Indian Journal of Applied Microbiology
ISSN (Online): 2454-289X, ISSN (Print): 2249-8400

Volume 21 Number 2
July - September 2018, pp. 29-38

Larvicidal Potential of Mycosynthesized Silver Nanoparticles against *Culex quinquefasciatus*

Poornima.M*, Jeevan.P and Prabha.T

PG and Research Department of Microbiology
J.J.College of Arts and Science (Autonomous) – Pudukkottai

Abstract: Understanding of biological processes at the nanoscale level is a strong driving force behind development of nanotechnology. Silver nanoaparticles have important applications in the field of biology. Stable silver nanoparticles were synthesized by biological reduction method. The objective of the present study was to evaluate the larvicidal activity of silver nanoparticles synthesized by a fungus named *Aspergillus flavus* against the larvae of *Culex quinquefasciatus* mosquito reared in stagnant water. The parasite larvae were exposed to varying concentrations of synthesized silver nanoparticles for 24 h as per WHO protocols. Distilled water served as control. Hundred percentage mortality was recorded. The synthesized nanoparticles exhibited significant larvicidal activity.

Keywords: Silver nanoparticles, larvicidal, *Culex quinquefasciatus*.

Introduction

Medical importance of mosquitoes vector for the transmission of serious diseases that cause morbidity, mortality, economical loss, and social disruption [1]. *Culex quinquefasciatus*, the primary carrier for nematode parasites that cause filariasis and are widespread over large areas of the tropic and subtropics. Reducing the incidence of this disease is by mosquito control, which is frequently dependent on applications of conventional synthetic insecticides [3]. Chemical measures in public health programs were initially considered likely to decrease mosquito populations, but these have failed because the constant use of chemical insecticides has often led to disruption of natural biological control system and outbreaks of insect species. Moreover, problems created by using synthetic insecticide include the development of mosquito resistance,

*Author for Correspondence. E-mail: poornimageetha89@gmail.com

environmental pollutions and undesirable effects of humans, mammals and other non-target organisms [2]. Currently, fungi are being utilized in nanotechnology for the production of nanoparticles. Synthesis using fungi has shown that this environmentally benign and renewable source can be used as an effective reducing agent for synthesis of silver nanoparticles (AgNPs). This biological reduction of metal could be utilized for a clean, nontoxic, and environmentally acceptable "green" approach to producing metal nanoparticles. It is well known that some microbes such as bacteria, yeast and fungi are potentially useful in the preparation of metal nanoparticles under normal air pressure and at room temperature. Many species of fungi have been used in nanotechnology for nanoparticle production, including *Fusarium oxysporum*, *Aspergillus fumigatus*, *Verticillium* spp., and *Chrysosporium tropicum*. The AgNPs formed are highly stable and have significant mosquito larvicidal activity. Hence, in the present study described the synthesis of fungal (*Aspergillus flavus*) nanoparticles for larvicidal and mode of action were studied.

Nanoparticles, generally considered as particles with a size of upto 100 mm, exhibit completely new or improved properties as compared to the larger particles of the bulk material that they are composed of based on specific characteristics such as size, distribution, and morphology. Nanoparticles have attracted considerable attention owing to their various applications. The silver nanoparticles are reported to possess anti-bacterial [10], anti-viral [8]. Anti-fungal activity[6]. Synthesis of nanoparticles using plants or microorganisms can potentially eliminate this problem by making the nanoparticles more bio-compatible. Indeed, over the past several years, plants, algae, fungi, bacteria, and viruses have been used for low-cost, energy-efficient, and nontoxic production of metallic nanoparticles [12]. Hence, in this present study made to attempt on laboratory evaluation of mycosynthesized AgNPs were tested against larvae of filarial vector, *Cx. quinquefasciatus*.

Materials and Methods

Production of mass culture

The fungus, *Aspergillus flavus* was isolated from soil samples of Sirumalai Hills, Dindigul district, Tamilnadu. Inoculum was prepared by using the one loopful of culture suspension in Potato Dextrose medium incubated at 28°C for 24 h in the environmental shaker at 120 rpm in dark condition. To prepare mass culture for AgNP biosynthesis studies, the fungi, *Aspergillus flavus* was grown aerobically in a Potato Dextrose medium media containing 0.5 % Peptone, 0.3 % beef extract, 1.5 % agar, 0.5% NaCl. The final pH was adjusted to 6.5±0.2. The flasks were inoculated, incubated on orbital shaker at 29°C and agitated at 120 rpm. After 48 h of incubation, the cell mass was separated by whatman No. 1 paper for filtration and to remove any medium component from the cell mass.

Synthesis of silver nanoparticles

Culture filtrate (wet weight) (10 g) was brought into contact with 1mM of 100ml silver nitrate solution. The flasks were incubated in the incubator shaker at 28°C with shaking speed of 120

rpm in dark condition. Silver nanoparticles were gradually obtained during the incubation period. Simultaneously, controls with cell biomass of fungal filtrate with Milli-Q deionized water and with silver nitrate solution were maintained under same conditions, separately.

Characterization of silver nanoparticles

UV-visible spectrophotometer analysis

After observing colour change, the sample was subjected to mild sonication for 10 minutes. The sample was subjected to scan (300 nm-600 nm) using Thermo-Biomate 3 UV-visible spectrophotometer. Distilled water was taken to adjust the baseline.

FTIR analysis

FTIR spectroscopic studies were carried out to find possible bio-reducing agent present in the fungal culture. The wavelength spectrum of the cell free extracts before and after the addition of AgNo3, the spectra were recorded using Perkin Elmer make model spectrum RX1 (wavelength range between 4000cm−1 and 400cm−1).

SEM analysis

The images of nanoparticles were obtained in the scanning electron microscope (JOEL, Japan, Model- 6360, in Department of Geology, UOM, Guindy). The details regarding applied voltage, magnification used and the size of the content of the images were implanted on the photographs itself.

Morphogenetic variation, behavioral changes and Mode of action

In continuation of bioassays done in respect of above experiments, larval deformities and inhibition of adult emergence, changes in morphological features and other behavioral aspects were also recorded. The mode of action of fungal toxin has only been studied in mosquito larvae. After introducing the larvae with required concentration of fungal nanoparticle the quick entry happens in the cuticle and anterior stomach. We noted 12 hrs. Observation of action of nanoparticles in the mosquito larvae of 4th instar of *Cx. quinquefasciatus.* The morphological variations were observed under Confocal Laser Scanning Microscope (Zeiss Model: LSM 700).

Statistical analysis

Probit analysis 16 was used for determination of LC_{50} andLC_{90} data from mortality and effect of concentrations were subjected to analysis of variance. Difference between the treatments was determined by Tukey's multiple range test ($P < 0.05$). The DMRT test was carried out two different variance of the sample.

Results

Formation of the silver nanoparticles exhibit reddish brown in water [9]. Figure. 1 .shows the color changes before (a) and after (b) the process of reduction of Ag+ to Ag nanoparticles. After

the conversion process silver nanoparticles in solution. These colors arise due to excitation of surface Plasmon vibrations in the silver metal nanoparticles [5].

Characterization of AgNPs

UV-Visible spectrophotometer

The Surface plasmon resonance (SPR) of the AgNPs produces a peak at 430nm which indicates the dispersion of silver nanoparticles (figure 2). The another peak was observed at 220 nm but commonly Silver nanoparticle formation is confirmed between the 400 – 450 nm.

FTIR interpretation

FTIR absorbance spectrum were observed at 3437cm-1, 2077cm-1, 1637cm-1, 1444cm-1, 1350cm-1 and 681 cm-1which indicates the functional group (Table.1) of the fungus component involves in the reduction and act as capping agent (Figure 3). FTIR results clearly indicate that water soluble compounds present in the fungal metabolites principally involved in the reduction and stabilization of AgNPs. Biosynthesis of silver nanoparticles using fungi currently under exploitation. The development of biologically inspired experimental process for the synthesis of silver nanoparticles is evolving into an important branch of nano technology.

SEM analysis

The SEM micrographs of nanoparticle obtained in the filtrate (Figure 4) showed that silver nanoparticles are spherical to roughly spherical shaped in the size range 2µm.

Figure 1: Extraction fungal nanoparticle (Left-Control, Right-Synthesized Silver nanoparticles)

Figure 2: UV-Visible spectra

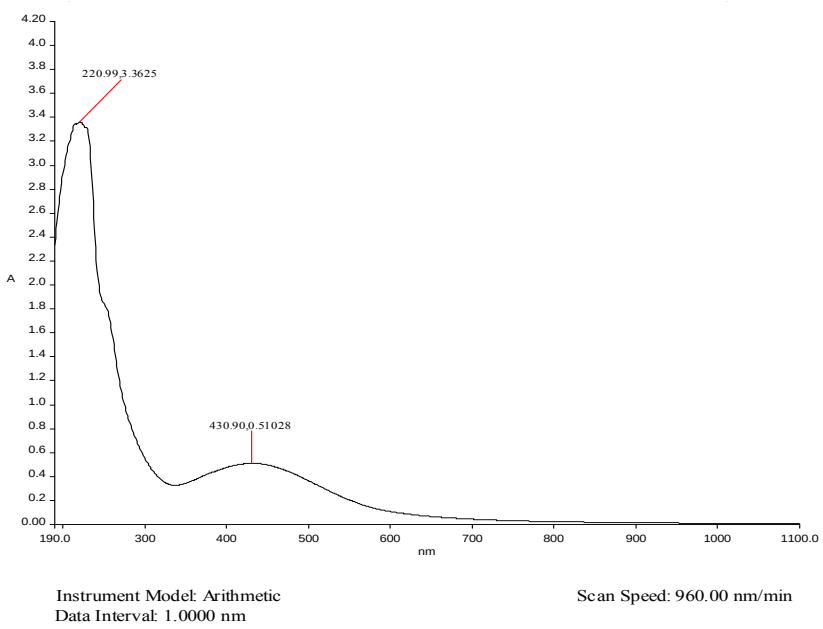


Figure 3: FT-IR analysis of biomolecules involved in reduction $AgNO_3$ into Ag ions using *Aspergillus flavus*

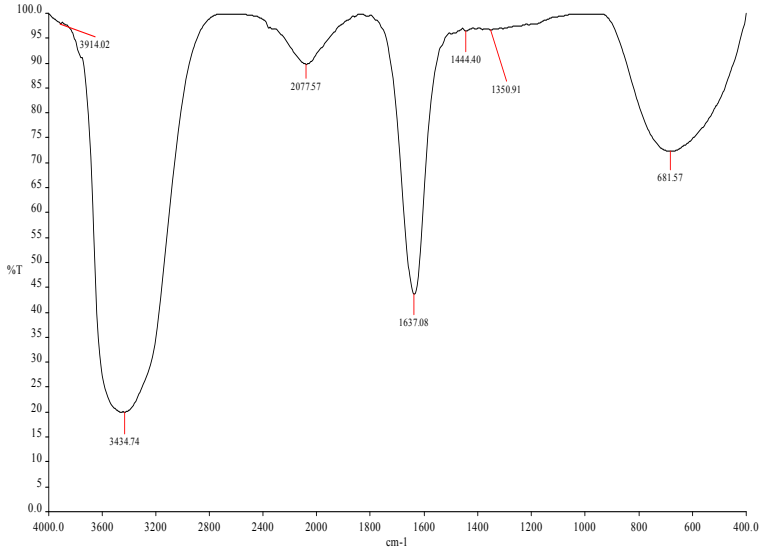


Figure 4: SEM image of nanoparticle.

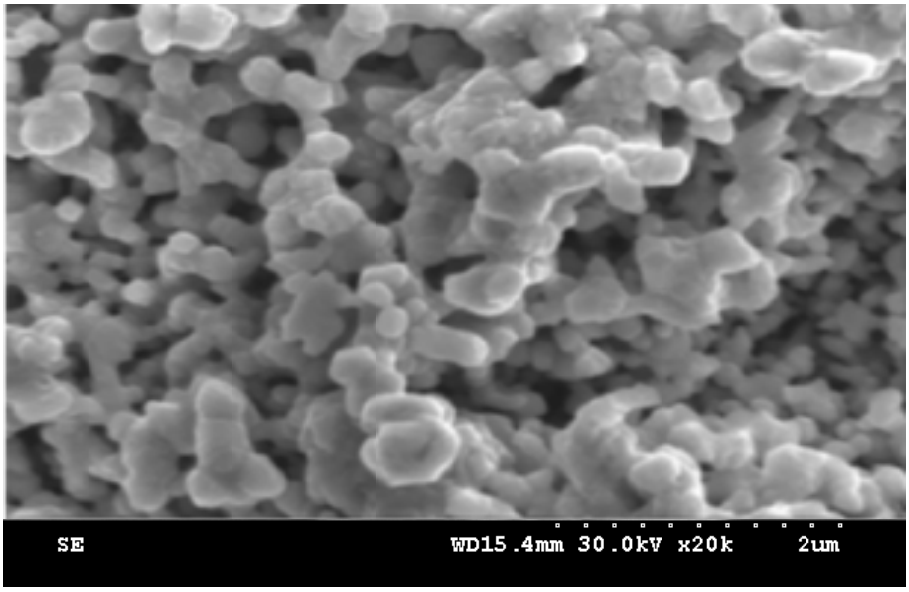


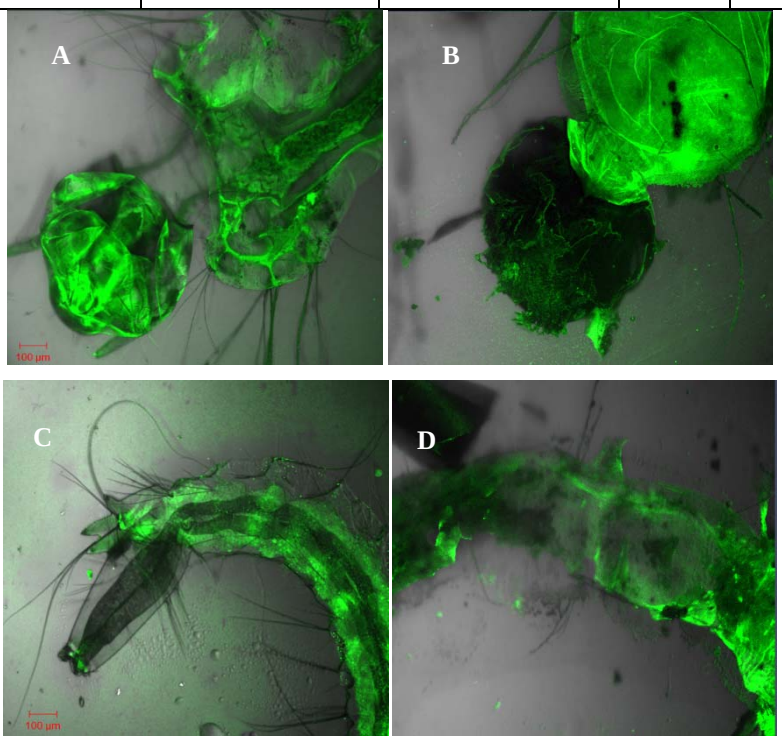
Table 1: Functional Groups analysis

Wave number (cm^{-1})	Bond	Functional group
3434.74	O-H stretch, H-bonded	Alcohols, Phenols
2077.57	-NCS	Isothiocyanate
1637.08	N-H bend	Primary amines
1444.40	C-C Stretch (in ring)	Aromatics
1350.91	C-H rock	Alkanes
681.57	C-Br Stretch	Alkyl halides

Table 2: Probit equations and susceptibility of *Cx. quinquefasciatus* against silver nanoparticles generated by *Aspergillus flavus*.

INSTAR	TIME (Hrs)	% VALUE OF LC_{50}	% VALUE OF LC_{90}	LCL	UCL
I	1	0.7758	4.7171	0.0001	1.6965
	8	0.6775	3.7744	0	1.5604
	16	0.4376	2.8904	0.044	4.3553
	24	0.6958	2.6962	0	1.5071

II	1	0.9338	6.9511	0	2.0117
	8	0.8019	4.9927	0.0002	1.7402
	16	0.6775	3.7744	0	1.5604
	24	0.6958	2.6962	0	0.5071
III	1	1.3588	8.2337	0.0066	2.4747
	8	1.0112	6.389	0.002	2.0173
	16	0.9969	4.8502	0.0264	1.848
	24	0.6775	3.7744	0	1.5604
IV	1	1.7092	10.643	0.0001	3.0246
	8	1.0149	7.6101	0	2.1317
	16	0.7187	6.1871	0	1.7873
	24	0.5177	4.1574	0.0664	4.0373



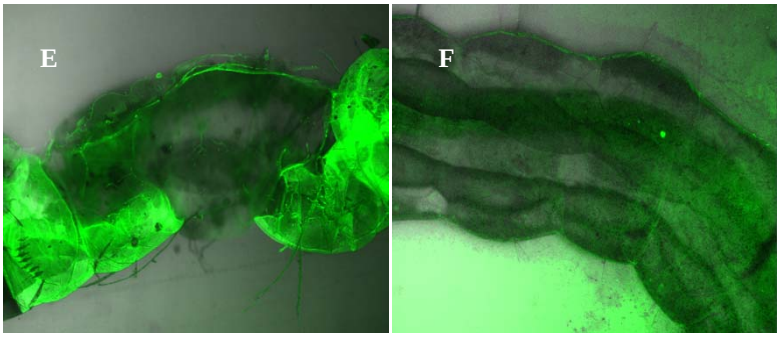


Figure 5: Confocal microscopy view (10X) of 4th Instar Larvae A) Untreated larvae B) Treated Head region C) Untreated Tail region D) Treated Tail region E) Untreated Anterior region and F) Treated Anterior region for epithelial layer damage for the treatment of *Aspergillus flavus* mediated silver nanoparticles for 24 hrs`2q1; EP-Epithelial layer.

Larvicidal activity

The table 2 provides the fungal synthesized nanoparticle (*A. flavus*) on various stages (I, II, III, IV and pupae) of *Cx. quinquefasciatus.* Considerable mortality was evident after the treatment of silver nano-particle for all larvae and pupae. Mortality was increased as concentration increased.

Morphological variation

After treatment with a lethal dosage (LC_{50}) of each fungal nanoparticles synthesis, the dead larvae were studied for morphological alterations under confocal microscopy. Morphological changes in body segments including the head, thorax, and abdomen, and other organs such as the eyes, antennae, mouth brushes, setae, saddle, and anal gills were observed, Photographed. For symptomatological observations on the larvae treated with the fungal nanoparticles. 4th instar larvae were still active immediately after exposure to LC_{50} of each nanoparticles concentration, and the feeding process and normal zigzag motion of these treated larvae were clearly seen. However, after 5 min of exposure, abnormal evidence of excitation, restlessness, and sluggishness was initially observed. Excitation and restlessness persisted for between 30-60 min, and other anomalous motions were seen such as a coiling movement. The treated larvae frequently sank down and floated up again quickly. During the period of 24 hrs, some larvae showed more toxic symptoms. Observations on the morphological alterations of treated 4th instar larvae revealed that the head, anterior region and tail region were damaged except anal papillae (gills), had a normal structural appearance. Under optical microscopes both treated and control larvae showed similarities in morphological architecture and cuticular sculpturing of the head, thorax, and abdomen segments, and other organs such as the eyes, antennae, mouth brushes, setae, saddle, siphon, and ventral brushes. A distinct difference, however, was the cuticle layer damage observed in the treated larvae. These results therefore indicated that the toxic effect of nanoparticles is predominantly on the cuticle layer, leading to their morphological deformation. In histological study, the nanoparticles treated 4th instar larval midgut was sectioned under microscopy. These

histology observations clearly indicate that the fungal toxic nanoparticles affect specifically on the cuticles epithelial layer of *Cx. quinquefasciatus.*

Similarly [7] investigated biosynthesis of silver nanoparticles using fungus *Trichoderma harzianum*. These silver nanoparticles were characterized by means of UV–Vis spectroscopy, transmission electron microscopy (TEM). UV–visible spectrum of the aqueous medium containing silver ion showed a peak at 420 nm corresponding to the Plasmon absorbance of silver nanoparticles.

The work of [4] indicated that the extracellular production of silver nanoparticles by the fungus *Aspergillus* species was investigated. It was found that exposure of *Aspergillus* species to silver ion leads to the formation of silver nanoparticles. The silver nanoparticles were in the range of 1-10 μm in dimension.

The larvicidal activities to determine the efficacies of synthesized silver nanoparticles using aqueous leaf extract of *V. rosea* against the larvae of malaria vector *An. stephensi* Liston and filariasis vector *Cx. quinquefasciatus* has been tested [11]. Their results showed that the maximum efficacy was observed in synthesized AgNPs against the fourth instar larvae of *An. stephensi* (LC_{50} 12.47 and 16.84 mg/mL and LC_{90} 36.33 and 68.62 mg/ mL) on 48 and 72 h of exposure and against *Cx. quinquefasciatus* (LC_{50} 43.80 mg/mL and LC_{90} 120.54 mg/mL) on 72-h exposure, and aqueous extract showed 100 % mortality against *An. stephensi* and *Cx. quinquefasciatus* (LC_{50} 78.62 and 55.21 mg/mL and LC_{90} 184.85 and 112.72 mg/ mL) on 72-h exposure at concentrations of 50 mg/mL, respectively. The AgNPs did not exhibit any noticeable toxicity on *Poecilia reticulata* after 24, 48, and 72 h of exposure. These results suggest that the synthesized AgNPs have the potential to be used as an ideal eco-friendly approach for the control of the *An. Stephensi* and *Cx. quinquefasciatus.* Here, the results showed that the efficacies after a long time of exposure. Whereas, in our study the synthesized NPs have shown the efficacies after short time of exposure.

Conclusion

The prospect of utilizing natural products for synthesizing silver nanoparticles and testing its efficacy in controlling mosquitoes as larvicides was a recent phenomenon facilitating the development of a more potent and environmentally safe pesticide. Identification of the bioactive principles involved and their mode of action and field trials are necessary to recommend an effective formulation as an anti-mosquito product in control programs**.** Thus the timing of treatments depends on the knowledge of the biology of targets as well as non-target species. We conclude that the application of fungal mediated SNP to kill larva, pupa of filarial vector could significantly reduce parasite transmission and therefore lead to reduced filarial risk.

Conflict of interest:

There is no conflict of interest.

References:

1. Becker N, Petric D, Zgomba M, Boase C, Dahl C, Lane J,Kaiser A, 2003. Mosquitoes and their control, *New York: Kluwer Academic/ Plenum Publishers*.
2. Lee SE, Kim JE, Lee HS, 2001. Insecticide resistance in increasing interest, *Agric Chem Biotechnol,* 44, 105-112.
3. Malavige GN, Fernando S, Fernando DJ, Seneviratne SL, 2004. Dengue viral infections, *Post grad Med J*, 80, 588-601.
4. Mohammadian, A., shojaosadati, SA., Habibi Rezaee, M., 2007. *Fusarium oxysporum* Mediates Photogeneration of silver Nanoparticles, *Sci Iran*, 14: 323-332.
5. Mulvaney P, 1996. Surface plasmon spectroscopy of nanosized metal particles, Surface Plasmon Spectroscopy of Nanosized Metal, *Langmuir*, 12, pp. 788- 800.
6. Panaceka A, Milan K, Renata V, Robert P, Jana S, Vladimır K, Petr H, Radek Z, Libor K, 2009. Antifungal activity of silver nanoparticles against Candida spp., *Biomaterials*, 30, 6333–6340.
7. Prashant Singh., and Balaji Raja, R., 2011. Biological Synthesis and Characterization of Silver Nanoparticles Using the Fungus *Trichoderma Harzianum, Asian J. Exp. Biol. Sci,* 2(4): 600-605.
8. Rogers JV, Parkinson CV, Choi YW, Speshock JL, Hussain SM, 2008. A preliminary assessment of silver nanoparticle inhibition of monkey pox virus plaque formation, *Nanoscale Res Lett*,3, 129–133.
9. Sastry M, Ahmad A, Khan IM, Kumar R, 2003. Biosynthesis of metal nanoparticles using fungi and actinomycete, *Curr Sci*, 85, 162-170.
10. Sathish kumar M, Sneha K, Won SW, Cho CW, Kim S, Yun YS, 2009. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bactericidal activity, *Colloids Surf B: Biointerfaces*, 73, 332–338.
11. Subarani, S., Sabhanayakam, S., Kamaraj, C. 2012. Studies on the impact of biosynthesized silver nanoparticles (AgNPs) in relation to malaria and filariasis vector control against *Anopheles stephensi* Liston and *Culex quinquefasciatus* Say (Diptera: Culicidae), *Parasitol Res*, 112(2), 487-499.
12. Thakkar KN, Mhatre SS, Parikh RY, 2010. Biological synthesis of metallic nanoparticles, *Nanomedicine,* 6, 257-262.
13. WHO, 1981. Instructions for determining susceptibility or resistance of mosquito larvae to insecticides, *WHO/VBC* 81, 80.