CHAPTER : 9

AN ATTEMPT AT SEPARATION OF THE HEXANE BODY-WASH OF THE FEMALE TOBACCO BEETLE, LASIODERMA SERRICORNE (F.) COLEOPTERA : ANOBIIDAE, CONTAINING OVIPOSITION DETERRING PHEROMONAL ACTIVITY BY THIN LAYER CHROMATOGRAPHY

The existence of a female-produced sex-pheromone in the tobacco beetle, L. serricorne has been reported by Burkholder (1970). Later, in 1972, Coffelt and Burkholder reported that female L. serricorne releases a sex-pheromone which attracts and excites males of the same species and the pheromone was named as serricornin. Levinson et al. (1983) discovered a pheromone-producing gland in the second abdominal segment of the female tobacco beetle. The gland was found to be provided with a duct that leds to an orifice beneath the genital pore. The structural elucidation and the synthesis of the serricornin (Chuman et al., 1979 a and b), determination of the stereochemical configuration (Chuman et al., 1981; Mori et al., 1981 and 1982; Mori et al., 1982 a and b) and structure-pheromone activity relationship of serricornin (Chuman et al. 1982 a and b) have already been reported. Levinson and Levinson (1987) held the opinion that the behavioural responses of male L. serricorne induced by the hexane extract of intact pheromone glands were more intense than those

obtainable with hexane extract of homogenized pheromone gland. They mentioned that probably the reason behind this difference was that the extract of intact glands provides mainly serricornin, the main component, and anhydroserricornin as a minor component (an antagonist) of the sex pheromone, while the extract of homogenized gland may provide alongwith serricornin one or more of pheromonal antagonists.

A chemical study of sex pheromone of tobacco beetle was carried out by Chuman et al. (1985) wherein as many as seven components were isolated from the active fractions of column chromatography of the sex pheromone. The structures of all these compounds were elucidated by gas chromatographic-mass spectroscopic evidences and confirmed through synthesis. According to Chuman et al. (1985) the pheromonal activities of these components revealed that each component was having a different role with respect to the parameters of bio-assay system viz.- (a) attractiveness (b) sex stimulation (c) reactivity. The sex pheromone was extracted from L. serricorne beetles (mixed population, F/M ratio = 1 : 1) with hexane. In the present study also hexane was utilized for preparing female body-wash, and it was found to possess a strong oviposition deterring activity. While testing the responsiveness of the adult male and female beetles to such a body-wash, it was found that the adult males were strongly attracted (Chapter 4).

A careful perusal of the available literature on the biology and chemistry of sex pheromone of L. serricorne points to the possibility of oviposition deterrent activity being present as a component of the sex pheromone itself of the beetle. It is also probable that some of the components of the sex pheromone individually or in combination may be responsible for the presently observed oviposition deterring function. The present study was, therefore, undertaken to investigate the possible roles of chemical fractions with regard to the oviposition deterring pheromonal property of the female L. serricorne (F.) body wash.

MATERIAL AND METHODS

Sufficient amount of body wash was collected by washing required number of female insects in the same way as described in Chapter 2. To separate the wash into its components thin layer chromatography (TLC) was carried out following the procedure described by Touchstone and Dobbine (1978).

Various solvent systems were tested and a mixture of petroleum ether, di-ethyl ether and acetic acid was found to be most effective. For distinct and wider separation of components/ fractions, various ratios of the above mentioned solvents were tested and it was found that 70 : 20 : 2 ratios of petroleum ether, di-ethyl ether and

acetic acid yielded six (6) distinct fractions. Visualization was achieved by exposure to Iodine fumes. The Rf values of different fractions (Fr.) were calculated in the following way:-

$$Rf = \frac{\text{distance of the fractions from origin}}{\text{distance of the solvent front from origin}}$$

The upper most spot was marked as fraction 1 and thus the lower most spot was marked as fraction 6.

For further work, preparative thin layer chromatography (PTLC) was carried out to collect sufficient quantities of all the six fractions. An original 5 ml of body-wash was concentrated to 1 ml. This was applied onto three plates. The chromatogrammes were developed as mentioned earlier, different bands were located, scraped of the plates in separate beakers. Each of the fraction thus collected was washed thrice with 5 ml aliquots of hexane (15 ml), centrifuged at 2500 r.p.m. for 15 minutes and the supernatent were collected, allowed to evaporate to a final volume of 5 ml in each case representing the equivalent of respective fraction present in original 5 ml wash.

The oviposition deterring activity of the different fractions was tested by applying a dose of 40 FE (0.2 ml) on each of leaf disc-stacks, prepared as per the procedure described earlier (Chapter 4). Another set of experiments was conducted to determine the influence of combinations of the three active fractions i.e. Fr. 1, 2 and 6. The

fractions were isolated through PTLC as described earlier. Each fraction was applied at a dose of 20 FE per disc-stacks and the combinations tested were as follows:-

Combinations	Fr. 1	Fr. 2	Fr. 6
i	0.1 ml	0.1 ml	0.1 ml
ii	0.1 ml	Nil	0.1 ml
iii	Nil	0.1 ml	0.1 ml
iv	0.2 ml	Nil	0.1 ml
v	Nil	0.2 ml	0.1 ml
vi	0.1 ml	0.1 ml	Nil

RESULTS AND DISCUSSION

The distances in cms travelled by the different fractions from origin were as follows:- Fr.1 = 14.9, Fr. 2 = 13.3, Fr. 3 = 10.0, Fr. 4 = 8.8, Fr. 5 = 6.4 and Fr. 6 = 5.4. The calculated Rf values of fraction 1 = 0.876, 2 = 0.782, 3 = 0.588, 4 = 0.518, 5 = 0.376 and 6 = 0.318.

The percentage egg distribution in respect of different fractions and the respective deterrence percentage are presented in Table 9.1. It was noted that the fraction number 6 was found to show the highest oviposition deterring activity (60.78%). Fraction 1 and 2 were weekly active in this respect and perhaps acted in complimentary way.

When a second chromatogram was run with the combined (1 : 1) male and female body-wash employing the same procedures and solvent system as described earlier, it was found that this was showing 7 fractions. The calculated Rf values of those fractions were as follows: Fr. 1 = 0.876, Fr. 2 = 0.797, Fr. 3 = 0.635, Fr. 4 = 0.532, Fr. 5 = 0.385, Fr. 6 = 0.362 and Fr. 7 = 0.038. It can be seen by way of cross comparison that the observed Rf values for fractions 1 to 6 were closely similar to those of the hexane female body-wash. Further, it was also observed that there were 7 fractions in the chromatogram of hexane male body-wash. It was therefore, possible to suggest that this 7th fraction (Rf = 0.037) may have originated from the male body.

Chuman et al. (1985) studied the sex-pheromone chemistry of the same species of tobacco beetle and isolated seven (7) compounds from the active fractions off column chromatography. They determined the structures of the compounds by mass spectroscopic evidences confirmed by partial synthesis to be (4S,6S,7S)- 4,6- dimethyl-7-hydroxynonan-3-one (serricornin) (i), 2,6-diethyl-3,5-dimethyl-3,4-dihydro-2H-pyran (anhydroserricornin) (ii) 4,6-dimethylnonan-3,7-dione (iii), 4,6-dimethylnonan-3,7-diol (iv), 4,6-dimethyl-7-hydroxy-4-nonen-3-one (v), (2S,3S)-2,3-dihydro-3,5-dimethyl-2-ethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one (serricorone) (vi) and (2S,3R)-2,3-dihyrdo-3,5-dimethyl-2-ethyl-6-(1-methyl-2-hydroxybutyl)-4H-pyran-4-one (serricorole) (vii).

In the light of the detailed chemical analysis of the 7 fractions, sited above, it could be suggested that the 7 fractions obtained in the present study(mix sexes wash) were apparently similar to those mentioned by Chuman et al. (1985). However, the points of reference in the two study were sex pheromonal and oviposition deterring properties. It may therefore be conjectured that the unexpected paralellism of fractionation points to the fact that the sex pheromone emanating from L. serricorne (F.) female body may act as a sex pheromone as well as a oviposition deterring pheromone. Perhaps variations in individual contributions of atleast 6 different fractions might be responsible for differential pheromonal activities.

The percentage distribution of eggs laid on the 'treated' and 'control' leaf disc-stacks in respect of the different combinations and their respective percentage deterrence are presented in Table 9.2. It was observed that the deterrent activity of the Fr.1 and Fr.6 were remarkably affected adversely due to the presence of the Fr.2. In this context, it is pertinent to mention here the findings of Levinson et al. (1981, and 1986) and Levinson and Levinson (1987) that anhydroserricornin (Fr.2) restrained the response of the male tobacco beetle to the sex pheromone.

It may, therefore finally be suggested that the

hexane female body-wash of the tobacco beetle, L.serricorne (F.) either as a whole or its fraction No.6 (based on present work) or serricorone - (2S, 3R)-2,3-dihydro-3, 5-dimethyl-2-ethyl-6-(1-methyl-2-oxobutyl)-4H-pyran-4-one (as per Chuman et al. 1985) alone may be used as a potent oviposition deterrent in the case of this beetle.

SUMMARY

An attempt was made to fractionate the hexane body-washes of females alone, 1: 1 mixed sex population and males alone by employing TLC techniques. Hexane body wash of female L. serricorne was resolvable into six fractions, whereas in mixed sexes or males alone, there were seven fractions. Apparently, the seventh fraction originated from male beetles. Fraction No.6 was found to exert more than 60% deterrent influence, that was probably complimented by fraction Nos. 1 and 2. According to Chuman et al., hexane body-wash exhibits sex-pheromone activity and that, that too, was resolved into 7 fractions. Assuming that by varying the proportions of different fractions alterations in pheromonal properties could be brought about, a study on recombinations of fraction 1, 2 and 6 (active as ODP) was conducted. The result indicated this to be possible. The implications and possible utility of findings has been discussed in the light of available literature.

Table 9.1. Oviposition deterring activity of the tobacco beetle, L. serricorne (F.) females against the different thin layer chromatographic fractions of the female body-wash

Fraction No.	Percentage egg distribution in respect of Treated stacks	Control	% deterrence
1	36.90	63.09	26.19
2	39.04	60.96	21.92
3	40.82	59.18	18.36
4	56.38	43.62	-12.76
5	46.30	53.70	7.40
6	19.61	80.39	60.78

Fractions applied at a dose of 40FE (0.2 ml) per leaf disc stacks.

Table 9.2. Oviposition response of the tobacco beetle, L. serricorne (F.) to the different combinations of the TLC fractions (1,2 and 6) of the hexane female body-wash

* Combinations	Percentage egg distribution in respect of Treated stacks	Control	% deterrence
i	41.38	58.62	17.24
ii	42.27	57.73	15.46
iii	57.14	42.86	-14.28
iv	39.76	60.24	20.48
v	57.35	42.65	-14.70
vi	55.56	44.44	-11.12

Each fraction applied at a dose of 20FE (0.1 ml) per disc stacks.

* Details of combinations are given in text.