Tropical Biomedicine 37(3): 791–802 (2020)

Biogenic larvicidal formulation of metabolites from *Steinernema saimkayi* symbiont *Xenorhabdus stockiae* KUT6 against dengue vector *Aedes aegypti*

Jissin, M.[1] and Vani, C.[1*]
Department of Biotechnology, Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India
[*]Corresponding author e-mail: vani@karunya.edu
Received 29 June 2019; received in revised form 27 March 2020; accepted 27 March 2020

Abstract. To characterize the production and larvicidal activity of *Xenorhabdus stockiae* KUT6 Petroleum ether extracts from Luria Broth and induced Quorum sensing medium containing N-3- oxododecanoyl Homoserine Lactone inducer against dengue vector *Aedes aegypti*. The *Galleria mellonella* larvae were reared for the isolation of *Steinernema saimkayi* symbiont *Xenorhabdus stockiae* KUT6 from Cucumber field soil sample in NBTA. Then for the extraction of compounds the KUT6 strains were cultured in Luria Broth and Quorum Sensing optimized media using N-3-oxododecanoyl homoserine lactone inducer. The larvicidal activity of *Xenorhabdus stockiae* KUT6 of petroleum ether extracts were bioassayed against 4th instar *Aedes aegypti* dengue vector. The maximum rate of mortality were recorded of the samples A-24h, B-48h, C-72h, A1-24h, B1-48h, C1-72h at different concentrations 50 µg/ml, 100 µg/ml and 150 µg/ml respectively for 24h to 72h of exposure treatment. The morphological characteristics of *Xenorhabdus stockiae* KUT6 in NBTA were red core colonies with blue background surrounded by zone of inhibition. After 24h exposure maximum rate of 100% mortality of *Aedes aegypti* 4th instar larvae was attained when treated with sample C1-72h 50 µg/ml of the petroleum ether extracts of quorum sensed medium whereas the sample C 72h petroleum ether extracts of KUT6 cultured in Luria broth recorded 100% mortality at 150 µg on 24h exposure indicates enhancement in the product yield. The study assures the use of *Xenorhabdus stockiae* KUT6 petroleum ether extracts as biocontrol agent could be beneficial for the control of dengue vectors.

INTRODUCTION

Research on microorganisms exists in symbiotic relationship with entomopathogenic nematodes are gaining much attention in these years emphasizing secured effects as biocontrol agents for sustainable environment (Chamaiporn *et al.*, 2017). This knowledge has contributed furnished hypothesis to our research on microorganism and its secondary metabolites on larvicidal activity against dengue vector *Aedes aegypti*. Foregoing research towards interpretation of specific interactions of microorganisms interlinking Integrated Pest Management environment friendly against dengue vector is paramount for its control. The spatial distribution of dengue vectors, *Aedes aegypti* and *Aedes albopictus* are affected by climatic factors (Yukiko, 2011). Tremendous researches proved the relation of dengue virus and its vectors *Aedes aegypti* and *Aedes albopictus* for their involvement in dengue transmission (Yukiko, 2011). Uncontrolled urbanization and global warming in developed countries exert an impact on vector mosquitoes causing vector-borne diseases (Goh *et al.*, 1985). Controlling vectors reduces its susceptibility to transmit dengue fever.

The primary vector *Aedes aegypti* is the vector mosquito responsible for dengue fever and dengue hemorrhagic fever. Dengue is the most common arthropod-borne viral infection

caused by dengue virus (DENV) a positive single stranded, positive sense enveloped RNA virus of Flaviviridae family and are mainly transmitted by *Aedes aegypti* and *Aedes albopictus*. Infection with any of the four dengue virus serotypes (DENV 1,2,3,4) leads to inapparent infection syndrome to classic dengue fever (DF), DHF and DSS with vascular leakage and shock (Mustafa *et al*., 2015). According to National Vector Borne Disease Control Programme (NVBDCP) report published in 2018 around 40886 cases in India including 2175 cases in Tamil Nadu alone dengue fever has been reported statistically (National Vector Borne Disease Control Programme 2018). According to WHO continuous usage of chemical insecticides such as synthetic pyrethroids, malathion and carbamates may results decreased susceptibility towards vector target (WHO, 2016). But the major disadvantage is that it can harm non-target beings in the environment and continuous usage of chemical insecticides causes health related issues and develops negative impact of resistance among mosquitoes (Guedes *et al*., 2014). Since no vaccines are available there is a need for developing eco-friendly biodegradable, low cost biological insecticides for the eradication.

The continuous usage of chemicals over a long period of time induces mosquito population resistance (Kovendan *et al*., 2011). The biocontrol agents with Integrated Vector management used for the control involves *Bacillus thuringiensis israelensis* or *Bacillus sphaericus* and their toxins, *Xenorhabdus/Photorhabdus* species, *Metarhizium anisopliae* and *Beauveria bassiana*, *Acanthamoeba polyphaga* and the copepod *Macrocyclops albidus* are commonly used (Park, 2015; Setha *et al*., 2016).

Xenorhabdus species are entomopathogenic bacteria symbiotically associated with insect parasitic nematodes of the family Steinernematidae. The entomopathogenic nematodes are obligate or facultative parasites of insects having soft and non-segmented body (Dilipkumar *et al*., 2017).

Reseach reviewed several gram negative bacteria communicates through synthesising and secreting small diffusible inducer homoserine lactone signal molecules by a special mechanism called Quorum sensing (Harshad *et al*., 2014). Since quorum sensing is also associated towards bacterial growth; bacterial compounds can intercept and modulates their virulence factor that enhances the pathogenicity (Chan *et al*., 2011; Lade *et al*., 2014). The future perspective of the existence of Acyl Homoserine Lactone-mediated QS in Gram negative bacteria and its potential role can be used as an alternative approach for the product yield formulation (Bhardwaj *et al*., 2012). In the present study inspired by the natural phenomena of entomopathogenic symbiotic bacteria we evaluated the larvicidal activity of crude extracts against *Aedes aegypti* 4th instar larvae from isolated *Xenorhabdus* species.

MATERIALS AND METHODS

Collection of soil samples for the isolation of Entomopathogenic Nematode by Insect Baiting Technique

Ten different agricultural field soil sample namely Tomato, Ginger, Cucumber, Turmeric, Coconut, Banana,Yam, Tapioca, Sugar Cane and Pumpkin were collected from the depth of at least 15cm (Vitta *et al*., 2017). Visual debris were removed from the collected soil samples to avoid saprobic contamination. To the 10 different soil sample container, five *Galleria mellonella* larvae were inoculated for baiting with particular nematodes. The containers were covered with black cloths to aid the infection at 25-28°C. The soil samples infected with Gallerial larvae were observed for up to 7 days and the cadaver infected with the nematode appeared black color indicating the presence of *Steinernema* species were rinsed thoroughly in sterile water (Boszormenyi *et al*., 2009).

Isolation of *Xenorhabdus* species

From the infected *Galleria mellonella*, hemolymph were taken and streaked on Nutrient agar Bromothymolblue 2,3,5-Triphenyltetrazolium chloride medium for incubation at 27°C for 24h. Bioluminiscent colonies observed under UV light at 365 nm and further cultured to establish pure isolates in Luria broth and stored at 4°C (San-Blas *et al*., 2017). The isolated bacteria were subjected to 16SrRNA sequencing.

Identification of *Xenorhabdus* species

The symbiotic bacterial isolates were represented as KUT6 (Karunya University Thadiyoor) based on the location of the soil samples and its symbiont entomopathogenic nematode as KUT1 which was confirmed by morphological (staining and motility) and cultural (NBTA medium) characterization methods. The 16SrRNA was done for species level identification of KUT6 strain.

Induction of Quorum sensing using N 3-Oxododecanoyl Homoserine Lactone

To enhance its product yield a loopful of culture was inoculated into 3000 ml of Luria broth with N 3-Oxododecanoyl homoserine lactone inducer and kept in rotary shaker incubator at 120 rpm for 24-72h. Aliquotes of culture broth were separated every 24h and centrifuged at 10000 rpm for 20 min (Hinchliffe *et al*., 2010; Harshad *et al*., 2014).

Extraction of secondary metabolites

Culture supernatant of *Xenorhabdus stockiae* KUT6 strain in Luria broth and Quorum sensing Luria broth medium at 24h, 48h and 72h were extracted with equal volume of the solvent such as petroleum ether, chloroform, ethyl acetate and butanol. The solvent fraction of petroleum ether was evaporated at 60°C at 100 rpm and the residue was stored at 4°C for further analysis (Hinchliffe *et al*., 2010).

Rearing of *Aedes aegypti* 4th Instar Larvae

The *Aedes aegypti* were collected from Centre for Research in Medical Entomology, Madurai, Tamil Nadu, India. At Laboratory condition the *Aedes aegypti* egg rafts were kept in tray containing tap water. An adequate amount of sterilized yeast powder and dog biscuit were added to the tray in the ratio of 4:1 ratio to enhance the growth of larvae. For the Bioassay 4th instar larvae of *Aedes aegypti* were used.

Larvicidal activity of secondary metabolites from Luria broth media and optimized media against *Aedes aegypti*

The extracts obtained from petroleum ether of Luria broth *Xenorhabdus stockiae* KUT6 culture supernatant and modified Luria broth with inducer were analyzed in different concentrations against 4th instar larvae of *Aedes aegypti*. From the stock standard of 1 mg/ml extracts with the concentration of 50 µg/ml, 100 µg/ml and 150 µg /ml were added into the disposable cup containing 20 ml of chlorine free tap water and 10 4th instar larvae of *Aedes aegypti* per cup were added in the bioassay cups in triplicates. The petroleum ether solvent without the extracts were kept as control. The rate of mortality was calculated from 24 to 72h duration using Abbott's formula (Padmanabhan *et al*., 2005; Abbot, 1925). All the experiments were carried out in Department of Biotechnology, Karunya University, Coimbatore.

Corrected Mortality = Observed mortality – Control Mortality/100 – Control Mortality x 100%

Statistical analysis

Statistical analysis was performed with graphed prism 6 software using one-way analysis of variance. The LC_{50} values were calculated using probit analysis (Guedes *et al*., 2014).

Histopathology

For the histopathological studies treated and untreated *Aedes aegypti* IVth instar larvae were stored in 10% formalin solution. The material was cut with glass knives in a rotary microtome and fixed with hematoxylin-eosin blue, which is used to study the morphological changes using phase contrast microscope and are compared with the control sample (Sugumar *et al*., 2014).

RESULTS

Isolation and screening of *Xenorhabdus* species associated entomopathogenic nematodes

Based on the hypothetical research, from the cucumber soil sample the black dead nematode infected *Galleria mellonella* were collected (Fig. 1A and 1B). The Gallerial hemolymph were inoculated in Nutrient Bromothymolblue 2,3,5 – Triphenyltetrazolium chloride Agar medium, the bacterial culture showed blue green background inhibit zone at 365 nm in the UV light. The morphological appearance of the isolated bacterial strain were, red coloured colonies with blue background inhibit zone, swamming and smooth free phase 1 colonies (Fig. 2A). The phase 2 colonies were appeared as dark red colonies. The phenotypic characterization of the bacterial strain recorded as swamming motility, and in Gram staining it was observed gram negative small rod shaped organism. Maximum growth was recorded at 27-30°C dark conditions for 24–48 hrs. The primary confirmed phase I colonies were given for 16S r RNA sequenicng. The 16SrRNA sequence of *Xenorhabdus stockiae* KUT6 strain has been submitted with NCBI (Accession Number- MF125441) (Fig. 3).

Induced Quorum sensing media and extraction of metabolites

The product yields of the compounds were induced by inoculating 24h culture in Luria broth containing N 3- Oxododecanoyl homoserine lactone QS inducer. After centrifugation out of the four solvents; the active secondary metabolites from Luria

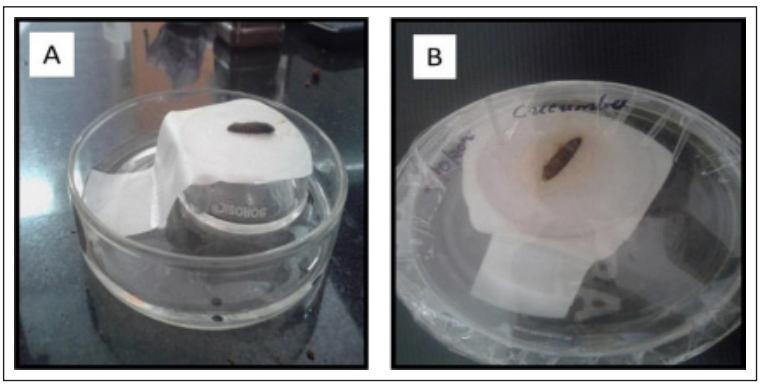

Figure 1. The entomopathogenic nematode infected black dead *Galleria mellonella* from cucumber field soil.

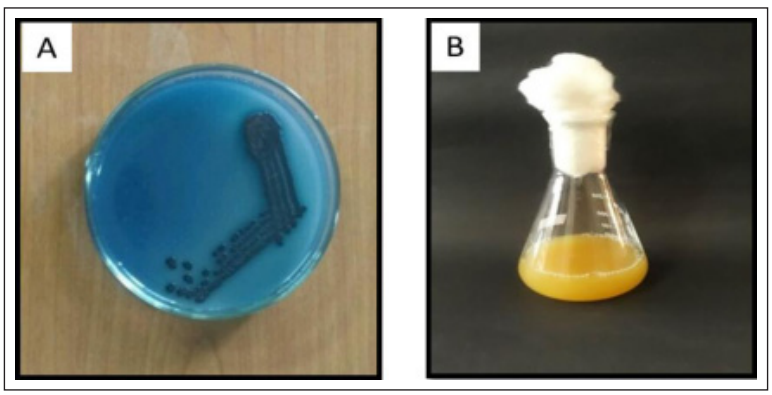

Figure 2a. Red core colonies with blue background on NBTA Medium. Fig. 2b. *Xenorhabdus* species grown in Luria Broth.

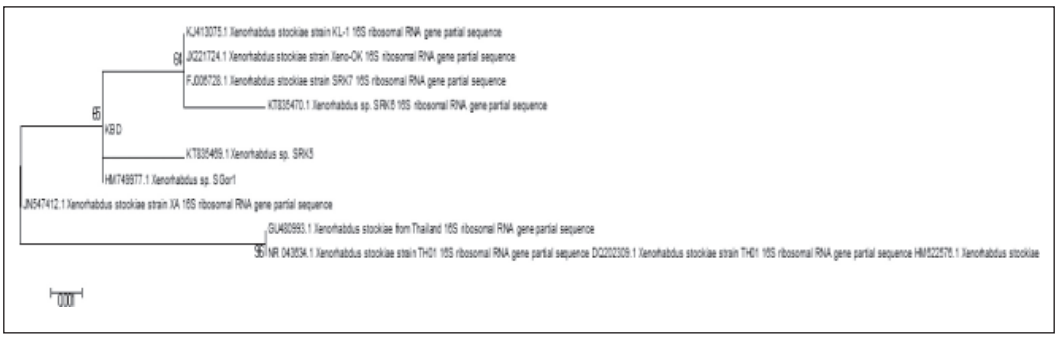

Figure 3. Maximum likelihood tree data of *Xenorhabdus stockiae* isolated from soil entomopathogenic nematodes. Bootstrap values >50% are displayed and the sequences obtained in this study are named as KUT6, reference sequence names are given followed by the GenBank Accession Number.

Figure 4a. The *Xenorhabdus stockiae* KUT6 were inoculated in Luria Broth for samples A (24h), B (48h) and C (72h) and are incubated at 28°C N 3- oxododecanoyl homoserine lactone quorum sensing inducer in Luria broth were prepared and incubated for samples A1 (24h), B1 (48h) and C1 (72h); Fig. 4b After centrifugation the secondary metabolites were extracted in Petroleum Ether; Fig. 4c The active compunds formed crystal structure and stored.

Table 1. The petroleum ether secondary metabolites from *Xenorhabdus stockiae* KUT6 strain in Luria Broth were weighed and calculated

Incubation time (hr)	Empty weight (g)	Dry weight (g)	Weight of compound (g)
A (24)	1.202	1.409	0.207
B (48)	1.202	1.335	0.133
C (72)	1.202	1.292	0.0903

Table 2. The petroleum ether secondary metabolites from *Xenorhabdus stockiae* KUT6 strain in QS induced Luria Broth medium were weighed and calculated

Incubation time (hr)	Empty weight (g)	Dry weight (g)	Weight of compound (g)
A1 (24)	24.438	24.645	0.207
B1 (48)	74.646	75.002	0.356
C1 (72)	74.646	73.646	1.000

broth (Fig. 2B) and modified medium were found in petroleum ether (Fig. 4A, 4B and 4C). The wet weight and dry weight of the petroleum ether extracts were given below (Table 1, 2).

Table 3. The relative quantitation of the *Xenorhabdus stockiae* KUT6 secondary metabolites against *Aedes aegypti*

Samples	24h Mortality			48h Mortality			72h Mortality		
	Concentration of secondary metabolites (µg/ml)			Concentration of secondary metabolites (µg/ml)			Concentration of secondary metabolites (µg/ml)		
	50 µg	100 µg	150 µg	50 µg	100 µg	150 µg	50 µg	100 µg	150 µg
Control	0.00±0	0.00±0	0.00±0	0.00±0	0.00±0	0.00±0	0.00±0	0.00±	0.00±0
Sample A	16±1.5 ^{a****}	23±0.5 ^{a****}	30±0 ^{a****}	30±1 ^{a****}	50±1.7 ^{a****}	56±0.5 ^{a****}	70±0 ^{a****}	76±0.5 ^{a****}	86±0.5 ^{a****}
Sample B	26±0.5 $^{a**** b****}$	33±0.5 $^{a**** b****}$	63±1.15 $^{a**** b****}$	46±1.15 $^{a**** b****}$	60±1 $^{a**** b****}$	80±1 $^{a**** b****}$	80±0 $^{a**** b****}$	86±0.5 $^{a**** b****}$	100±0 $^{a**** b****}$
Sample C	50±1 $^{a**** b**** c****}$	80±1 $^{a**** b**** c****}$	93±0.5 $^{a**** b**** c****}$	73±0.5 $^{a**** b**** c****}$	90±1 $^{a**** b**** c****}$	100±0 $^{a**** b**** c****}$	90±1 $^{a**** b**** c****}$	100±0 $^{a**** b**** c****}$	100±0 $^{a**** b**** c****}$
Sample A1	40±1 $^{a**** b**** c*** d****}$	46±1.15 $^{a**** b**** c*** d****}$	56±1.15 $^{a**** b**** c*** d****}$	66±0.5 $^{a**** b**** c*** d****}$	80±1 $^{a**** b**** c*** d****}$	86±0.5 $^{a**** b**** c*** d****}$	86±0.5 $^{a**** b**** c*** d****}$	90±0 $^{a**** b**** c*** d****}$	100±0 $^{a**** b**** c*** d****}$
Sample B1	50±1 $^{a**** b**** c****dns e****}$	63±0.5 $^{a**** b**** c****dns e****}$	86±1.5 $^{a**** b**** c****dns e****}$	80±1 $^{a**** b**** c****dns e****}$	80±1 $^{a**** b**** c****dns e****}$	93±0.5 $^{a**** b**** c****dns e****}$	93±0.5 $^{a**** b**** c****dns e****}$	100±0 $^{a**** b**** c****dns e****}$	100±0 $^{a**** b**** c****dns e****}$
Sample C1	90±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$	100±0 $^{a**** b**** c**** d**** e**** f****}$

The values are expressed in mean ± S.D; significant* $p \leq 0.05$; highly significant**** $p \leq 0.01$; not significant^{ns} $p \geq 0.05$; ^{a} – Control vs Sample A,B,C,A1,B1,C1; ^{b} – A vs B,C,A1,B1,C1; ^{c} – B vs C,A1,B1,C1; ^{d} – C vs A1,B1,C1; ^{e} – A1 vs B1,C1; ^{f} B1 vs C1.

Larvicidal activity of petroleum ether extracts against *Aedes aegypti*

Larvicidal activity of the secondary metabolite against 4th instar larvae of *Aedes aegypti* was recorded for 24h, 48h and 72h exposure (Fig. 5A and 5B) (Table 3). In Control bioassay cup no dead larvae were obtained. On 24h exposure, Petroleum Ether extracts of Sample A (24h) showed 16.6% mortality at 50 µg/ml; 23.3% mortality at 100 µg/ml and 23.3% mortality at 150 µg/ml. On 48h exposure, petroleum ether extract of Sample A (24h) showed 30% at 50 µg/ml; 50% mortality at 100 µg/ml and 76.6% at 150 µg/ml. On 72h exposure *in vitro*, recorded 23.3% mortality at 50 µg/ml; 50% mortality at 100 µg/ml and 86.6% mortality at 150 µg/ml (Fig. 6A).

Larvicidal activity of extracts of Sample B (48h) showed 26.6% mortality at 50 µg/ml; 30% at 100 µg/ml and 53.3% mortality at 150 µg/ml on 24h exposure. 48h exposure recorded 46.6% mortality at 50 µg/ml; 50% mortality at 100 µg/ml and 70% mortality at 150 µg/ml. On the 72h expsoure Sample B (48h) extracts showed 70% mortality at 50 µg/ml; 76.6% at 100 µg/ml and 100% at 150 µg/ml (Fig. 6B). Sample C (72h) petroleum ether extracts of 24h exposure showed 36.6% at 50 µg/ml; 66.6% mortality at 100 µg/ml and 66.6% mortality at 150 µg/ml. On the 2nd day of exposure the percentage of mortality was 46.6% at 50 µg/ml; 86.6% at 100 µg/ml and 86.6% mortality at 150 µg/ml. On 72h treatment 86.6% mortality at 50 µg/ml; 100% mortality was recorded at 100 µg/ml and 150 µg/ml concentrations (Fig. 6C).

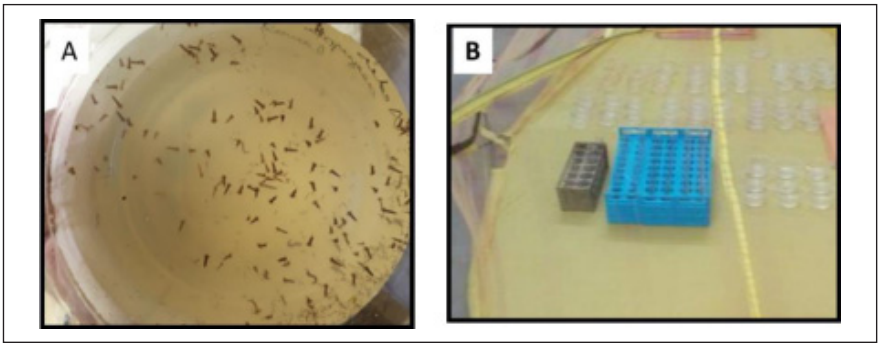

Figure 5a. The 4th instar larvae of *Aedes aegypti* hatched from the egg were inoculated in chlorine free tap water; Fig. 5b The Biolarvicidal activity of seconadary metabolites from *Xenorhabdus stokiae* KUT6 were performed under 24-72h exposure treatment.

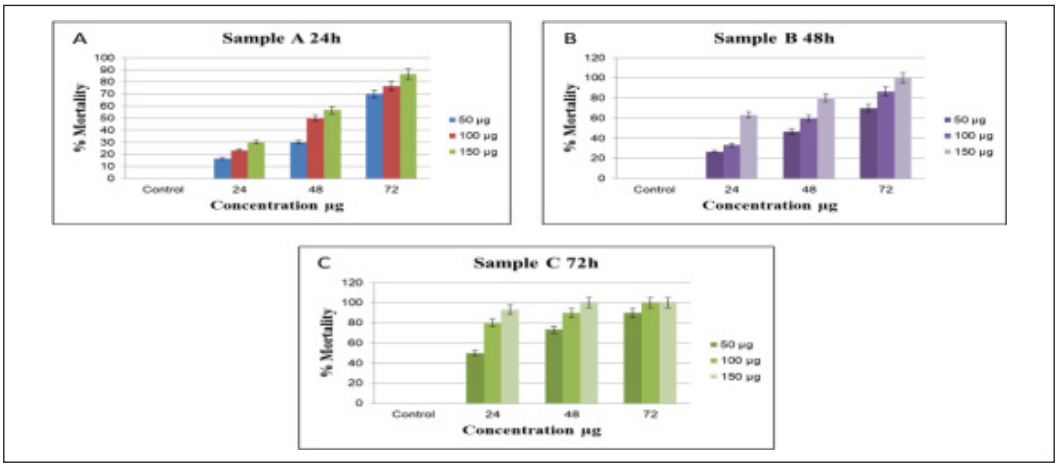

Figure 6a. Larvicidal Activity of Sample A 24h; Fig. 6b Larvicidal Activity of Sample B 48h; Fig. 6c Larvicidal Activity of Sample C 72h against *Aedes aegypti*.

QS Sample A1 (24h) extracts showed 33.3% moratlity at 50 µg/ml; 36.6% at 100 µg/ml and 36.6% mortality rate at 150 µg/ml on the 24h treatment. The 48h exposure of the larvicidal activity was 53.3% at 50 µg/ml and 100 µg/ml and 56.6% at 150 µg/ml. On the 3rd day of exposure 83.3% mortality was recorded at 50 µg/ml; 90% at 100 µg/ml and maximum mortality 100% was attained at 150 µg/ml (Fig. 7A).

QS Sample B1 (48h) extracts on 24h showed 40% at 50 µg/ml; 50% at 100 µg/ml and 63.3% mortality attained at 150 µg/ml. On the 48h exposure 56.6% mortality was observed at 50 µg/ml; 66.6% at 100 µg/ml and 76.6% rate of mortality was observed at 150 µg/ml. On the 3rd day 93.3% mortality was recorded at 50 µg and maximum rate of mortality of 100% attained on 100 µg and 150 µg (Fig. 7B).

The Sample C1 (72h) of quorum sensed extract showed 90%, 100% and 100% at 50 µg/ml, 100 µg/ml and 150 µg/ml on 24h exposure. On 48h and 72h treatment maximum rate of mortality was recorded on 50 µg/ml, 100 µg/ml and 150 µg/ml (Fig. 7C). Among the samples significance was observed $p \leq 0.01$ (Fig. 8). The LC_{50} value

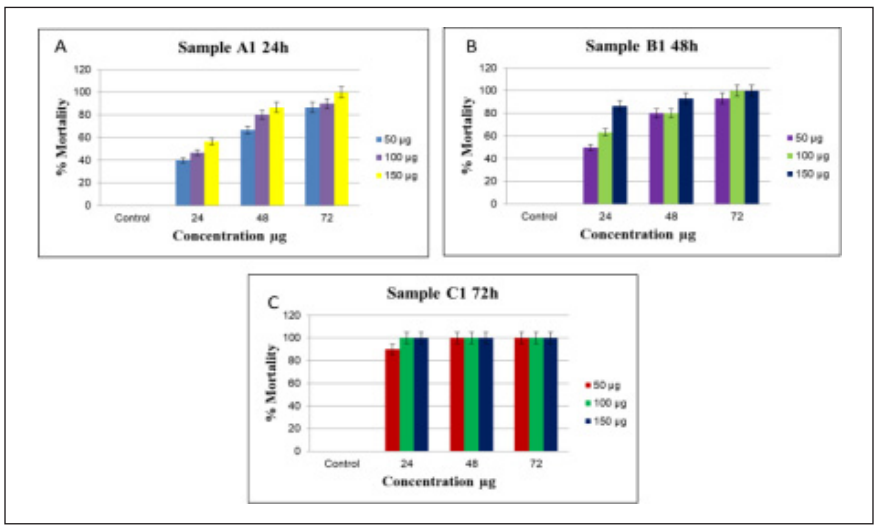

Figure 7a. Larvicidal Activity of Sample A1 24h; Fig. 7b Larvicidal Activity of Sample B1 48h; Fig. 7c Larvicidal Activity of Sample C1 72h against *Aedes aegypti*.

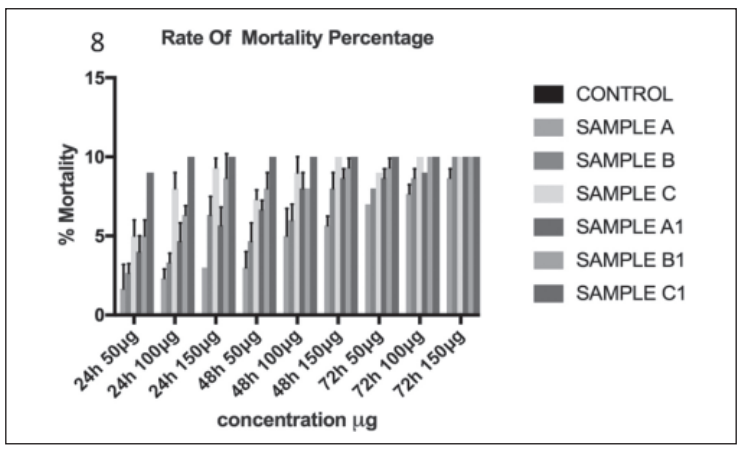

Figure 8. The larvicidal activity of the petroleum ether extracts against *Aedes aegypti* at different concentration (µg) for 24 to 72h exposure. Significance was measured at $p \leq 0.01$.

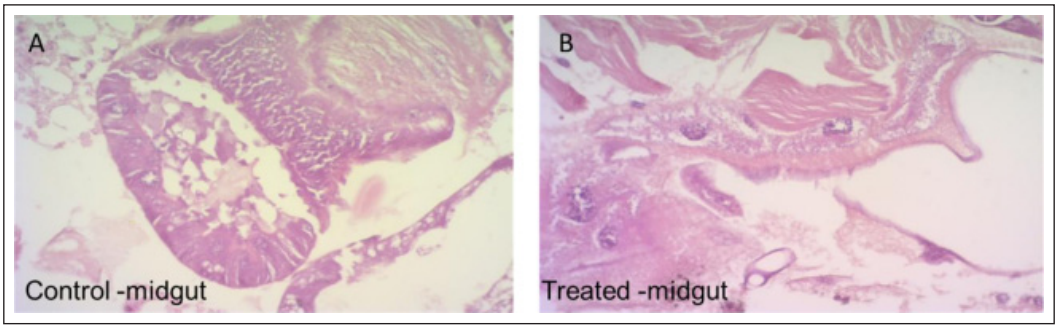

Figure 9a. Untreated *Aedes aegypti* larvae with intact midgut region; Fig. 9b Treated *Aedes aegypti* larvae with damaged midgut region.

were recorded as 2.38 µg/ml, 1.93 µg/ml, 1.73 µg/ml for 50 µg, 100 µg and 150 µg for Sample C and 1.77 µg/ml, 1.66 µg/ml, 1.66 µg/ml for 50 µg, 100 µg and 150 µg for Sample C1.

Histopathology

Toxicity of larvicidal compounds from *Xenorhabdus stockiae* KUT6 in the midgut of the 4th instar larvae of *Aedes aegypti* were studied by histopathology technique. Larvae treated with *Xenorhabdus stockiae* KUT6 secondary metabolites showed that the peritrophic membrane (PM), epithelial cells (EC) of the midgut have been damaged and there is also a leakage of the midgut contents (Figure 9B). Whereas in the control larvae midgut region is intact (Figure 9A).

DISCUSSION

In the present study *Xenorhabdus stockiae* KUT6 bacterial strain were isolated from agriculture soil field Kadayar, Kerala, India. The culture morphological characterization of the isolates were gram negative rod and highly motile. And on selective medium NBTA red core colonies surrounded by blue colour phase 1 colonies were developed. This assures the research similar to the *Xenorhabdus* species isolated from Nam Nao National Park, Thailand (Temsiri *et al.*, 2018). The isolates were further confirmed through 16SrRNA sequencing. The phylogenetic tree analysis of 16SrRNA sequencing confirmed KUT6 strain as *Xenorhabdus stockiae*. The bacterial strain was cultured in Luria Broth and product yield were optimised through Quorum sensing using N-3oxododecanoyl homoserine lactone inducer for further experimental studies.

Identification of the toxic compound from the culture supernatant of *Xenorhabdus stockiae* KUT6 was done using petroleum ether extraction. Larvicidal activity against *Aedes aegypti* was carried out with the Sample A (24h), Sample B (48h) and Sample C (72h) culture supernatant and Sample A1 (24h), Sample B1 (48h) and Sample C1 (72h) induced QS culture supernatant extracts and mortality rate were observed from 24h to 72h exposure. Among the six samples the Sample C (72h) culture supernatant extract of *Xenorhabdus stockiae* KUT6 strain showed 100% mortality against the *Aedes aegypti* larvae at 100 µg and 150 µg on 48h and 72h exposure. Sample C1 (72h) optimised extract of *Xenorhabdus stockiae* KUT6 strain showed 100% mortality against the *Aedes aegypti* larvae at 100 µg and 150 µg on 24h exposure. Petroleum ether at low concentration (50 µg – 73%) is more toxic to the *Aedes aegypti*. The result of our study was appreciable as 50 µg/ml of petroleum ether extracts due to its virulence enhancement in quorum sensing recorded 100% mortality of *Aedes aegypti* at 24h however, this result deviated with the earlier studies; on the solvent extracts of petroleum ether, chloroform and aqueous extracts obtained from *Acalypha indica*, *Aerva Lanta*, *Boerhaavia diffusa*, *Commelina benghalensis*, *Gomphrena* spp., *Datura stramonium*, *Euphoria hurt*, *Cynodon* spp., *Lantana camera* and *Tridax procumbens*.

It was reported that the larvicidal activity was higher in petroleum extracts of the above plant with a higher concentration of 1000 µg/ml at 48h exposure (Rajasekaran *et al*., 2012).

Based on our results the maximum mortality might be due to the absorbance of the toxic compounds through the cuticle of the larvae, as further development of the larvae were not observed (Brammacharry *et al*., 2012; Lalithambika *et al*., 2016). Untreated larvae in which the midgut region is intact. In the treated larvae after treatment shows that the peritrophic membrane (PM) and the epithelial cells (EC) of the midgut have been damaged and there is also a leakage of the midgut contents. Our result was in agreement with the result obtained by the research (Sugumar *et al*., 2014), it was noted that the *Culex quinquefasciatus* larvae when treated with eucalyptus oil nanoemulsions, showed that the damage of the peritrophic membrane and the epithelial cells of the midgut with leakage of the contents. Our results authenticated to be one of the best suitable biocontrol agent in containment of dengue fever. Further studies on the formulations will pave the way for control of *Aedes aegypti* which has developed resistance to DDT and synthetic pyrethroids reported recently (Kushwah *et al*., 2015; Jyotirmoy *et al*., 2006).

This research was effectuated to identify the maximal production of bioactive compounds from the culture supernatant. The production of compounds from Luria Broth and modified medium were recorded respectively. The larvicidal activity against 4th instar larvae of *Aedes aegypti* was maximum in the modified medium; this may be due to increased production of compounds in the modified medium (Harshad *et al*., 2014).

The innovation of biological insecticides succeeds synthetic insecticides showing promising effects on various factors such as stability of the compound, stability of its activity against pathogens, low cost feasible effective compound production and product yield. Majority of biocontrol agents takes more than 3 days or weeks to thwart pests/pathogens; but nematode associated bacterial complexes shows its larvicidal activity within 24-72h at maximal rate and minimal concentrations (Perez *et al*., 1990; Dilipkumar *et al*., 2017). Habitually Entomopathogenic nematodes functions as vector for transmitting its symbiotic bacteria to insect host; causing septicaemia within few days of infection. This parameter strengthens the strong specificity of our hypothesis against dengue vector *Aedes aegypti*.

CONCLUSION

In conclusion, the bacterial strains isolated from different agricultural soils were identified through 16SrRNA sequencing as *Xenorhabdus stockiae* KUT6 strain. Based on our bioassay, we defined the lethal larvicidal activity of *Xenorhabdus stockiae* KUT6 strain by examining the interaction against *Aedes aegypti* larvae within 24h to 72h treatment and is the best pathogen to the larvae. This indicates that *Xenorhabdus stockiae* KUT6 can be effective biocontol agent and the bioactive compounds should be further investigated for the application of the bacteria. The QS autoinducer induces the product yield but QS pathway studies will be carried out further.

Acknowledgements. This study was supported and funded by University Grant Commission – Maulana Azad National Fellowship (Grant Number – F1-17.1/2016-17/MANF-2015-17-KER-69816). We would like to thank Department of Biotechnology, Karunya Institute of Technology and Sciences. Many thanks are extended to Dr. Sankaranaryanan Department of Nematology, Sugar Cane Breeding Institute. We thank Poomalai D for his timely help; and also acknowledging our thanks to Mathew C Abraham, Susan Mathew, Blesson Mathew, Jeba Elis for all the supports.

Conflicts of Interests

The authors have declared no conflicts of interest.

REFERENCES

Abott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**: 265-267.

Acosta, E.G., Castilla, V. & Damonte, E.B. (2008). Functional entry of dengue virus into *Aedes albopictus* mosquito cells is dependent on clathrin-mediated endocytosis. *Journal of General Virology* **89**: 474-484.

Alves, S.B., Alves, L.F.A., Lopes, R.B. & Pereira, R.M. (2002). Potential of Some *Metarhizium anisopliae* Isolates for Control of *Culex quinquefasciatus* (Diptera, Culicidae). *Journal of Applied Entomology* **126**: 504-509.

Bhardwaj, A.K. & Mohanty, P. (2012). Bacterial Efflux Pumps Involved in Multidrug Resistance and their Inhibitors: Rejuvinating the Antimicrobial Chemotherapy. Rec. *Patents on Anti-Infective Drug Discovery* **7**: 73-89.

Boszormenyi, E., Ersek, T. & Fodor, A. (2009). Isolation and activity of *Xenorhabdus* antimicrobial compounds against the plant pathogens *Erwinia amylovora* and *Phytophthora nicotianae*. *Journal of Applied Microbiology* **107**: 746-759.

Bowatte, G., Perera, P., Seneviratne, G. & Meegaskumbura, S. (2013). Tadpoles as dengue mosquito (*Aedes aegypti*) egg predators. *Biological Control* **67**: 469-474.

Brammacharry, U. & Paily, K. (2012). Chitinase like activity of metabolites of *Pseudomonas fluorescens Migula* on immature stages of the mosquito *Culex quinquefasciatus* (Diptera: Culicidae). *African Journal of Microbiology Research* **6**: 2718-2726.

Chamaiporn, F., Thatcha, Y., Manawat, S. & Paramaporn, M. (2017). Isolation and identification of *Xenorhabdus* and *Photorhabdus* bacteria associated with entomopathogenic nematodes and their larvicidal activity against *Aedes aegypti*. *Parasites and Vectors* **10**: 440-450.

Chan, K., Atkinson, S. & Mathew, K. (2011). Characterization of N-acylhomoserine lactone-degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: co-existence of quorum quenching and quorum sensing in *Acinetobacter* and *Burkholderia*. *BMC Microbiology* **11**: 51-60.

Dilipkumar, A., Ramalingam, K.R., Govindhan, G. & Paramasivam, D. (2017). Antibacterial activities of extracellular metabolites of symbiotic bacteria, *Xenorhabdus* and *Photorhabdus* isolated from entomopathogenic nematodes. *International Biological and Biomedical Journal* **3**: 80-88.

Goh, K.N.S. & Kumarapathy, S. (1985). Disease-bearing insects brought in by international aircraft into Singapore. *Southeast Asian Journal. Tropical Medicine and Public Health* **16**: 49-53.

Guedes, E.A.C., De-Carvalho, C.M., Ribeiro-Junior, K.A.L. & Lisboa, R.T.F. (2014). Larvicidal activity against *Aedes aegypti* and molluscicidal activity against *Biomphalaria glabrata* of Brazilian marine algae. *Parasitolology* **1**: 1-6.

Harshad, L., Diby, P. & Ji, H.K. (2014). N-Acyl Homoserine Lactone-Mediated Quorum Sensing with Special Reference to Use of Quorum Quenching Bacteria in Membrane Biofouling Control. *BioMed Research International* **10**: 1-25.

Hinchliffe, S.J., Hares, M.C. & Dowling, A.J. (2010). Insecticidal toxins from the *Photorhabdus* and *Xenorhabdus* bacteria. *Open Toxinology Journal* **3**: 83-100.

Izabela, A., Rodenhuis-Zybert, J., Wilschut. & Jolanda, M.S. (2010). Dengue virus life cycle: viral and host factors modulating Infectivity. *Cellular and Molecular Life Sciences* **67**: 2773-2786.

Jyotirmoy, B., Jitendra, S., Mohan, C.J. & Shubhendu, G. (2006). The cytotoxic fimbrial structural subunit of *Xenorhabdus nematophila* is a pore-forming toxin. *Journal of Bacteriology* **10**: 87-113.

Kovendan, K., Murugan, K., Vincent, S. & Kamalakannan, S. (2011). Larvicidal efficacy of *Jatropha curcas* and bacterial insecticide, *Bacillus thuringiensis*, against lymphatic filarial vector, *Culex quinquefasciatus* say (Diptera: Culicidae). *Parasitology Research* **109**: 1251-1257.

Kushwah, R.B.S., Mallick, P.K., Ravikumar, H. & Dev, V. (2015). Status of DDT and pyrethroid resistance in Indian *Aedes albopictus* and absence of knockdown resistance (kdr) mutation. *Journal of Vector Borne Diseases* **52**: 95-98.

Lade, H., Paul, D. & Kweon, J.H. (2014). Isolation and molecular characterization of biofouling bacteria and profiling of quorum sensing signal molecules from membrane bioreactor activated sludge. *International Journal of Molecular Sciences* **15**: 2255-2273.

Lalithambika, B. & Vani, C. (2016). *Pseudomonas aeruginosa* KUN2, extracellular toxins – A potential source for the control of dengue vector. *Journal of Vector Borne Diseases* **53**: 105-111.

Modis, Y., Ogata, S., Clements, D. & Harrison, S.C. (2004). Structure of the dengue virus envelope protein after membrane fusion. *Nature* **427**: 313-319.

Mustafa, M.S., Rastoqi, V., Jain, S. & Gupta, V. (2015). Discovery of fifth stereo- type of dengue virus (DENV-5): A new public health dilemma in dengue control. *Medical Journal Armed Forces India* **71**: 67-70.

National Vector Borne Disease Control Programme (2017–2018). Dengue fever Annual Report. 1–38.

Padmanabhan, V., Prabakaran, G., Paily, K.P. & Balaraman, K. (2005). Toxicity of a mosquitocidal metabolite of *Pseudomonas fluorescens* on larvae and pupae of the house fly, *Musca domestica*. *Indian Journal of Medical Research* **121**: 116-119.

Park, Y. (2015). Entomopathogenic bacterium, *Xenorhabdus nematophila* and *Photorhabdus luminescens*, enhances *Bacillus thuringiensis* Cry4Ba toxicity against yellow fever mosquito, *Aedes aegypti* (Diptera: Culicidae). *Journal of Asia-Pacific Entomology* **18**: 459-463.

Perez, C., Pauli, M. & Bazerque, P. (1990). An antibiotic assay by the agar well diffusion method. *Acta Biologiae et Medicinae Experimentalis* **15**: 113-115.

Rajasekaran, A. & Geethapriya, D. (2012). Larvicidal activity of plant extracts on *Aedes aegypti* L. *Asian Pacific Journal of Tropical Biomedicine* **1**: 1578-1582.

San-Blas, E., Cubillán, N. & Guerra, M. (2017). Characterization of *Xenorhabdus* and *Photorhabdus* bacteria by Fourier transform mid-infrared spectroscopy with attenuated total reflection (FTIR/ATR). *Internaional Biological and Biomedical Journal* **3**: 20-27.

Setha, T., Chantha, N., Benjamin, S. & Socheat, D. (2016). Bacterial larvicide, *Bacillus thuringiensis israelensis* strain AM 65-52 water dispersible granule formulation impacts both dengue vector, *Aedes aegypti* (L.) population density and disease transmission in cambodia. *PLoS Neglected Tropical Diseases* **10**: 1-15.

Sugumar, S., Clarke, S.K., Nirmala, M.J., Tyagi, B.K., Mukherjee, A. & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against *Culex quinquefasciatus*. *Bulletin of Entomological Research* **104**: 393-402.

Temsiri, Y., Paramaporn, M., Raxsina, P. & Sarunporn, T. (2018). Identification of entomopathogenic nematodes and symbiotic bacteria from Nam Nao National Park in Thailand and larvicidal activity of symbiotic bacteria against *Aedes aegypti* and *Aedes albopictus*. *Plos One* **10**: 1-19.

Van Der, S., Rust, H.M. & Chen, J. (2008). Dissecting the cell entry pathway of dengue virus by single-particle tracking in living cells. *Journal of Pathology* **4**: 250-256.

Vitta, A., Fukruksa, C., Yimthin, T. & Deelue, K. (2017). Preliminary survey of entomopathogenic nematodes in upper northern Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **48**: 18-26.

WHO. (2016). www.who.int/mediacentre/factsheets/fs117/en/

Yukiko, H. (2011). Dengue Vectors and their Spatial Distribution. *Tropical Medicine and Health* **39**: 17-27.