLESTUS* (DIPTERA: CULICIDAE)

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In the recent years, as a result of the increase of anthropogenic influence on the environment, global ecological changes have taken place – climatic rise in temperature, soil erosion, deforestation, desertification etc. We face a rapid increase of pathogenic microorganisms which cause various infections such as malaria, yellow fever, dengue, rift causing great economical damage to many countries. Biological control with entomopathogens can provide a good control of pests, reduce the population of mosquitoes and decrease damage to human health. The efficacy of entomopathogenic nematodes (EPNs) of the species *Steinernema feltiae* and bacterial pesticides*:* Bitoxybacillin (BTB), Thuringin-2 and Dipel were evaluated in the control of two species of mosquitoes: *Anopheles maculipennis* and *Culex pipiens molestus* in the laboratory conduction. The mosquitoes mortality grew with increase of concentration of bacterial pesticides. On the 5th day with the high concentration of Thuringin-2 and Dipel, mortality rate of insects increased, although it did not reach 100%. The maximum mortality (100%) of mosquito larvae was observed only when exposed to *BTB*. It can be concluded that suspension of entomopathogenic nematodes of the species *S. feltiae* with dose 100 IJs /per larvae showed slight activity (36%–40%), compared with bacterial pesticides toward the larvae of the indicated mosquito species where the mortality ranged between 45 and 100%.

Keywords: entomopathogenic nematodes, bacterial pesticides, *Anopheles maculipennis*, *Culex pipiens molestus*.

INTRODUCTION

Among blood-feeding arthropods as carriers of causative agents of dangerous infectious diseases, mosquitoes have major importance. Mosquitoes (Diptera: Culicidae) are the most important groups of arthropods in medical and veterinary fields. They act as vectors of several diseases such as malaria, yellow fever, dengue, etc. Ecological changes connected with global warming produce an essential effect on the thermal conditions of the biotopes of blood-feeding arthropods. In recent years much attention has been given to the study of carriers of pathogenic microorganisms which cause various infections in men and animals.

South Caucasus (Georgia and Azerbaijan) – is characterized as a zone of great geographic and climatic diversity as well as diversity of fauna migrant and drift birds, bats that may cause significant variations in the virus circulation chain and in the forming of ecological niches for pathogenic agents. This paper will discuss the advances in understanding in the application of entomopathogenic nematodes (EPNs) species *Steinernema feltiae* and bacterial pesticides Bitoxybacillin (BTB), Thuringin-2 and Dipel against mosquitoes: *Anopheles maculipennis* Meigen, 1918 and *Culex pipiens molestus* Forskal, 1775 in laboratory. Generally, application of both pathogens showed encouraging results. The successful widespread use of biological control agents against mosquitoes requires a precise understanding of the ecology of predator/prey and pathogen/host relationships. The opportunistic characteristics of many species, including their ability to take advantage of temporary habitats, coupled with their short generation time, high natural mortality, great dispersal potential, and other R-strategist characteristics, pose difficult problems for any biological control agent (Garcia & Legner, 1999). Mosquitoes typically exploit many aquatic habitats. Often a good biological control agent will have a much narrower range of environmental activity than the target species. Thus, in many situations a number of different biological control agents and/or appropriate methods are necessary to control even one species of mosquito across its range of exploitable breeding sources.

Nematodes. Entomopathogenic nematodes (EPNs) are extraordinarily lethal to many important insect pests, yet they are safe for plants and animals. This high degree of safety means that unlike chemicals, nematode applications do not require masks or other safety equipment; and re-entry time, residues, groundwater contamination, chemical trespass, and pollinators are not issues. Most biological pesticides require days or weeks to kill, yet nematodes, working with their symbiotic bacteria, can kill insects within 24–48 hours. Two families – the steinernematids and the heterorhabditids – are obligate parasites of insects used for microbial control. Juvenile nematodes parasitize their hosts by directly penetrating the cuticle or through natural openings. They then introduce symbiotic bacteria, which multiply rapidly and cause death by septicemia. The symbiotic bacteria break down the insect body, which provides food for the nematodes. After the insect has died, the juvenile nematodes develop to adults and reproduce. A new generation of infective juveniles emerges 8–14 days after infection. Dozens of different insect pests are susceptible to infection, yet no adverse effects have been shown against beneficial insects or other nontargets in field studies (Georgis *et al*., 1991; Akhurst & Smith, 2002). The entomopathogenic members of the genera *Photorhabdus* and *Xenorhabdus* are represented by endo-symbionts of insecticidal nematodes. The first which are typically associated with entomopathogenic nematodes in the genus *Heterorhabditis*, while the other is the species of genus *Steinernema*. The pathogenic action usually involves the release of symbiotic bacteria in the insect hemocoel once the nematodes have actively entered the insect body. Here the bacteria proliferate producing various antimicrobial compounds to contrast the growth of other microorganisms. They also release different enzymes that contribute to the degradation processes in the hemocoel, thus creating an ideal environment for the development of the nematode population (Luca, 2015). A variety of bacterial virulence factors are involved in the interaction with the susceptible host. Different *Photorhabdus* and *Xenorhabdus* species producing an insecticidal toxin complex (Tc) have high potential for pest management (Waterfield *et al*., 2001). Generally, the Tcs are high-molecular weight and multisubunit proteins that include three components, A, B and C, orally active against different insects (French-Constant & Waterfield, 2006). All these components are normally needed to achieve full toxicity (French-Constant *et al*., 2007).

Bacterial pesticides. Over 90 species of naturally occurring, insect-specific (entomopathogenic) bacteria have been isolated from insects, plants, and the soil, but only a few have been studied intensively. Much attention has been given to *Bacillus thuringiensis*, a species that has been developed as a microbial insecticide. *B. thuringiensis* (*Bt)* occurs naturally in the soil and on plants. Different varieties of this bacterium produce a crystal protein that is toxic to specific groups of insects. *Bt* have an excellent safety record and can be used on crops until close to the day of harvest. *Bt* can be applied using conventional spray equipment but, because the bacteria must be eaten to be effective, good spray coverage is essential. *B. thuringiensis* the majority of bacterial pathogens of insects, widespread in soil, is a lethal pathogen of a range of orders and the most widely used as biological control agent (Hoffmann & Frodsham, 1993). Bitoxybacillin (BTB) has been widely used for a long period of time in forestry and agriculture all over the world. The active components of the bitoxybacillin (BTB) are beta-exotoxin and crystal endotoxin. Dipel delivers outstanding control of more than 30 species of insects, did not harm any other organism and has long been a favorite of organic gardeners and commercial growers. Dipel is biodegradable and has minimal effect on humans, non-target animals or the environment (Grishechina, 2015).

Mosquitoes. Mosquitoes (Diptera: Culicidae) are the most important groups of arthropods in medical and veterinary fields. They act as vectors of several diseases such as malaria, yellow fever, dengue, filariasis, setariasis and encephalitis, causing serious health problems to humans (Service, 2003, Almeida *et al*., 2008).

The study has shown that in respect of feeding mosquitoes of west Georgia may be divided into three groups: 1) Species characterized by a wide range of feeders, i.e. these preferring to feed on domestic ungulates, humans, and more or less birds (*Anopheles plumbeus, An. elaviger, An. maculipennis, An. hyrcanus, Mansonia richiardii, Aedes vexans, Ae. cinereus, Ae. caspius, Ae. geniculatus*); 2) Species which seldom bite humans, but feed mainly on domestic ungulates and poultry (*Culex hortensis, Cx. mimeticus, Cx. theileri, Culiseta annulata, Cs. setivalva*) as well as on birds and cold-blooded vertebrates (*Culex territans*); 3) Species feeding in the countryside on birds and domestic ungulates (*Culex pipiens pipiens*) and in towns on humans, birds and carnivores (*Culex pipiens molestus, Cx. pipiens pipiens*) (Sichinava, 1978; Gugushvili, 2002).

In the recent time larvae and adults collections were carried out from different habitats using the standard methods in twenty-five localities of seven counties across West Azerbaijan Province (Iran) near Georgia. Overall, 1569 mosquitoes including 1336 larvae and 233 adults were collected from 25 localities. The details of geographical properties were recorded. Five genera with 12 species were collected and identified: *Anopheles claviger, An. maculipennis s.l., An. superpictus, Culex pipiens, Cx. theileri, Cx. modestus, Cx. hortensis, Cx. mimeticus, Culiseta longiareolata, Ochlerotatus caspius s.l., Oc. geniculatus and Uranotaenia unguiculata.*

Due to the geographical location of the West Azerbaijan Province, it comprises different climatic conditions which provide a suitable environment for the establishment of various species of mosquitoes. The solidarity geographical, cultural and territorial exchanges complicate the situation of the province and its vectors as a threat for future and probable epidemics of mosquito-borne diseases (Farahnaz Khoshdel-Nezamiha *et al*., 2014). The objective of this study was to evaluate the susceptibility of entomopathogenic nematodes of the species of *S. feltiae* and action of bacterial pesticides: Bitoxybacillin (BTB), Thuringin-2 and Dipel against Mosquitoes – *Anopheles maculipennis* and *Culex pipiens molestus* in the laboratory conduction.

MATERIAL AND METHODS

Larval collection. The larvae of mosquitoes were collected from June to August 2017, in the village Chiauri on the border of Georgia – Azerbaijan, 08.00 to 11.00 AM by using standard dipper (350 ml) and eye dropper. After that mosquitoes were transferred into a closed container, sent to the laboratory and placed within a few cups into cages to obtain F1 generation (Silver, 2008). In urban areas, larvae were collected from barrels and open sewage reservoirs. Rural populations came from flood plains, ditches and ground pools in forests and meadows. The samples were mounted and identified by systematic keys (Shahgudian, 1960; Zaim & Cranston, 1986; Azari-Hamidian & Harbach, 2009). Mosquitoes *Anopheles maculipennis* and *Culex pipiens molestus* were counted and identified using standard identification keys of Harbach *et al*., 1985; Cranston *et al*., 1987; Harbach, 1988).

Entomopathogenic nematodes (EPNs) of the species *S. feltiae* were reared at 22 ± 2°C in last instars of the wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae), following the method of Kaya and Stock (1997). IJs were stored at 4°C in sterile distilled water (SDW) and were less 3 weeks old when used in the experiments. The nematode suspensions allowed to acclimatize at ambient room temperature for 24 h prior to exposure to mosquitoes. Quantification of nematodes in the suspension was done using the dilution method (Kaya and Stock, 1997).

For the control of *Anopheles maculipennis* and *Culex pipiens molestus* was used *S. feltiae* with concentration 5000 IJs/mL water (i.e. 100 per larva) and solution of bacterial insecticides BTB, Thuringin-2 and Dipel with dose 0.1, 0.2 and 0.5 separately.

I. Experiment – fifteen larvae of the 3rd–4th stages of both species of mosquitoes were placed individually on a wet filter paper in 10×10 cm diameter Petri dishes. Suspensions of 100 IJs/ per insect were treated in each Petri dish. II. Experiment – solutions of bacterial pesticides with dichlorinated water were placed in glass jars. In the jars the amount of liquid was 300 ml. Fifteen Mosquito larvae of both species were introduced into those solutions.

In the case of biomass control, the larvae were placed only in water. White bread was used as a nutrient (1.5 g per 15 larvae). Each treatment was replicated four times including the untreated control. The dead larvae of both species of mosquitoes were removed from Petri dishes and glass jars, washed with sterile water, put individually onto10 White traps and incubated at 25°C until the emergence of a new generation of IJs. The emerging IJs were harvested and counted after 11 to 15 days.

Experiments were carried out in the laboratory conditions at a temperature of 22 ± 2°C and 80% RH. The mortality percentage was recorded and corrected by means of the Abbott formula (Abbott, 1925). One-way ANOVA was used to compare the mortality of mosquitoes. Means were compared at the $P=0.05$ level, and Tukey's test was used to separate means (SPSS, 1999). Arcsine transformation was carried out on mortality (%) before analyses.

RESULTS AND DISCUSSION

The estimate of insect's mortality was recorded on days 3, 4, 5 and 6 after application of pathogens.

Within 4-day exposure to the dose 100/IJs per larvae of *Anopheles maculipennis* mortality was not observed, larvae were infected on days 5 and 6, the mortality rate was 15.6 and 20.5% (Table 1). There was no significant difference between the 5th and 6th days $P < 0.05$. Larval mortality % of *Culex pipiens molestus* ranged from 55.2 to 100.

Table 1

Larval mortality % of *Anopheles maculipennis* after exposure to *S. feltiae* and Bacterial insecticides Bitoxybacillin (BTB), Thuringin-2 and Dipel

Anopheles maculipennis					
Pathogens	Concentration	Mortality %			
		Day 3	Day 4	Day 5	Day 6
S. feltiae	5000 IJs mL/water	–	–	15.6	20.5
	Biological pesticides %				
BTB	0.1	0	30.3	60.2	
	0.2	17.2	44.5	68.5	
	0.5	30.2	76.5	100	
Turingin-2	0.1	0	18	37.1	
	0.2	11.5	33	66	
	0.5	22.8	74.3	95.3	
Dipel	0.1	0	10	35.5	
	0.2	0	19.2	38.3	
	0.5	10.2	32.3	55.2	
Control	–	–	–	–	–

Using bacterial insecticides BTB, Thuringin-2 and Dipel at a concentration of 0.1%, the first 2 days larvae mortality was also not recorded, although on day 3 larvae mortality rate reached 17.2–30.2 (BTB), 11.5–22.8 (Thuringin-*2*) and 10.2% (Dipel) caused at 0.2 and 0.5% concentration of bacterial insecticide. However, efficacy of 0.1 and 0.2% concentration of Dipel was not noted.

On the following 4th and 5th days under applying higher concentrations (0.5%) of bacterial insecticides BTB, Thuringin-*2* and Dipel mortality rates reached 76.5–100%, 74.3-95.3% and 32.3–55.2%, respectively. The result shows that insecticidal activity of *BTB* of 0.5% concentration against larvae of *Anopheles maculipennis* is much more effective (76.5–100% mortality) than of Thuringin-2 and Dipel. There was no significant difference on days 4, 5 between BTB and Turingin-*2* at high concentration (0.5%). A significant difference was observed between BTB and Dipel (76.5–100% and 32.3–55.2%). All bacterial insecticides produced significantly more mortality than the control (Table 1).

The data of activities of *S. feltiae* suspension (100/IJs per larvae) and bacterial insecticides BTB, Thuringin-2 and Dipel against *Culex pipiens molestus* is presented in Table 2.

Table 2

Larval mortality % of *Culex pipiens molestus* after exposure to *S. feltiae* and bacterial insecticides Bitoxybacillin (BTB), Thuringin-2 and Dipel

Culex pipiens molestus					
Pathogens	Concentration	Mortality %			
		Day 3	Day 4	Day 5	Day 6
S. feltiae	5000 IJs/mL water	–	–	13.4	18.5
	Biological pesticides %				
BTB	0.1	0	33	59.2	
	0.2	18.5	39.2	69.3	
	0.5	35.2	81.1	95.5	
Thuringin-2	0.1	0	22.3	56.2	
	0.2	0	40.1	63.4	
	0.5	19.8	72.2	80.5	
Dipel	0.1	0	21.0	32.2	
	0.2	5.5	44.5	37	
	0.5	8.2	34.5	51.2	
Control	–	–	–	–	–

In both experiments the same concentrations of nematode suspension and bacterial insecticides were used. Larvae of *Culex pipiens molestus* were less sensitive against toxin of nematode suspension; the larval mortality reduced about 10.3–10.0% compared with larvae of *Anopheles maculipennis*, while activities of bacterial insecticides at higher concentration (0.5%) against the both insects were almost equal, 81.1–95.5, 72.2–80.5; 34.5–51.2. There was no significant difference on days 4, 5 between BTB and Turingin-*2* at high concentration (0.5%). A significant difference was observed between *BTB* and *Dipel* (81.1–95.5; 34.5–51.2). However, BTB, Turingin-2 and Dipel at high concentration (0.5) produced significantly more mortality than in control (Table 1, 2). It is clear that with the increase of concentration of bacterial insecticides, the mortality of insects increases significantly. Larvae of both species are more susceptible to BTB, than to Thuringin-2 and Dipel. When testing high concentration (0.5%) of bacterial insecticides, larval mortality on the day 4 reached 81.1%, 72% and 34.5%.

On the next 5th day with the higher dose of Dipel and Thuringin-2, mortality rate of insects increased as well, although it did not reach 100%. Larval mortality % of *Culex pipiens molestus* ranged from 51.2 to 95.5.

The maximum mortality (100%) of mosquito larvae was observed only when exposed to BTB. In control experiments all survived larvae developed up to the pupa; control pupas were stored in separate cells, where flight of normal imago was observed.

CONCLUSIONS

The action of entomopathogenic nematode *S. feltiae* and bacterial pesticides BTB, Dipel and Thuringin-2 has been studied against mosquitoes *Anopheles maculipennis* and *Culex pipiens molestus*. It can be concluded that a suspension of *S. feltiae* with a dose 100 IJs /per larvae showed a slight activity (36–40%) toward the larvae of the indicated mosquito species compared with bacterial pesticides where the mortality ranged from 51.2–100%. More effective was the action of bacterial pesticides BTB, Thuringin-2 and Dipel, the mosquito's mortality grew with the increase of concentration. The mosquito's larvae were most sensitive to the highest (0.5) concentration of bacterial pesticide BTB.

Our results indicate that generally biological agents are regarded as one of the best means for biological control and successfully used against different species of mosquitoes.

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