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Bacillus thuringiensis israelensis VCRC B650 culture filtrate useful for mosquito oviposition attractant and larvicidal action

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ABSTRACT: Oviposition attractants hold the potential attention for monitoring and controlling mosquitoes by enticing them to deposit eggs at specific locations. In this study, the bacterial culture filtrate of *Bacillus thuringiensis* var. *israelensis* VCRC B650, previously isolated from clay soil at an agricultural site in U.T of Puducherry, was investigated for the first time. This larvicidal bacterium was assessed for oviposition attractancy against dengue and filarial vectors, namely, gravid females of *Aedes aegypti* and *Culex quinquefasciatus*, respectively. The investigation revealed that, at 1:1 dilution of bacterial culture filtrate and water, *Cx. quinquefasciatus* exhibited significant attraction, with an oviposition activity index (OAI) value of +0.92, surpassing the standard threshold value of +0.30. The OAI of *Bti* culture filtrate at a 1:1 dilution was also compared with a standard oviposition attractant, p-cresol, at a concentration of 10ppm. It was observed that the *Bti* culture filtrate demonstrated a significant oviposition attractant effect, with 80 per cent more egg rafts laid, compared to p-cresol at 20 per cent. GC-MS analysis showed that there were 25 compounds present in the *Bti* culture supernatant, and out of those, only one compound, namely, benzaldehyde, was effective in showing oviposition attraction.

KEY WORDS: *Bti* culture filtrate, oviposition attractant, oviposition activity index, mosquitocidal activity, *Aedes aegypti, Culex quinquefasciatus*

INTRODUCTION

Mosquito species exhibit a wide range of behaviors when selecting suitable locations to deposit their eggs. Choosing the right site is a crucial aspect of the mosquito's reproductive cycle, given the potential risks associated with factors, such as, temporary water bodies, prolonged droughts, harsh winter conditions, and insufficient nourishment for larvae. The careful selection of an egg-laying site becomes paramount for the survival and successful development of mosquito offspring. These behaviors ensure that eggs are placed in environments conducive to the thriving development of mosquito larvae, ultimately leading to the emergence of the next mosquito generation (Eitam and Blaustein,

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2004). Understanding these diverse, oviposition behaviors is essential for devising effective strategies to monitor and control specific mosquito species that carry diseases (Wong *et al*., 2012). To produce a batch of eggs, female mosquitoes require a blood meal for the necessary protein. The breakdown of this blood meal is influenced by temperature, requiring two to three days in tropical areas and five to eight days in temperate zones. During the digestion process, byproducts like aminoacids are taken up by the fat-body, where the synthesis and release of vitellogenin (Vg), a glycophospholipoprotein, occur into the haemolymph. Subsequently, Vg is conveyed to the ovaries, where oocytes in the follicular epithelia absorb it (Wu *et al*., 2021). Various meteorological factors, such as, temperature, rainfall, relative humidity, wind, and others impact the flight and oviposition of gravid females. These females employ visual and olfactory cues while in flight to locate potential oviposition sites. The determination of a suitable oviposition site involves the use of visual, olfactory, and tactile signals (Beehler *et al.,* 1993; Day 2016). In the quest for suitable oviposition sites, mosquitoes rely on long-range infochemicals like pheromones for detecting their presence (Okal *et al*., 2013). As they approach a potential site, mosquitoes switch to short-range infochemicals to distinguish between suitable and unsuitable breeding locations for their upcoming generation. Once at an oviposition site, infochemicals become pivotal for gravid females in assessing the chemical characteristics of the potential habitats for immature stages. Mosquitoes employ contact stimuli to evaluate factors such as, water quality before engaging in oviposition (Mwingira *et al.,* 2020).

Chemical cues can elicit effective oviposition responses across mosquito species, with some species-specific cues identified in previous studies (McCall and Eaton 2001). Variations in breeding habitat preferences exist among different mosquito species, and these ecological distinctions influence the approach to their surveillance and control (Allgood and Yee, 2017). Microorganisms generate chemical cues that interact with chemosensory receptors (such as, antennae and mouth parts) and subsequently engage with other factors like pH and alkalinity when choosing locations for egg laying (Ponnusamy *et al*., 2008). The chemical cues from microorganisms may either act as repellents or stimulants for mosquito oviposition, depending on their concentration (Seenivasagan *et al*., 2014). Numerous studies have explored the potential of different plant substances, such as, cow grass, bamboo leaf infusions, barmuda grass infusion and hay infusions, to attract for oviposition (Millar *et al*., 1992; Ponnusamy *et al*., 2010). Natural attractants also include the temperature of humans, which has been identified as more appealing to mosquitoes (Schreck *et al.*, 1990; Ellwanger *et al*., 2021). The effectiveness of oviposition attractants is largely determined by the concentration of the infusion (Iyyappan *et al*., 2022). Studies indicate that bacteria obtained from hay infusion, specifically *Aerobacter aerogenes*, release volatile compounds that stimulate the oviposition of *Ae. aegypti* and *Cx. quinquefasciatus* gravid females (Hazard *et al*., 1967). Likewise, gravid *Cx. quinquefasciatus* has exhibited an attraction to protein hydrolysate solutions contaminated with bacteria (Beehler *et al*., 1994). The cell-free filtrate from *B. cereus*, *B. thuringiensi*s, and *Pseudomonas fluorescens* has demonstrated attractancy for gravid female mosquitoes of *Cx. quinquefasciatus* (Poonam *et al*., 2002). The chemical cues generated by microorganisms, in terms of quantity, compound nature, and composition, are likely to vary among bacterial species, influencing mosquito oviposition (Ponnusamy *et al*., 2015). The propensity of *Culex* mosquito species to deposit eggs in freshwater pools which are highly organic has been utilized to design effective gravid traps, for monitoring mosquito groups. Likewise, the preference of *Aedes* mosquito species for laying eggs in synthetic/plastic vessels has been leveraged to create ovitraps for monitoring and management purposes (Day, 2016).

A deeper comprehension of the diverse range of oviposition behavior of mosquitoes can enable the creation of innovative surveillance and management tools aimed at addressing other significant mosquito vectors that transmit diseases affecting both humans and animals. In the current study, explorative research was carried out to evaluate the oviposition attractancy of culture filtrate of

newly isolated *B. thuringienesis israelensis* VCRC B650 against gravid mosquitoes.

MATERIALS AND METHODS

Bacillus thuringiensis var. *israelensis* VCRC B650, a bacterial strain known for its mosquito larvicidal properties, was used for this study. Formerly, the bacterial strain was isolated from the clay soil from agricultural field of Bahour village, U.T of Puducherry, India. Nutrient Yeast Extract Mineral Salt Medium (NYSM) with the following constitutions per 100ml (wt./v %): 500mg of D-glucose, 500mg of peptone, 500mg of sodium chloride, 300mg of HM peptone, 500mg of yeast extract, and 1ml of a salt solution (containing magnesium chloride (20.3g), manganese chloride (1g), and calcium chloride (10.2g) mixed in one liter distilled water with pH adjusted to 7) was used for culturing the bacteria VCRC *Bti* B650 (Yousten *et al*., 1984; Hemaladkshmi *et al*., 2023). The bacterial culture derived from glycerol stock/agar slant was inoculated into 10 ml of the NYSM medium in a test tube using a loop. Subsequently, it was placed on a rotary shaker and incubated for 8 hours at 250-300 rpm and 28 to 30°C. There after, the bacterial culture was inoculated into a 250 ml a flask containing 50ml of NYSM medium and then incubated for a duration of 10 hours. Finally, 10ml of the inoculum was transferred to a 2 L flask containing 500ml of production medium, and it was then subjected to a 72 hour incubation period. The cell pellet was collected through centrifugation at 10,000 rpm for 10 minutes. After discarding the cell pellet, the culture filtrate was utilized as the test substance for oviposition attractancy evaluations.

Mosquito eggs collected from Rearing and Colonization Facility (RCF) in the parent institution, were placed in trays filled with dechlorinated water for hatching. The resulting mosquito larvae were raised in water and nourished with a combination of finely ground dog biscuits and yeast. After undergoing metamorphosis into pupae, they were collected and transferred to a bowl of water, before being introduced into mosquito cages measuring 23L×23B×23H cm, where the adult mosquitoes emerged. Subsequently, the adult mosquitoes were reared in cages and were provided with freshly soaked raisins as their primary diet. Later on, female mosquitoes were given a diet of fresh chicken blood on the third day after emerging. These gravid female mosquitoes were then used for the experimental procedures.

Optimal oviposition attraction: Three to five days old females of *Cx. quinquefasciatus* were fed on chicken blood and maintained on raisins soaked with water for 48 hours, at a temperature of 28 ± 2°C and a humidity level ranging from 70 to 80 per cent. Mature blood fed female mosquitoes were selected for evaluating the oviposition attraction to different substances. Various dilutions (1:1, 1:10, 1:20, and 1:50) of the study samples were concocted with water. 200 ml of each test dilution was poured into wax coated disposable paper cups with a capacity of 250ml, these cups were then placed inside a mosquito cage measuring 55L x 55B x 55Hcm. The Nutrient Yeast Extract Salt (NYSM) medium served as a control. In each cage, 100 fully gravid female mosquitoes were released. Three cages were utilized for each test and five disposable cups were consistently positioned in the cage for all the experiments. Four cups, each containing a distinct concentration of the bacterial culture filtrate, were arranged at the corners, while the cup, containing NYSM (used as a control), was positioned at the center of the cage. The cages were kept at a temperature of 28 ± 2°C and a relative humidity of 70-80 per cent. The study was initiated at 4pm (16:00 hour) and monitoring for the egg rafts, were conducted at 10am (10:00 hour), the following day. The count of egg rafts deposited in each cup was recorded, and the percentages deposited in various cups were determined based on the overall number laid, which included the control. The procedure was replicated thrice in different days, with changes in the position of disposable cups on each occasion (Geeta *et al*., 2003).

Oviposition-activity index (OAI): OAI of the test sample was assessed by positioning it inside a cage, alongside an additional cup containing NYSM as a control sample. The bacterial culture filtrate

was tested at various dilutions (1:1, 1:10, 1:20 and 1:50). As a standard oviposition attractant, p-cresol (10 ppm), was used (Bentley *et al*., 1979). The OAI was determined utilizing the following formula (Hwang *et al*., 1982):

OAI = Nt - Ns/Nt + NS

Nt represents, total-number-of -egg-rafts in-the-test-sample; Ns represents, total-number-of -egg-rafts in-the control Compounds exhibiting OAI of +0.3 and higher are categorized as attractants, whereas those with -0.3 and lower are classified as repellents (Hwang et al., 1982).

Comparative analysis with p-cresol: The attractancy of the different dilutions of bacterial culture filtrate for oviposition was individually assessed, comparing each with p-cresol by assessing them at concentrations where their attractancy is optimal. In each case, a cup with the bacterial culture filtrate and another cup with p-cresol were positioned at diagonally opposite corners of the cage. The counting and recording of the egg rafts were conducted in accordance with the previously specified procedures. The percentage of egg rafts deposited on each culture filtrate was computed in relation to the overall count, encompassing both the culture filtrate and p-cresol.

The data underwent statistical assessments using the Mann-Whitney U test to evaluate the significance of variation in oviposition attractancy. The Mann-Whitney U-test was used to evaluate the distinction between data of different groups. The level of significance (P values) less than 0.05 was considered as statistically significant. Complete statistical studies were done using STATA 14.2. These analyses aimed to determine whether there were significant variations in attractancy between the test preparations and p-cresol.

The aforementioned procedure was replicated with *Ae. aegypti* to investigate their attractancy to *Bti* VCRC B650.

Gas Chromatography- Mass Spectrometry: The bacteria culture filtrate of *Bti* VCRC B650 was lyophilized in freeze dryer until it formed dry powder. The powder obtained was then dissolved in methanol. The sample was sent for analysis for volatile component present in the filtrate which causes the mosquito to attract and enable them to oviposit. The analysis was done using gas chromatography- mass spectrometry with NIST library search in Sophisticated analytical-instrumentation facility (SAIF) at Indian-Institute-of-Technology, Madras. The components present in the culture filtrate were identified. A comprehensive literature review was conducted to identify compounds that are known to attract mosquitoes for oviposition. Through this review, a specific compound was pinpointed that has been documented to influence mosquito behavior, particularly in the context of egg-laying. This identification was crucial in understanding the potential role of this compound in guiding mosquito oviposition, thereby providing insights into its application in research or mosquito control strategies.

Mosquito larvicidal activity of the culture filtrate: Bioassay was conducted using bacterial culture filtrate at various dilutions (1:1, 1:10, 1:20, and 1:50) to evaluate its toxicity against first and second instar larvae of three mosquito species: *Cx. quinquefasciatus*, *Anopheles stephensi*, and *Ae. aegypti*. The experiment was set up in wax-coated paper cups, each containing 100 ml of the test dilutions. For comparison, a nutrient yeast salt medium (NYSM) was used as the control. In each bioassay, 25 larvae from each species were introduced into the cups containing the different culture filtrate dilutions. Four replicates were prepared for each test dilution, as well as for the control. Mortality rates of the larvae were observed and recorded at 24 hours post-exposure. The experiments were performed under controlled laboratory conditions, ensuring consistent relative humidity and temperature. To ensure the reliability of the results, the bioassay was repeated three times on different days.

Additionally, an experiment was conducted to assess the oviposition and subsequent larvicidal activity of the bacterial culture filtrate dilutions. Female mosquitoes were attracted to lay their eggs

in the various culture filtrate dilutions, once the eggs or egg rafts were deposited in the diluted culture filtrate, the containers were left undisturbed to allow the eggs to hatch naturally. The development and mortality of the larvae were closely monitored and recorded at 24 hours after the eggs had hatched.

RESULTS AND DISCUSSION

Optimum concentration for oviposition attractancy: From the initial experiment, the most effective bacterial culture filtrate dilution was found out to be 1:1 dilution in case of *Cx. quinquefasciatus* and 1:50 in case of *Ae. aegypti*. In case of *Culex* maximum number of 47 egg rafts were found in 1:1 dilution, 15 in 1:10 dilution, 7 in 1:20 dilution, 6 in 1:50 dilution and 1 in NYSM (control). In case of *Ae. aegypti* the higher oviposition was observed in 1:50 dilution with about 1862 eggs, 652 eggs in 1:20 dilution, 517 eggs in 1:10 dilution and 92 eggs in 1:1 dilution (Table 1) (Fig. 1).

Oviposition activity index (OAI): The analysis of OAI for *Bti* VCRC B650 bacterial culture filtrates revealed that all four dilutions exhibited oviposition attractancy against *Culex.* mosquitoes. The indices were 0.94, 0.83, 0.71, and 0.54 for dilutions 1:1, 1:10, 1:20, and 1:50, respectively (Fig. 2). These values exceeded the threshold of 0.3, indicating that these dilutions are considered attractants. However, for *Aedes*, the OAI values were 0.23, -0.26, -0.36, and -0.84, all of which were below the required threshold of 0.3 to be considered attractants (Fig. 3). Consequently, in the case of *Aedes*, the *Bti* B650 culture filtrate dilutions did not demonstrate oviposition attractancy.

Comparative analysis of test sample with p-cresol: When the oviposition attraction of culture filtrate with dilution 1:50 of *Bti* VCRC B650 was assessed against p- cresol which is a known oviposition attractant, at 10ppm the former was found to be more attracting for oviposition in case of *Cx. quinquefasciatus* with 80 per cent egg laying than the later with 20 per cent egg laying (Fig. 4). The proportion of eggs deposited in the culture filtrates for *Aedes aegypti* was lower than the quantity laid in p-cresol.

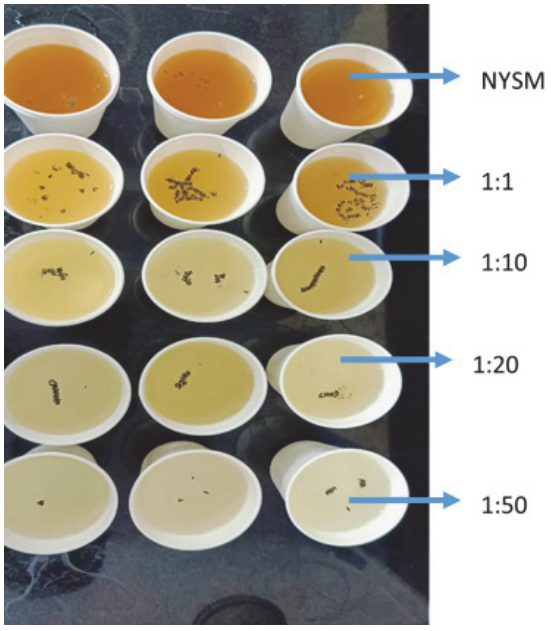

Fig. 1 *Culex quinquefasciatus* egg rafts in different concentrations of bacterial culture filtrate

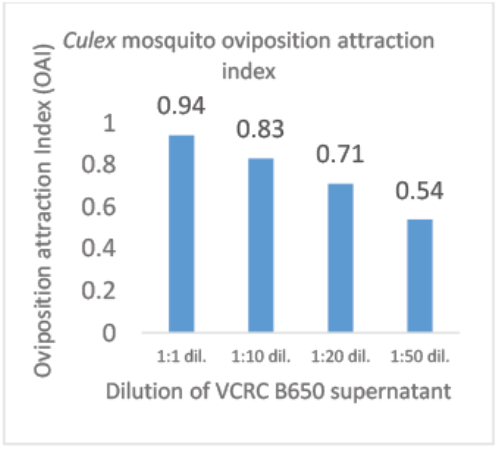

Fig. 2 Oviposition attraction index (OAI) against *Culex quinquefasciatus*

Gas chromatography- mass spectrometry: The analysis of *Bti* VCRC B650 bacteria culture filtrate through GC-MS revealed the presence of nine prominent compounds.

Through an extensive literature survey, it was determined that benzaldehyde has been documented in scientific literature as an attractant for *Aedes aegypti* and *Culex pipiens* (Dormont, 2021; Otienoburu *et al*., 2012). This finding suggests that the identified compound holds promise

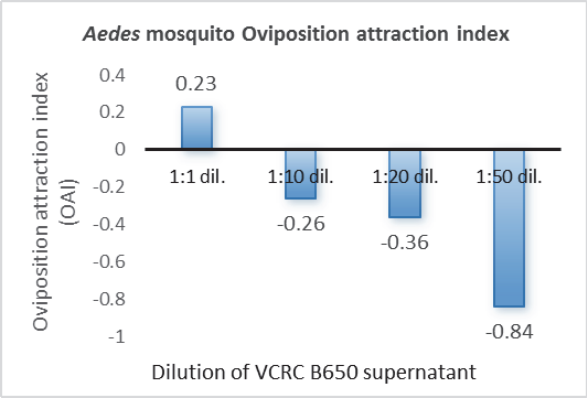

Fig. 3 Oviposition attraction index against *Aedes aegypti*

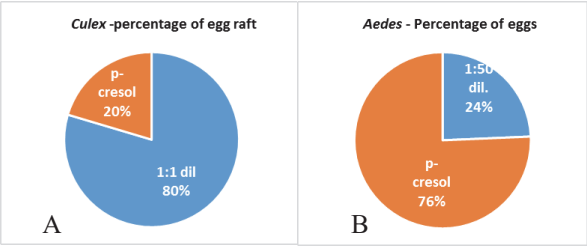

Fig. 4 Comparative analysis of the oviposition attractancy of the Bti. VCRC B650 in relation to p-cresol (A) with Culex mosquito (B) with Aedes mosquito

as a potential oviposition attractant for *Culex* species, indicating its potential for further exploration in mosquito behavior studies and control strategies, particularly in areas susceptible to vector-borne diseases (Table 3) (Fig. 5).

Mosquito larvicidal activity of the culture filtrate: The culture filtrate dilutions demonstrated significant mosquito larvicidal activity. In all dilutions tested, the larvae mortality was observed within few hours of hatching, indicating a potent mosquito larvicidal effect. In contrast, the control group (NYSM), showed no significant larval mortality, confirming that the observed lethality was directly attributable to the culture filtrate. In 1:1 dilution and 1:10 dilution the 1st instar larvae died within few hours of exposure. After 24 hours of exposure 100 per cent mortality was observed in all the bioassay cups (Table 4). The experiment demonstrated that even the lowest dilution, where 2 ml of culture filtrate was mixed with 100 ml of water, resulted in

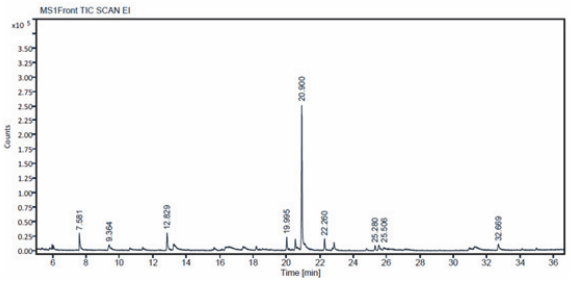

Fig. 5 Total ion chromatogram of Bti VCRC B650 culture filtrate

complete mortality of 1st and 2nd instar larvae within just 24 hours of exposure. This indicates the high potency of the culture filtrate, as it effectively caused 100% mortality at a relatively low concentration, proving its larvicidal activity against the early larval stages.

This high mortality rate among the larvae is likely due to the presence of secondary metabolites in the supernatant of the bacterial culture. These compounds, potentially produced during bacterial growth, appear to be highly effective in disrupting the development of mosquito larvae immediately after hatching. This experiment provided additional insights into the efficacy of the bacterial filtrate in preventing larval development, thus furthering the understanding of its potential as a mosquito control agent.

An attempt was made using the bacterial culture filtrate (culture supernatant), which is usually discarded as waste after harvesting the cell mass. These extracts significantly attract the gravid mosquitoes. In addition to this, the newly emerged first instar larvae died immediately. Literatures says that the chemical ecology of mosquito oviposition behavior has the potential to enhance our ecological knowledge regarding the source, function, and importance of natural organic compounds that play a role in interactions among mosquito species and their environment. Observing these interactions in their natural setting and understanding the compounds involved could pave the way for innovative strategies in the control and surveillance of mosquitoes and the diseases they transmit. Many current mosquito control methods primarily target

Table 1. Eggs deposited by *Culex quinquefasciatus* in different dilutions of *Bti.* VCRC B650 culture filtrate

Group	Number of egg rafts			P-value[1]	P-value[2]
	Mean(SD)	Median	Min – Max		
1:1	48.67(10.44)	47	41 – 75	0.0001	0.0003
1:10	14.56(3.09)	15	10 – 20		0.0003
1:20	7.44(1.51)	7	5 – 10		0.0003
1:50	4.89(2.57)	6	1 – 9		0.0037
NYSM	1.22(1.09)	1	0 – 3		Nil
Group	No. of egg rafts				P-value[2]
1:1	87.56(6.12)	85	79 – 98	0.0003	
P-cresol	22.44(2.50)	23	19 – 26	Nil	

[1]Kruskal-wallis test;[2]Mann-whitney U test

behaviors like biting and resting, and delving into the chemical ecology of oviposition could provide additional insights for more effective management approaches (Pates and Curtis, 2005). Various mosquito species demonstrate distinct preferences for laying their eggs by carefully choosing specific larval sites. Typically, mosquitoes steer clear of depositing eggs in locations already inhabited by competing species or potential predators. Their inclination is to lay eggs in environments where conspecific larvae are present, as this signifies the habitat's suitability for the survival of the upcoming generation. Consequently, mosquitoes exhibit a discerning approach when selecting sites for egg-laying, as they occupy a non-random assortment of aquatic habitats (Mwingira *et al.,* 2020). In present study, the prime aim of oviposition attractants has the potential for both surveillance and management of mosquitoes, as they entice them to deposit eggs at selected locations. Accordingly, the present study was focused on the novel mosquitocidal bacteria *Bti* VCRC B650 culture filtrate and it was tested for oviposition attractancy in gravid female of filarial and dengue vectors of *Cx. quinquefasciatus* and *Ae. aegypti* mosquitoes. Among the tested concentrations, 1:1 and 1:50 dilution has shown attractancy for *Cx. quinquefasciatus* and *Ae. aegypti* respectively. When their oviposition activity index (OAI) was evaluated, the culture filtrate of *Bti* VCRC B650 exhibited oviposition attractancy with different dilution (1:1, 1:10 and 1:20) for *Cx. quinquefasciatus* and *Ae. aegypti.* For *Cx. quinquefasciatus,* the OAI was found at 1:1 dilution (0.73) which was more than 0.3 required, thus it can be considered as a potential oviposition attractant. Hence, the mosquitoes are attracted to it to lay eggs and when the egg hatches, the larvae were immediately killed, thus this bacterial culture filtrate is also mosquitocidal in nature. However, for *Ae. aegypti,* the OAI was found for 1:50 dilution to be 0.23, thus OAI is below the required threshold of 0.3, indicating that they are not considered attractants. When the attractiveness of bacterial culture filtrates for oviposition was contrasted with a known oviposition attractant (p-cresol), at 10 and 3ppm respectively, the culture filtrate of *Bti* VCRC B650 1:1, 1:10, 1:20 and 1:10 were found to be more attractant than p-cresol. From the current study, it was identified that a compound *i.e.* Benzenaldehyde, which is reported to be attractant for *Culex* spp. in the culture filtrate of *Bti* VCRC B650 that could be investigated further for its attractiveness. This could involve synthesizing the compound or obtaining the synthesized form and testing it for its ability to attract mosquito oviposition. This approach may serve as a potential control method for vector mosquitoes.

The results corroborate the pioneer workers, wherein, the *Bti* (wild and mutants) and *B. sphaericus* culture filtrates proved attractancy at 2000 ppm with OAI of 0.71, 0.59, and 0.68, respectively. The cell-free filtrate from *B. cereus*, *B. thuringiensi*s, and *Pseudomonas fluorescens* has demonstrated attractancy for gravid female mosquitoes of *Cx. quinquefasciatus* (Poonam *et al.,* 2002). While certain cues associated with bacteria prompt oviposition at specific concentrations, elevated levels of the same cues, such as, tetra decanoic acid, or other cues produced by either the same or different bacteria, like hexadecenoic acid methyl ester, act as deterrents to oviposition (Ponnusamy *et al*., 2008). Gravid mosquitoes are also known to be attracted to a variety of volatile compounds generated by microbes, which helps them to find oviposition locations for depositing eggs (Girard *et al*., 2021).

Table 2. Eggs deposited by *Aedes aegypti* in different dilutions of *Bti.* VCRC B650 culture filtrate

Group	Number of eggs			P-value[1]	P-value[2]
	Mean(SD)	Median	Min – Max		
1:1	92.89(7.75)	96	80 – 103	0.0001	0.0003
1:10	517.56(76.38)	495	425 – 658		0.0003
1:20	652.33(109.68)	629	507 – 875		0.0003
1:50	1862.11(281.51)	1725	1599 – 2445		0.0003
NYSM	1121.89(112.16)	1088	1032 – 1379		Nil

Group	Number of egg rafts			
	Mean(SD)	Median	Min – Max	P-value2
1:50	889.11(67.88)	879	802 – 976	0.0003
P-cresol	2767.11(106.95)	2780	2600 – 2889	Nil

Burgeoning literatures indicate that bacteria obtained from hay infusion, specifically *Aerobacter aerogenes*, release volatile compounds that stimulate the oviposition of *Ae. aegypti* and *Cx. quinquefasciatus* gravid females (Hazard *et al*., 1967). Similarly, gravid *Cx. quinquefasciatus* has exhibited an attraction to protein hydrolysate solutions contaminated with bacteria (Beehler *et al*., 1994). The chemical cues generated by microorganisms, in terms of quantity, compound nature, and composition, are likely to vary among bacterial species, influencing mosquito oviposition (Poopathi, 2008). The propensity of *Culex* mosquito species to deposit eggs in freshwater pools which are highly organic has been utilized to design effective gravid traps, for monitoring mosquito groups. Likewise, the preference of *Aedes* mosquito species for laying eggs in synthetic/plastic vessels has been leveraged to create ovitraps for monitoring and management purposes (Day, 2016). Hazaed *et al*. (1967) conducted a study examining the impact of chemicals on attracting mosquitoes to breeding sites and triggering egg-laying behaviors. In a laboratory setting, gravid female mosquitoes of both *Aedes* and *Culex* species were exposed to options of water and moist substrate for oviposition. The findings revealed that 66 per cent of the females showed a preference for the odor of hay infusion over distilled water, and 78 per cent favored the bacterial solution over distilled water. The results of the present study indicate that the bacterial culture supernatant lured gravid females of *Cx. quinquefasciatus* and *Ae. aegypti* to deposit eggs and this is the first report that a mosquitocidal bacteria (*Bti* VCRC B650) isolated from clay soil from Pondicherry showed substantial effect in attracting and killing the mosquitoes.

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Table 3. Compounds identified through GCMS analysis

No	Retention time	Area %	Compound Name	Class of compound	Molecular formula
1	7.581	7.2	2-Piperidinone	Piperidinones	C5H9NO
2	9.364	2.34	2-Phenylacetamide	Benzenoids	C8H9NO
3	12.829	9.47	Benzaldehyde	Benzenoids	C7H6O
4	19.995	5.55	1,4-diazabicyclo [4.3.0]nonan-2,5-dione 3-methyl	Piperazine	C8H12N2O2
5	20.900	64.01	Pyrrolo [1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	Pyrrolopyrazine	C11H18N202
6	22.260	5.29	Cyclo(L-prolyl-L-valine)	Diketopiperazine	C10H16N2O2
7	25.280	2.21	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	Piperazine	C11H18N2O2
8	25.506	2.25	5,10-Diethoxy- 2,3,7,8-tetrahydro -1H,6H dipyrrolo[1,2-a:1',2-d] pyrazine	Pyrazine	C14H22N2O2
9	32.669	1.67	2'-Hydroxy-2,3,5'-trimethoxychalcone	Chalcone	C18H18O5

Table 4. Mosquito larvicidal activity of culture filtrate dilutions

Culture filtrate dilution	Composition for 100ml	mortality after 24 hours
1:1	50 ml culture filtrate + 50 ml water	100%
1:10	10 ml culture filtrate + 40 ml water	100%
1:20	5 ml culture filtrate + 45 ml water	100%
1:50	2 ml culture filtrate + 48 ml water	100%
NYSM (control)	NYSM	No mortality

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