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Studies on the ovitraps baited with hay and leaf infusions for the surveillance of dengue vector, *Aedes albopictus* in northeastern India

Gopalakrishnan, R.[1], Das, M.[1], Baruah, I.[1], Veer, V.[1] and Dutta, P.[2]
[a]Defence Research Laboratory (Defence Research & Development Organisation), PB No. 2, Tezpur - 784 001 (Assam) India
[b]Regional Medical Research Centre, North East Region (Indian Council of Medical Research), PB No. 105, Dibrugarh 786 001 (Assam) India
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Abstract. Ovitraps baited with hay and leaf infusions were evaluated for enhancing the oviposition response of gravid females of the dengue vector *Aedes albopictus*. The egg density per trap (mean ± SE_{mean}) was the highest with 30% infusions of *Pennisetum* grass hay (623.6 ± 41) and rice straw (580 ± 51.3), which corresponded to oviposition activity index (OAI) of 0.62. Infusions (5-50%) of mango and banana leaves with OAI ranging from -0.36 to 0.39 were not observed to enhance the oviposition response significantly over control. Rice straw and *Pennisetum* grass hay are available round the year in northeastern India and the use of these infusions can be a cost effective way to augment the ovitrap surveillance of dengue vectors.

INTRODUCTION

The 'tiger mosquito', *Aedes albopictus* (Skuse), which has invaded many countries outside its natural region, is an important vector of several arboviral diseases (Aranda *et al*., 2006). This invasive species of mosquito is commonly dispersed through the eggs in used tyres (Knudsen, 1995) and its introduction to new areas is perceived as a serious public health threat (Gratz, 2004). *Aedes albopictus* along with *Aedes aegypti* are vectors of chikungunya and were involved in the 2005-2006 outbreak in the Indian Ocean region (Martin *et al*., 2010). *Aedes albopictus* has been shown to play a role in dengue transmission in Kerala and Delhi in India (Das *et al*., 2004; Kumari *et al*., 2011).

Aedes mosquitoes, especially *Ae. albopictus* are widely present in the northeastern region of India where the weather conditions are favourable for mosquito proliferation. Surveillance of disease vectors is an important component in the management of vector-borne diseases. Ovitrap surveillance is advantageous due to its high sensitivity, ease of management in the field and low material cost (Carrieri *et al*., 2011). Baiting the ovitraps with plant infusions was found to increase the sensitivity of surveillance efforts (Trexler *et al*., 1998). Rice, banana and mango are commonly cultivated crops in this part of the country and the plant debris are available throughout the year. Hence, the present study was carried out to establish the suitability of these plant infusions in enhancing the efficacy of ovitrap surveillance. The optimum concentrations of the plant infusions were determined and the efficacy of different infusions in attracting gravid females of *Aedes albopictus* for oviposition was compared.

MATERIALS AND METHODS

Plant infusions

Rice (*Oryza sativa*) straw, grass (*Pennisetum sp.*) hay, mango (*Mangifera indica*) leaves and banana (*Musa paradisiaca*) leaves collected from Tezpur, Assam were used for preparation of the plant infusions. The plant materials were washed in water to remove organic matter if any and dried under shade for 2 days. The infusions were prepared by soaking 30 g each of the plant materials in 300 ml of distilled water for 7 days in a sealed polyethylene container. The infusions thus obtained were filtered through sterile cotton gauze to obtain the stock solutions, which were stored for 48 hours under ambient temperature conditions (25-30ºC). A range of concentrations from 5 to 50% of the infusions were made by diluting the stock solutions in tap water, whereas traps containing plain tap water were used as control.

Oviposition response

Black polyethylene cups (350 ml) with strips (24 x 8 cm) of blotting paper taped on the inner side were used as the ovitraps. The cups were filled with 5, 10, 20, 30, 40 and 50% concentrations of the plant infusions (300 ml each). There were five replicates for each concentration of the infusions and control. The ovitraps with the four plant infusions and plain tap water as control were placed separately in four wire mesh cages of 60 cm^3 size. Adult gravid female *Ae. albopictus* mosquitoes (50-60 numbers) obtained from the mosquito rearing facility of the Defence Research Laboratory, Tezpur were released in to each cage. Cotton pads soaked in 10% sucrose solution were provided in the cages for nutrition of the mosquitoes. The number of eggs laid in each ovitrap was recorded 72 hours after the release of the mosquitoes.

Data analysis

The mean number of eggs (density) per ovitrap (± standard error) of eggs was determined for each treatment. Oviposition activity index (OAI) for each treatment was calculated as per the formula, OAI = (NT - NC) / (NT + NC), where NT and NC are the total number of eggs in the test solution (infusion) and control (plain water) respectively (Phamsokosolsil & Soonwera, 2012). OAI values range from -1 to +1 and 0 indicates neutral response, whereas positive values indicate that more eggs were laid in the test solution than in control. The egg counts were subjected to square root transformation and the egg densities corresponding to different strengths of each infusion were compared using one-way ANOVA.

RESULTS

The number of eggs laid per trap (per trap density) in the ovitraps enhanced with 5 and 10% rice (*O. sativa*) straw infusion were 218.6 ± 73.8 and 423 ± 110.2, which were not significantly different from the control ovitraps with plain water (136.5±61.5; $P > 0.05$). The per trap density of eggs was the highest in the ovitraps enhanced with 30% (580 ± 51.3) followed by 20% (546 ± 103.8) rice straw infusions. The egg density in the ovitraps containing 40 and 50% infusions of rice straw were 146.7 ± 29.8 and 130.7 ± 12.2, which were significantly lower than that for 30% infusion ($P < 0.05$). The egg density in the ovitraps with 5% grass infusion (228 ± 17.6) did not differ significantly ($P > 0.05$) from the control traps with plain water (144.5 ± 61.5). The highest number of eggs laid were in the ovitraps with 30% (623.6 ± 41) followed by 20% (547.2 ± 38.7) and 10% (462.2 ± 41.6) grass infusions. The density of eggs in traps with 40% (310.7 ± 137) and 50% (334.3 ± 62) infusions were significantly lower than the traps with 30% infusion ($P < 0.05$) (Table 1).

The egg density in the ovitraps with 5% and 10% mango (*M. indica*) leaf infusions were 149 ± 26.1 and 183 ± 11.4, which were not significantly different from the control traps (178.5 ± 29.5; $P > 0.05$). The highest number of eggs was observed in the traps with 20% mango leaf infusion (403.2 ± 57.4) although it was not significantly higher than the traps with plain tap water. The number of eggs in ovitraps with 30, 40 and 50% infusions were 296.2 ± 33.4, 198.3 ± 83.4 and 142.3 ± 50 respectively. The ovitraps with banana

Table 1. Response of *Aedes albopictus* to ovitraps enhanced with rice (*Oryza sativa*) and grass (*Pennisetum* sp.) hay infusions

Hay infusion		Number of eggs laid per trap (Mean± SE_{mean})[1]
Oryza sativa	5%	218.6±73.8[abc]
	10%	423±110.2[abc]
	20%	546±103.8[bc]
	30%	580±51.3[c]
	40%	146.7±29.8[ab]
	50%	130.7±12.2[a]
	Control	136.5±61.5[a]
	F	5.785
	p	0.001
Pennisetum sp.	5%	228±17.6[ab]
	10%	462.2±41.6[bcd]
	20%	547.2±38.7[cd]
	30%	623.6±41[d]
	40%	310.7±137[abc]
	50%	334.3±62[abcd]
	Control	144.5±61.5[a]
	F	10.054
	p	0.000

[1] ANOVA followed by Tukey HSD. Values in same column followed by same letters are not significantly different (p>0.05)

Table 2. Response of *Aedes albopictus* to ovitraps enhanced with mango (*Mangifera indica*) and banana (*Musa paradisiaca*) leaf infusions

Leaf infusion		Number of eggs laid per trap (Mean± SE_{mean})[1]
Mangifera indica	5%	149± 26.1[a]
	10%	183±11.4[ab]
	20%	403.2±57.4[b]
	30%	296.2±33.4[ab]
	40%	198.3±83.4[ab]
	50%	142.3±50[a]
	Control	178.5±29.5[ab]
	F	5.170
	p	0.002
Musa paradisiaca	5%	175.3±77.1[abc]
	10%	301.6±22.1[bc]
	20%	328.6±18.7[c]
	30%	132.3±43.4[ab]
	40%	125.3±18.5[ab]
	50%	92.3±18.8[a]
	Control	198±20[abc]
	F	7.333
	p	0.001

[1] ANOVA followed by Tukey HSD. Values in same column followed by same letters are not significantly different (p>0.05)

(*M. paradisiaca*) leaf infusions (5 and 10%) were having 175.3 ± 77.1 and 301.6 ± 22.1 eggs per trap respectively, which were not significantly different from the traps with plain water. The highest number of eggs per trap (328.6 ± 18.7) was observed in the ovitraps containing 20% banana leaf infusion. The densities of eggs in the ovitraps with 30, 40 and 50% banana leaf infusions were 132.3 ± 43.4, 125.3 ± 18.5 and 92.3 ± 18.8 respectively (Table 2).

Comparison of the oviposition activity index (OAI) among different concentrations of rice straw infusion indicated that the OAI was the highest in 30% (0.62) followed by 20% infusion (0.6), whereas the lowest was in 50% infusion (-0.02). In the case of grass infusions, the highest OAI was in 30% (0.62) followed by 20% (0.58), whereas the lowest was in 5% infusion (0.22). Among the mango leaf infusions, the OAI at the highest was observed with 20% (0.39) followed by 30% (0.25), whereas the lowest was with 50% (-0.11). The OAI among the banana leaf infusions was the highest in 20% (0.25) followed by 10% (0.21) infusions, whereas the lowest was in 50% infusion (-0.36). Overall, the OAI was the highest in 30% infusions of grass hay and rice straw, whereas the lowest in 50% banana leaf infusion (Fig. 1).

DISCUSSION

The selection of oviposition sites by mosquitoes is based on the physical characteristics such as colour, substrate texture, odorants and other chemicals (Bentley & Day, 1989). *Aedes albopictus* and *Ae. aegypti* are container-breeding mosquitoes, which develop in water-filled containers having organic detritus and the associated microbial fauna, which the larvae utilise as food (Ponnusamy *et al*., 2008). Hence, the environments that contain an abundance of decaying leaf litter generally

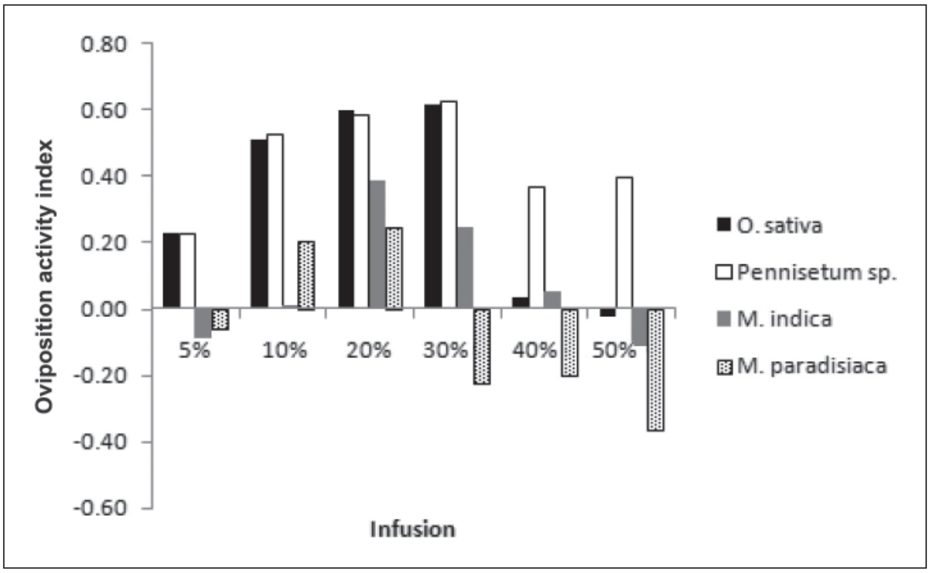

Figure 1. Oviposition response of *Aedes albopictus* to hay and leaf infusions

support mosquito growth, resulting in increased larval development and subsequent adult populations (Dieng *et al.*, 2002). However, the leaf detritus present in such container habitats supporting mosquito growth varies in microbial fauna and decomposition rates. Microbial activity on detritus results in the production of many metabolites which in turn attract gravid females for ovipositon in the containers (Ponnusamy *et al.*, 2008). Although, the effectiveness of ovitraps in general was found to be enhanced by the addition of infusions, the level of attraction to a species depends on the type of plant materials used. Hay and bamboo infusions were attractive to *Ae. aegypti* while oak and maple leaf infusions to *Ae. albopictus* (Holck *et al.*, 1988; Obenauer *et al.*, 2009).

Ovitrap surveillance will be helpful in understanding the seasonal prevalence and distribution of *Aedes* mosquitoes in this region. The use of plant infusions as oviposition attractants in ovitraps would help in better surveillance by attracting the gravid females to the ovitraps. In the present study, the suitability of the infusions made using the plant materials widely available in northeastern India such as rice straw, grass, mango leaf and banana leaf infusions for enhancement of the ovitraps was evaluated. The oviposition response was the highest with 30% concentrations of rice straw and grass hay infusions wherein the number of eggs received was more than 4 times than plain water. Ovitraps with 10% rice straw infusion received twice as many eggs as plain water in field trials conducted in Cambodia (Polson *et al.*, 2002), whereas thrice as many eggs in the present study. However, the infusions with concentrations beyond 30% had a deterrent effect on the ovipositing females. The compounds isolated from hay infusion such as 3-methylindole and 4-ethylphenol were shown to induce positive oviposition response in *Ae. albopictus*. However, these compounds induced repellency or oviposition deterrence at high concentrations (Allan & Kline 1995) as observed in the present study. The infusions of mango and banana leaves (5-50%) were not suitable for enhancing the ovitraps since there was no significant increase in oviposition response with these infusions. Hence, 30% solutions of rice straw and grass hay infusions were observed to be the most suitable for use in ovitrap surveillance.

The oviposition response of *Ae. aegypti* and *Ae. albopictus* to plant infusions vary with the plant species, biomass and the duration of fermentation (Ponnusamy *et al.*, 2010). The studies carried out in Brazil revealed that the ovitraps with *P. maximum* infusions were effective for the surveillance of *Aedes* mosquitoes (Santana *et al.*, 2006). Oak leaf infusions, regardless of the concentration or age, enhanced the oviposition response of *Ae. albopictus* (Trexler, 1998). Ovitrap pairs with hay infusion and a 10% dilution of the infusion in tap water was observed to yield 8 times more eggs of *Ae. aegypti* and such enhanced pairs were more attractive than other combinations of infusion, water or methyl propionate (Reiter *et al.*, 1991). Infusions (50%) of cashew (*Anacardium occidentale*) leaves were found to stimulate oviposition in *Ae. aegypti* (Santos *et al.*, 2010). The response to oviposition stimuli such as hay infusion and larval water was stronger in *Ae. albopictus* than in *Ae. aegypti* (Allan & Kline, 1995).

The chemical stimuli for mosquito oviposition include pheromones associated with eggs, carboxylic acids, methyl esters and bacteria present in larval water (Obenauer *et al.*, 2010). *Aerobacter aerogenes* present in hay infusion was found to be responsible for enhancing the attractiveness of ovitraps to gravid females of *Ae. aegypti* and *Culex quinquefasciatus* (Hazard *et al.*, 1967). *Acinitobacter calcoaceticus* and *Enterobacter cloacae* present in larval rearing water were identified to be stimulating the oviposition response in *Ae. aegypti* (Benzon & Apperson, 1988). The oviposition pheromone n- heneicosane, identified and characterised from the larval cuticle of *Ae. aegypti*, attracted gravid females for oviposition (Seenivasagan *et al.*, 2009). Propyl octadecanoate, a fatty acid ester, enhanced oviposition activity in females of *Ae. aegypti* and can potentially be used in ovitrap surveillance (Seenivasagan *et al.*, 2012). Similarly, tetradecanoic, dodecanoic and hexadecanoic acids induced positive oviposition response in *Aedes aegypti* (Sivakumar *et al.*, 2011).

Ovitraps are practically useful surveillance tools as they are inexpensive, non-intrusive and do not require specialized skills to operate (Polson *et al.*, 2002). Ovitraps enhanced with plant infusions attract more gravid females in comparison to traps with plain water by the release of volatiles produced through microbial fermentation (Trexler *et al.*, 2003). Strong infusions contribute to long range attraction, whereas mosquitoes prefer to oviposit in weaker solutions and hence pairs of strong and weak infusions were found to be highly effective for *Aedes* surveillance (Reiter *et al.*, 1991). Since transporting the prepared ovitraps may not be feasible, the bottles containing the prepared infusions can be taken to the field (Polson *et al.*, 2002). The results of the present study indicated that rice straw and grass infusions can be used for enhancing the efficacy of ovitrap surveillance. Since these plant materials are available in plenty in northeastern India, the infusions can be easily prepared and used for field surveillance of *Ae. albopictus* in this region.

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