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SHORT COMMUNICATION

OVIPOSITION RATES, LARVAL AND PUPAL SURVIVAL RATES OF *CULEX QUINQUEFASCIATUS* IN DIFFERENT PLANT LEAF INFUSIONS.

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Animals select ovipositional sites that maximize their fitness. The ovipositional site determination of mosquitoes is based on a complex array of factors including visual ones and those mediated by semio-chemicals. Positive response to hay infusion has been reported in several species of *Aedes* and *Culex*.[1] Bacteria present in fermenting hay infusion produce compounds that attract gravid mosquitoes. In previous studies on such organic infusions, hay and grass had been used. In a natural system, mosquitoes oviposit in pools containing leaves of different plant species and other wastes from the surrounding area. Attractiveness of the ovipositing medium can differ according to chemicals found in the plant species. The aim of the study was to test whether the ovipositional rates vary among infusions of leaves of different species. As there were differences in oviposition rates in the different plant leaf infusions, we also studied the functional significance of preferences measured by reproductive success. A preliminary investigation to establish a protocol for ovitrap and peak time of oviposition was also done. The four plant species used in the study were: Neem (*Azadirachta indica*) which is known to have antifeedant, insect growth regulator and oviposition deterrent qualities; water fern *Azolla* and *Lemna* - commonly found in temporary ponds; *Cycas ficalensis* (based on a preliminary screening) and a grass (*Axonopus affinis*).

All experiments were done at the Eastern University premises (07° 43'N, 81° 42'E) in the East Coast of Sri Lanka. This area is flat not exceeding 7.62 m in elevation with a tropical climate; temperature ranges from 23.6 to 35.1°C and annual rainfall ranges from 864 to 2897 mm (from October to January). The experiments were conducted during the rainy season from October 1999 to January 2000. A variety of locally used containers (cleaned and of about the same size) were tested first viz: a glass beaker (transparent, 600 ml), plastic container (transparent) and greenish discarded soft drink bottle (cut to exclude the narrow end), empty coconut shell (household refuse) and wide mouthed clay pot (unglazed). The containers were arranged in linear transect clusters approximately 30 cm apart and 1 m between the clusters; 5 clusters were placed each day; this was repeated

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for 6 days. The containers were filled with 250-300 ml of mildly chlorinated water obtained from a drinking water well and were placed at 1800 hours and were observed each hour. During each hour egg clusters if any, were removed carefully, containers were well cleaned and refilled with fresh tap water. In a second experiment, leaf infusions were prepared by soaking 1000 g of chopped leaf in 5 *l* of tap water and fermenting for 4-5 days; 200 ml of the infusion was used in each clay container; the control group had 200 ml tap water. Six replicates of each leaf type were placed each day; the trials were repeated for 8 d. In a third experiment, the same four types of leaf material were cut into small pieces of about 1 cm and dried for 48 h at 60° C; 12 g of leaf were placed in a 600 ml beaker with 400 ml tap water for 3 d. Leaves can provide a substrate for growth of microbes, the primary food of larvae. One field collected egg raft was placed in each beaker. The total number of eggs in each egg raft was counted under a binocular microscope prior to placement in the container (six replicates for each). These beakers were covered with mosquito netting secured with a rubber band and arranged near a window in natural light with approximately 12:12 dark:light cycle. Ambient temperature ranged from 25-29 °C during the study. The observations of life cycle included: percentage of hatching (from the number of larvae counted on day 1 post hatch and the total number of eggs in the raft); larval survival (the number of larvae counted on day 6 post hatch in relation to the number of larvae found on day 1 post hatch); pupal size (two pupae were separated from each container n = 12 for each leaf type) and the length of pupae was measured from the base of trumpet to the end of wing pad with a stage microscope and percentage pupation was calculated from cumulative pupae on a particular day divided by the sum of remaining larvae and the cumulative pupae. The behaviour of the larvae in the different leaf infusions was also monitored by observing thirty larvae from each type of leaf infusion and their behaviour was recorded every 5 min, using instantaneous scan censuses for 3 h. Activities included: thrashing, browsing, filtering and resting. All egg rafts collected in the field were taken to the laboratory and reared separately to adult stage to confirm identification. Data on number of egg rafts were log (n + 1) transformed and analysed for significant difference by Student's t test and ANOVA. The percentage data were arcsine transformed and subjected to ANOVA. Data were subjected to Duncan's Multiple Range Test (DMRT) if a significant interaction was found.

Only one species *C. quinquefasciatus* oviposited in the containers. Ninety four percent of the population in the study area were *C.quinquefasciatus*; other species were *C. fuscocephala*, *C. sitiens*, *Mansonia* spp. and *Aedes* spp. Obviously the abundance of a species in an area will also influence oviposition using attractants. Clay container attracted more eggs rafts (total 12) than the others ($F_{3,23} = 8.03$, $p = 0.001$); two egg rafts were found in the coconut shell; plastic and glass containers had 1 egg raft each. Comparison can be drawn to the study on *Armigeres* species[5], which prefer water with a brick in it for oviposition than water alone. The possibility of a chemical factor in the clay containers attracting

mosquitoes needs to be further investigated. Egg laying periodicity showed a bimodal pattern with a peak of 2 egg rafts per day between 0700-0800 h and 0800-0900 h and a much smaller peak in the evening 1700-1800 h (overall ANOVA $F_{1,143}$ = 1.58; p = 0.06). The peak at 0700-0800 h was significantly higher than the peak at 800-900 h ($F_{1,11}$ = 8.04; p = 0.017). In a recent study in Tanzania two peaks of oviposition were observed for the species *C. quinquefasciatus* but the highest peak was in the evening. Morning ovipositional activity has been reported for *Anopheles gambiae* and *Anopheles funestus*.

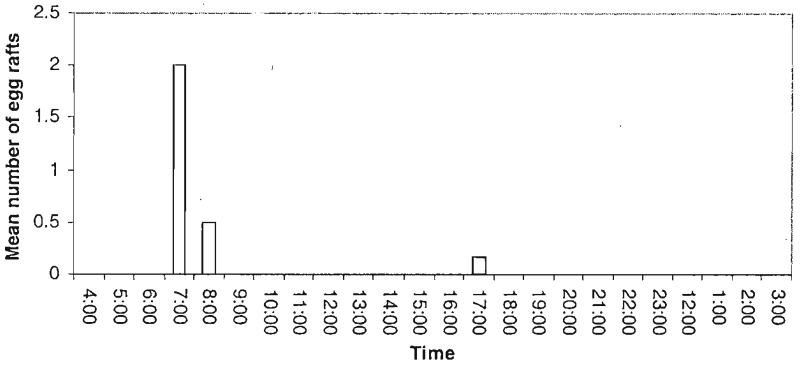

Figure 1: Circadian oviposition pattern of *Culex quinquefasciatus*

The number of egg clusters collected in the different types of leaf infusions varied significantly ($F_{4,29}$ = 6.01; p = 0.001). 28, 8 and 2 eggs rafts were collected in *Cycas*, grass and duckweed infusions respectively. Egg rafts were not found from Neem infusion and control. Neem leaves seems to act an oviposition deterrent in addition to Neem seeds. Egg hatching rates in the four types of leaf infusions were different ($F_{3,23}$ = 14.01, p<0.0001). Hatching rates were not significantly different between grass and *Cycas* spp leaf infusions. Larval survival was not significantly different between those reared in grass and *Cycas* spp leaf infusions. Larval mortality in Neem infusion was 100 % on day 8. Pupation started on day 6 post hatch and continued till day 8 post hatch in *Cycas* and grass medium (Table.1). The larvae surviving on day 6 showed 100% cumulative pupation in *Cycas* and grass medium by day 8 ($F_{3,23}$ = 11.37, p = 0.001). Pupation of larvae in duckweed was prolonged and continued up to day 15 post hatch; the remaining larvae that did not pupate died. In *Cycas* spp and grass there was 100% (with reference to larva present on day 6 post hatch) adult emergence on day 9. The overall percentage of total adult that emerged on day 16 with reference to the number of larvae on day 1 was not significantly different between the three groups: *Cycas* spp. grass and duckweed ($F_{2,17}$ = 0.168, p = 0.85). The ratio of adult females that emerged on day 9 was low in duckweed ($F_{2,18}$ = 13.8, p<0.0001). Pupal length was more in those reared in the *Cycas* medium followed by that of grass and the lowest was recorded in duckweed ($F_{2,35}$ = 29.43, p<0.001). There was an overall significant difference

Table 1: Percentage (SE) of eggs hatched, larval survival on day 6 post hatch, cumulative pupation on day 8 and 11, the percentage of adult emerged and the ratio of females emerged in *Culex quinquefasciatus* in the different leaf infusions (N=6). Means with a different letter are significantly different at P<0.05.

	Duckweed	Grass	*Cycas spp*	Neem
Mean Percentage of hatching (%) ± SE	94.9 ± 0.48 a	86.0 ± 2.1 b	87.0 ± 2.9 b	44.7 ± 11.6 c
Mean percentage larval survival on day 6 (%) + SE	84.3 ± 15.7 a	31.8 ± 9.9 b	18.9 ± 6.4 b	12.9 ± 13.0 b
Cumulative percentage pupation on day 8 (%) ± SE	24.1 ± 11.0 b	100.0 ± 0.0 a	100.0 ± 0.0 a	-
Cumulative percentage pupation on day 11 (%) ± SE.	52.3 ± 14.9 b	100.0 ± 0.0 a	100.0 ± 0.0 a	-
Pupal length (mm) ± SE.	4.6 ± 0.57 c	5.7 ± 0.56 b	6.3 ± 0.45 a	-
Percentage of adult emerged on day 9 (ref. larvae on day 6)	12.3 ± 3.8 b	100.0 ± 0.0 a	100.0 ± 0.0 a	-
Percentage of adult emerged (%) ± SD (ref. initial larva)	32.3 ± 14.9 a	31.8 ± 10.8 a	23.9 ± 6.4 a	-
Ratio of female emerged on day 9 post hatch	22.9 ± 5.7 b	39.2 ± 5.2 a	43.5 ± 3.3 a	-
Ratio of females emerged on day 15	38.7 ± 3.1 a	39.2 ± 5.2 a	43.5 ± 3.3 a	-

between the behaviour of larvae in the four groups ($F_{2,359}$ = 26.84; p<0.001) with significant interaction ($F_{6,359}$ = 26.37; p<0.001). Filtering activity was high in *Cycas* (65.3 ± 4.1 %) and duckweed (64.0 ± 3.8%). This was followed by grass infusion (31.3 ± 4.8) and larvae in the Neem infusion only spent 6.0 (± 2.2) % time in filtering activity. Browsing was only observed in duckweed medium. Larvae reared in Neem infusion spent their time between being inactive and thrashing with a minimum time spent in feeding. Inactivity was also high in grass infusion (45.3%). The egg hatching rate and early larval survival was unexpectedly high in duckweed. In conclusion, it could be said that in addition to grass other types of fermented leaf can be used in designing traps. Neem leaf acts as an oviposition deterrent and larvicide. Thrashing seems to occur in an unsuitable condition in Neem. The larvae in the Neem infusion were also inactive for most of the time. Feeding activity was also low in the grass medium. Even though *Cycas* spp leaf infusion was preferred over grass infusion for egg laying there did not seem to be any tangible functional advantage as far as reproductive success is concerned. In a natural situation, ground pools with Neem leaf may help in the reduction of mosquito populations.

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