VOLUME: 1
ISSUE: 1
ISSN: xxxx-xxxx
Jul - Sep 2018

KAMARAJ JOURNAL OF ACADEMIC RESEARCH

An International, Online, Open Access, Peer Reviewed, Multi-disciplinary Journal

Subject:

MICROBIOLOGY

Title of Original Research Paper:

Mosquito Larvicidal Activity of Partially Purified Chitinase Enzyme against the Human Vector Aedes aegypti.

Author:

Dr. S. ABIRAMI

Assistant Professor, Kamaraj College, Thoothukudi – 628 003, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, Tamil Nadu, India.

ABSTRACT

Vector borne diseases are serious problems in tropical and subtropical regions. Mosquito borne diseases are diseases caused by viruses, bacteria and parasitic organisms transmitted by mosquito vectors. The indiscriminate use of chemically synthesized pesticides in controlling mosquitoes causes major environmental problems. Finding the better bipesticide compounds are challenging work in the world. The chitinolytic bacteria *Bacillus licheniformis* (SSCL10) was isolated from shrimp shell waste dump site soil environment. The partially purified chitinase enzyme was tested against *Aedes aegypti* mosquito larvae (III instar). Based on the mortality of *Aedes aegypti* mosquito larvae (III instar), it was confirmed that the larvicidal activity of chitinase enzyme. There was 100% of mortality recorded in 500µl/ml of partially purified chitinase enzyme within 14 hours. It is evident that the chitinolytic *Bacillus licheniformis*

(SSCL10) was potential for controlling mosquito borne diseases through controlling of mosquito larvae effectively for human welfare.

Key words: chitinase enzyme, *Aedes aegypti*, Vectors, mortality etc.

INTRODUCTION

The most common dreadful diseases associated with mosquitoes are malaria, yellow fever, filariasis, schistosomiasis, japanese encephalitis (Das and Ansari,2003) and the worst means, dengue hemorrhagic fever was caused by *Aedes aegypti* (Udonsi, 1986). Filariasis is carried by the mosquito *Culex quinquefasciatus*, which is a pantropical pest and urban vector of *Wuchereria bancrofti* (Samuel *et al.*, 2007). *Aedes aegypti* is a vector for transmitting several tropical fevers. Its native place is Africa but now it is found in tropical and subtropical regions. It occurs in insects as a major component of the cuticle and of the peritrophic membrane, a protective sleeve lining the gut of many insects (Kramer and Koga 1986).

The *Ae. aegypti* populations were controlled mostly based on the application of chemical insecticides. However, the overuse of compounds such as DDT, malathion, and temephos has led to the development of insecticide resistance (Ocampo *et al.*, 2011). Another issue to be considered is the negative ecological effect of these insecticides (Lin *et al.*, 2017). Hence, biological control can be a more specific and safer alternative in maintaining low levels of mosquitoes populations and as such reducing the incidence of vector-borne diseases (WHO,2009).

Several research works is going on to find the novel source for controlling the *Ae. aegypti* larvae. El-Khawagh *et al*. (2011) did research to control larva of *Culex sp* through culture filtrates of actinomycetes as the bioinsecticide source. Dhanasekaran *et al*. (2010) tried to control the *Anopheles* mosquitoes through culture filtrates of actinomycetes as the larvicidal activity. Janaki (2016) reported that mosquito larvicidal activity of *Streptomyces cacaoi* subsp. *cacaoi*-M20 culture filtrate against *Ae.aegypti* due to the isolate M20 was chitinase positive and it inhibits the growth and kills the mosquito larva effectively.

Chitinase is an enzyme used by insects to degrade the structural polysaccharide chitin during the molting process (Zhang *et al.*, 2002).The largest chitinase activity among bacteria has been observed in *Streptomycetes*, *Serratia*, *Vibrio* and *Bacillus*. Chitinase enzyme is very important in the biological control of insects (Reguera and Leschine, 2001). Chitin is a major component of the cuticle, the peritrophic membrane, and also functions as sleeve lining the gut of many insects. Hydrolysis of chitin to disaccharides and larger oligomeric saccharides usually takes place extra-cellular by the action of chitinases. In this paper deals with the chitinase enzyme produced by *B. licheniformis* and also showed excellent larvicidal activity against *Aedes aegypti* mosquito. There is a lack of published information with regard to the use of *Bacillus licheniformis* as biocontrol agents for mosquito larvae.

MATERIALS AND METHODS

Collection of Soil Sample:

The chitin extracted from shrimp shell wastes as detailed in the previous paper, was used in the experiments. All other chemicals used in the experiment were of analytical grade. *Bacillus licheniformis* (SSCL10), chitinolytic bacterial strain isolated from shrimp shell dumping soil was used for the studies.

Mass production and purification of chitinase enzyme:

The strain *Bacillus licheniformis* (SSCL10), selected due to its highest activity ratio of chitin hydrolysis, was studied for optimal conditions for enzyme production. The bacterial isolate *B. licheniformis* (SSCL10) strain was grown in 100 ml of minimal medium (0.1% KH_2PO_4; 0.05% $MgSO_4.7H_2O$; 50 mM sodium phosphate buffer, pH 7.0) and chitin (shrimp shell extracted chitin), as a major carbon source for chitinase production, was added to the liquid medium at 20 g/l. In addition, incubation temperature (37^oC) and pH 7 were maintained for enzyme production in a 250 ml Erlenmeyer flask. The culture was grown with agitation (180 rpm) and samples were taken every day (upto 7 days) for checking reducing sugar and chitinase activity (Mejia Saules *et al.*, 2006).

Partial Purification of Chitinase Enzyme by Dialysis:

The culture supernatant fluid was subjected to precipitation with ammonium sulphate to 80% saturation and stirred for 2 h. Then the precipitate was allowed to stand overnight and then collected by centrifugation at 10,000 rpm at 4^{o}C for 30 min. The precipitated pellets were dissolved in phosphate buffer, centrifuged and dialyzed against the same buffer to partially purify chitinase enzyme (Kavikarunya *et al.*, 2011).

Measurement of Enzyme Activity:

Chitinase activity was measured with colloidal chitin as the substrate. 0.5 ml of partially purified enzyme was added to 1.0 ml of reaction mixture with 1.5% colloidal chitin in phosphate buffer (50 mM, pH 7.0). The mixture was incubated at 37^{o}C for 15 minutes. After centrifugation, the amount of reducing sugars produced in the supernatant was determined by the Dinitrosalicylic acid method (Miller, 1972) using N-acetyl glucosamine as a reference compound (Imoto and Yagishita, 1971). One unit of chitinase activity was defined as the amount of the enzyme that produced 1 µmol of reducing sugar per minute (Mathur *et al.*, 2011).

***Aedes aegypti* larvae maintainance:**

The mosquitocidal activity was tested with larvae of *Aedes aegypti* mosquito in the laboratory. Egg cards of *Aedes aegypti* were collected from ICMR Institute in Madurai, Tamilnadu, India and kept under controlled conditions at temperature of 27 ± 2°C.

The larvae of *Aedes aegypti* were collected in a plastic bucket that had fresh water without chlorine, kept outside the window of laboratory for one week without disturbing it. Larvae of *Aedes* were collected examined under the microscope for its morphological features and they were concluded to belong to larvae of *Aedes aegypti*.

Bioassay of crude chitinase enzyme against the larvae of *Aedes aegypti:*

Five sterile cups containing each with 50 ml of sterilized tap water without any chlorine were taken and different concentrations of chitinase enzyme of 25µl, 75µl, 125µl, 250µl and 500µl was added and 10 larvae of *Aedes aegypti* were transferred to each cup. Cups were kept

under controlled conditions. The number dead larvae were counted and % mortality was calculated after 24hours (Sundarapandian *et al*., 2002, El-Khawagh, *et al*., 2011). Distilled water was used as negative controls. Each experiment was performed in duplicate. The larvae were observed daily until pupation. The following formula was used

Larval mortality % = Y / X × 100

where X = number of tested larvae

and Y = number of dead larvae

Larvicidal bioassay of *B. licheniformis* against the larvae of *Aedes aegypti:*

The larvicidal bioassay was done by slightly modified method of El-Khawagh *et al*. (2011). Seven sterile cups containing each with 50 ml of sterilized tap water without any chlorine were taken. Dilutions of *B. licheniformis* cultures ranging from (1to 5 ml/g)OD value of 600 were well mixed with the melted diet at about 50^0C and applied into separate cups .10 larvae of *Aedes aegypti* were transferred to each cup and a plastic cover was used to confine the larvae. Cups were kept under controlled conditions. The number live larvae were counted and % mortality was calculated after 24hours .Distilled water was used as negative controls. Each experiment was performed in duplicate. The larvae were observed daily until pupation. The following formula was used.

Larval mortality % = Y / X × 100

where X = number of tested larvae

and Y = number of dead larvae

RESULTS

The ability of extracellular chitinase enzyme production by the bacterial isolate *Bacillus licheniformis* (SSCL10) was carried out by the minimal medium with shrimp shell extracted chitin as a substrate. The first day showed negligible amount of chitinase production (0.2U/ml). After that, gradual enhancement of chitinase enzyme production was observed (Fig.1). The 2nd day of fermentation showed 1.5 U/ml, followed by 2.1 U/ml on the 3rd day, 2.8U/ml on the 5th day and 2.2 U/ml on the 6th day respectively. The 4th day produced the maximum amount of chitinase enzyme of 3.1 U/ml. After that, chitinase enzyme production slightly decreased to 1.8 U/ml on the 7th day.

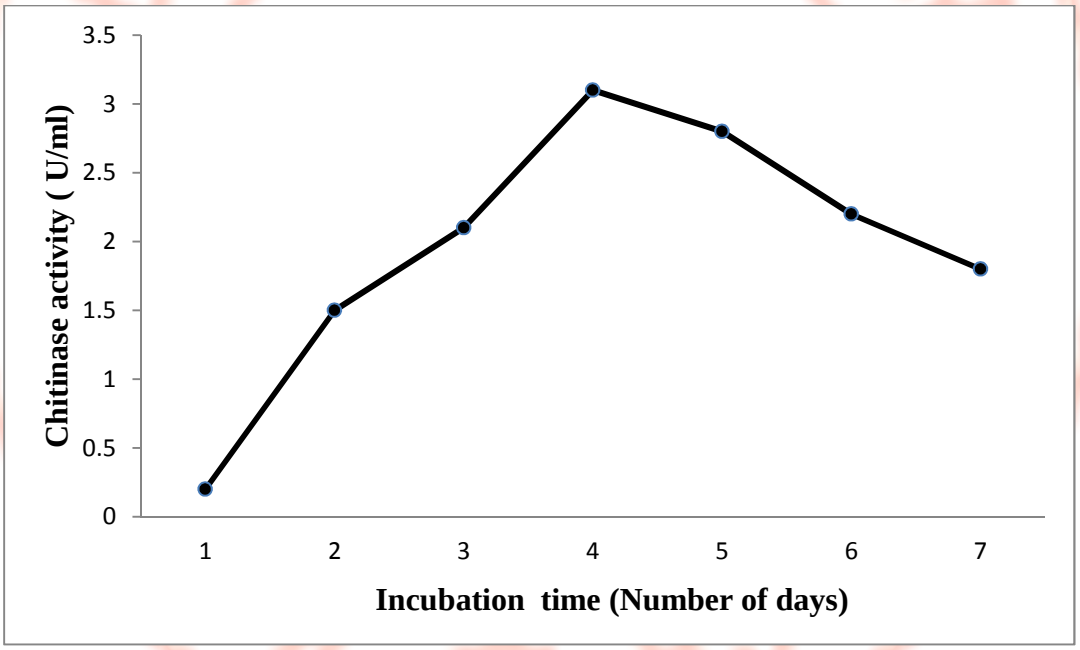

Fig. 1: Production of chitinase enzyme by *Bacillus licheniformis* (SSCL10) containing shrimp shell extracted chitin

After first day of incubation, the reducing sugar concentration had gradually decreased. The chitinolytic *Bacillus licheniformis* (SSCL10) had slowly degraded the shrimp shell extracted chitin for chitinase enzyme production. The 2nd day of fermentation showed 15.3% of reducing

sugar, followed by 14% on the 3rd day, 10% on the 4th day, 7.2% on the 5th day, 5.9% and 4% on the 6th day and 7th day of fermentation respectively (Fig.2).

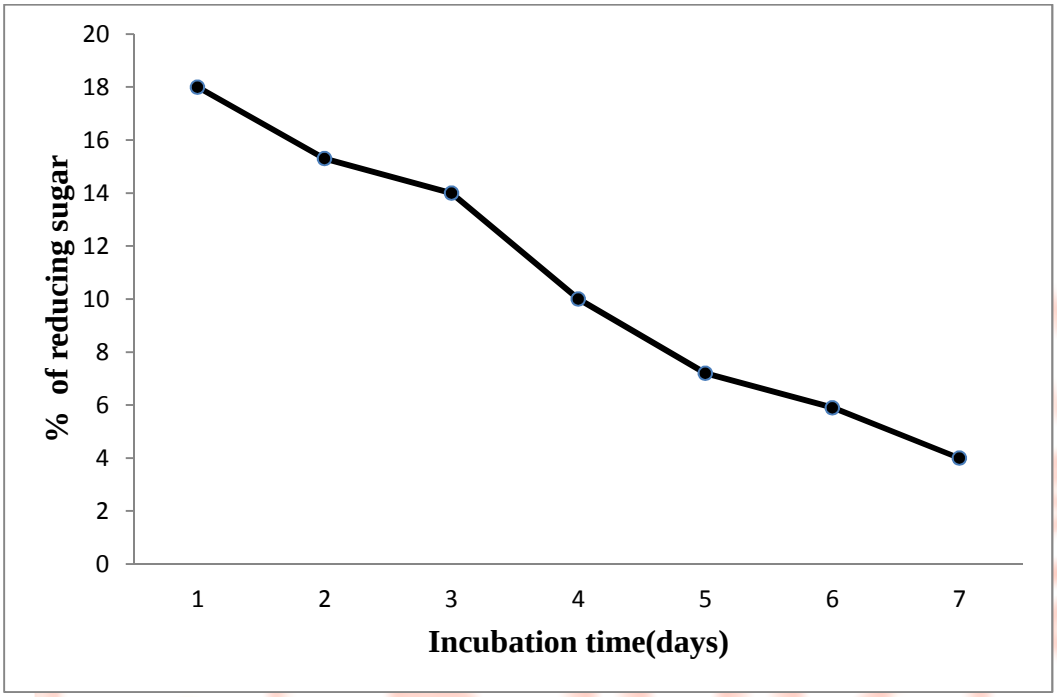

Fig.2 : Reducing sugar analysis on chitinase enzyme production by using *Bacillus licheniformis* (SSCL10)

The maximum chitinase production (3.1 U/ml) was observed on the 4th day with pH 7 and temperature of 37°C. The reducing sugar concentration had also gradually decreased after a day of incubation. The reducing sugar concentration was indirectly proportional to the chitin utilization. Most of the chitin had been utilized on the 7th day, which was indicated by the fact that reducing sugar concentration had greatly reduced (4%) because chitin was a substrate for chitinase enzyme production.

The toxicity of various concentrations of chitinase enzyme from culture supernatant of *B. licheniformis* was assayed toward *Aedes aegypti* larvae. It was found that 500 µ1 of chitinase enzyme exhibited the elimination of all larvae after exposure of 10 hours. No larvicidal activity occurred in cup treated with portions of 25µ1 of chitinase enzyme after exposure for 14 hours.

The activity of chitinase enzyme of the highest concentration was 2.5U/ml (500 µ1). Chitinase enzyme from *Bacillus licheniformis* showed greater larvicidal activity against 3rd instar larvae of the mosquito species *Aedes aegypti.* Table 1 shows larvicidal activity of chitinase enzyme of *B. licheniformis* against larvae of *Aedes aegypti* after exposure for 14 hours. It infers that chitinase produced by *B. licheniformis* might play a role in the activity of *Aedes aegypti* larvicidal. The mosquitoes have chitinous covering in their body as a protective layer and they need chitin for completing its metamorphosis. Without chitin, the larvae cannot form pupa.

Table 1

Larvicidal Activity of the Chitinase Enzyme of *B. Licheniformis*

against Larvae of *Aedes aegypti*

Concentration of chitinase enzyme	% of larval mortality of *Aedes aegypti* with after exposure of 14 hours.							
	2h	4h	6h	8h	10h	12	14	Control
25µl	0	0	0	0	0	0	0	0
75	0	0	0	0	20	50	50	0
125	0	20	40	60	60	70	80	0
250	0	40	60	80	90	100	100	0
500	0	50	70	90	100	100	100	0

The isolate *Bacillus licheniformis* SSCL10 was actively degrading the chitin of larval covering due to its chitinase activity. The *B. licheniformis* SSCL10 was larvicidal activity against the larvae of *Aedes* showed excellent larval mortality with dilution of 5ml/g *B. licheniformis* mixed with larval feed. But 1ml/g culture mixed feed showed very poor larval mortality, but 4ml/g showed moderate larval mortality (Table 2). Compare to chitinase enzyme, culture mixed feed showed less larvicidal activity.

Table 2

Larvicidal Activity of B. Licheniformis Culture against Larvae of Aedes aegypti

Dilution of *B. licheniformis* mixed with larval feed (ml/g)	control	% of larval mortality of *Aedes aegypti* (observation of 14 hours)						
		2h	4h	6h	8h	10h	12h	14h
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	20
3	0	0	0	0	0	20	20	30
4	0	0	0	30	60	70	80	90
5	0	10	30	50	70	100	100	100

Therefore, the use of this chitinase enzyme from *B. licheniformis* for larvicidal property offers a safer alternative method against synthetic chemical insecticides. Some polluted environment chitinase enzyme did not exhibit suitable larvicidal activity because catalytic activity of enzyme was very sensitive. Other methods were need to protect the enzyme for using larvicidal such as immobilization, lyophillisation etc. To avoid this problem, *B. licheniformis* culture directly sprayed on environment because it was a spore-forming, aerobic, entomopathogenic bacterium.

DISCUSSION

Insects of dipteran species such as *Culex* spp., *Anopheles* spp., and *Aedes* spp. are responsible for the transmission of many infectious disease agents. Mosquito control at the larval stage is an effective practice for controlling the mosquito born diseases (Nandita *et al.*,2009). Synthesis of chitin is very essential for insects to get full development and cuticle formation for their protection. Some of the insects act as vectors for spreading harmful diseases on environment where they are surviving, such type of insects can be controlled with the help of

chitin lysing enzyme secreted by other organisms that are not harmful. Janaki(2016) reported that the isolate M20-*Streptomyces cacaoi* subsp. *cacaoi* synthesis such enzyme extracellularly, the compound from the isolate M20 was subjected for mosquito larvicidal activity.

Chitin, an insoluble polymer of N-acetyl-D-glucosamine (GlcNAc), is an important component of insect exoskeletons, crustacean shells and fungal cell walls. Chitinases, and chitobiase, play important physiological and ecological roles in chitin metabolism (Flach *et al.*, 1992;Felse and Panda, 1999). Many microbial species produce chitinase and digest chitin as a carbon and energy source.

Enzyme technology is an interdisciplinary field and enzymes are routinely used in many environment friendly industrial sectors. Chitinase, a group of enzymes, is capable of degrading chitin directly to low molecular weight product (Yong *et al.,* 2005). Many bacteria and fungi produce extracellular chitinolytic enzymes, known as chitinases, able to convert chitin into compounds that can be of industrial interest, mainly N-acetyl-D-glucosamine. There is an increasing interest in the use of chitinases for the control of moulds, insects, nematodes and production of different chitin oligomers (Mejia Saules *et al*., 2006).

The present study revealed that the chitinase enzyme produced by *Bacillus licheniformis* (SSCL10) from shrimp shell chitin acts as an excellent enzyme inducer and carbon source. Krishnaveni and Ragunathan (2014) have reported that *Bionectria* CBNR BKRR is an efficient fungus which produces highly active chitinase when grown in statistically optimized medium containing crab shell, fish scales and shrimp shells as substrates. The great majority of these organisms are able to effectively breakdown chitin and use it as a source of carbon and energy and to synthesize chitinases during this process (Shubakov and Kucheryavykh, 2004). It is believed that chitinases are induced enzymes, which means that the expression of encoding genes is induced by a specific factor or factors. This expression is regulated by the repressor/inducer system. Zhong *et al.*(2015) reported that bacteria in particular can produce high levels of chitinolytic enzymes. Similarly, in the present work *Bacillus licheniformis* produced excellent amount of chitinase enzyme.

Poopathi and Abidha (2010) reported that the toxicity of various concentrations from culture supernatant of the studied strain was assayed toward *Culex pipiens* larvae. It was found that 500µlof culture supernatant cause 100% mortality after 3 h while 50µl did not cause mortality, where activity of the chitinase enzyme of the highest culture supernatant concentration was 3.5U/ml. In the present work, also exhibited that 500µlof culture supernatant cause 100% mortality after 10 h while 50µl did not cause mortality, where activity of the chitinase enzyme of the highest culture supernatant concentration was 2.5U/ml. Meanwhile, chitinase could potentially be used as a larvicidal agent III instar larvae of *Ae. Aegypti.* . The World Health Organization suggests that instar three is the proper stage to evaluate the larvicidal effect of a new substance (WHO ,2005).

Studies by Wiwat *et al.*(2000) had indicated that the toxicity of *B.thuringiensis* ssp. *kurstaki* HD-1(G) for diamondback moth larvae was increased when in combination with its supernatant. Expression of a chitinase gene, *chiAC*, from *Bacillus thuringiensis* in *B. sphaericus* 2297 using the binary toxin promoter yielded a recombinant strain that was 4,297-fold more toxic than strain 2297 against resistant *Culex quinquefasciatus*. These results show that this chitinase can synergize the toxicity of the binary toxin against mosquitoes and thus may be useful in managing mosquito resistance to *B. sphaericus***(**Cai *et al.*, 2007). Enzymatic cleavage occurs randomly at internal locations over the entire length of the chitin microfibril, leading to the impairment of the insect midgut (Kramer and Koga 1986; Kramer *et al.*,1997). Hypothetically, chitinase causes perforations in the gut peritrophic membrane, which facilitate entry of the pathogens into the haemocoel of susceptible insects (Brandt *et al.*, 1978). Charles and Nielsen-Le Roux (1996) reported that *Bacillus sphaericus* has been successfully used for mosquito control in the last decade because it was a gram-positive, spore-forming, aerobic, entomopathogenic bacterium. Its activity against target mosquito larvae is mainly due to the crystal toxin, commonly referred to as the binary toxin, as it consists of equimolar amounts of two proteins of 51 and 42kDa

In the present study, *Bacillus licheniformis* was used in the control of *Ae. Aegypti. Bacillus licheniformis* persistence and recycling is possible because of its ability to grow under polluted environments because of its spore forming ability. Same work supported that *L.*

sphaericus was a bacterium widely used in the control of *Ae. aegypti* and other culicids (Savini and Fazii 2016). Meanwhile, the vegetative cells of *L. sphaericus* can persist and recycle in the environment and its persistence and recycling is possible because of its ability to grow under polluted environments and UV light resistance. Likewise, bacteria go through a cycle where vegetative cells germinate from a dormant spore. Other advantages of *L. sphaericus* include humans and reductions of pesticide residues in the environment. Likewise, further studies have developed an easy way of industrial production of *Bacillus licheniformis* which is a concern when a formulation is accepted for a large treatment. A number of reports are available on the cloning of chitinases either to increase biocontrol efficiency of *Bacillus thuringiensis* to prepare highly active chitinase preparation or to produce transgenic plants for increased resistance against insects (Dahiya *et al.*, 2006).

CONCLUSION

These are the some of the reasons for isolating the novel chitinolytic bacteria from the shrimp shell waste dumpsite soil environment and its chitinase enzyme to control the vector borne diseases with the help of their bioinsecticide properties. Chitinase could enhance chitinolytic process in the presence of mosquito larvae and might be used to improve the mosquito larvicidal activity of *B. licheniformis.*

REFERENCES

1. Cai Y., J. YanXiaomin Hu, B. Han and Z. Yuan 2007. Improving the insecticidal activity against resistant *Culex quA. Appl. Environmental microbial.*, **73(23):** 7744–7746.
2. Charles J. F and C. Nielsen-LeRoux 1996. BACILLUS SPHAERICUS TOXINS: Molecular Biology and Mode of Action. *Anna. Rev. Entornol.*, **41:** 451-72.
3. Dahiya N., R.Tewari and G.S Hoondal 2006. Biotechnological Aspects of Chitinolytic Enzymes: A Review. *Appl. Microbio. Biotechnol.*, **71**:773-782
4. Das M.K and M.A. Ansari 2003. Evaluation of repellent action of *Cymbopogan martinii* martinii Stapf var sofia oil against *Anopheles* sundaicus in tribal villages of Car Nicobar Island, Andaman and Nicobar Islands. *India. J. Vector Borne Dis.*, **40:**100-104.

5. Dhanasekaran D., V. Sakthi, N. Thajuddin, N. Panneerselvam, 2010. A Preliminary evaluation of anopheles mosquito larvicidal efficacy of mangrove actinobacteria. *Int. J. Appl.Biol. Pharmaceut. Technol.*, **1(2):** 374-381.
6. El-Khawagh M.A., K.S Hamadah and T.M El-Sheikh 2011. The insecticidal activity of actinomycete metabolites, against the mosquitoe *Culex pipiens*. Egypt. *Acad. J biolog. Sci.*, **4(1):**103-113.
7. Felse P.A and T. Panda 2000a. Submerged culture production of chitinase by *Trichoderma harzianum* in stirred tank bioreactors: The influence of agitator speed. *Biochem. Eng. J.*, **4:** 115-120.
8. Flach J., P.E Pilet and P. Jolles 1992. What's new in chitinase research? *Experientia.*, **48(8):** 701-716.
9. Imoto T and K. Yagishita 1971. A simple activity measurement by lysozyme. *Agric. Biological. Chem.*, **35:** 1154-1156.
10. Janaki T. 2016. Larvicidal activity of *Streptomyces cacaoi* subsp. *cacaoi*-M20 against *Aedes aegypti*. *Int. J. Botany Stud.*, **1(2):** 47-49.
11. KaviKarunya S., D. Reetha, P. Saranraj and D. John Milton 2011. Optimization and purification of chitinase produced by *Bacillus subtilis* and its antifungal activity against plant pathogens. *Int. J. Pharmaceut. Biol. Arch.*, **2(6):** 1680-1685.
12. Kramer K.J. and D. Koga 1986. Insect chitin: physical state, synthesis, degradation and metabolic regulation. *Insect Biochem.*, **16:** 851–877.
13. Kramer K.J., S. Muthukrishnan, L. Johnson and F. White 1997. Chitinases for insect control. In Advances in Insect Control: the Role of Transgenic Plants ed. Carozzi, N. and Koziel, M. pp. 185–193.
14. Krishnaveni B and R. Ragunathan 2014. Chitinase production from seafood wastes by plant pathogen *Bionectria* CBNR BKRR *sps.* and its application in bioremediation studies. *Int. Res. J. Med. Sci.*, **2(11):** 15-19.
15. Lin Y., W. Tsen, N. Tien and Y. Luo 2013. Biochemical and molecular analyses to determine pyrethroid resistance in *Aedes aegypti*. *Pestic. Biochem. Phys.*, **107:** 266–76.
16. Mathur A., A. Rawat, G. Bhatt, S. Baweja, F. Ahmad, A. Grover, K. Madhav, M. Dhand, D. Mathur, S.K Verma, S.K Singh and V.K Dua 2011. Isolation of *Bacillus*

producing chitinases from soil: Production and purification of chito-oligosaccharides from chitin extracted from fresh water crustaceans and antimicrobial activity of chitinases. *Recent Res. Sci. Technol.,* **3 (11):**1-6.

17. Mejia Saules J.M., K.N Waliszewski, M.A Garcia and R. Cruz Camarillo 2006. The use of crude shrimp shell powder for chitinase production by *Serratia marcescens* WF. *Food Technol. Biotechnol.,* **44(7):** 646-651.
18. Nandita C., L. Subrata and C. Goutam 2008. Mosquito larvicidal and antimicrobial activity of protein of *Solanum villosum* leaves. *BMC Complement Altern. Med.,* **8:** 62.
19. Ocampo C., M. Salazar-Terreros, N. Mina, J. McAllister and W. Brogdon 2011. Insecticide resistance status of *Aedes aegypti* in 10 localities in Colombia. *Acta Trop.,***118:** 37–44.
20. Poopathi S and S Abidha 2010. Mosquitocidal bacterial toxins (*Bacillus sphaericus* and *Bacillus thuringiensis serovar israelensis):* mode of action, cytopathological effects and mechanism of resistance. *J. Physiol. Pathophysiol*., **1:** 22–38.
21. Reguera G and S.B2001. Leschine Chitin degradation by cellulolytic anaerobes and facultative aerobes from soils and sediments. *FEMS Microbiol Lett.,* **204:** 367-374.
22. Samuel T., M. Jayakumar and S.J William 2007. *Culex* mosquito: An overview. In: William SJ, editors. Defeating the Public Enemy, the Mosquito: A Real Challenge. Chennai: Loyola Publications;pp. 95-116.
23. Savini V and P. Fazii 2016. *Bacillus thuringiensis* Insecticide Properties. In: Savini, V., Ed., The Diverse Faces of *Bacillus cereus*, Elsevier Inc., Amsterdam, 139-155.
24. Shubakov A.A and P.S Kucheryavykh 2004. Chitynolytic activity of filamentous fungi. *Biochem. Microbiol.,* **5:** 445.
25. Sundarapandian S., M.O Sundaram, P. Tholappian and V. Balasubramanian 2002. Mosquitocidal properties of indigenous fungi and actinomycetes against Culex quinquefasciatus say. *J. Biol. cont.,* **16:** 89-91.
26. Udonsi J.K1986. The status of human filariasis in relation to clinical signs in endemic areas of the Niger Delta. *Ann. Trop. Med. Parasitol.,* **80:** 425-32.

27. WHO, 2005. Guidelines for laboratory and field testing of mosquito larvicides. http://apps.who.int/iris/bitstream/10665/69101/1/WHO_CDS_WHOPES_GCDPP_2005.13.pdf. Accessed 14 Apr 2017.
28. WHO, 2009. Dengue. Guidelines for the diagnosis, treatment, prevention and control. http://www.who.int/tdr/publications/documents/denguediagnosis.pdf Accessed 12 Apr 2017.
29. Wiwat C., S. Thaithanum, S. Pantuwatana and A. Bhumiratana 2000. Toxicity of chitinaseproducing *Bacillus thuringiensis ssp. Kurstaki* HD1 (G) toward *Plutella xylostella. J. Inverebr. Pathol.,* **76:** 270-277.
30. Zhang H., X. Huang, T. Fukamizo, S. Muthukrishnan and K.J Kramer 2002. Site directed mutagenesis and functional analysis of an active site tryptophan of insect chitinase. *Insect Biochem Molec.,* **32:**1477-1488.
31. ZhongW., S. Ding and H. Guo 2015. The chitinase C gene PsChiC from *Pseudomonas sp.* and its synergistic effects on larvicidal activity. *Genetics and Molecular Biol.,* **38(3):** 366-372.