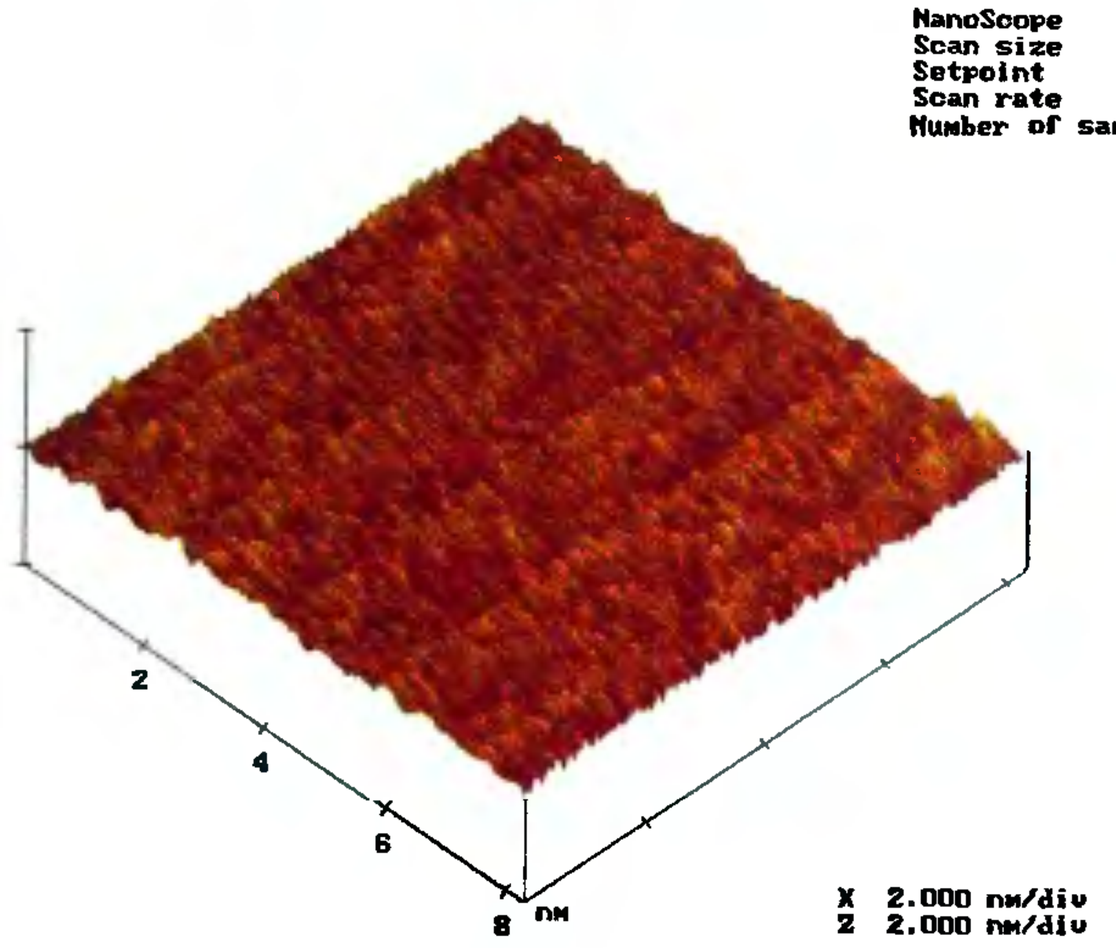


**Figure 2.** Seed coat surface, Lalgola strain.

lished) reports at least 6 different molecules. Together with the chemical studies an ultrastructural atlas of various strains could be prepared.

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# Heneicosane: An oviposition-attractant pheromone of larval origin in *Aedes aegypti* mosquito

Oviposition aggregation pheromone can specifically influence many insect females to lay eggs in the same site resulting in more eggs deposition. The first unequivocal evidence for an oviposition pheromone occurrence in an insect vector mosquito was in *Culex*[1]. However, studies on the influence of eggs of conspecific and heterospecific larval stages on the site selection by various *Aedes* species have given conflicting results[2,3] Surprisingly, in *Anopheles* mosquito the presence of conspecifics may actually be a deterrent[4].

*Aedes aegypti* prefers to oviposit on water containing the larvae of the same species[5]. This larval conditioned water (LCW) is found to be effective after removing the larvae by filtration and the attractant activity is retained for several weeks. Many groups earlier tried to identify the oviposition-attractant factors present in the LCW, but the extremely small amount released by the larvae thwarted its characterization[3,6]. Here we report the chemical primarily responsible for the oviposition activity of the LCW using gas chromatography coupled with mass spectrometry (GC/MS) followed by biological evaluation in the laboratory.

For these studies, water used for rearing *A. aegypti* larvae only for twenty days continuously was taken after filtration as the LCW. We extracted this LCW with hexane and ether (HPLC grade) sequentially, combined the extracts, concentrated and analysed by GC/MS. Similarly control water was extracted for comparison (blank). GC/MS analyses were per-

**Table 1.** Fragmentation pattern of the additional components in LCW

| Peak retention time (min) | Compounds identified | MW | Fragmentations |
|---|---|---|---|
| 17.25 | Octadecane | 254 | 254 ($M^+$), 57 (100), 71, 85, 99 |
| 17.63 | Isopropyl myristate | 270 | 270 ($M^+$), 43 (100), 228, 102, 60 |
| 20.20 | Heneicosane | 296 | 296 ($M^+$), 57 (100), 71, 85, 99 |
| 21.10 | Docosane | 310 | 310 ($M^+$), 57 (100), 71, 85, 99 |
| 26.82 | Nonacosane | 408 | 408 ($M^+$), 57 (100), 71, 85, 99 |

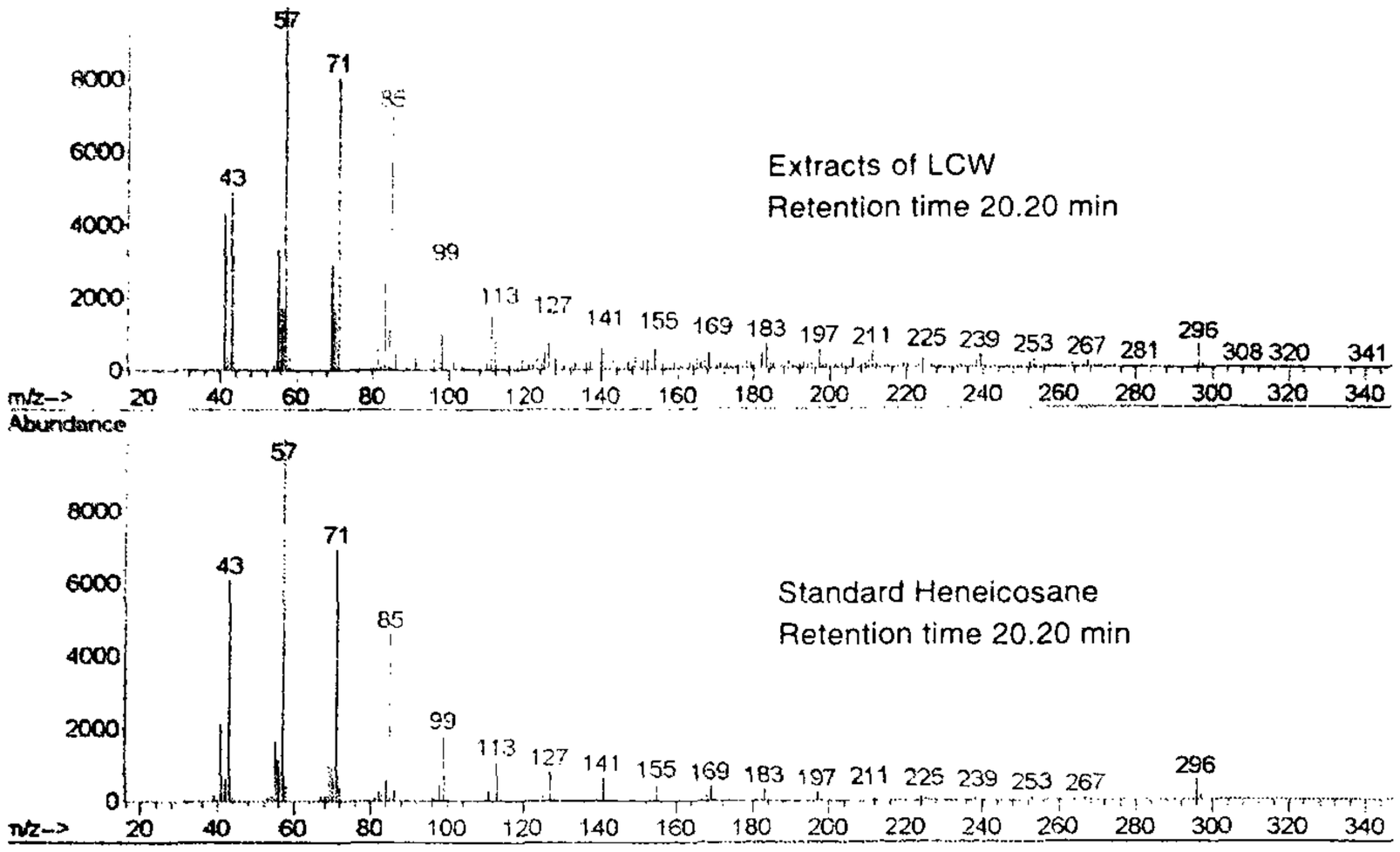


**Figure 1.** Comparison of the mass spectra of heneicosane (standard) and one of the additional components present in the solvent extract of LCW of *Aedes aegypti* mosquito larvae.

formed on a HP 6890 gas chromatograph coupled to a 5973 quadrupole mass spectrometer using capillary column coated with 5% phenyl polydimethyl siloxane stationary phase (HP-5). The column temperature was programmed as follows: 50°C, 2 min, isothermal, then 10°C/min to 280°C ramp., 5 min isothermal (total 30 min run). Comparison between total ion chromatogram of the LCW and the control revealed that the LCW extract contained additional five peaks which could be of larval origin. The compounds were identified as heneicosane, docosane, nonacosane, octadecane and isopropyl myristate on the basis of comparison of their retention times and fragmentation patterns with the commercially available authentic samples (Table 1). The mass spectrum of heneicosane from the LCW and the authentic sample are shown in Figure 1 as a representative example. A GC flame ionization detector however could not identify these tiny amounts.

In order to confirm the above identified compounds to be of larval origin, GC/MS analysis of the hexane extract of the cuticular components from the *A. aegypti* larvae was performed under identical conditions using selected ion monitoring procedure corresponding to the *m/z* values 254, 270, 296, 310 and 408. The retention times and mass spectra of the peaks were in agreement with the compounds obtained from the LCW extract as well as with the authentic samples. From this it is evident that the origin of heneicosane and other identified components must be from the larvae of *A. aegypti* in the LCW.

We evaluated in the laboratory the oviposition attractancy essentially based on the method described by Allan and Kline[3] with slight modification of the above five commercially available chemicals either individually at various concentrations or as a mixture. We varied the concentration of heneicosane (6.9, 69, 690; 50, 69, 90 ppm) in these experiments. This showed that heneicosane is the most promising oviposition attractant for *A. aegypti* females. The eggs laid per replicate (mean ± SE, $n = 50$) were 67.83 ± 10.79 in control water whereas eggs laid per replicate were 121.00 ± 16.26 ($P < 0.01$) in heneicosane-treated water at 69 ppm. Further comparison of egg laying was made with tap water, yeast water and heneicosane-treated water. While there was no statistical difference between the first two, there was 2-fold increase in heneicosane-treated water in comparison to the other two controls. However the eggs laid per replicate were comparatively less than in the LCW (eggs per replicate 179.00 ± 15.55, mean ± SE, $n = 30$) suggesting the possibility of the presence of some minor undetectable components.

Oviposition attractants may have several benefits for the insect species. However, the oviposition behaviour of vectors[7] is not yet fully studied. The oviposition-attractant pheromone identified can be combined with novel vector control strategies (Shri Prakash *et al.*, unpublished).

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