Short Communication

Vorticella sp: Prospective Mosquito Biocontrol Agent

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Abstract

Background: Considering the disadvantages of chemical insecticides, we aimed to evaluate *Vorticella* parasites for control of mosquito larvae of *Anopheles stephensi* and *Aedes aegypti* at different larval stages.

Methods: *Vorticella* sp infected mosquito larvae were crushed in the 0.85% saline and homogenized well to get *Vorticella* in suspension. The effects of *Vorticella* sp infections on larval development were investigated by inoculating protozoan on different larval instars of *An. stephensi* and *Ae. aegypti* and observed under light microscope. Lethal time of the *Vorticella* infected larvae at different stages was calculated.

Results: First and 2nd larval instars of both *An. stephensi* and *Ae. aegypti* did not show signs of infection by *Vorticella* sp., whereas 3rd instars of *An. stephensi* showed more *Vorticella* infection than those of *Ae. aegypti*. However, 4th larval instars of both mosquitoes were heavily infected with *Vorticella* parasite which was responsible for sluggish movements of larvae and eventually death. Moreover, parasites (*Vorticella* spp) were responsible for more than 90% reduction in adult emergence for both infected *An. stephensi* and *Ae. aegypti*.

Conclusion: This study provides insights for mosquito larvicidal action of surface parasite *Vorticella* on different larval stages of *An. stephensi* and *Ae. Aegypti*. It could be suggested as a potential candidate in mosquito biocontrol programs.

Keywords: *Anopheles stephensi*, *Aedes aegypti*, *Vorticella*, Biocontrol

Introduction

Pathogenic agents of diseases like malaria, dengue fever, yellow fever and filariasis are mainly transmitted by mosquitoes and therefore are responsible for human mortality and morbidity throughout the tropics (WHO 2004). As there is less development in drugs controlling pathogenic agents, mosquito vector control has been recognized as an effective tool for controlling the spread of these tropical diseases.

Several synthetic chemical insecticides such as organophosphate, temephos, etc. have been used during the past several decades for the mosquito control. However, their use has been greatly impeded due to development of insecticide resistant vectors (WHO 2013). Besides the use of chemical insecticides raised issues of environmental pollution which im-

part harmful effects on non-target animals (Rao et al. 1995). Due to such reasons, there has been an increasing interest in developing biopesticides as a useful substitute to chemical insecticides.

Bacillus thuringiensis subsp *israelensis* (*Bti*) has been extensively used due to its specificity and high toxicity to a variety of mosquito larvae. Although *Bti* based strategies represent an environmentally friendly approach to mosquito suppression with the potential for area-wide implementation, it is still lagging behind chemical insecticides, presumably due to its high cost and geometrical variation in *Bti* activity. In addition, the development of *Bti* resistance has made it difficult to control insect pests with *Bti* alone (Margot et al. 2011). Several insect control agents have been with-

drawn for economic or regulatory reasons resulting in greater selection pressure and more rapid resistance development to the remaining materials (Phillip et al. 2011). Thus, it is necessary to search new alternatives to develop promising biological control against mosquitoes.

Although various biocontrol measures are in vogue, their effective control is yet to be highlighted. In continuation of our previous studies (Chandrashekhar et al. 2011a, 2011b, Rahul et al. 2011, Suryawanshi et al. 2014, 2015) on searching of insecticidal agents for mosquito borne disease control, this study was conducted to isolate new insecticidal agents against mosquito larvae. Various samples of dead mosquito larvae were collected from different mosquito breeding sites. We found extracellular protozoan parasite associated with these larvae. The parasite was identified as *Vorticella*, a genus of peritrich protozoan. It is one of the very first protists seen by Antony van Leeuwenhoek who observed it through the lens of his simple microscope in the late seventeenth century. *Vorticella* has a bell shaped body with a cilia lined in oral cavity at one end and a long stalk on the other. *Vorticella* reproduces asexually by mitotic cell division (Noland and Finley 1931). They are sessile fresh water forms reported from different habitats like moist soil, mud and plant roots (Neumann and Martinoia 2002).

In this study we aimed for i) collection of the *Vorticella* sp in suspension form ii) collection of mosquito larvae from local breeding sites, iii) evaluation of *Vorticella* as a future biocontroling agent for mosquito larvae iv) calculation of lethal time of mosquito larvae for *Vorticella* suspension and v) to study the target larval selection by *Vorticella* sp.

Materials and Methods

Aedes aegypti and *An. stephensi* larvae (1st, 2nd, 3rd and 4th instar) were collected from nearby marshy places or stagnant ponds of Jalgaon area, at geographical coordinates of 21°2 54 N, 76°32 3 E, elevation 209 m and used as experimental mosquitoes. Key factor for primary identification was swimming behavior of Aedes and Anopheles species (e.g. *Anopheles* larvae rests parallel while *Aedes* larvae attach to water surface with their body pointing downwards). From such samples, few larvae (5–10 depending on population size) were taken randomly for identification purpose. Identification of mosquito larvae was done by observing body parts like antenna, cephalon, mesothorax, metathorax and abdominal segments (Du Bose and Curtin 1965, Ramachandra 1984, Subbarao et al. 1988, Amerasinghe 1990).

For maintenance, the larvae were kept in plastic trays containing dechlorinated tap water. The experiments were carried out at 28± 2 °C and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed with a diet of finely ground brewer's yeast and dog biscuits (3:1) (Chandrashekhar et al. 2011a, Suryawanshi et al. 2014).

Larvae were observed under microscope (Labomade LB341), heavily infected larvae were identified by presence of attached parasites all over the body surface, more populated at the junction of all segments and sluggish movements of infected larvae. Five heavily infected mosquito larvae were selected. The larvae were further crushed in the 0.85% saline (5 ml) and homogenized well to prepare *Vorticella* suspension. The presence of *Vorticella* in the suspension was confirmed by observation under microscope. This suspension (5 ml) was inoculated into glass trays each containing the test 300 mosquito larvae (each instars) of *An. stephensi* or *Ae. aegypti* in one liter tap water containing glass tray and observations were made after each 24 h.

Moreover, the effects of *Vorticella* sp infections on larval development were investigated by inoculating protozoan on the different larval instars of *An. stephensi* and *Ae. aegypti*.

603

Similar experiment was done for observing the preference of mosquito species by *Vorticella* by infecting *Vorticella* suspension to the mixture of both *An. stephensi* and *Ae. aegypti* larvae at their 4th larval instar.

Mortality was Corrected using Abbott's formula (Abbott 1925). The dose–response data was subjected to probit regression analysis (Finney 1971). Lethal time 50 (LT_{50}) (the time at which 50% larvae died) of the larvae at different stages was calculated by probit analysis.

Results

After 24 h, around five to ten *Vorticella* sp stalk bodies could be observed on first segment and 20–25 stalk bodies on last anal segment of only 4th instar larvae of *An. stephensi*. Only a few stalked bodies of parasites were observed on 3rd instar larvae. While no any infection was observed on 1st and 2nd larval instars of *An. stephensi*. Similarly, *Ae. aegypti* larvae were not infected by protozoan up to 48 h and only few stalk bodies were observed on the first segment of 4th instars after 50 h after which infection was found to increase during the progression of life cycle. In contrast, the whole body surfaces of *An. stephensi* larvae were infected by the long stalked *Vorticella* sp up to 48 h (Fig. 1). *Anopheles stephensi* larvae did not showed abnormal changes in motility behavior for the first 48 h. However, the larvae became sluggish and then immotile after 48 h. During this stage, larvae showed movement of only upper mouthpart and siphon. When mixed lar-val populations of *Anopheles* and *Aedes* were inoculated with *Vorticella* the parasites preferred only *Anopheles* larvae for infections. However, they heavily infect the *Aedes* larvae after the death of *Anopheles* larvae.

The effects of *Vorticella* sp infections on larval development were investigated by inoculating the parasite into different larval instars of *An. stephensi* and *Ae. aegypti*. The 50% of 3rd and 4th instars population of *An. stephensi* larvae were killed after 72 h and 59 h, respectively (LT_{50}). The 1st and 2nd instars larvae of both the mosquito species did not show any mortality up to72 h. The 3rd instar of *Ae. aegypti* showed the infection but did not cause mortality. However, 4th instar larvae showed 50% mortality after 84 h. Only first segment of larvae was infected by parasites (Table 1).

In adult immergence assay, out of the total 300 larvae, 244 larvae of *Ae. aegypti* died within 84 h, 56 went to pupal stage and 23 adult mosquitoes were emerged. Upto 255 out of 300 *An. stephensi* larvae were killed, and 43 developed to pupal stage and 17 emerged to adult mosquitoes. The emergence rate of control *An. stephensi* and *Ae. aegypti* without *Vorticella* treatment was 73% and 76.1%, respectively (Table 2). Only 18.66% and 10% larvae of *An. stephensi* and *Ae. aegypti* attained pupal stages, respectively and died thereafter. Only 5.86% and 7.43% emerged as adult mosquitoes (Table 2). However, the emerged mosquitoes died within 4–5 days after their transfer and culture on the artificial diet.

Table 1. Effect of *Vorticella* sp infection on mortality of mosquito larvae

Mosquito species	Larval instars stage	Infection	LT_{50} (Hours)
Aedes aegypti	I	Nil	--
	II	Nil	--
	III	D	--
	IV	++	82.3±8.6

Table 1. Continued…

Anopheles stephensi	I	Nil	--
	II	Nil	--
	III	+++	72±7
	IV	++++	59±8.5

LT_{50}= the time at which 50% larvae died
D= detectable
++= infected only first segment of larvae and very few *Vorticella* parasites were observed
+++= *Vorticella* parasites infected the whole larval body and many *Vorticella* parasites were observed
++++= very high infection throughout larval body and larvae became sluggish

Table 2. Effect of *Vorticella* sp infection on emergence of adult mosquitoes

Mosquito species	Measure	4th instars larval mortality (number/ 300)	Pupae emerged (number/300)	Adults emerged (number/300)
Aedes aegypti	Control[a]	39.3±4.04	260±15	228.3±12.6
	Test[b]	244.67±15	56.6±9	22.3±5
Anopheles stephensi	Control[a]	29.6±4.5	271.6±17.5	219.3±17.7
	Test[b]	255.7±10	43.6±6	17.6±5

[a]Control- larvae without *Vorticella* infection
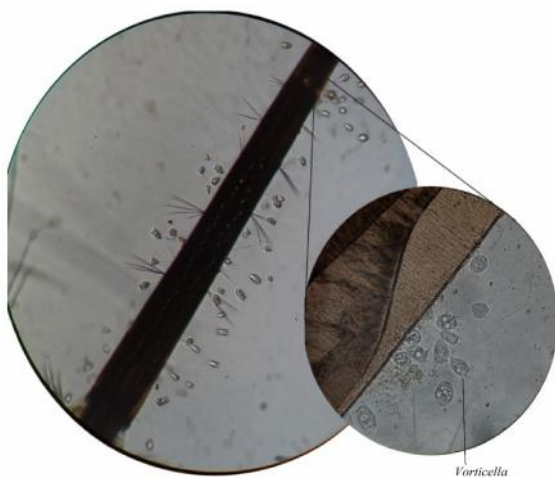[b]Test- larvae with *Vorticella* infection

Fig. 1. Infection of parasite (*Vorticella* spp) to the 4th larval instars of *Anopheles* mosquito (10X magnification)

Discussion

Vorticella spp. are largely known as bacterial feeders and have also been reported to parasitize nematodes with the help of their integument, in *Rana pipiens* and *Rana sylvatica* tadpoles it cause tegumentary impairment while in fresh water prawns *Acrobrachiumrosen bergii Vorticella* sp infect the eye stalk, antenna, uropod and egg masses (Tonguthai 1997). Ciliates and *Vorticella* sp have been found occasionally infecting mosquito larvae (Muspratt 1945, Micks 1950, 1955, Schober 1967). Vorticellids of *Vorticella* can cause infection in mosquito species at risk (Micks 1950).

In the present study, the effects of *Vorticella* sp on all larval instars of *An. stephensi*

605

and *Ae. aegypti* were investigated. The study on life cycle of the infected *An. stephensi* and *Ae. aegypti* larvae has revealed the inhibition of larval growth, development and adult emergence of both the test mosquito species. Micks (1950) reported *Vorticella* infection in *Anophele*s and death of 7000 infected larvae. However, the mechanism behind this inhibition is still unknown since more than 74 years of first report. The foot of the stalk attaches to a substrate, such as a rock, plant, or even an aquatic animal. The stalks of these organisms are capable of contracting at astounding speeds. The mechanism by which the adhesive pad of *V. convallaria* adheres to so many different substrates is also unknown. The organism secrets a some sort of "glue", probably a biopolymer. It may be possible that organism secretes some biochemical substances with these glue to fix itself on substrate and those substances may damaging some surface sensory system or causing pore formation in larval body. Either it may also be possible that the metabolic and secretary products of *Vorticella* are toxic to the mosquito larvae, polluting its natural environment. As per Schober (1967) this association is benefited by *Vorticella* as it may be using host as a transportation vehicle to spread in new environment, while as per Mick (1955) the larval death may be apparently due to the inability of the infected larvae to remain on the water surface, thereby interfering with respiration.

Previously it was observed that *Vorticella* infection was found only in *Anophele*s and it did not show either infection or mortalities in *Aedes aegypti*. In the present study, although *Vorticella* sp has first preference to *Anophele*s but it can attack other mosquito species like *Ae. aegypti*. The observations from this study can be important to design strategy to control of mosquito larvae. Further studies on non-target organisms and persistence in different environment will be needed for use of *Vorticella* sp as potential biocontrol agent in the mosquito larval habitat.

Conclusion

The *Vorticella* sp found in the present study showed potential of infection and mortality of *An. stephensi* and *Ae. aegypti* mosquito larvae. Being major aquatic living species, *Vorticella* sp alone can be valuable for devising easy, effective and eco-friendly strategy for mosquito biocontrol. This may be a new species and need to do the morphological study to be identified.

Acknowledgements

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