



Effects of *Xenorhabdus* and *Photorhabdus* bacterial metabolites on the ovipositional activity of *Aedes albopictus*

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Abstract

Viral diseases like yellow fever, dengue, and Zika have an alarming impact on public health. These diseases can be transmitted by *Aedes* mosquito species, such as *Ae. albopictus*, which is now found in many countries outside its original range. *Xenorhabdus* and *Photorhabdus* spp. are enteric bacterial symbionts of insect-preying nematodes and are known to produce an array of natural products with various activities including larvicidal activity. In this study, the effects of natural products produced by four *Xenorhabdus* and one *Photorhabdus* bacteria on the ovipositional behavior of *Ae. albopictus* mosquitoes were assessed. Utilizing a binary choice assay in insect cages, gravid female mosquitoes were presented with two oviposition cups containing water supplemented with varying concentrations of bacterial supernatants (50–1% concentrations) versus control medium. After 72 h, the eggs deposited on filter papers were counted. The oviposition attractant index (OAI) feature of the bacterial supernatant was evaluated using the number of eggs laid in the cups. Notably, all tested supernatants exhibited concentration-dependent deterrence of oviposition. *Xenorhabdus cabanillasii* displayed the strongest deterrent effect, inhibiting egg-laying at 50–5% concentrations (OAI: – 0.87 to – 0.35), followed by *X. nematophila* (50–10%, OAI: – 0.82 to – 0.52). *Xenorhabdus szentirmaii*, *X. doucetiae*, and *P. kayaii* showed significant deterrence at $\geq 20\%$ concentrations. Using promoter exchange mutants generated by the easyPACId approach, fabclavine from *X. szentirmaii* was identified as the bioactive compound with evident deterrent effects. Such deterrents targeting egg-laying could be valuable for controlling populations by disrupting their breeding in suitable habitats.

Keywords Oviposition · Deterrence · Mosquito · *Xenorhabdus* · *Photorhabdus* · Fabclavines · easyPACId method

Key message

- Nematode-associated bacteria produce natural products with various biological activities

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- Mosquitoes abstained from laying eggs in containers with *Xenorhabdus* and *Photorhabdus* metabolites
- Fabclavines are peptide–polyketide–polyamines from *Xenorhabdus* sp. with oviposition deterrent effects
- Such compounds could be useful in mosquito control programs

Introduction

Aedes albopictus (Diptera: Culicidae), distinguished by white bands on its legs and body, is a synanthropic *Aedes* species originally endemic to tropical and subtropical regions of South Asia (Schaffner and Mathis 2014; Laporta et al. 2023). Due to its invasive and highly adaptive nature, it is now widely distributed in at least 30 countries throughout the tropics, subtropics, and temperate regions of the world outside Asia (Benedict et al. 2007; Fonseca et al. 2013; Touray et al. 2023). This spread has been greatly facilitated by rising temperatures due to climate change and transport of its immature stages (diapausing eggs and larvae) which are capable of withstanding cold conditions in temperate regions. These stages are transported in bamboo plants, used tires, and artificial containers during global shipping activities. Drastic deviations in global climate have also made its establishment in new habitats easier (Kraemer et al. 2015; Faraji and Unlu 2016; Beaty et al. 2016; Messina et al. 2019). Importantly, *Ae. albopictus* is a daytime-biting species that can take blood meals from a variety of host including humans, domestic animals and wildlife and along with *Ae. aegypti*, is a prominent vector of Zika, Chikungunya, dengue, and yellow fever—viral diseases whose epidemic potential and incidence has increased globally since the 1950s (Godfray 2013; Messina et al. 2019; Romanello et al. 2021; Bursali and Simsek 2023). Foremost among these arthropod infections, dengue is a predominantly urban disease of tropical and sub-tropical climates that has an alarming impact on public health with more than 3 billion people living in endemic areas and around 100 million cases, and at least 40,000 deaths occur annually in 195 countries (Zeng et al. 2021; Ahebwa et al. 2023). Symptoms include a characteristic skin rash, high fever, headache, muscle and joint pains. Severe cases may be life-threatening by causing serious bleeding and even death (Gubler 2002; Vos et al. 2015; Zeng et al. 2021). The economic cost related to *Aedes* diseases, particularly dengue, is estimated to at least US \$87.3 billion between 1975 and 2020 (Roiz et al. 2023) and funds dedicated to vector control and surveillance programs ranged from \$5.62–73.5 million (Thompson et al. 2020).

Aside from yellow fever, these *Aedes*-transmitted diseases have no vaccine or effective therapeutics (Smith et al. 2016; Huang et al. 2023); therefore, mosquito control professionals around the world monitor *Aedes* populations and evaluate novel control strategies to mitigate pathogen transmission (Schwab et al. 2018; Caputo and Manica 2020). A well-organized monitoring program aims to assess the occurrence, dispersal, and abundance of the target populations and includes inspections of potential breeding sites, employing ovitraps and adult traps, and human-landing-collections (Lühken et al. 2023). Control strategies include elimination of mosquito breeding sites or use of chemical larvicides in these breeding sites or insecticides against adult mosquitoes (Beaty et al. 2016; Weeratunga et al. 2017). Chemical adulticides (e.g., organophosphates such as malathion and naled, and pyrethroids such as prallethrin, etofenprox, pyrethrins, permethrin, resmethrin) are used. They are applied in indoor residual spraying, impregnated into bed nets or ultralow-volume aerial sprays in open areas using truck-mounted sprayers (Ochomo et al. 2024). Use of numerous insecticides is banned or restricted in most developed countries in America, Asia and Europe due to health and environmental issues, with exceptions granted only under strict conditions (Damico 2017). Nevertheless, *Ae. albopictus* mosquitoes are not well-controlled by the above strategies as location and complete elimination of breeding sites, especially artificial containers, in urban and poorly developed areas is often a challenging task (Faraji and Unlu 2016). Also, the effects of chemical insecticides are short-termed and continuous use of chemical insecticides has resulted in development of resistance to insecticides in many countries and most insecticides have damaging impacts on the environment (Smith et al. 2016). These issues have encouraged the use of environmentally friendly biological alternatives like the fungus, *Metarhizium anisopliae* (Aguilar-Durán et al. 2023; Perumal et al. 2023; Gomes et al. 2023) and the bacteria, *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Lysinibacillus sphaericus* as well as insect growth regulators (methoprene, diflubenzuron, pyriproxyfen) in breeding sites inspected for mosquito developing stages (Lacey 2007; Scholte et al. 2007; Benelli et al. 2016). These bio-control agents are eco-friendly, prevent water pollution, and do not harm humans and other useful organisms in the aquatic habitats (Pathak et al. 2022; Barathi et al. 2024). They have had successes in implemented countries and despite been used extensively for many years, there are no or few reports of reports of field resistance (Tetreau et al., 2013; Ferreira and Silva-Filha 2013). Over the years, much effort has been invested in the search for many more.

Xenorhabdus and *Photorhabdus* (Fam: Morganellaceae) are enteric Gram-negative bacterial symbionts of soil-dwelling and insect-preying nematodes in genera *Steinernema* and

Heterorhabditis, respectively (Hazir et al. 2022). These nematode-bacteria complexes have a near-global distribution, and as biological control agents are applied to soil to control pestiferous arthropods that are a menace to agriculture and public health (Shapiro-Ilan et al. 2019). *Xenorhabdus* and *Photorhabdus* bacteria are known to produce a startling array of enzymes and natural products (NP) that can suppress insect immunity, rapidly kill host, and disintegrate tissue, as well as perform defense functions against competitors and natural enemies through the use of polyketide synthases (PKSs) or non-ribosomal peptide synthetases (NRPS) (Bode 2009; Tobias et al. 2017; Cimen et al. 2022). So far, an important body of literature exists detailing the antimicrobial activities of these products from these bacteria against bacterial (Webster et al. 2002; Furgani et al. 2008), fungal (Fang et al. 2014; Shapiro-Ilan et al. 2014; Hazir et al. 2016; Chacón-Orozco et al. 2020; Cimen et al. 2021; Otoyá-Martínez et al. 2023), and protozoal pathogens (Antonello et al. 2018, 2019; Gulsen et al. 2022) as well as insect and mite pests (Sergeant et al. 2006; Hinchliffe et al. 2010; Incedayi et al. 2021) of medical, veterinary and agricultural importance. For instance, several recent works (da Silva JLR et al. 2013; Wagutu et al. 2017; da Silva JLR et al. 2017; Vítta et al. 2018; Shah et al. 2021) have demonstrated the larvicidal efficacy of cell-free bacterial supernatants and/or bacterial cell suspensions of *Xenorhabdus* and *Photorhabdus* on different mosquito species. Attempts to identify the bioactive natural product as potential larvicidal agents from these bacteria are ongoing. Furthermore, the calliphorid fly, *Chrysomya albiceps*, did not lay eggs on treating lamb meat with *P. luminescens* supernatant (Gulcu et al. 2012). Additionally, *Lobesia botrana* laid 55–95% fewer eggs on grapes treated with *X. nematophila* and *P. laumondii* supernatants when compared with untreated grapes (Vicente-Díez et al. 2023).

Ovipositing mosquitoes are fastidious creatures. After digesting a blood meal, gravid females seek out suitable aquatic habitats rich in nutrients for her offspring and devoid of potential predators based on the myriad of olfactory, visual, gustatory, and tactile signals emanating from breeding sites as well as environmental factors that can influence ovipositional behavior (Bentley and Day 1989; Day 2016; Parker et al. 2020; Baik and Carlson 2020; Girard et al. 2021). The literature on mosquito oviposition behavior is vast and several studies have established that factors such as presence of con- and heterospecific immature stages, microbes, predators and chemical cues associated with these organisms attract, repel or deter gravid mosquitoes from depositing eggs (Eitam and Blaustein 2004; Afify and Galizia 2015; Russell et al. 2022). Application of potential deterrent compounds to breeding sites might influence the ovipositional behavior of mosquitoes. Numerous compounds obtained from plants have been reported to have oviposition

deterrent activity against mosquitoes (Waliwitiya et al. 2009; Cheah et al. 2013; Dias and Moraes 2014). Development of deterrent compounds could be a powerful tool in mosquito control programs. Such deterrents can prevent breeding in certain habitats and as such help reduce mosquito population densities and dispersion in conducive areas (Cimen 2023).

The aim of this study was to assess the effects of metabolites of *Xenorhabdus* and *Photorhabdus* on the ovipositional behavior of *Ae. albopictus*. We investigated the effects of cell-free supernatant (CFS) of *Xenorhabdus* and *Photorhabdus* on mosquito ovipositional behavior and aimed to identify bioactive deterrent compounds.

Material and method

Maintenance of *Aedes albopictus* colony

Adult *Ae. albopictus* were reared in insect cages (40×40×40 cm) in a controlled insectary with a temperature of 30 °C, 70±10% relative humidity and a 12 h light: 12 h dark photoperiod. Mosquitoes were fed every 2–3 days on defibrinated sheep blood using an artificial blood feeder (Shah et al. 2021). Sugary water (10%) was available at all times. Mosquitoes laid eggs on filter papers in water filled cups. Eggs were hatched in tap water in small plastic containers (10×15×15 cm). Emerged larvae fed on crushed fish scales (TetraMin®) and maintained at 24±1 °C. Newly emerged adults (~1 week old) were used in the experiments.

Preparation of *Xenorhabdus* and *Photorhabdus* supernatants

Xenorhabdus cabanillasii JM26-1, *X. doucetiae* DSMZ 17909, *X. nematophila* ATCC 19061, *X. szentirmaii* DSMZ16338 and *Photorhabdus kayaii* DSMZ 15194 were used in this study. Cell free supernatants of these bacteria were obtained as described in Hazir et al. (2017). Briefly, bacteria were cultured on Luria–Bertani (LB) (10 g peptone, 5 g yeast extract, 5 g NaCl) agar (Merck, Darmstadt, Germany) for 24 h at 28 °C, and then, a single colony was inoculated into a fresh LB broth (20 ml) to prepare an overnight culture. One ml of this overnight culture was then inoculated into a fresh 100 ml LB medium which was incubated at 28 °C for 72 h. These cultures were then centrifuged (Eppendorf AG 22331, Germany) at 10,000 rpm at 4 °C for 10 min and the supernatants were separated into new Falcon tubes.

Generation of *Xenorhabdus* spp. Δ hfq pCEP-KM promoter exchange supernatants

To identify bioactive ovipositional deterrent compound(s) in the bacterial supernatants, Δ hfq promoter exchange mutants

of *Xenorhabdus* bacteria were used (Table 1). These mutants were generated using the easyPACId approach (easy Promoter Activated Compound Identification) (Bode et al. 2019, 2023) and have been used in previous studies (Incedayi et al. 2021; Cimen et al. 2021; Gulsen et al. 2022). Briefly, *X. szentirmaii* Δ hfq mutants were generated which results in no NP production, and subsequently, the native promoter regions of selected NP BGCs listed in Table 1 were replaced with a chemically inducible promoter P_{BAD} via the integration of the plasmid pCEP-KM; the biosynthesis of the selected BGC and subsequent selective (over) production of the associated single natural product (NP) compound class can be activated by the addition of L-arabinose (Bode et al. 2015, 2019, 2023; Tobias et al. 2017). HPLC/MS analysis of mutant cultures shows higher production of the selected compounds as compared to wildtype cultures. Detailed information about this mutant generation technique can be found in Bode et al. (2023).

Generated mutants were first cultured in LB agar + kanamycin (50 μ g/ml final concentration) and incubated at 30 °C for 48 h according to Cimen et al. (2021). Then, a single colony was transferred into 10 ml LB medium + kanamycin (50 μ g/ml final concentration) and incubated at 150 rpm and 30 °C to obtain an overnight culture which was transferred into 100 ml fresh LBs and the final optical density (OD_{600nm}) was adjusted to 0.1. These newly inoculated cultures were incubated for 1 h at 30 °C and afterward induced with 0.2% L-arabinose (Wenski et al. 2020). These induced cultures were incubated for 72 h at 150 rpm and 30 °C, and then, CFS was obtained and used in the oviposition experiments.

Table 1 *Xenorhabdus szentirmaii* Δ hfq pCEP-KMxy mutants used in this study

Bacteria species	Mutant name	Compound name
<i>X. szentirmaii</i>	Wildtype DSM 16338	–
	Δ hfq_pCEP_KM_3460	Szentiamid
	Δ hfq_pCEP_KM_3680	Xenobactin
	Δ hfq_pCEP_KM_3942	Rhabdusein
	Δ hfq_pCEP_KM_fclC	Fabclavine
	Δ hfq pCEP-KM-0377	PAX-short
	Δ hfq PCEP 3663	Xenoamicin
	Δ hfq Pcep-KM-5118	Pyrollizixenamamide
<i>X. cabanillasii</i>	Δ hfq_128-129	Fabclavine
<i>X. hominickii</i>	Δ hfq_130-131	Fabclavine
<i>X. budapestensis</i>	Δ hfq_pCEP_fclC	Fabclavine
<i>X. stockiae</i>	Δ hfq_pCEP_fclC	Fabclavine

Effects of wild-type and mutant bacterial supernatants on the ovipositional activity of *Aedes albopictus*

Experimental design was based on (Kramer and Mulla 1979) using a binary choice design with 10 newly blood-fed female mosquitoes in insect cages. After blood feeding, females were transferred to new cages from stock colonies using an aspirator and blood digestion and ovarian development was allowed for 4 days post-blood meal. Sugary water (10%) was available at all times. For the two choice experiments, two 100 ml-plastic cups with 40 ml distilled water containing CFS of wild-type *X. szentirmaii*, *X. cabanillasii*, *X. nematophila*, *X. doucetiae* or *P. kayaii* or LB (control) were introduced into the cages. Bacterial supernatants were tested at 50, 20, 10, 5 and 1% concentrations. Edges of the cups were lined with Whatman No. 2 filter papers as a substrate for collection of deposited eggs. These plastic cups were placed equidistant from each other at the corner of the cages, and their positions were alternated between replicates. The cages were placed in the insectarium at 27 ± 1 °C temperature, 70% relative humidity and 12 h photoperiod. After 72 h, the filter papers in each plastic cup were collected, and the eggs deposited on filter papers were counted under a stereomicroscope. Each treatment had four replicates and the experiments were conducted three times ($n = 12$).

Besides, commercial larvicidal compounds were compared with distilled water to determine if they have any ovipositional deterrent activity. The active ingredient in the larvicides used was Spinosad, *Lysinibacillus sphaericus*, or *Bacillus thuringiensis* subsp. *israelensis* (Table 2). These larvicides were added to distilled water at doses recommended by the manufacturer (Table 2), and then, 40 ml of prepared solution was added to oviposition cups used in the experiments. Field-collected water was also assessed against distilled water.

The oviposition attractant/deterrence feature of the bacterial supernatant was evaluated by calculating the oviposition activity index (OAI) using the number of eggs laid in the cups (Kramer and Mulla 1979; Hwang et al. 1982). The OAI was determined using the formula.

$$OAI = \frac{(\text{Number of eggs in treated water} - \text{number of eggs in control})}{(\text{Number of eggs in treated water} + \text{number of eggs in control})}$$

And the scores were used for analysis of variance ($p = 0.05$). An OAI score close to -1 shows a high deterrence, between $+0.3$ and -0.3 shows neutrality, and $+1$ shows a strong attraction (Hwang et al. 1982). The same method was used with cell-free supernatants obtained from promoter exchange mutants shown in Table 1 to identify the compound responsible for the attractant/deterrence activity. Different derivatives of the bioactive compound obtained from different bacteria (i.e., *X. hominickii*, *X. cabanillasii*, *X.*

Table 2 Commercial larvicidal products used in the study

Larvicide	Commercial product	Recommended concentration	Formulation
<i>Bacillus thuringiensis</i> subsp. <i>israelensis</i>	Vectobac® 12AS	0.19 ml/L	SC
Spinosad	Moskill 120SC	3.3 ml/L	SC
<i>Lysinibacillus sphaericus</i>	Vectolex WDG	5 g/L	WDG

SC suspension concentrate, WDG water-dispersible granule

budapestensis, *X. szentirmaii*, *X. bovienii*, and *X. stockiae*) were also assessed using the binary choice method.

Performance of bioactive oviposition deterrent compounds

After identifying the bioactive compound/s, multiple choice experiments were also conducted. In this case, four plastic cups (100 ml) with the components below were added to cages with 10 female mosquitoes (a week old):

Choice experiment-1 (baseline experiment) cup A-Field collected water, cup B-clean water, cup C-distilled water with 10 larvae (3rd stage), and cup D-LB-water (control).

Choice experiment-2 cup A-Field collected water with supernatant (20%) from Fabclavine producing *X. szentirmaii* (Δ hfq_pCEP_KM_fclC), cup B-clean water, cup C-distilled water with 10 larvae, and cup D-LB-water (control).

Choice experiment-3 cup A-Field collected water, cup B-clean water with supernatant (20%) from Fabclavine producing *X. szentirmaii* (Δ hfq_pCEP_KM_fclC), cup C-distilled water with 10 larvae, and cup D-LB-water (control).

Choice experiment-4 cup A-Field collected water, cup B-clean water, cup C-distilled water with larvae (10) treated with supernatant (20%) from Fabclavine producing *X. szentirmaii* (Δ hfq_pCEP_KM_fclC), and cup D-LB-water (control).

The total liquid volume in each cup was 40 ml. The plastic cups insides lined with filter paper were placed in the four corners of the insect cages. Eggs were counted after 3 days, and the OAI was calculated as described previously. The position of the cups was rotated daily during the experiments. These experiments had 5 replicates, and the experiments were conducted twice. This multiple choice design allows mosquitoes to choose between options, reflecting natural behavior (choosing oviposition sites).

Statistical analyses

Analysis of variance with Tukey's test ($p = 0.05$) was used to compared OAI as well as a number of eggs laid in multiple choice experiments in the statistical analysis using SPSS program (version 23).

Results

Effects of wild-type and mutant strain bacterial supernatants on the ovipositional activity of *Aedes albopictus*

The oviposition deterrence activity of CFS from different wild-type *Xenorhabdus* and *Photorhabdus* bacteria against *Ae. albopictus* is presented in Fig. 1. All bacteria supernatants effectively deter oviposition of female mosquitoes in a concentration-dependent manner with significant difference between all treatments ($F = 9.328$; $df = 29,303$; $p < 0.001$). Supernatant from *X. cabanillasii* displayed deterrent effects at concentrations 50–5%. Oviposition activity index values ranged between -0.87 and -0.35 . Index values for *X. nematophila* were -0.82 , -0.85 , and -0.52 at 50, 20 and 10% concentrations, respectively. *Xenorhabdus szentirmaii*, *X. doucetiae*, and *P. kayai* were effective at concentrations $\geq 20\%$. Among the controls, the mosquitoes only abstained from treatments with *L. sphaericus* with an OAI of -0.47 . *Aedes albopictus* females were neutral to effects of *B. thuringiensis israelensis* and Spinosad on egg laying. Field collected water was significantly more attractive than distilled water (Fig. 1).

Various mutants generated from *X. szentirmaii* were used to identify the respective deterrent compounds present in supernatants. Fabclavine (OAI = -0.72) as the only compound with evident deterrent effects in *X. szentirmaii* (Figs. 2, 3).

The OAI of fabclavine derivatives obtained from *X. hominickii*, *X. cabanillasii*, *X. budapestensis*, *X. szentirmaii*, *X. bovienii*, and *X. stockiae* on *Ae. albopictus* were -0.89 , -0.88 , -0.86 , -0.75 , -0.57 and -0.53 , respectively. There was a statistical difference only in the deterrent effects between *X. hominickii* and *X. stockiae* ($F = 3.266$; $df = 5, 46$; $P = 0.14$) (Fig. 4).

Performance of bioactive ovipositional deterrent compounds

There was a statistically significant difference in the number of eggs laid by *Ae. albopictus* females in the multiple-choice experiments with 4 different containers that had either field

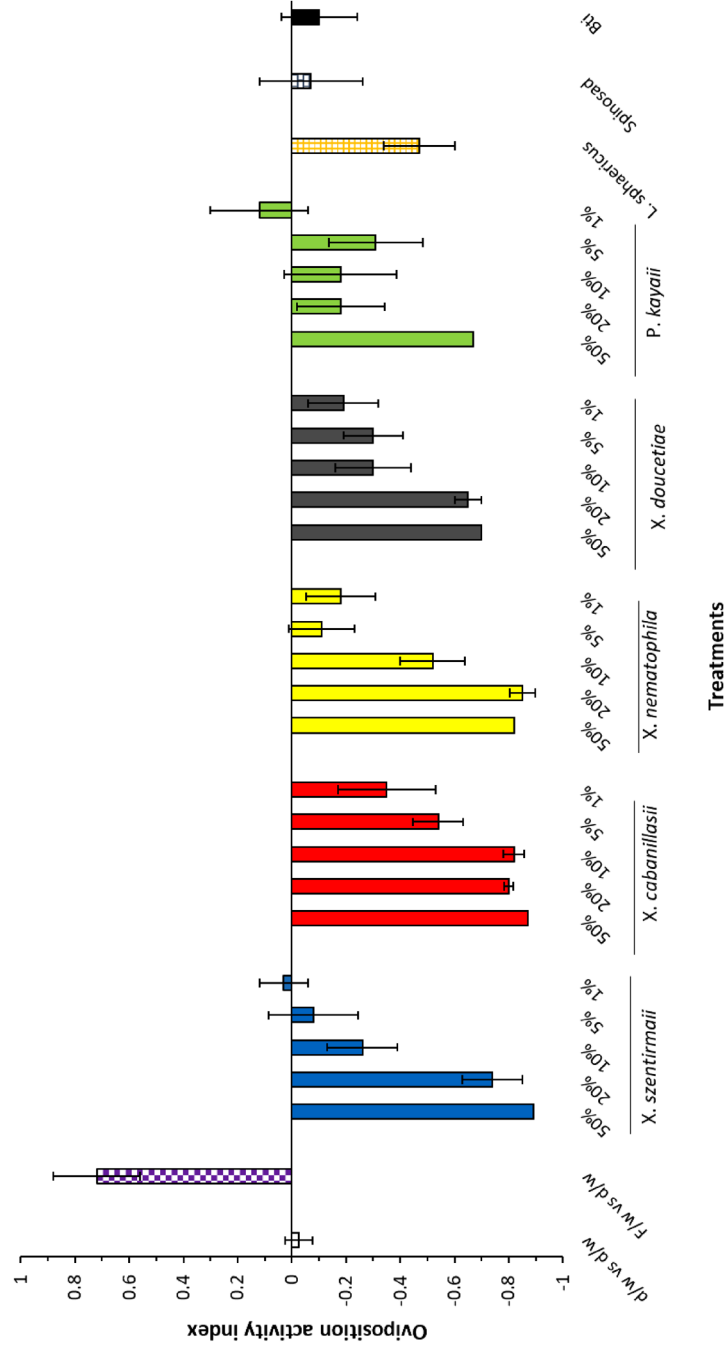


Fig. 1 The ovipositional deterrence effects of *Xenorhabdus* and *Photobacterium* bacteria on *Aedes albopictus*. d/w = distilled water; f/w = field collected water; Bti = *Bacillus thuringiensis israelensis* (0.19 ml/L); Spinosad (3.3 ml/L) and *L. sphaericus* (5 g/L). An OAI score close to -1 shows a high deterrence, between +0.3 and -0.3 shows neutrality, and +1 shows a strong attraction

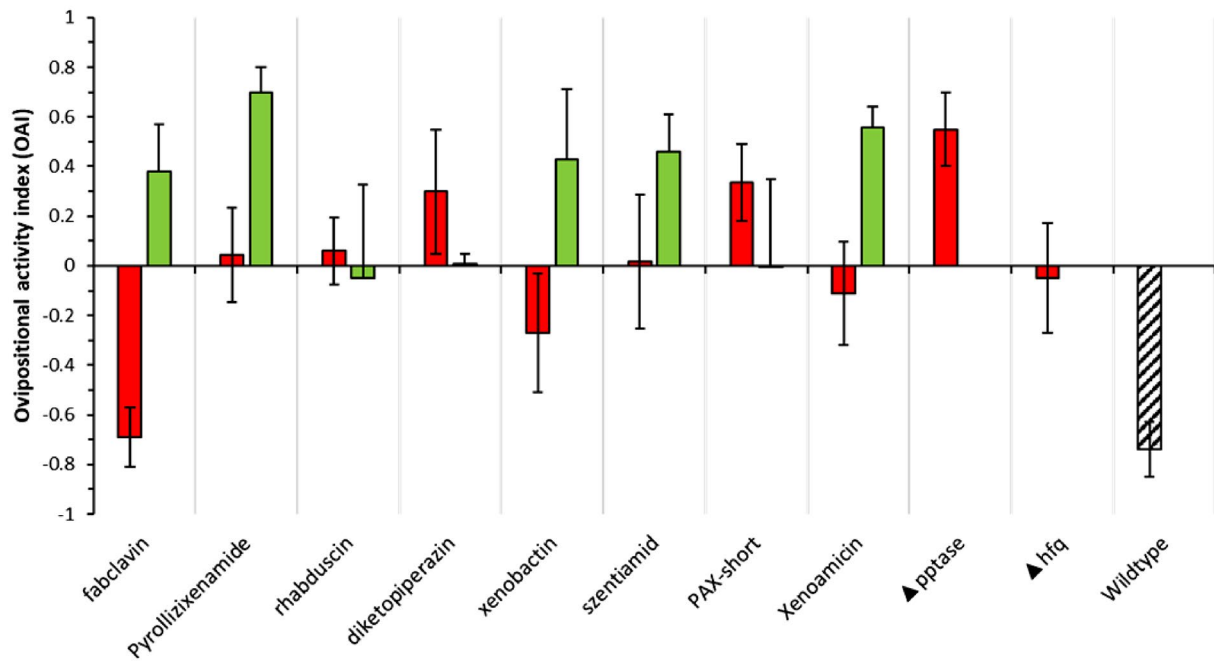
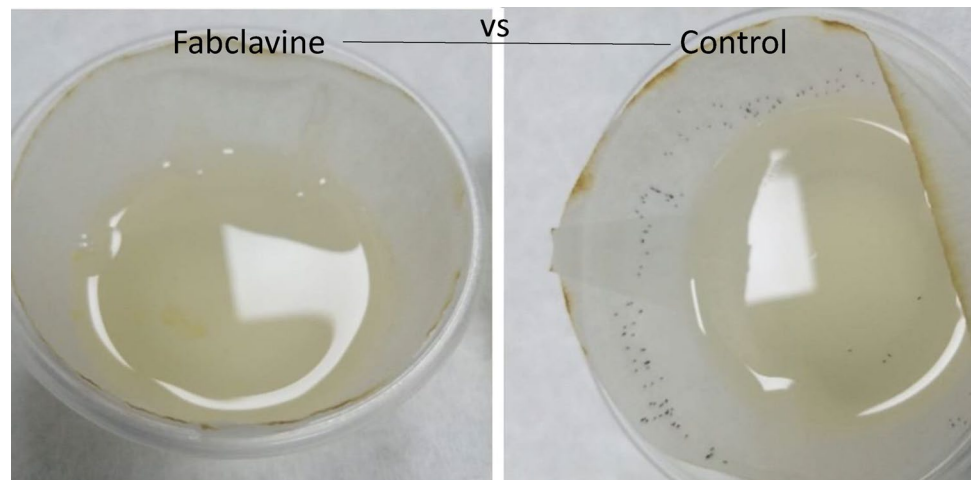


Fig. 2 Activity of different secondary metabolites from *Xenorhabdus szentirmaii* on the ovipositional behavior of *Aedes albopictus*. An OAI score close to -1 shows a high deterrence, between $+0.3$ and -0.3 shows neutrality, and $+1$ shows a strong attraction

Fig. 3 Ovipositional deterrence effects of fabclavine from *Xenorhabdus szentirmaii* on *Aedes albopictus* females



collected water, supernatant (treated), water with larvae (10), LB-water (control) or clean water (Fig. 5). In the first combination (choice experiment-1), which served as a control, field water was found to be the most attractive breeding site for the female with at least 140 eggs laid here. In choice experiments 2 and 4, females were observed to abstain from containers where fabclavine had been added (Fig. 5), even reducing the attractiveness of field collected water. The number of eggs laid in the containers with field collected water and fabclavine in the choice experiment-3 was nearly halved (73 eggs) compared to the other combinations.

Discussion

Our data show that *Ae. albopictus* abstained from laying eggs in containers with supernatants of *Xenorhabdus* and *Photorhabdus*. All bacteria supernatants effectively deterred oviposition of female mosquitoes in a concentration-dependent manner.

Several reports have highlighted the importance of the bioactive natural product of *Xenorhabdus* and *Photorhabdus* as potential larvicidal agents on different mosquito species (da Silva JLR et al. 2013; Wagutu et al. 2017; da Silva JLR

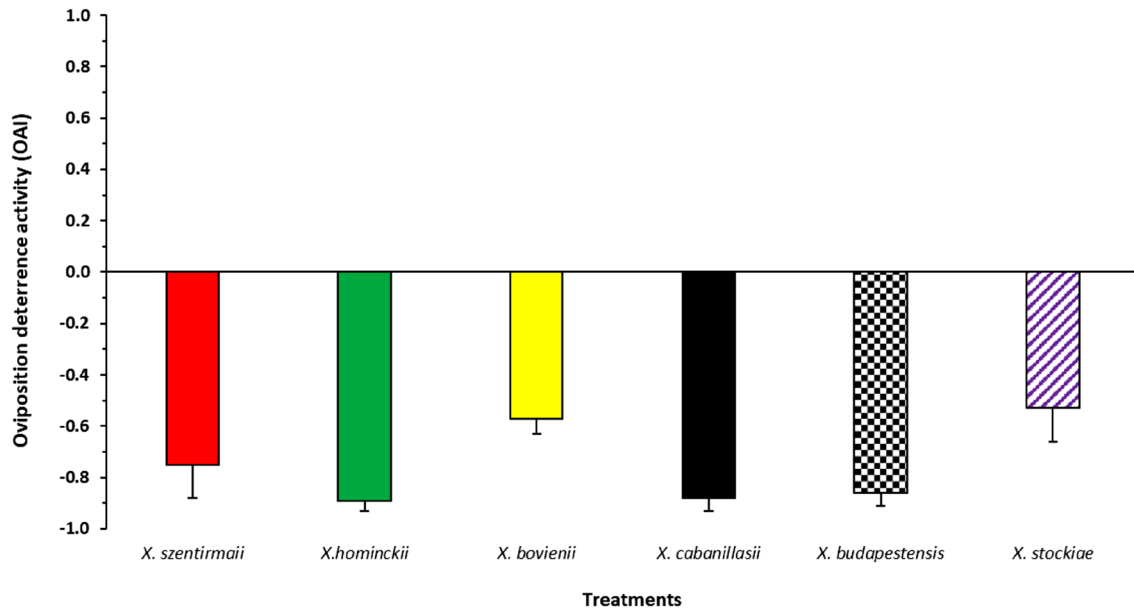


Fig. 4 The percentage deterrent effects of different fabclavine derivatives from different *Xenorhabdus* spp. An OAI score close to -1 shows a high deterrence, between $+0.3$ and -0.3 shows neutrality, and $+1$ shows a strong attraction

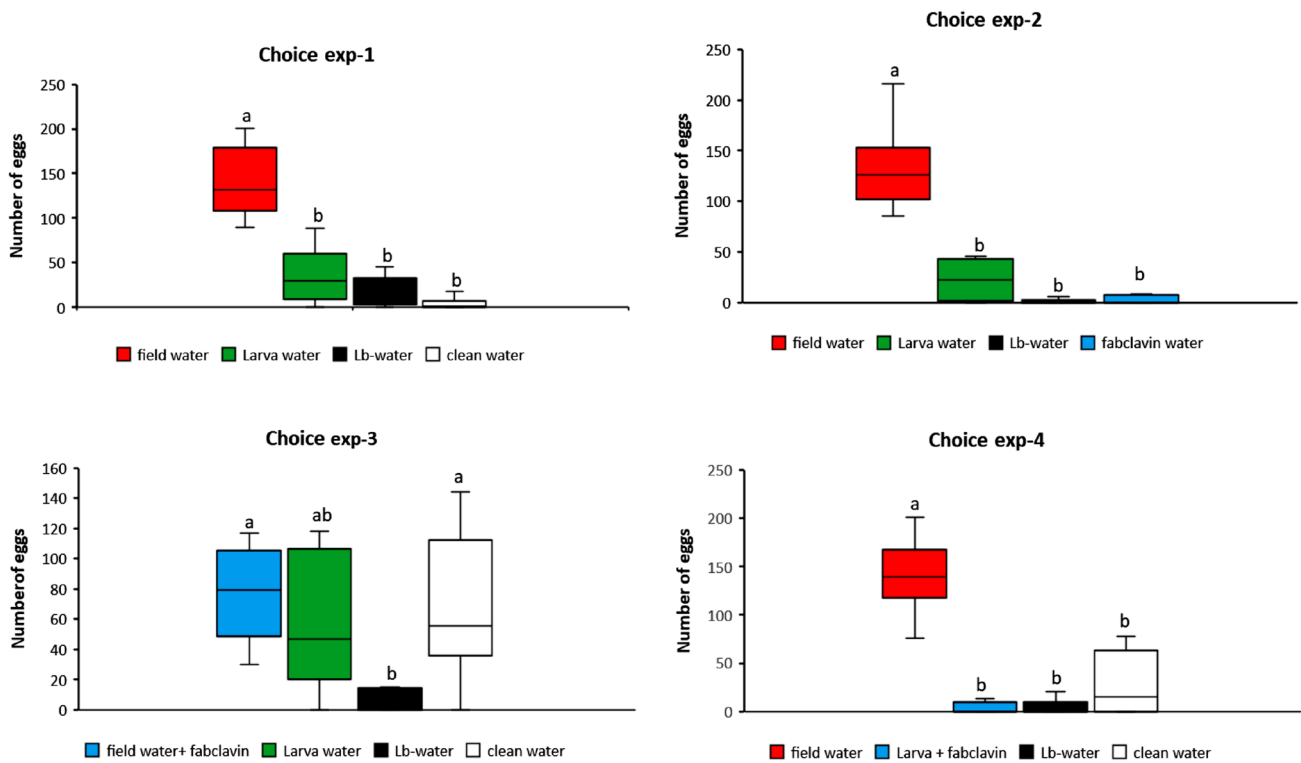


Fig. 5 Deterrent effects of fabclavine from *Xenorhabdus szentirmai* on *Aedes albopictus* females in the multiple-choice experiments. Letters above boxplots indicate differences according to analysis of variance ($p=0.05$)

et al. 2017; Vitta et al. 2018; Shah et al. 2021; Subkrasae et al. 2022; Yüksel et al. 2023). Our study, as a first, demonstrates that *Xenorhabdus* and *Photorhabdus* spp. produce highly effective ovipositional deterrent compounds against mosquito *Ae. albopictus*. The easyPACId method showed clearly that the bioactive deterrent compound was fabclavine, a non-ribosomally synthesized peptide/polyketide with polyamine moieties and broad-spectrum bioactivities (Wenski et al. 2020; Duan et al. 2024). Studies have shown that derivatives from different species can differ greatly in structure and bioactivity, e.g., *X. bovienii* produces derivatives with only the polyamine part (Wenski et al. 2020, 2021). An important body of literature exists detailing the possible application of fabclavines against various bacterial (Donmez Ozkan et al. 2019), fungal (Cimen et al. 2021; Yuan et al. 2023), and protozoal organisms (Gulsen et al. 2022) of medical, veterinary, and agricultural importance. Fabclavines are produced to kill adversaries of the EPN-bacteria complex and help maintain a monoxenic environment within the infected host (Fuchs et al. 2014; Wenski et al. 2020, 2021).

Female mosquitoes are highly sensitive to environmental factors. They can sense the myriad of olfactory, visual, gustatory, and tactile signals emanating from breeding sites using the sensilla in antennae, maxillary palps, proboscis, and taste organs including tarsal segments of the legs (Baik and Carlson 2020; Girard et al. 2021). Ovipositional behavior is also influenced by various environmental factors such as fluctuations in temperature, rainfall, relative humidity (Bentley and Day 1989; Day 2016; Parker et al. 2020; Girard et al. 2021). Mosquitoes generally seek out suitable aquatic habitats rich in nutrients for their offspring and devoid of potential predators (Bentley and Day 1989; Day 2016; Baik and Carlson 2020). These flies can even sense the presence of con- and heterospecific immature stages and predators and chemical cues associated with these organisms, and they are attracted, repelled or deterred from depositing eggs in such sites (Afify and Galizia 2015; Russell et al. 2022). Our study demonstrates that the application of fabclavine and *L. sphaericus* influences the ovipositional behavior of mosquitoes. The effects of *B. thuringiensis israelensis* and spinosad were neutral. Likewise, Nazni et al. (2009) showed that *Ae. albopictus* females have not been repelled from Bti treated containers. However, we detected that the females did not lay eggs or laid a few in containers where fabclavine had been added. Fabclavine derivatives obtained from *X. hominickii*, *X. cabanillasii*, *X. budapestensis*, *X. szentirmaii*, *X. bovienii*, and *X. stockiae* had varied deterrent activities on *Ae. albopictus*. Fabclavine from *X. szentirmaii* even reduced the attractiveness of field collected water.

Mosquitoes landing on the wet filter papers on the sides of treated containers likely sensed fabclavines, a highly polar and water-miscible compound, through sensilla in tarsal segments of their legs, prompting them to abstain from egg laying and move away. The mode of action of fabclavine on female mosquitoes needs to be elucidated.

Interestingly, despite lacking the biosynthesis gene cluster responsible for fabclavine production *X. nematophila*, *X. doucetiae* and *P. kayaii* bacteria still exhibit oviposition deterrence activity (Fig. 1) (Tobias et al. 2017). This suggests the presence of another, yet unidentified, deterrent compound.

As far as we are aware this is the first report of the oviposition deterrent effects of bacterial secondary metabolite on mosquitoes. Poonam et al. (2002) investigated the effects of CFS of *Azospirillum brasilense*, *B. cereus*, *B. megaterium*, *Pseudomonas fluorescens*, *B. thuringiensis* var. *israelensis* and *B. sphaericus*, on the oviposition activity of *Culex quinquefasciatus* females. They observed that depending on concentration CSF of these bacteria exhibited attractancy comparable to p-cresol, a known oviposition attractant. On the other hand, numerous compounds mainly from plants have been reported to have ovipositional deterrent activity against mosquitoes (Waliwitiya et al. 2009; Cheah et al. 2013; Dias and Moraes 2014). Waliwitiya et al. (2009) reported that *Ae. aegypti* laid fewer eggs in cups with eugenol, pulegone, thymol, trans-anethole or citronellal extracted from rosemary (OAI ranged between -0.5 and -0.8). Studies show that essential oils from *Piper marginatum* leaves, stems, and flowers exhibit moderate oviposition deterrence against *Ae. Aegypti* (OAI = -0.4) (Autran et al. 2009).

In conclusion, this study clearly demonstrated that bacterial metabolites from *Xenorhabdus* and *Photorhabdus* species are potent deterrents for oviposition in *Aedes albopictus*. Specifically, supernatants from these bacteria, particularly *X. cabanillasii* and *X. nematophila*, exhibited strong concentration-dependent inhibition of egg-laying by the mosquitoes. Notably, fabclavine isolated from *X. szentirmaii* was identified as the key bioactive compound responsible for this deterrent effect. Development of compounds with deterrence effects could be useful in mosquito control programs as such compounds can prevent gravid mosquito females from breeding in certain habitats. Choice of breeding site can greatly influence mosquito species establishment, population densities and dispersion in conducive areas. Use of oviposition deterrent compounds in specific sites would make the mosquitoes find other oviposition sites; these attractive sites can be used for target killing, hence reducing the amount of pesticides used.

Author contributions

MT and SH designed the experiments, analyzed the data and wrote the manuscript; MT and HC conducted the experiments; HBB and EB generated the mutants strains; and all authors reviewed the manuscript.

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Data availability The data that support the findings of this study are available from the corresponding author, [MT], upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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