Journal of Pest Science (2024) 97:2203–2215
https://doi.org/10.1007/s10340-024-01760-7

ORIGINAL PAPER

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

Mustapha Touray[1] · Harun Cimen[2] · Edna Bode[3] · Helge B. Bode[3,4,5,6,7] · Selcuk Hazir[1,8]

Received: 9 October 2023 / Revised: 5 February 2024 / Accepted: 12 February 2024 / Published online: 21 March 2024

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 ≥ 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

---

Communicated by Andre Wilke.

✉ Mustapha Touray
mtpha.touray@gmail.com

[1] Department of Biology, Faculty of Science, Aydin Adnan Menderes University, Aydin, Turkey

[2] Recombinant DNA and Recombinant Protein Center, Aydın Adnan Menderes University, Aydin, Turkey

[3] Department of Natural Products in Organismic Interactions, Max-Planck-Institute for Terrestrial Microbiology, 35043 Marburg, Germany

[4] Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, Max-Von-Laue-Str. 9, 60438 Frankfurt, Germany

[5] Center for Synthetic Microbiology (SYNMIKRO), Phillips University Marburg, 35043 Marburg, Germany

[6] Department of Chemistry, Phillips University Marburg, 35043 Marburg, Germany

[7] Senckenberg Gesellschaft für Naturforschung, 60325 Frankfurt am Main, Germany

[8] Department of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu 602105, India

- 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 biocontrol 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 starling 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; Otoya-Martinez 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; Vitta 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 albicep*s, 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 deterrence 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 deterrence 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. Δhfq pCEP-KM promoter exchange supernatants

To identify bioactive ovipositional deterrent compound(s) in the bacterial supernatants, Δhfq 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* Δhfq 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 µg/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 µg/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* Δhfq pCEP-KMxy mutants used in this study

Bacteria species	Mutant name	Compound name
X. szentirmaii	Wildtype DSM 16338	–
	Δhfq_pCEP_KM_3460	Szentiamid
	Δhfq_pCEP_KM_3680	Xenobactin
	Δhfq_pCEP_KM_3942	Rhabduscin
	Δhfq_pCEP_KM_fclC	Fabclavine
	Δ*hfq* pCEP-KM-0377	PAX-short
	Δhfq PCEP 3663	Xenoamicin
	Δhfq Pcep-KM-5118	Pyrollizixenamide
X. cabanillasii	Δhfq_128-129	Fabclavine
X. hominickii	Δhfq_130-131	Fabclavine
X. budapestensis	Δhfq_pCEP_fclC	Fabclavine
X. stockiae	Δhfq_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.

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

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
Bacillus thuringiensis subsp. *israelensis*	Vectobac® 12AS	0.19 ml/L	SC
Spinosad	Moskill 120SC	3.3 ml/L	SC
Lysinibacillus sphaericus	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* (Δhfq_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* (Δhfq_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* (Δhfq_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. kayaii* were effective at concentrations ≥ 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

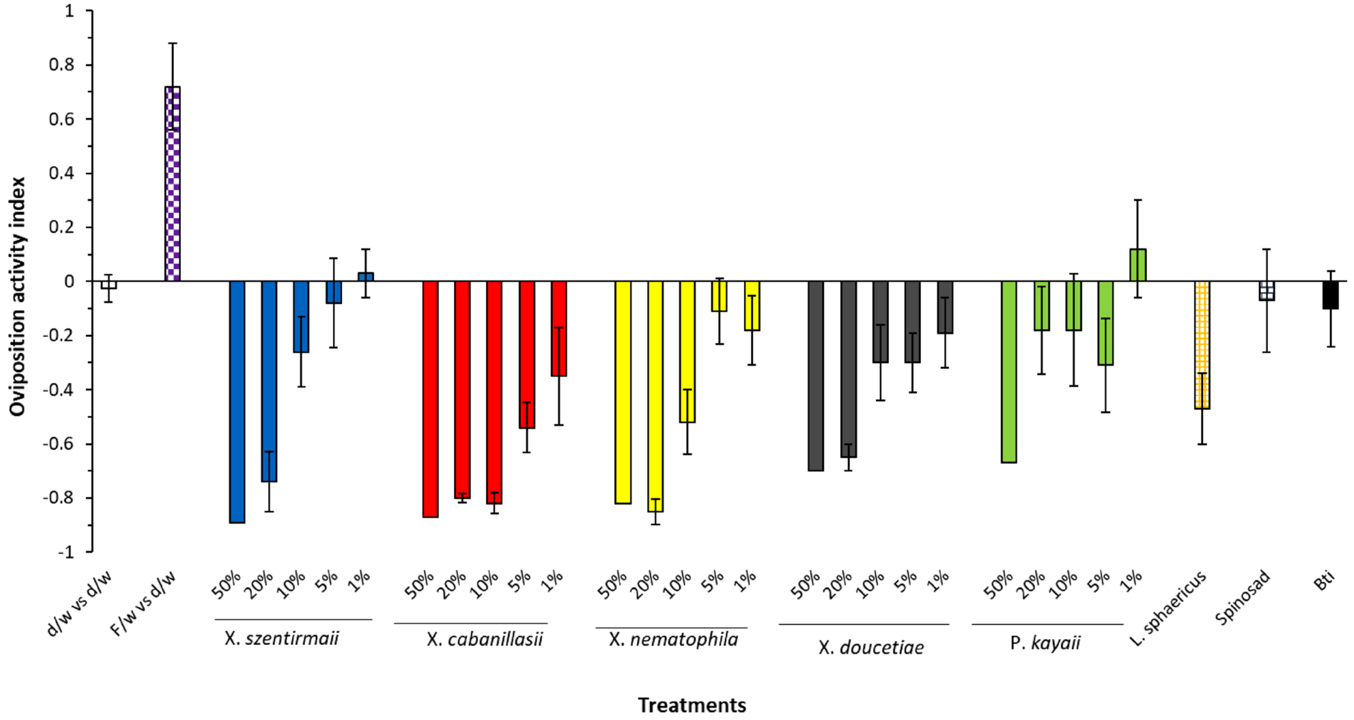


Fig. 1 The ovipositional deterrence effects of *Xenorhabdus* and *Photorhabdus* bacteria on *Aedes albopictus.* d/w = distilled water; f/w = field collected water; Bti = *Bacillus thuringiensis israelensis* (0.19 ml/L)*; Spinosad* (3.3 ml/L) and *L. sphaericus* (5 g/L). An OAI score close to − 1 shows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

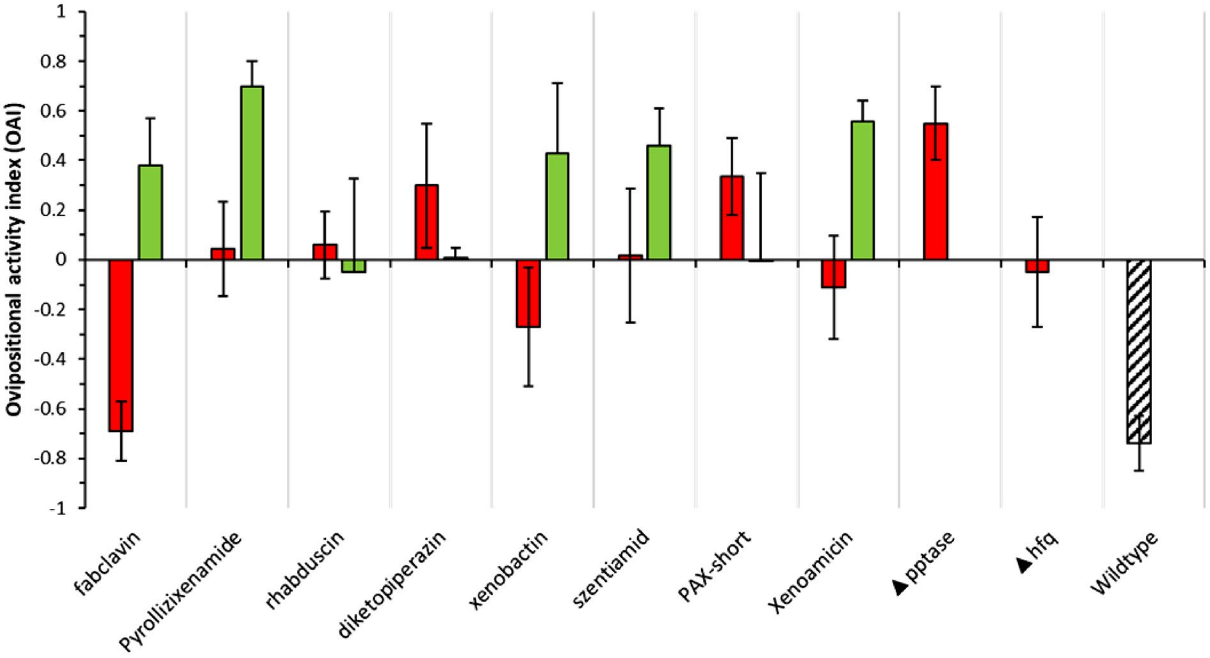
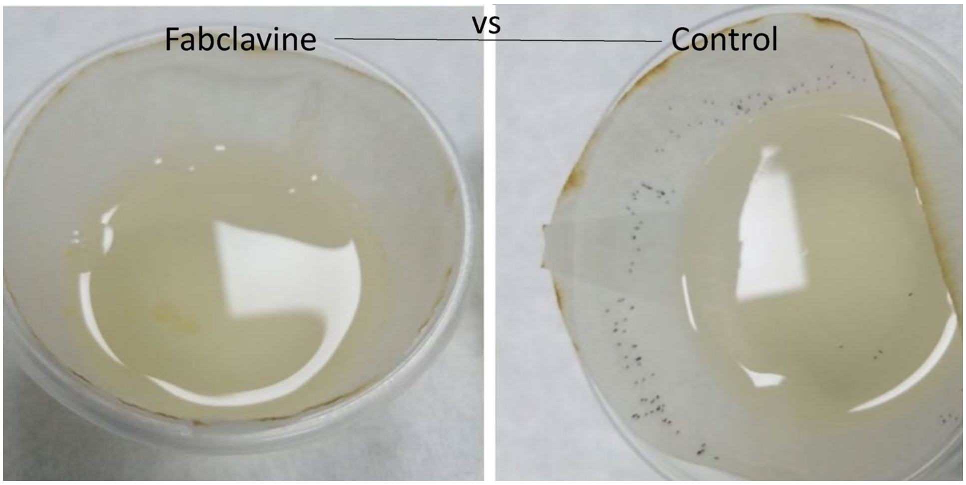


Fig. 2 Activity of different secondary metabolites from *Xenorhabdus szentirmaii* on the ovipositional behavior of *Aedes albopictus*. An OAI score close to − 1 shows a high deterrence, between + 0.3 and −0.3 shows neutrality, and + 1 shows a strong attraction

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

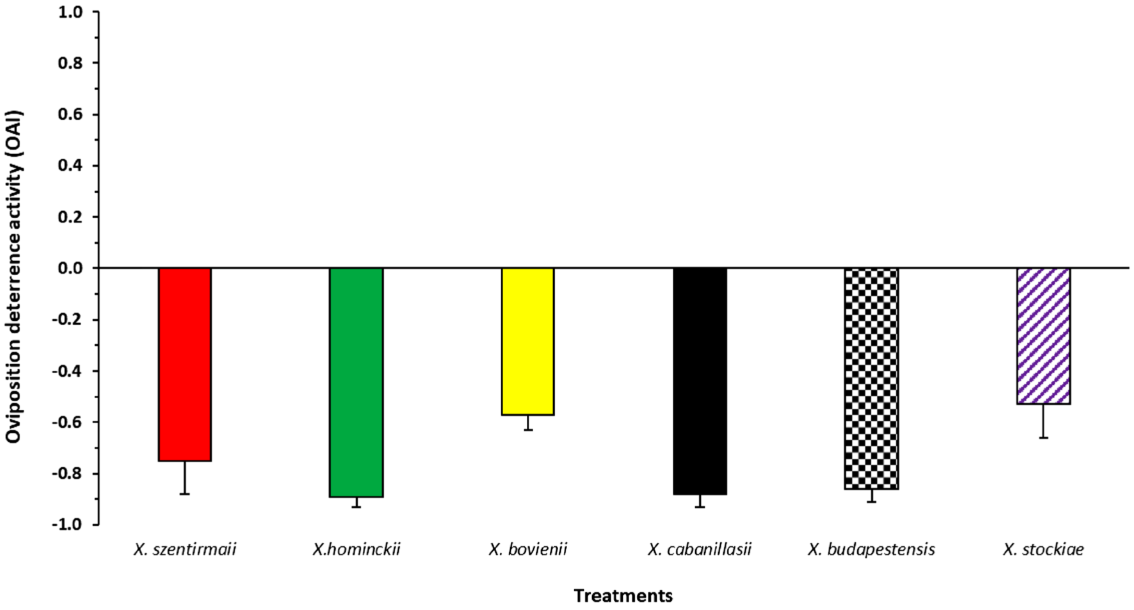


Fig. 4 The percentage deterrent effects of different fabclavine derivatives from different *Xenorhabdus* spp. An OAI score close to − 1 shows a high deterrence, between + 0.3 and −0.3 shows neutrality, and + 1 shows a strong attraction

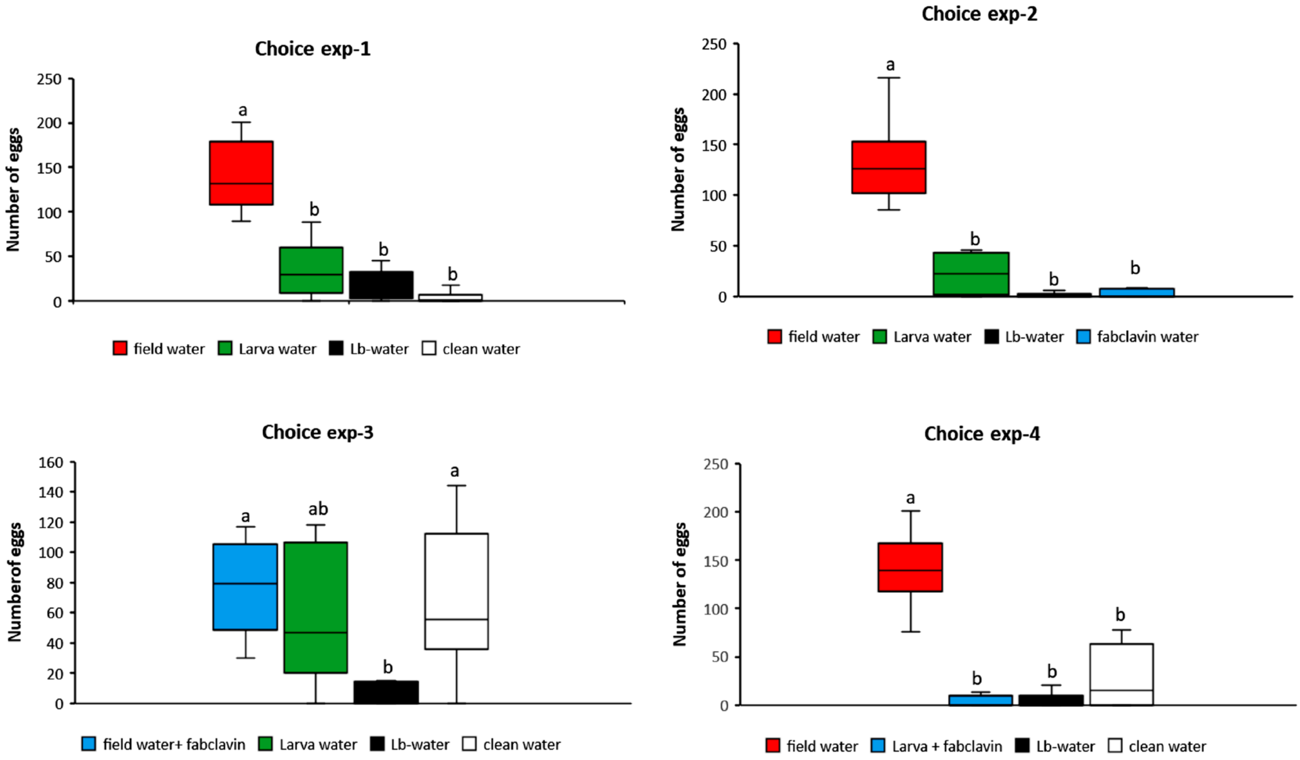


Fig. 5 Deterrent effects of fabclavine from *Xenorhabdus szentirmaii* on *Aedes albopictus* females in the multiple-choice experiments. Letters above boxplots indicate differences according to analysis of variance ($p = 0.05$)

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-anithole 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.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). Work in Hazir lab was funded by Aydin Adnan Menderes University (project no. FEF-22014) and TUBITAK-116Z074. Work in Bode lab was supported by LOEWE TBG funded by the state of Hesse and an ERC Advanced Grant (835108).

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.

References

Afify A, Galizia CG (2015) Chemosensory cues for mosquito oviposition site selection. J Med Entomol 52:120–130. https://doi.org/10.1093/jme/tju024

Aguilar-Durán JA, Villarreal-Treviño C, Fernández-Santos NA, Hamer GL, Rodríguez-Pérez MA (2023) Virulence of entomopathogenic fungi isolated from wild mosquitoes against *Aedes aegypti*. Entomol Res 53:158–166. https://doi.org/10.1111/1748-5967.12640

Ahebwa A, Hii J, Neoh K-B, Chareonviriyaphap T (2023) *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) ecology, biology, behaviour, and implications on arbovirus transmission in Thailand: review. One Health 16:100555. https://doi.org/10.1016/j.onehlt.2023.100555

Antonello AM, Sartori T, Folmer Correa AP, Brandelli A, Heermann R, Rodrigues Júnior LC, Peres A, Romão PRT, Da Silva OS (2018) Entomopathogenic bacteria *Photorhabdus luminescens* as drug source against *Leishmania amazonensis*. Parasitology 145:1065–1074. https://doi.org/10.1017/S0031182017002001

Antonello AM, Sartori T, Silva MB, Prophiro JS, Pinge-Filho P, Heermann R, da Silva OS, Romão PRT (2019) Anti-*Trypanosoma* activity of bioactive metabolites from *Photorhabdus luminescens* and *Xenorhabdus nematophila*. Exp Parasitol 204:107724. https://doi.org/10.1016/j.exppara.2019.107724

Autran ES, Neves IA, da Silva CSB, Santos GKN, da Câmara C, a. G, Navarro DM a. F, (2009) Chemical composition, oviposition deterrent and larvicidal activities against *Aedes aegypti* of essential oils from *Piper marginatum* Jacq. (Piperaceae). Bioresour Technol 100:2284–2288. https://doi.org/10.1016/j.biortech.2008.10.055

Baik LS, Carlson JR (2020) The mosquito taste system and disease control. Proc Natl Acad Sci U S A 117:32848–32856. https://doi.org/10.1073/pnas.2013076117

Barathi S, Sabapathi N, Kandasamy S, Lee J (2024) Present status of insecticide impacts and eco-friendly approaches for remediation-a review. Environ Res 240:117432. https://doi.org/10.1016/j.envres.2023.117432

Beaty BJ, William C. Black IV, Eisen L, Flores AE, García-Rejón JE, Loroño-Pino M, Saavedra-Rodriguez K (2016) The intensifying storm: domestication of *Aedes aegypti*, urbanization of arboviruses, and emerging insecticide resistance. In: Global health impacts of vector-borne diseases: workshop summary. National Academies Press. http://dx.crossref.org/10.17226/21792 (US)http://dx.crossref.org/10.17226/21792

Benedict MQ, LEVINE RS, HAWLEY WA, LOUNIBOS LP, (2007) Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector Borne Zoonotic Dis 7:76–85. https://doi.org/10.1089/vbz.2006.0562

Benelli G, Jeffries C, Walker T (2016) Biological control of mosquito vectors: past, present, and future. InSects 7:52. https://doi.org/10.3390/insects7040052

Bentley MD, Day JF (1989) Chemical ecology and behavioral aspects of mosquito oviposition. Annu Rev Entomol 34:401–421. https://doi.org/10.1146/annurev.en.34.010189.002153

Bode HB (2009) Entomopathogenic bacteria as a source of secondary metabolites. Curr Opin Chem Biol 13:224–230. https://doi.org/10.1016/j.cbpa.2009.02.037

Bode E, Brachmann AO, Kegler C, Simsek R, Dauth C, Zhou Q, Kaiser M, Klemmt P, Bode HB (2015) Simple "on-demand" production of bioactive natural products. ChemBioChem 16:1115–1119. https://doi.org/10.1002/cbic.201500094

Bode E, Heinrich AK, Hirschmann M, Abebew D, Shi Y-N, Vo TD, Wesche F, Shi Y-M, Grün P, Simonyi S, Keller N, Engel Y, Wenski S, Bennet R, Beyer S, Bischoff I, Buaya A, Brandt S, Cakmak I, Çimen H, Eckstein S, Frank D, Fürst R, Gand M, Geisslinger G, Hazir S, Henke M, Heermann R, Lecaudey V, Schäfer W, Schiffmann S, Schüffler A, Schwenk R, Skaljac M, Thines E, Thines M, Ulshöfer T, Vilcinskas A, Wichelhaus TA, Bode HB (2019) Promoter activation in Δhfq mutants as an efficient tool for specialized metabolite production enabling direct bioactivity testing. Angew Chem Int Ed Engl 58:18957–18963. https://doi.org/10.1002/anie.201910563

Bode E, Assmann D, Happel P, Meyer E, Münch K, Rössel N, Bode HB (2023) easyPACId, a simple method for induced production, isolation, identification, and testing of natural products from proteobacteria. Bio Protoc 13:e4709. https://doi.org/10.21769/BioProtoc.4709

Bursali F, Simsek FM (2023) Effects of different feeding methods and hosts on the fecundity and blood-feeding behavior of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). Biologia. https://doi.org/10.1007/s11756-023-01514-3

Caputo B, Manica M (2020) Mosquito surveillance and disease outbreak risk models to inform mosquito-control operations in Europe. Curr Opin Insect Sci 39:101–108. https://doi.org/10.1016/j.cois.2020.03.009

Chacón-Orozco JG, Bueno CJ, Shapiro-Ilan DI, Hazir S, Leite LG, Harakava R (2020) Antifungal activity of *Xenorhabdus* spp. and *Photorhabdus* spp. against the soybean pathogenic *Sclerotinia sclerotiorum*. Sci Rep 10:20649. https://doi.org/10.1038/s41598-020-77472-6

Cheah S-X, Tay J-W, Chan L-K, Jaal Z (2013) Larvicidal, oviposition, and ovicidal effects of *Artemisia annua* (Asterales: Asteraceae) against *Aedes aegypti*, *Anopheles sinensis*, and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res 112:3275–3282. https://doi.org/10.1007/s00436-013-3506-0

Cimen H (2023) Mosquito oviposition deterring and larvicidal effect of cinnamaldehyde and eugenol. J Anatol Environ Anim Sci 8:322–325. https://doi.org/10.35229/jaes.1313226

Cimen H, Touray M, Gulsen SH, Erincik O, Wenski SL, Bode HB, Shapiro-Ilan D, Hazir S (2021) Antifungal activity of different *Xenorhabdus* and *Photorhabdus* species against various fungal phytopathogens and identification of the antifungal compounds from *X. szentirmaii*. Appl Microbiol Biotechnol 105:5517–5528. https://doi.org/10.1007/s00253-021-11435-3

Cimen H, Touray M, Gulsen SH, Hazir S (2022) Natural products from *Photorhabdus* and *Xenorhabdus*: mechanisms and impacts. Appl Microbiol Biotechnol 106:4387–4399. https://doi.org/10.1007/s00253-022-12023-9

da Silva OS, Prado GR, da Silva JLR, Silva CE, da Costa M, Heermann R (2013) Oral toxicity of *Photorhabdus* luminescens and *Xenorhabdus nematophila* (Enterobacteriaceae) against *Aedes aegypti* (Diptera: Culicidae). Parasitol Res 112:2891–2896. https://doi.org/10.1007/s00436-013-3460-x

da Silva JRL, Undurraga Schwalm F, Eugênio Silva C, da Costa M, Heermann R, Santos da Silva O (2017) Larvicidal and growth-inhibitory activity of entomopathogenic bacteria culture fluids against *Aedes aegypti* (Diptera: Culicidae). J Econ Entomol 110:378–385. https://doi.org/10.1093/jee/tow224

Damico T (2017) Biopesticides are in high demand in today's pest management programs

Day JF (2016) Mosquito oviposition behavior and vector control. InSects 7:65. https://doi.org/10.3390/insects7040065

Dias CN, Moraes DFC (2014) Essential oils and their compounds as *Aedes aegypti L*. (Diptera: Culicidae) larvicides: review. Parasitol Res 113:565–592. https://doi.org/10.1007/s00436-013-3687-6

Donmez Ozkan H, Cimen H, Ulug D, Wenski S, Yigit Ozer S, Telli M, Aydin N, Bode HB, Hazir S (2019) Nematode-associated bacteria: production of antimicrobial agent as a presumptive nominee for curing endodontic infections caused by *Enterococcus faecalis*. Front Microbiol 10:2672. https://doi.org/10.3389/fmicb.2019.02672

Duan J, Yuan B, Jia F, Li X, Chen C, Li G (2024) Development of an efficient and seamless genetic manipulation method for *Xenorhabdus* and its application for enhancing the production of Fabclavines. J Agric Food Chem 72:274–283. https://doi.org/10.1021/acs.jafc.3c04136

Eitam A, Blaustein L (2004) Oviposition habitat selection by mosquitoes in response to predator (*Notonecta maculata*) density. Physiol Entomol 29:188–191. https://doi.org/10.1111/j.0307-6962.2004.0372.x

Fang X, Zhang M, Tang Q, Wang Y, Zhang X (2014) Inhibitory effect of *Xenorhabdus nematophila* TB on plant pathogens Phytophthora capsici and *Botrytis cinerea* in vitro and in planta. Sci Rep 4:4300. https://doi.org/10.1038/srep04300

Faraji A, Unlu I (2016) The eye of the tiger, the thrill of the fight: effective larval and adult control measures against the asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America. J Med Entomol 53:1029–1047. https://doi.org/10.1093/jme/tjw096

Ferreira LM, Silva-Filha MHNL (2013) Bacterial larvicides for vector control: mode of action of toxins and implications for resistance. Biocontrol Sci Technol 23(10):1137–1168. https://doi.org/10.1080/09583157.2013.822472

Fonseca DM, Unlu I, Crepeau T, Farajollahi A, Healy SP, Bartlett-Healy K, Strickman D, Gaugler R, Hamilton G, Kline D, Clark GG (2013) Area-wide management of *Aedes albopictus*. Part 2: gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. Pest Manag Sci 69:1351–1361. https://doi.org/10.1002/ps.3511

Fuchs SW, Grundmann F, Kurz M, Kaiser M, Bode HB (2014) Fabclavines: bioactive peptide-polyketide-polyamino hybrids from *Xenorhabdus*. ChemBioChem 15:512–516. https://doi.org/10.1002/cbic.201300802

Furgani G, Böszörményi E, Fodor A, Máthé-Fodor A, Forst S, Hogan JS, Katona Z, Klein MG, Stackebrandt E, Szentirmai A, Sztaricskai F, Wolf SL (2008) *Xenorhabdus* antibiotics: a comparative analysis and potential utility for controlling mastitis caused by bacteria. J Appl Microbiol 104:745–758. https://doi.org/10.1111/j.1365-2672.2007.03613.x

Girard M, Martin E, Vallon L, Raquin V, Bellet C, Rozier Y, Desouhant E, Hay A-E, Luis P, Valiente Moro C, Minard G (2021) Microorganisms associated with mosquito oviposition sites: implications for habitat selection and insect life histories. Microorganisms 9:1589. https://doi.org/10.3390/microorganisms9081589

Godfray HCJ (2013) Mosquito ecology and control of malaria. J Anim Ecol 82:15–25. https://doi.org/10.1111/1365-2656.12003

Gomes SA, Carolino AT, Teodoro TBP, Silva GA, de Bitencourt R, Silva CP, Alkhaibari AM, Butt TM, Samuels RI (2023) The potential of *Metarhizium anisopliae* blastospores to control *Aedes aegypti* larvae in the field. J Fungi 9:759. https://doi.org/10.3390/jof9070759

Gubler DJ (2002) Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol 10:100–103. https://doi.org/10.1016/s0966-842x(01)02288-0

Gulcu B, Hazir S, Kaya HK (2012) Scavenger deterrent factor (SDF) from symbiotic bacteria of entomopathogenic nematodes. J Invertebr Pathol 110:326–333. https://doi.org/10.1016/j.jip.2012.03.014

Gulsen SH, Tileklioglu E, Bode E, Cimen H, Ertabaklar H, Ulug D, Ertug S, Wenski SL, Touray M, Hazir C, Bilecenoglu DK, Yildiz I, Bode HB, Hazir S (2022) Antiprotozoal activity of different *Xenorhabdus* and *Photorhabdus* bacterial secondary metabolites and identification of bioactive compounds using the easyPACId approach. Sci Rep 12:10779. https://doi.org/10.1038/s41598-022-13722-z

Hazir S, KAYA H, Touray M, Cimen H, ILAN DS (2022) Basic laboratory and field manual for conducting research with the entomopathogenic nematodes, Steinernema and Heterorhabditis, and their bacterial symbionts. Turkish J Zool 46:305–350. https://doi.org/10.55730/1300-0179.3085

Hazir S, Shapiro-Ilan DI, Hazir C, Leite LG, Cakmak I, Olson D (2016) Multifaceted effects of host plants on entomopathogenic nematodes. J Invertebr Pathol 135:53–59. https://doi.org/10.1016/j.jip.2016.02.004

Hazir S, Shapiro-Ilan DI, Bock CH, Leite LG (2017) Trans-cinnamic acid and *Xenorhabdus* szentirmaii metabolites synergize the potency of some commercial fungicides. J Invertebr Pathol 145:1–8. https://doi.org/10.1016/j.jip.2017.03.007

Hinchliffe SJ, Hares MC, Dowling AJ, ffrench-Constant RH (2010) Insecticidal toxins from the *Photorhabdus* and *Xenorhabdus* bacteria. Open Toxinol J. https://doi.org/10.2174/1875414701003010083

Huang Z, Zhang Y, Li H, Zhu J, Song W, Chen K, Zhang Y, Lou Y (2023) Vaccine development for mosquito-borne viral diseases. Front Immunol 14:1161149. https://doi.org/10.3389/fimmu.2023.1161149

Hwang Y-S, Schultz GW, Axelrod H, Kramer WL, Mulla MS (1982) Ovipositional repellency of fatty acids and their derivatives against *Culex*1 and *Aedes*1 mosquitoes. Environ Entomol 11:223–226. https://doi.org/10.1093/ee/11.1.223

Incedayi G, Cimen H, Ulug D, Touray M, Bode E, Bode HB, Orenlili Yaylagul E, Hazir S, Cakmak I (2021) Relative potency of a novel acaricidal compound from *Xenorhabdus*, a bacterial genus mutualistically associated with entomopathogenic nematodes. Sci Rep 11:11253. https://doi.org/10.1038/s41598-021-90726-1

Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Van Bortel W, Hendrickx G, Schaffner F, Elyazar IRF, Teng H-J, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GRW, Golding N, Hay SI (2015) The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. Elife 4:e08347. https://doi.org/10.7554/eLife.08347

Kramer WL, Mulla MS (1979) Oviposition attractants and repellents of mosquitoes: oviposition responses of *Culex*1 mosquitoes to organic infusions 2. Environ Entomol 8:1111–1117. https://doi.org/10.1093/ee/8.6.1111

Lacey LA (2007) *Bacillus thuringiensis* serovariety israelensis and *Bacillus sphaericus* for mosquito control. J Am Mosq Control Assoc 23:133–163. https://doi.org/10.2987/8756-971X(2007)23[133:BTSIAB]2.0.CO;2

Laporta GZ, Potter AM, Oliveira JFA, Bourke BP, Pecor DB, Linton Y-M (2023) Global distribution of *Aedes aegypti* and *Aedes albopictus* in a climate change scenario of regional rivalry. InSects 14:49. https://doi.org/10.3390/insects14010049

Lühken R, Brattig N, Becker N (2023) Introduction of invasive mosquito species into Europe and prospects for arbovirus transmission and vector control in an era of globalization. Infect Dis Poverty 12:109. https://doi.org/10.1186/s40249-023-01167-z

Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, Pigott DM, Shearer FM, Johnson K, Earl L, Marczak LB, Shirude S, Davis Weaver N, Gilbert M, Velayudhan R, Jones P, Jaenisch T, Scott TW, Reiner RC, Hay SI (2019) The current and future global distribution and population at risk of dengue. Nat Microbiol 4:1508–1515. https://doi.org/10.1038/s41564-019-0476-8

Nazni WA, Lee HL, Wan Rozita WM, Lian AC, Chen CD, Azahari AH, Sadiyah I, Sadiyah I (2009) Oviposition behaviour of Aedes albopictus in temephos and Bacillus thuringiensis israelensis-treated ovitraps. WHO Regional Office for South-East Asia. https://iris.who.int/handle/10665/170958

Ochomo E, Rund SSC, Mthawanji RS, Antonio-Nkondjio C, Machani M, Samake S, Wolie RZ, Nsango S, Lown LA, Matoke-Muhia D, Kamau L, Lukyamuzi E, Njeri J, Chabi J, Akrofi OO, Ntege C, Mero V, Mwalimu C, Kiware S, Bilgo E, Traoré MM, Afrane Y, Hakizimana E, Muleba M, Orefuwa E, Chaki P, Juma EO (2024) Mosquito control by abatement programmes in the United States: perspectives and lessons for countries in sub-Saharan Africa. Malar J 23:8. https://doi.org/10.1186/s12936-023-04829-3

Otoya-Martinez N, Leite LG, Harakava R, Touray M, Hazir S, Chacon-Orozco J, Bueno CJ (2023) Disease caused by *Neofusicoccum parvum* in pruning wounds of grapevine shoots and its control by *Trichoderma* spp. and *Xenorhabdus szentirmaii*. Fungal Biol 127:865–871. https://doi.org/10.1016/j.funbio.2022.12.002

Parker AT, McGill K, Allan BF (2020) Container type affects mosquito (Diptera: Culicidae) oviposition choice. J Med Entomol 57:1459–1467. https://doi.org/10.1093/jme/tjaa045

Pathak VM, Verma VK, Rawat BS, Kaur B, Babu N, Sharma A, Dewali S, Yadav M, Kumari R, Singh S, Mohapatra A, Pandey V, Rana N, Cunill JM (2022) Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Front Microbiol 13:962619. https://doi.org/10.3389/fmicb.2022.962619

Perumal V, Kannan S, Alford L, Pittarate S, Mekchay S, Reddy GVP, Elangovan D, Marimuthu R, Krutmuang P (2023) Biocontrol effect of entomopathogenic fungi *Metarhizium anisopliae* ethyl acetate-derived chemical molecules: An eco-friendly anti-malarial drug and insecticide. Arch Insect Biochem Physiol 114:e22037. https://doi.org/10.1002/arch.22037

Poonam S, Paily KP, Balaraman K (2002) Oviposition attractancy of bacterial culture filtrates: response of Culex quinquefasciatus. Mem Inst Oswaldo Cruz 97:359–362. https://doi.org/10.1590/s0074-02762002000300015

Roiz D, Pontifes P, Jourdain F, Diagne C, Leroy B, Vaissière A-C, Tolsá MJ, Salles J-M, Simard F, Courchamp F (2023) The rising global economic costs of Aedes and Aedes-borne diseases. In Review. https://doi.org/10.21203/rs.3.rs-2679030/v1

Romanello M, McGushin A, Di Napoli C, Drummond P, Hughes N, Jamart L, Kennard H, Lampard P, Solano Rodriguez B, Arnell N, Ayeb-Karlsson S, Belesova K, Cai W, Campbell-Lendrum D, Capstick S, Chambers J, Chu L, Ciampi L, Dalin C, Dasandi N, Dasgupta S, Davies M, Dominguez-Salas P, Dubrow R, Ebi KL, Eckelman M, Ekins P, Escobar LE, Georgeson L, Grace D, Graham H, Gunther SH, Hartinger S, He K, Heaviside C, Hess J, Hsu S-C, Jankin S, Jimenez MP, Kelman I, Kiesewetter G, Kinney PL, Kjellstrom T, Kniveton D, Lee JKW, Lemke B, Liu Y, Liu Z, Lott M, Lowe R, Martinez-Urtaza J, Maslin M, McAllister L, McMichael C, Mi Z, Milner J, Minor K, Mohajeri N, Moradi-Lakeh M, Morrissey K, Munzert S, Murray KA, Neville T, Nilsson M, Obradovich N, Sewe MO, Oreszczyn T, Otto M, Owfi F, Pearman O, Pencheon D, Rabbaniha M, Robinson E, Rocklöv J, Salas RN, Semenza JC, Sherman J, Shi L, Springmann M, Tabatabaei M, Taylor J, Trinanes J, Shumake-Guillemot J, Vu B, Wagner F, Wilkinson P, Winning M, Yglesias M, Zhang S, Gong P, Montgomery H, Costello A, Hamilton I (2021) The 2021 report of the lancet countdown on health and climate change: code red for a healthy future. Lancet 398:1619–1662. https://doi.org/10.1016/S0140-6736(21)01787-6

Russell MC, Herzog CM, Gajewski Z, Ramsay C, El Moustaid F, Evans MV, Desai T, Gottdenker NL, Hermann SL, Power AG, McCall AC (2022) Both consumptive and non-consumptive effects of predators impact mosquito populations and have implications for disease transmission. Elife 11:e71503. https://doi.org/10.7554/eLife.71503

Schaffner F, Mathis A (2014) Dengue and dengue vectors in the WHO European region: past, present, and scenarios for the future. Lancet Infect Dis 14:1271–1280. https://doi.org/10.1016/S1473-3099(14)70834-5

Scholte E-J, Takken W, Knols BGJ (2007) Infection of adult *Aedes aegypti* and *Ae. albopictus* mosquitoes with the entomopathogenic fungus *Metarhizium anisopliae*. Acta Trop 102:151–158. https://doi.org/10.1016/j.actatropica.2007.04.011

Schwab SR, Stone CM, Fonseca DM, Fefferman NH (2018) The importance of being urgent: the impact of surveillance target and scale on mosquito-borne disease control. Epidemics 23:55–63. https://doi.org/10.1016/j.epidem.2017.12.004

Sergeant M, Baxter L, Jarrett P, Shaw E, Ousley M, Winstanley C, Morgan JAW (2006) Identification, typing, and insecticidal activity of *Xenorhabdus* isolates from entomopathogenic nematodes in United Kingdom soil and characterization of the xpt toxin loci. Appl Environ Microbiol 72:5895–5907. https://doi.org/10.1128/AEM.00217-06

Shah FA, Abdoarrahem MM, Berry C, Touray M, Hazir S, Butt TM (2021) Indiscriminate ingestion of entomopathogenic nematodes and their symbiotic bacteria by *Aedes aegypti* larvae: a novel strategy to control the vector of Chikungunya, Dengue and Yellow Fever. Turkish J Zool 45:372. https://doi.org/10.3906/zoo-2107-2383

Shapiro-Ilan DI, Bock CH, Hotchkiss MW (2014) Suppression of pecan and peach pathogens on different substrates using *Xenorhabdus bovienii* and *Photorhabdus* luminescens. Biol Control 77:1–6. https://doi.org/10.1016/j.biocontrol.2014.05.010

Shapiro-Ilan D, Hazir S, Glazer I (2019) Advances in use of entomopathogenic nematodes in integrated pest management. In:

Integrated management of insect pests. Burleigh Dodds Science Publishing

Smith LB, Kasai S, Scott JG (2016) Pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus*: important mosquito vectors of human diseases. Pestic Biochem Physiol 133:1–12. https://doi.org/10.1016/j.pestbp.2016.03.005

Subkrasae C, Ardpairin J, Dumidae A, Janthu P, Muangpat P, Polseela R, Tandhavanant S, Thanwisai A, Vitta A (2022) Larvicidal activity of *Photorhabdus* and *Xenorhabdus* bacteria isolated from insect parasitic nematodes against *Aedes aegypti* and *Aedes albopictus*. Acta Trop 235:106668. https://doi.org/10.1016/j.actatropica.2022.106668

Tetreau G, Stalinski R, David JP, Després L (2013) Monitoring resistance to Bacillus thuringiensis subsp. israelensis in the field by performing bioassays with each Cry toxin separately. Mem Inst Oswaldo Cruz 108(7):894–900. https://doi.org/10.1590/0074-0276130155

Thompson R, Martin Del Campo J, Constenla D (2020) A review of the economic evidence of Aedes-borne arboviruses and Aedes-borne arboviral disease prevention and control strategies. Expert Rev Vaccines 19:143–162. https://doi.org/10.1080/14760584.2020.1733419

Tobias NJ, Wolff H, Djahanschiri B, Grundmann F, Kronenwerth M, Shi Y-M, Simonyi S, Grün P, Shapiro-Ilan D, Pidot SJ, Stinear TP, Ebersberger I, Bode HB (2017) Natural product diversity associated with the nematode symbionts *Photorhabdus* and *Xenorhabdus*. Nat Microbiol 2:1676–1685. https://doi.org/10.1038/s41564-017-0039-9

Touray M, Bakirci S, Ulug D, Gulsen SH, Cimen H, Yavasoglu SI, Simsek FM, Ertabaklar H, Ozbel Y, Hazir S (2023) Arthropod vectors of disease agents: their role in public and veterinary health in Turkiye and their control measures. Acta Trop. https://doi.org/10.1016/j.actatropica.2023.106893

Vicente-Díez I, Pou A, Campos-Herrera R (2023) The deterrent ability of *Xenorhabdus nematophila* and *Photorhabdus laumondii* compounds as a potential novel tool for *Lobesia botrana* (Lepidoptera: Tortricidae) management. J Invertebr Pathol. https://doi.org/10.1016/j.jip.2023.107911

Vitta A, Thimpoo P, Meesil W, Yimthin T, Fukruksa C, Polseela R, Mangkit B, Tandhavanant S, Thanwisai A (2018) Larvicidal activity of *Xenorhabdus* and *Photorhabdus* bacteria against *Aedes aegypti* and *Aedes albopictus*. Asian Pac J Trop Biomed 8:31. https://doi.org/10.4103/2221-1691.221134

Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, Charlson F, Davis A, Erskine H, Feigin VL (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 386:743–800. https://doi.org/10.1016/S0140-6736(15)60692-4

Wagutu GK, Mwangi W, Waturu CN (2017) Entomopathogenic bacteria: *Xenorhabdus* Spp and *Photorhabdus* Spp from Steinernema Karii and *Heterorhabditis Indica* for the control of mosquito larvae. J Agric Sci Technol 18:21–38. https://doi.org/10.4314/jagst.v18i2

Waliwitiya R, Kennedy CJ, Lowenberger CA (2009) Larvicidal and oviposition-altering activity of monoterpenoids, *trans* -anithole and rosemary oil to the yellow fever mosquito *Aedes aegypti* (Diptera: Culicidae). Pest Manag Sci 65:241–248. https://doi.org/10.1002/ps.1675

Webster J, Chen G, Hu K, Li J (2002) Bacterial metabolites. Entomopathogenic nematology. CABI International, London, pp 99–114

Weeratunga P, Rodrigo C, Fernando SD, Rajapakse S (2017) Control methods for *Aedes albopictus* and *Aedes aegypti*. Cochrane Database Syst Rev. https://doi.org/10.1002/14651858.CD012759

Wenski SL, Cimen H, Berghaus N, Fuchs SW, Hazir S, Bode HB (2020) Fabclavine diversity in *Xenorhabdus* bacteria. Beilstein J Org Chem 16:956–965. https://doi.org/10.3762/bjoc.16.84

Wenski SL, Berghaus N, Keller N, Bode HB (2021) Structure and biosynthesis of deoxy-polyamine in Xenorhabdus bovienii. J Ind Microbiol Biotechnol 48:kuab006. https://doi.org/10.1093/jimb/kuab006

Yuan B, Li B, Shen H, Duan J, Jia F, Maimaiti Y, Li Y, Li G (2023) Identification of fabclavine derivatives, Fcl-7 and Fcl-8, from *Xenorhabdus budapestensis* as major antifungal natural products against *Rhizoctonia solani*. J Appl Microbiol 134:lxad190. https://doi.org/10.1093/jambio/lxad190

Yüksel E, Yıldırım A, İmren M, Canhilal R, Dababat AA (2023) *Xenorhabdus* and *Photorhabdus* bacteria as potential candidates for the control of *Culex pipiens L.* (Diptera: Culicidae), the principal vector of west nile virus and lymphatic filariasis. Pathogens 12:1095. https://doi.org/10.3390/pathogens12091095

Zeng Z, Zhan J, Chen L, Chen H, Cheng S (2021) Global, regional, and national dengue burden from 1990 to 2017: a systematic analysis based on the global burden of disease study 2017. EClinicalMedicine 32:100712. https://doi.org/10.1016/j.eclinm.2020.100712