Oviposition Attractancy of Bacterial Culture Filtrates – Response of *Culex quinquefasciatus*

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Oviposition attractants could be used for monitoring as well as controlling mosquitoes by attracting them to lay eggs at chosen sites. In the present study, culture filtrates of seven bacterial species were tested for their attractancy against gravid females of Culex quinquefasciatus*. When their oviposition active indices (OAI) were studied, the culture filtrates of* Bacillus cereus *and* Pseudomonas fluorescens *exhibited oviposition attractancy (OAI = >0.3) at 100 ppm and the OAI were respectively 0.70 and 0.47. Culture filtrates of* B. thuringiensis *var.* israelensis *(wild type),* B. t. *var.* israelensis *(mutant) and* B. sphaericus *showed attractancy at 2000 ppm with OAI of respectively 0.71, 0.59 and 0.68. However, the OAI of* B. megaterium *as well as* Azospirillum brasilense *was 0.13 (at 2000 ppm), which was less than 0.3 required to be considered them as attractants. When the oviposition attractancy of the bacterial culture filtrates were compared with that of a known oviposition attractant,* p-*cresol (at 10 ppm), the culture filtrates of* B. t. *var.* israelensis *(wild type) and* B. cereus *were found to be more active than p-cresol, respectively with 64.2 and 54.3% oviposition.*

Key words: bacteria - *Culex quinquefasciatus* - oviposition attractant - metabolites

Biocontrol agents and oviposition attractants are promising alternate tools/adjuncts for mosquito control. Using oviposition attractants, monitoring of vector populations, especially *Aedes* species, could be carried out so as to plan control measures, or to attract gravid females to lay eggs at chosen sites and kill the emerging larvae by combining a larvicide. Microorganisms inhabiting mosquito-breeding sites (Androsov et al. 1986) play a major role in the decomposition of detritus present in the habitats leading to the production of several metabolites. Some of these metabolites are likely to act as oviposition attractant and/or stimulants for mosquitoes. Therefore, in the present study culture filtrates containing metabolites of a few bacteria were examined for oviposition attractancy and the results are presented hereunder.

MATERIALS AND METHODS

Bacterial strains - *Bacillus cereus, Pseudomonas fluorescens, B. megaterium, Azospirillum brasilense, B. thuringiensis* var. *israelensis* (wild type), *B. t.* var. *israelensis* (mutant) and *B. sphaericus,* obtained from the culture collection of Vector Control Research Centre (VCRC), Pondicherry, were used for the study.

Cultivation of bacteria - Nutrient broth (NB) containing (wt/v %) glucose (0.5), beef extract (0.5), sodium chloride (0.5), and peptone (0.5) in distilled water at a pH 7.5 was used to grow *P. fluorescens*, *B. cereus* and *B. megaterium*. Nutrient yeast extract mineral salt medium (NYSM) [composition (wt/v %): glucose, 0.5; peptone, 0.5; sodium chloride, 0.5; beef extract. 0.3; yeast extract, 0.5; magnesium chloride, 0.020; manganous chloride, 0.001; and calcium chloride, 0.010 in distilled water, pH 7.2] was used to grow wild type as well as mutant strains of *B. t.* var. *israelensis* (Yousten et al. 1980). And to grow *B. sphaericus* the medium NYSM without glucose was used. For the cultivation of *A. brasilense* a medium containing (wt/v %) yeast extract, 1.0 in distilled water, pH 7.5 was used. A loop full of bacterial growth from an agar slope was transferred to 10 ml of growth medium (in a boiling tube) and incubated for 8 h on a rotatory shaker at 250 rpm and 28 ± 2°C. The culture was then transferred to 50 ml of growth medium (in 250 ml capacity Erlenmeyer flask) and incubated, as stated above, for 10 h. Five ml of this inoculum was transferred to 500 ml of production medium (in 2 l flask) and incubated for 48 h. Then, the cell-mass from the culture was harvested by spinning at 10,000 rpm for 10 min. The cell-mass was discarded and the cell-free supernatant was used as test material for oviposition attractancy tests.

Determination of optimum concentration of bacterial culture filtrates for oviposition attractancy test - Three-day-old *Culex quinquefasciatus* females, obtained from a colony maintained at VCRC, were fed on fowl blood and maintained for two days on raisins at 28 ± 2°C and 70-80% RH. Gravid females were used for determination of oviposition attractancy of various compounds. Different concentrations (5-3000 ppm) of the test materials were prepared in tap water. Two hundred ml of each test preparation held in disposable cups (capacity, 250 ml) was placed in a mosquito cage (size, 55 x 55 x 55 cm). Tap water was used as a control. One hundred fully gravid female mosquitoes were released into the cage. For each test, one cage was used and at any given time, not more than five disposable cups were kept on the floor of the cage. Four cups with four different concentrations of the culture filtrate were on four corners and the fifth cup with tap water (control) was at the center of the cage. The cages were kept at 28 ± 2°C and 70-80% RH. Experiments were set up

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at 16:00 h and observations for the presence of egg rafts, if any, were made at 10:00 h the next day. Number of egg rafts laid in each cup was counted and the percentages laid in different cups were calculated from the total number laid, including the control. Each experiment was repeated four times and on each occasion the position of the disposable cups were changed.

Oviposition active index (OAI) of test materials - OAI of any test material was determined by placing in a cage, one cup containing the pre-determined optimum concentration of a test material and another cup containing tap water as a control. The culture filtrates of different bacteria were tested at the following concentrations (ppm): *B. cereus*: 100; *P. fluorescens*: 100; *B. megaterium*: 2000; *A. brasilense*: 2000; *B. t.* var. *israelensis* (wild type): 2000; *B. t.* var. *israelensis* (mutant): 2000; *B. sphaericus*: 2000. A known oviposition attractant, *p*-cresol (10 ppm) was used as a standard.

The oviposition active index (Hwang et al. 1982) was calculated by using the formula:

$$OAI = \frac{Nt\text{-}Ns}{Nt+Ns}$$

where, Nt = total number of egg rafts in test solution, Ns = total number of egg rafts in control.

As suggested by Hwang et al. (1982), compounds with an OAI of +0.3 and above are considered as attractants, while those with -0.3 and below are considered as repellents.

Oviposition attractancy of test materials in comparison with p-cresol - The oviposition attractancy of the test preparations were compared with *p*-cresol, individually, by testing them at their optimum concentrations for attractancy.

On each occasion, one cup containing the bacterial culture filtrate and another cup containing *p*- cresol were kept on the opposite corners of the cage. The numbers of egg rafts laid were counted and recorded according to the procedures mentioned earlier. Percentage of egg rafts laid in each culture filtrate was calculated from the total number laid, both in the culture filtrate and *p*-cresol.

Statistical analysis of data - The data were subjected to student's "t" test to find out the significance of any differences in the oviposition attractancy between the test preparations and *p*-cresol, between different concentrations of each test preparations, and between test preparations and control.

RESULTS

Optimum concentration for oviposition attractancy - In the case of *B. cereus* and *P. fluorescens*, significantly higher oviposition was observed at 100 ppm and the percentage of egg rafts laid was respectively 54 ($P < 0.001$; $t = 16.44$) and 42.8 ($P = 0.006$; $t = -4.22$) as against other concentrations and control (Fig. 1). In the case of the remaining five bacterial species, the optimum concentration was higher (2000 ppm) and the percentage oviposition was, respectively, 40.3 ($P = 0.003$; $t = -4.71$), 38.3 ($P = 0.001$; $t = -5.70$), 54.5 ($P < 0.001$; $t = 9.80$), 34.5 ($P = 0.004$; $t = -4.53$) and 50.5 ($P < 0.001$; $t = -8.28$) for *B. megaterium*, *A. brasilense*, *B. t.* var. *israelensis* (wild type), *B. t.* var. *israelensis* (mutant) and *B. sphaericus*. For *p*-cresol, the optimum concentration was 10 ppm and the percentage oviposition was 33.8 ($P = 0.032$; $t = 2.78$).

Oviposition active index - The data on the OAI of the seven bacterial culture filtrates in comparison with *p*-cresol are illustrated in the Fig. 2. The culture filtrates of five bacteria viz., *B. t.* var. *israelensis* (wild type), *B. cereus*, *B. sphaericus*, *B. t.* var. *israelensis* (mutant) and *P. fluorescens* exhibited oviposition attractancy as their indices were > 0.3. These were respectively 0.71, 0.70, 0.68, 0.59, and 0.47 for these bacterial culture filtrates. However, the OAI of *B. megaterium* as well as *A. brasilense*

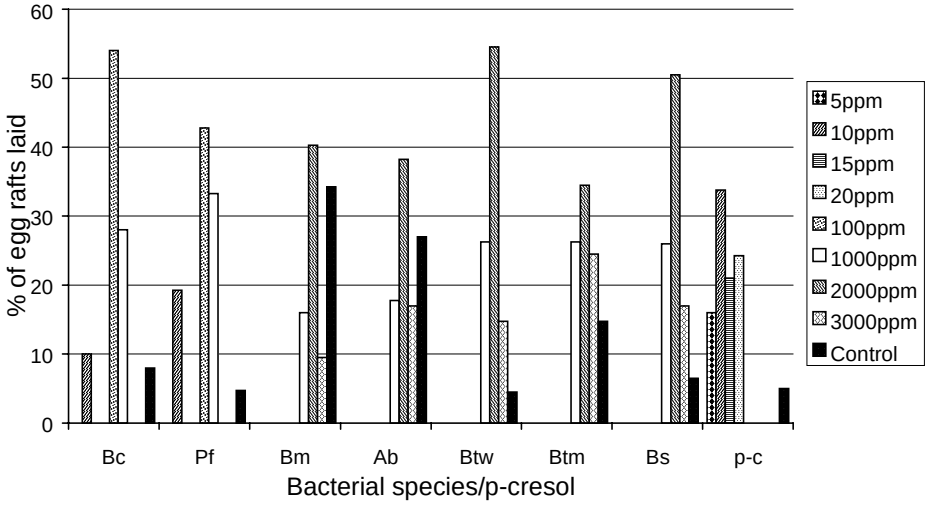

Fig. 1: oviposition attractancy of different concentrations of bacterial culture filtrates (Bc: *Bacillus cereus*, Pf: *Pseudomonas fluorescens*, Bm: *B. megaterium*, Ab: *Azospirillum brasilense*, Btw: *B. thuringiensis* var. *israelensis* (wild type), Btm: *B. t.* var. *israelensis* (mutant), Bs: *B. sphaericus*), and *p*-cresol (p-c)

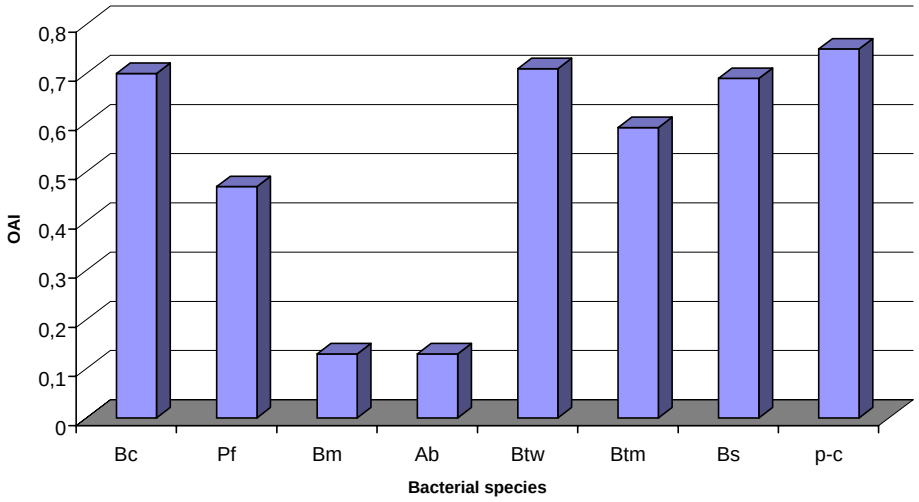

Fig. 2: oviposition active indices (OAI) of bacterial culture filtrates (Bc: *Bacillus cereus* at 100 ppm, Pf: *Pseudomonas fluorescens* at 100 ppm, Bm: *B. megaterium* at 2000 ppm, Ab: *Azospirillum brasilense* at 2000 ppm, Btw: *B. thuringiensis* var. *israelensis* (wild type) at 2000 ppm, Btm: *B. t.* var. *israelensis* (mutant) at 2000 ppm, Bs: *B. sphaericus* at 2000 ppm, and *p*-cresol (p-c) at 10 ppm

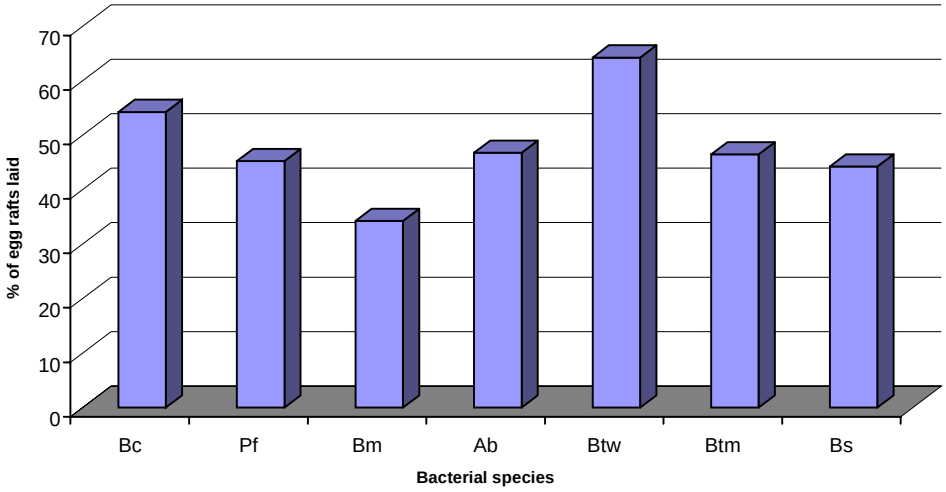

Fig. 3: egg rafts laid (%) by *Culex quinquefasciatus* in bacterial culture filtrates (Bc: *Bacillus cereus* at 100 ppm, Pf: *Pseudomonas fluorescens* at 100 ppm, Bm: *B. megaterium* at 2000 ppm, Ab: *Azospirillum brasilense* at 2000 ppm, Btw: *B. thuringiensis* var. *israelensis* (wild type) at 2000 ppm, Btm: *B. t.* var. *israelensis* (mutant) at 2000 ppm, Bs: *B. sphaericus* at 2000 ppm) when tested along with *p*-cresol at 10 ppm

was 0.13, which was less than 0.3 required to be considered them as attractants. The OAI of *p*-cresol (0.75) was higher than that of the culture filtrates of all the bacteria tested.

Oviposition attractancy of test materials in comparison with p-cresol - The data on the percentage of eggs laid by *Cx. quinquefasciatus* females in various culture filtrates, compared to that of *p*-cresol are shown in the Fig. 3. The observations indicate that the culture filtrate of *B. t.* var. *israelensis* (wild type) had highest oviposition attractancy (64.2%) followed by that of *B. cereus* (54.3%). Also the percentages of eggs laid in these two culture filtrates were higher than that in the *p*-cresol. The percentage of eggs laid in the culture filtrates of other bacteria was less than that laid in *p*-cresol.

DISCUSSION

Microorganisms, especially bacteria, have often been encountered in mosquito larvae and mosquito breeding sites (Chao & Wistreich 1959, 1960, Wistreich & Chao 1960, 1961, Kuno 1975, Androsov et al. 1986, Biswas et al. 1988, Manonmani et al. 1987, 1990, Vasanti & Hoti 1992). The detritus present in mosquito breeding sites has been found to be decomposed by the microorganisms leading to evolution of volatile secondary metabolites (Kennedy 1942, Millar et al. 1992). Consequently, gravid female mosquitoes of *Cx. peus*, *Cx. tarsalis* and *Anopheles freeborni* (Gerhardt 1959) and *Cx. quinquefasciatus* (Ikeshoji 1966) are attracted to such habitats and stimulated to lay eggs.

In the present study cell-free culture filtrates of seven bacteria were tested for oviposition attractancy to gravid

females of *Cx. quinquefasciatus*. Observations indicated that the culture filtrates of *B. cereus* and *P. fluorescens* had maximum oviposition attractancy at 100 ppm; the oviposition indices of the culture filtrates of *B. cereus* and *B. t.* var. *israelensis* (wild type) are comparable to that of *p*-cresol; and a greater number of eggs were laid in the former than the latter. The results of the present experiments confirm the speculation of earlier studies that the decomposition of organic matter present in aquatic bodies by bacteria lead to the evolution of certain volatile compounds, which attract and/or stimulate gravid female mosquitoes to lay eggs.

The study leads to the conclusion that the bacterial culture filtrates attracted and/or stimulated *Cx. quinquefasciatus* gravid females to lay eggs, however, their efficacy as oviposition attractants varied, and the efficacy of some of them was equivalent to, or more than, that of *p*-cresol, a known oviposition attractant.

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