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Short Communication

Larvicidal Activity of *Bacillus laterosporus* Against Mosquitoes

Farzana Bashir,* Sadia Aslam, Rauf Ahmed Khan and Romana Shahzadi
Centre for Environmental Protection Studies, Pakistan Council of Scientific and Industrial Research, Labs. 54600, Ferozepur Road, Lahore

ABSTRACT

In present study *Bacillus laterosporus* isolated from a commercial formulation showed larvicidal activity against second instar larvae of *Aedies aegypti*, *Anophles stephense* and *Culex qunquefasciatus*. The % mortality without heat treatment was 37.34 against *Anophles* and 18.67 against *Culex*. The toxin was heat stable and its toxicity increased with inoculum size and incubation time. The study reveals that this strain of *B. laterosporus* has limited potential as an agent for mosquito vector control.

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Authors' Contributions
FB performed bioassays, analyzed the data and wrote the article. SA and RS isolated and characterized the bacteria. RAK provided research facilities as Principal Investigator.

Key words
***Bacillus laterosporus*, entomopathogenicity, *Aedes aegypti*, mosquitoes.**

The use of entomopathogenic bacteria like *Bacillus* sp., *Pseudomonas* sp., *Serratia* sp. and *Yersinia entomophaga* is a viable safe alternative for pest control that can reduce ecological damage caused by the use of chemical insecticides (De Oliveira *et al.,* 2004; Charles *et al.,* 1996; Gonzalez *et al.,* 2013). Among these microbes *B. laterosporus* is new and its toxicity against the larvae of mosquitoes *Aedes aegypti*, *Anopheles stephensi*, and *Culex pipiens* has been reported by Favret and Yousten (1985). The *B. thuringiensis* is more efficient larvicidal strain than other bacillis species like *B. sphaericus* and other (Jahan and Hussain, 2011). It was reported that toxicity level of *Bacillus laterosporus* was though lesser than that of *B. thuringiensis* had higher insecticidal activity at stationary phase or at sporulated cultures against *Ae. aegypti* and *An. stephensi* larvae (Rivers *et al*., 1991; Ruiu *et al.,* 2007; Orlova *et al.,* 1998). *B. laterosporus* exhibits non-pathogenic effects towards non-target species (Ruiu *et al.,* 2013).

B. laterosporus a spore-forming bacterium characterized by its ability to produce a canoe-shaped parasporal inclusion adjacent to the spore, has been isolated from a wide range of materials including soil (De Oliveira *et al.,* 2004), insect bodies (Smirnova *et al.,* 1993), leaf surfaces (Sarkar *et al.,* 2002), compost (Adegunloye *et al*., 2007), honey (Iurlina and Fritz, 2005), starchy foods (Fangio *et al*., 2010), olive mill wastewater (Aguilera *et al.,* 2001) and fresh water (Laubach, 1916). Some strains of *B. laterosporus* exhibit antimicrobial activity against bacteria and fungi and have been used in medicines (Umezawa and Takeuchi, 1986).

B. laterosporus is used as probiotics for mammals and birds (Hong *et al*., 2005; Duc *et al*., 2004). It has application for the treatment of soil to improve soil properties like maintenance of an alkaline pH, fixation of nutrients, neutralization of odors and to improve the microbial status of soil (Canbolat *et al.*, 2006; O'donnell, 1997). Though *B. laterosporus* strains are ecofriendly, they are rarely reported for their use in bioremediation. It has potential for azo dye decolorization and successfully used for the treatment of different textile wastewater (Kurade *et al.*, 2013; Gomare and Govindwar, 2009).

In present study *B. laterosporus* was isolated from a commercial formulation that is being applied as microbial soil conditioner. They can upgrade the supply level of nutrients substances in soil and improve its physical and chemical properties. Soil conditioner gives higher yield when applied to crops and reduce the dosage of fertilizer (Guangdong Detection Centre of Microbiology, China). As reported in literature (Favret and Yousten, 1985; Ruiu *et al*., 2007) some species of *B. laterosporus* have larvicidal activities against mosquitoes larvae. Present study examines the larvicidal activity of *B. laterosporus* against second instar larvae of *Ae. aegypti*, *An. stephensi* and *Cx. pipiens.*

Materials and methods

In an Erlenmeyer flask one gram of the sachet material (UNIGROW, Soil Conditioner By Winnerway, P.O. Box 523070, Guangdong, P.R.C) was suspended in 100 mL autoclaved distilled water, shaken for one hour

* Corresponding author: beefarzana@gmail.com
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on an orbital shaker and then heat shocked at 80ºC for 10 min. Heating was done to kill other effective live microbes present in sachet material. Suspension (10 ml) was transferred to Nickerson Broth (Orlova *et al.,* 1998), to which some other supplements *i.e.,* glucose (1%), methionine (10 µg/mL) and thiamine (10 µg/mL) were added. pH adjusted at 7, agitated in rotary shaker for two hours, plated on Nickerson agar, and incubated at 30ºC for 48 h. The colonies were identified and characterized as *B. laterosporus* according to Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994).

After confirmation pure culture was grown in Nickerson Broth for 72 h at 30ºC, then harvested and viability counts was performed to determine the cell numbers. Final whole culture (FWC) was used for further bioassay studies.

The bioassay for larvicidal activity was conducted according to WHO protocols (WHO, 1985). Second instar larvae of *Ae. aegypti, An. stephense* and *Cx. pipiens* were taken from Malaria Research Centre, Lahore. Twenty five larvae of each mosquitoe in triplicate were added in 100 mL glass beakers containing dechlorinated tap water and a drop of yeast extract. The beakers were incubated at 25-28ºC with three different concentrations *i.e.* 1×10^3, 1×10^6 and 1×10^8cells/ml of final whole culture. A blank was prepared along with each dilution, having same number of larvae but without final whole culture. Normally, larvae were very motile, those which show no motility when they are probed with a needle were considered as dead after 24, 48 and 72 h. Each bioassay was performed two times with larvae from different batches and mortality was calculated.

The % mortalities were analyzed using Student's t-test to compare means of treated and control group. The tests having P values greater than 0.05 are considered significant while less than 0.05 are considered non-significant.

Results and discussion

The colonies of isolated *B. laterosporus* were off white, circular, raised and mucoid. The isolated bacteria are motile, rod shape and Gram positive, which produced acid from glucose, had catalase activity, reduced nitrate, showed positive lecithinase and indoles tests, could not hydrolyze starch, and had negative mannitol and VP tests.

Table I shows effect of *B. laterosporus* on second instar larvae of *Ae. aegypti, An. stephense* and *Cx. pipiens*. The larvicidal activity of *B. laterosporus* against mosquitoes is related to protein crystals, formed when sporulation occurs (Orlova *et al*., 1998). The toxicity of *B. laterosporus* against *Ae. aegypti* and *Anophles* was almost the same *i.e.* 34.67 % and 37.34%, respectively. *Culex* showed 18.67% mortality against this toxin. The statistical analysis showed that *B. laterosporus* without heat treatment was significantly toxic against all the three species, while after heat treatment toxicity results were non-significant against *Culex* and significant against *Ae. aegypti* and *An. stephense.*

The previous data supports the findings that *B. laterosporus* has less larvicidal activity (De Oliveira *et al*., 2004) compared to *B. thruingiensis and B. sphaericus* (Favret and Yousten, 1985; Rivers *et al*., 1991). However, its pathogenicity against other insects like Coleopetra and other organisms such as nematodes and house flies has been studied, and also used for their control (Singer *et al*., 1997; Schnepf *et al*., 2003; Singer, 1996). Xiaowei *et al*. (2005) have shown that extracellular protein of *B. laterosporus* can kill and destroy the nematode to greater extent than previously reported parasporalcrystalline (Orlova *et al*., 1998).

Figure 1 shows percentage mortality varied with concentration of microbial suspension and also the incubation time. Other studies also support the findings that entomopathogenic activity of *Bacillus* species varies with incubation time (Eswarapriya *et al*., 2010).

The heat stability of the toxin for larvicidal activity was tested. It was observed that the number of viable spores was reduced after a heat shock at 96°C for 10 min. It was also observed that heat shock slightly decreased the mortality of *Ae. aegypti* (4%), *Culex* (5.33%) and *Anopheles* (1%). This observation has also been corroborated by Favret and Youstan (1985).

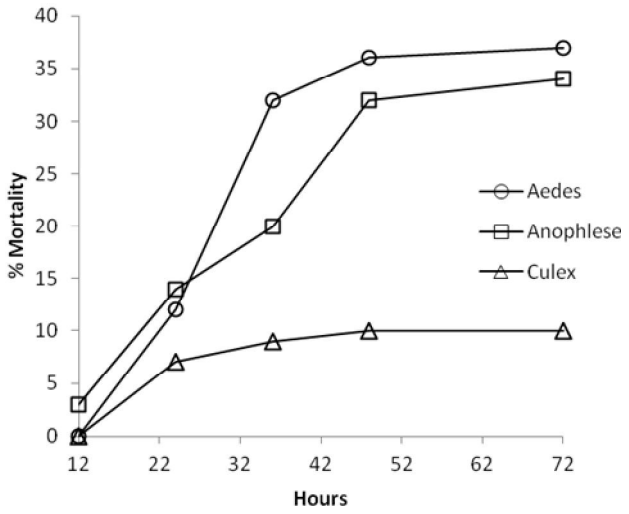

Fig. 1. Effect of incubation time on percentage mortality.

Sabrina *et al.* (2011) treated the entamopathogenic

bacillus culture at 80, 90 and 100ºC for 15 min before conducting bioassay against mosquitoe larvae. All the three treatments showed 100% mortality against *Ae. aegypti* and *Culex*.

Table I.- Toxicity of *Bacillus latrosporous* against mosquito larvae during bioassay. The percent mortality is shown in brackets.

Mosquitoes	No of larvae died out of 75 larvae (n=3)		
	Control	Without heat treatment	After heat treatment
Aedes aegypti	2 (2.66±0.21[≈])	26 (34.67±1.45*)	23 (30.67±1.20*)
Culex sp.	4 (5.33±0.33)	14 (18.67±0.67*)	10 (13.34±0.5**)
Anopheles sp.	3 (4.0±0.58)	28 (37.34±1.15*)	27(36.33±0.89*)

[≈]Mean ± SEM; Student 't' test: *P < 0.05, **P > 0.05; n, number of replicates.

Rivers *et al.* (1991) and Ruiu *et al.* (2007) have however, who found that autoclaving or heating the spores at 121°C for 15 min caused a significant reduction in their toxicity and also in a loss of the most larvicidal activity. So high temperature can degrade the toxin and affect its toxicity level.

The entamopathogenicity of different strains of *B. laterosporus* have been demonstrated against other insects like black flies (Favret and Yousten, 1985), lepidoptera (De Oliveira *et al.*, 2004), Coleoptera, nematodes and mollusks (Singer, 1996) and also towards house flies (Ruiu *et al.*, 2007).

The present isolates have moderate mortality against mosquitoe larvae, however it encourages that there is need to isolate, study and use other biological control agents as ecofriendly approach against mosquitoe vector and also toward other natural enemies.

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References

Adegunloye, Adetuyi, D., Akinyosoye, F. and Doyeni, M. O., 2007. *Pak. J. Nutr.*, **6**:506-510.

Aguilera, M. M. S., Suajrez, M., Guerra, A., Lizama, V., Bennasar, C. and Alberto, A.R.C., 2001. *Int. J Syst. Evol. Microbiol.*, **51**:1687-1692.

Canbolat, M.Y., Bilen, S.R., Cakmakci, F., Sahin and Aydin, A., 2006. *Biol. Fert. Soils*, **42**:350-357.

Charles, J., Nielson-Leroux, C. and Delecluse, A., 1996. *Annu. Rev. Ent.*, **41**:451-472.

De Oliveira, E.J., Rabinovitch, L., Monnerat, R. G., Passos, L.K.J. and Zahner, V., 2004. *Appl. environ. Microbiol.*, **70**: 6657-64.

Duc, L.H., Hong, H. A., Barbosa, T. M., Henriques, A. O. and Cutting, S. M., 2004. *Appl. environ. Microbiol.*, **70**:2161-2171.

Eswarapriya, B., Gopalsamy, B., Kameswari, B., Meera, R. and Devi, P., 2010. *Int. J. Pharm. Tech. Res.*, **2**: 2048-2053.

Fangio, M. F., Roura, S. I. and Fritz, R. A., 2010. *J. Fd. Sci.*,**75**:218-221.

Favret, M.E. and Yousten, A.A., 1985. *J. Inverteb. Pathol.*, **45**: 195-203.

Gomare, S. and Govindwar, S., 2009. *J. appl. Microbiol.*, **106**: 993-1004.

Gonzalez, A., Rodraguez, G., Bruzan, R.Y., Dnzaz, M., Companions, A., Menaondez and Renao, Z., 2013. *J. Vect. Ecol.*, **38**:46-52

Holt, J.K., Sneath, P. and Wiliams, S., 1994. *Bergey's Manual of determinative bacteriology.* Baltimore, Williams and Wilkins.

Hong, H.A., Duc, L.H. and Cutting, S.M., 2005. *FEMS Microbiol. Rev.*, **29**:813-835.

Iurlina, M.O. and Fritz, R., 2005. *Int. J. Fd. Microbiol.*,**105**:297-304.

Jahan, N. and Hussain , N., 2011. *Pakistan J. Zool.*, **43**:915-919.

Kurade, M.B., Waghmode, T.R., Kabra, A.N. and Govindwar, S.P., 2013. *Biotech. Lett.*, **35**:1593-1598.

Laubach, A.C., 1916. *J. Bact.*, **1:505**-512.

O'donnell, B., 1997. Treatment of soil and plants with a composition containing *Bacillus laterosporus. Patents No.5,702,701.*

Orlova, M.V., Smirnova, T.A., Ganushkina, L.A., Yacubovich, V.Y., Azizbekyan. and Roudolf, R., 1998. *Appl. environ. Microbiol.*, **64**:2723-2725.

Rivers, D.B., Vann, C.N., Zimmack, H.L. and Dean, D.H., 1991. *J. Inverteb. Pathol.*, **58**:444-447.

Ruiu, L., Floris, I., Satta, A. and Ellar, D.J., 2007a. *Biol. Contr.*, **43**: 136-143.

Ruiu, L., Satta, A. and Floris, I., 2007b. *Biol. Contr.*, **43**:188–194.

Ruiu, L., Satta, A. and Floris, I., 2013. *Bull. Insectol.*, **66**:181-186.

Sabrina R. Hayes, Hudon, M. and Park, H-W., 2011. *J. Inverteb. Pathol.,* **107**:79-81.

Sarkar, P., Hasenack, B. and Nout, M., 2002. *Int. J. Fd. Microbiol.*, **77**:175-186.

Schnepf, H.E., Narva, K.E., Stockhoff, B.A., Lee, S.F., Walz, M. and Sturgis, B., 2003. Pesticidal toxins and genes from *Bacillus laterosporus* strains. *U.S. Patent 6,956,116, 18 Oct 2005.*

Singer, S., 1996. *Adv. appl. Microbiol*.,**42**:219-61.

Singer, S., Van, F.A., Viel, J. and Genevese, E.E., 1997. *J. indust. Microbiol. Biotechnol.,* **18**:226-231.

Smirnova, T., Shamshina, Konstantinova, G., Ganvshkina, L.A., Kuznetsova, N.I., Minenkova, I.B., Znikolaenko, M.A. and Azizbekyan, R.R., 1993. *Biotechnology (Russia),* **3**:11-15.

Umezawa, K. and Takeuchi, T., 1986. *Biomed. Pharmacother*., **41**: 227-232.

Xiaowei, H., Baoyu, T., Qiuhong, N., Jinkui, Y., Lemin, Z. and Keqin, Z., 2005. *Res. Microbiol***., 156**:719–727.

World Health Organization, 1987. *Report of an informal consultation on the detection, isolation, identification and ecology of biocontrol agents disease vectors*. TDR/BCV/IC-GE/87- 3,annex. II, p. 12. World Health Organization, Geneva, Switzerland.