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OPERATIONAL NOTE

EVALUATION OF THE EFFICIENCY OF BEETROOT PEEL (*BETA VULGARIS*) IN OVITRAPS AS AN ATTRACTANT FOR SURVEILLANCE OF ARBOVIRUS VECTORS IN THE MUNICIPALITY OF AGRESTINA, STATE OF PERNAMBUCO, BRAZIL

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ABSTRACT. It is estimated that there are over 500 species of arboviruses worldwide, with more than 150 of them directly associated with human diseases, the majority of which are zoonotic. Among the main arboviruses circulating in Brazil, dengue, Zika, and chikungunya stand out, all transmitted through a common vector, *Aedes aegypti*. Given this scenario, the development and implementation of more efficient surveillance strategies become urgent. This study aims to compare and evaluate the efficiency of beetroot peel, *Beta vulgaris*, as an attractant for *Ae. aegypti* oviposition under field conditions in the municipality of Agrestina, State of Pernambuco, Brazil. Beetroot peel extract is a cheap and accessible source of geosmin, which holds significant potential as an attractant for mosquitoes due to its resemblance to microbial volatiles found in water bodies rich in organic material. During the study period (November 2023 to April 2024), 40 traps were set in each neighborhood, with 20 of each attractant. Two traps were used per property, one following the traditionally used model (beer yeast infusion) and another containing beetroot peel. Overall, in both analyzed neighborhoods (Campo Novo and Cohab), beetroot peel appeared to be a more efficient attractant for the oviposition of culicids. Beetroot peel showed the highest values in egg abundance and in the analyzed indices.

KEY WORDS *Ae. aegypti*, attractant, beetroot peel, dengue, oviposition, ovitrap

The mosquito *Aedes aegypti* (L.) poses a major impact on public health in Brazil and has been the primary vector of the dengue virus for over 30 years. It is also responsible for the transmission of recently introduced emerging arboviruses in the New World, including dengue (DENV), chikungunya (CHIKV), and Zika (ZIKV). These arbovirus infections are considered significant challenges for public health. Dengue is already endemic in almost the entire Brazilian territory, which favors its dissemination (Azevedo et al. 2015).

Uncontrolled urbanization, inadequate collection and treatment of solid waste, lack of basic sanitation and climatic factors such as temperature conditions throughout the year contribute to the continuous proliferation of *Ae. aegypti* (Ministério da Saúde do Brasil MS, 2024). Oviposition traps are an efficient and economically viable way of detecting the presence of *Ae. aegypti* and *Aedes albopictus* (Skuse) through deposited eggs (Acero-Sandoval et al. 2023, Schultes et al. 2021). Thus, it is possible to identify regions with a high occurrence of these vector species. Efficient monitoring and better targeting of actions help prevent mosquito proliferation and the use of insecticides, contribute to the prevention of new epidemic outbreaks. Beer yeast solution is known to increase attractiveness and stimulate egg deposition by female mosquitoes (Serpa et al. 2013, Snetselaar et al. 2014, James et al. 2022). Geosmin, communly found in beetroot peel has shown to be an attractant for honeybees, ants, mosquitoes, and springtails (Garbeva et al. 2022). The smell of geosmin is attractive to *Ae. aegypti* females for oviposition (Melo et al. 2020). Geosmin, a volatile organic compound previously identified in cyanobacteria, has shown attractive properties in laboratory assays with increased number of mosquito eggs relative to control traps (Mulatier et al. 2022). In this study we aimed to evaluate the efficiency of beetroot peel (*Beta vulgaris* L.) as an attractant for *Ae. aegypti* oviposition under field conditions. Ovitraps used in this study consisted of 1-liter, labeled black plastic containers, containing 300 ml of water supplemented with an attractant. Each trap included a numbered and partially submerged Eucalyptus paddle (width: 2.5 cm; height: 12.5 cm). The ovitraps were set in the peridomestic, shaded, and rain-protected areas, free from constant human and animal movement. They were positioned at a height of 70 to 100 cm above the ground, with placement directly on the ground avoided to minimize accident risks. Field tests were conducted in urban areas with the setting up of ovitraps in two neighborhoods located in zones susceptible to *Ae. aegypti*, previously selected based on entomological survey data in the municipality of Agrestina, state of Pernambuco (Soares, 2018). The two neighborhoods were in the municipality of Agrestina,

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Table 1. Ovitrap indices of *Aedes aegypti* for beetroot peel and beer yeast infusion in the municipality of Agrestina, state of Pernambuco, Brazil.[1]

Neighborhood	Treatment	Cycles	Inspected	Positive	No. of eggs	OPI (%)	EDI (n)	MEI (n)
Campo Novo	BP	1	20	15	1405	75	93.66	70.25
		2	20	11	996	55	90.54	49.8
		3	20	11	1005	55	91.36	50.25
		4	20	17	1843	85	108.41	92.15
		5	20	15	912	75	60.8	45.6
	BYI	1	20	9	722	45	80.22	36.1
		2	20	12	809	60	67.41	40.45
		3	20	13	918	65	70.61	45.9
		4	20	16	1112	80	69.5	55.6
		5	20	10	847	50	84.7	42.35
Cohab	BP	1	20	18	1840	90	102.22	92
		2	20	13	702	65	54	35.1
		3	20	14	1064	70	76.0	53.20
		4	20	20	2063	100	103.15	103.15
		5	20	19	1228	95	64.63	61.4
	BYI	1	20	13	1015	65	78.07	50.75
		2	20	10	1023	50	102.3	51.15
		3	20	10	1003	50	100.3	50.15
		4	20	16	1402	80	87.62	70.1
		5	20	12	1021	60	85.08	51.05

[1] OPI, ovitrap positivity index; EDI, egg density index; MEI, mean egg index; BP, ovitraps treated with beetroot peel; BYI, ovitraps treated with beer yeast infusion.

Pernambuco, Brazil: Campo Novo (8°27'32.3"S 35° 56'22.3"W), and Cohab (8°27'00.6"S 35°57'07.8"W). In each neighborhood, a total of 40 traps were set, split into two treatments: 1) 20 ovitraps containing a 0.04% concentration of beer yeast infusion (BYI) (Marylene et al. 2023) and 2) 20 ovitraps containing water and 5 grams of beetroot peel (BP). We maintained ovitraps for 5 days in the households before collecting the wooden paddles for counting and remove all ovitraps. Subsequently, the paddles were sent to the laboratory for egg counting, and viability was assessed through microscopic analysis. The quantity of eggs per each paddle in each ovitrap was recorded individually. Positive paddles were immersed in water for hatching in plastic basins. After the larvae hatched and developed into adults, the specimens were identified by direct observation of morphological characteristics under a stereoscopic microscope (Leica DMD108® Germany) and using the dichotomous key elaborated by Consoli and Lourenço-de-Oliveira, 1994.

To assess the effectiveness of attractants in mosquito oviposition, the following indices were used: 1) ovitrap positivity index (OPI) = (number of positive traps/number of inspected ovitraps x 100); 2) egg density index (EDI) = (total number of eggs on paddles/total number of positive traps); 3) mean egg index (MEI) = (number of eggs collected/number of inspected ovitraps) (Silva and Limongi, 2018). We used Fisher's exact test to determine differences between the proportion of positive ovitraps between treatments (Contigency table 2x2; Variables: ovitrap treatment; Outcome: positive vs. negative), calculated by the Prism 10.2.0 (GraphPad Software, CA, USA). Normality for the number of eggs per household was tested using 4 standard tests available in Prism 10.2.0 (i.e. D'Agostino and Pearson test, Anderson-Darling test, Shapiro-Wilk test, and Kolmogorov-Smirnov test). The paired Student's t-test in Prism 10.2.0 was used to compare the number of eggs from ovitraps with different treatments, with a significance level of $P < 0.05$. The oviposition activity index (OAI) used for inferring the attractivity/stimulation vs. repellence/deterrence of substances to oviposition was calculated by Kramer and Mulla equation (Kramer and Mulla 1979). The oviposition index (OAI) was calculated between ovitraps with BP (considered as the treatment) and ovitraps with BYI (considered as the control). This index was calculated for the overall number of eggs in each neighborhood and for each household. The Prism 10.2.0 was used to calculate the mean and 95% confident intervals for OAI calculations.

All adult mosquitoes obtained from the 5 collections cycle were identified as *Ae. aegypti.* In the locality of Campo Novo, the OPI for beetroot peel (BP) ranged from 55% to 85%, while for BYI, it ranged from 45% to 80% (Fisher's exact test, $P =$ 0.2370). Although there was no statistically significant difference in ovitrap positivity in this locality. The EDI showed higher values for BP, ranging from approximately 90 to 110 eggs per positive trap, compared to BYI with approximately 60 to 85 eggs per positive trap. Moreover, the MEI was consistently higher for BP, ranging from approximately 45 to 92 eggs per trap, compared to BYI of approximately 36 to 56 eggs per trap (Table 1). In the locality of Cohab, BP exhibited a significantly higher OPI, ranging from 65% to 100%, compared to 50% to 80% for

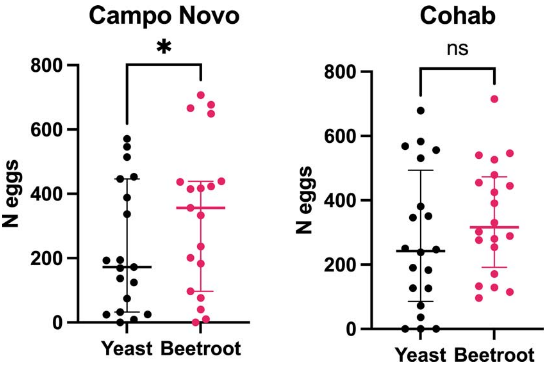

Fig. 1. Distribution of egg number in ovitraps treated with beetroot peel against the standard beer yeast infusion. Black dots = number of eggs per ovitraps with beer yeast infusion; pink dots and lines = number of eggs per ovitraps with beetroot peel; black lines = median and interquartile range for beer yeast infusion; pink lines = median and interquartile range for beetroot peel; ns: $P > 0.05$ and *: $P < 0.05$.

BYI (Fisher's exact test, $P = 0.0003$). Additionally, the EDI was also consistently higher for BP, with values ranging from 54 to 103 eggs per positive trap, compared to 50 to 87 eggs for BYI. Furthermore, the MEI consistently indicated a greater attraction to BP, with counts ranging from 35.1 to 103.15 eggs per trap, compared to 50.75 to 70.1 eggs for BYI (Table 1).

When we compared the number of eggs per household for both treatments, Campo Novo presented a significantly higher number of eggs per household for BP than BYI (Mean difference = 102.8, 95%CI [16.89–188.8]; paired Student's t-test, $P = 0.0217$), while Cohab did not present significant differences between both treatments (Fig. 1). Parametric tests were used to compare the number of eggs per household for ovitraps with BP and BYI because the analysis suggested that both datasets follow normal distribution. Both neighborhoods presented positive $OAI_{Cohab} = 0.116$; $OAI_{CampoNovo} = 0.181$), indicating a potential attractive response for BP when compared with BYI. When we analyzed OAI per household, we observed a higher OAI mean for Cohab ($OAI_{mean} = 0.244$, 95%CI [0.048, 0.441]) significantly different for "0," suggesting an attractive response for BP when compared with BYI, while Campo Grande did not show a significant attractive signal ($OAI_{mean} = 0.124$, 95%CI [–0.112, 0.360]).

It is generally accepted that, *Ae. aegypti* prefers to breed in clean water however some studies demonstrated that these mosquitoes deposited a higher number of eggs more frequently in ovitraps with hay infusion than in those with plain water (Chadee et al. 1993, Reiter et al. 1991). Geosmin is one of the most recognizable and common microbial smells on the planet and has a porimissing potentional as an attractant to *Ae. aegypt* while Beetroot peel extract is a cheap and accessible source of geosmin (Melo et al. 2020). The higher proportion of positive ovitraps with a consistently positive OAI average in Cohab and the greater number of eggs in Campo Novo for beetroot peel suggest that this attractant performs better than the standard beer yeast infusion. The density/positivity indexes and OAI fluctuate between showing similar attraction or a slightly better performance for beetroot peel in attracting *Aedes* mosquitoes. These results highlight the potential of beetroot peel as an alternative attractant for mosquito surveillance and control efforts.

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