CHAPTER 4.1

AN ATTEMPT AT ISOLATION AND IDENTIFICATION OF DETERRENT COMPONENTS FROM THE EXTRACTS OF THREE SELECTED PLANT SPECIES AND THEIR ACTION ON ADULT AND LARVAL FORMS OF CIGARETTE BEETLE, *LASIODERMA SERRICORNE* F.

Protection of stored commodities from insect pests by application of repellent or deterrent chemicals has received increased attention in recent years (Bernays, 1983; Hare, 1983; Raffa, 1986). Many scientists have earlier tried to control the insect pests with the help of synthetic insecticides (Childs, 1966). In the recent past, several researchers have tried to introduce some chemicals obtained from plants having repellent or deterrent properties for the management of insect pests of stored products, as these are less toxic and are also biodegradable in nature. So, the application of knowledge of biologically active chemicals from natural plant products has provided some powerful tools for insect pests management. First such application involved notably the pyrethroids (Elliot, 1977 and Menn, 1983). Synthetic chemical repellents have also been used effectively against haematophagous arthropods that affect man and domestic animals (Dethier, 1956 and

Khan,1977). Synthetic and naturally occuring chemicals have been shown to possess repellent activities against Blattella germanica L. and Periplanata americana L. (Goodhue and Tissol,1952; Burden and Eastein,1960; Goodhue, 1960; Goodhue and Howell,1960; Bodenstein and Fales,1976; Cornwell,1976 and Inazuka,1982b). Such synthetic chemicals that act as repellents include - amides (McGovern,et al., 1974b and 1975a), sulfonamides (McGovern,et al., 1974a and 1975b), carboxamides (McGovern and Burden,1985) and cyano acetic acid related compounds (Schwartz,et al., 1970).

Many reports are available on several non-toxic plant chemicals which are effective against various insect pests. Raffa (1987) reported that azadirachtin, a derivative of neem, caused more inhibition of fall armyworm, Spodoptera frugiperda J.E.Smith., larval feeding. Azadirachtin a tetranortriterpenoid product of Azadirachta indica A. Juss., (Meliaceae) and Melia azedarach L., has relatively broad spectrum insect feeding deterrent property (Nakanishi,1974; Warthen,et al., 1978; Kubo and Klocke, 1982 and Arnason,et al., 1985). Citrus limonoids such as limonin, nomilin, and obacunone have been the subject of recent studies on antifeedants (Klocke and Kubo,1982; Koul,1983; Alford and Bentley,1986). The repellent properties of Pinus roxburgii needles have been studied against the termites-Odontoternis obescus by Zaheer et al.,(1987). Kocke et al.,(1987) have tested a monoterpenoid compound, 1,8-cineole (Eucalyptol) isolated from the volatile oil of the plant Hemozonia fitchi (Asteraceae) against the adult yellow fever mosquito, Aedes aegypti, which was found to be effective as feeding deterrent/repellent. Derivatives of natural or synthetic clerodane diterpenoids were assayed by Blaney et al.,(1988), behaviourally and electrophysiologically, as antifeedants against larvae of Spodoptera exempta Walker., S. littoralis B., S. frugiperda J.E. Smith., Heliothes armigera Hubner. and H. virescens F. Subash et al.,(1982) have studied the insect repellents from vetiver oil on the basis of spectral data and partial synthesis.

From the above cited literature, it became obvious that no reports are available on deterrent chemicals obtainable from the three locally available plants viz.— A. lamarkii, T. stans and V. rosea being used against Lasioderma serricorne F. The purpose of present work was, therefore, to isolate and identify deterrent fractions in different extracts of three named plant species, which would be effective against the cigarette beetle, L. serricorne F.

MATERIAL AND METHODS

Thin layer chromatography (TLC) technique was employed for seperation of alkaloids, carboxylic acids, essential oils, lipids (general), glycerides, phospholipids, sugars, amino acids, steroids and sterols and terpenoids present in different plant extracts (details indicated at appropriate places). Silica gel-G was used as the sorbent for the chromatography. The slurry was prepared by mixing 30 gm of silica gel-G powder with 70 ml of redistilled water, homogenized for 30 seconds and then spread as 1.0 mm thick uniform layer with spreader on 20 X 20 cm glass plates. The coated plates were dried at ambient temperature, then activated an air oven at 110°C for 60 minutes. Then the activated plates were allowed to stand at room temperature for cooling and then stored in dust free cabinets till utilization. The different plant leaf extracts were prepared from _T. stans_ and _A. lamarkii_ as hot water extracts and from the leaves of _V. rosea_ as alkaline extract. Microcapillary pippettes were used for application of samples onto the activated plates.

The solvent systems employed, as per methods given by Touchstone & Dobbine (1978), for different separations were as follows:- Benzene - Ethanol (9:1) for alkaloids, Benzene - Methanol - Acetic acid (45 : 8 : 8) for carboxylic acids, Benzene - Chloroform (1 : 1) for essential oils, Petroleum ether - Diethyl ether - Acetic acid (90 : 10 : 1) or (70 : 20 : 4) for lipids, Chloroform - Benzene (7 : 3) for Glycerides, Chloroform - Methanol - Water (65 : 25 : 4) for phospholipids, Ethylacetate - Isopropanol (2 : 1) for sugars, Butanol - Acetone - Glacial acetic acid - Water (35 : 35 : 10 : 20) for aminoacids, Benzene - Ethylacetate (9 : 1) for steroids and sterols, Isopropyl ether - Acetone (5 : 2) for terpenoids. The required proportion of above mentioned solvents were kept in the developing chamber and the plates were placed vertically into the chamber. The solvents were allowed to run upto 15 cm. The travelling time for each of the solvents was recorded. The separated fractions were vizualized by exposure to iodine vapours. The flavonoids were separated by paper chromatography (PC) with 15% acetic acid in distilled water as the solvent. The dried paper chromatogrammes were vizualised by their fluorescence in UV (360 nm) light and the fluorescent bands were marked. A strip(¼) of the chromatogramme was sprayed with 10% sodium carbonate solution and the bands giving yellow colour were noted. The flavonoid bands were then cut out from the unsprayed chromatogrammes by comparison, eluted in spectroscopic grade methanol and the UV absorption spectra were determined on Schimadzu UV-240 recording spectrophotometer. The identification of the flavonoid was done using their absorption maxima, colour reactions and co-chromatography with authentic samples. For identification of clearly separated components, iodine chamber and the spraying reagents like Ethanol - H_2SO_4 (conc.) - Anisaldehyde (9 ml : .5ml : .5 ml), Dragendorffs stock solution with 20% tartaric acid, Ninhydrine with 0.1% solution in acetone were used. Vapours of iodine and above said spraying reagents clearly indicated the separated components whatsever components were separated by the selected solvent systems. The separated components from the hot water leaf extracts of the plant T. stans were 2-alkaloids, 3-sugars, 3-aminoacids, 2-flavonoids and 1-carboxylic acid. Hot water leaf extracts of A. lamarkii was separated into 2-alkaloids, 2-sugars, 4-amonoacids and 1-flavonoid component. Alkaline leaf extract of V. rosea indicated the presence of 2-alkaloids, 1-sugar, 3-aminoacids (including peptides bonds) and 2-flavonoids components. The details of chemical identities of separated fractions are given in Table - 1. Later, preparative thin layer chromatography (PTLC) was carried out to obtain by isolation enough quantities of previously confirmed (TLC) component of leaf extracts with respect to the influence of some components on the behaviour of larval as well as adult stages of L. serricorne F.

The components to be tested for their influence on behaviour of insect species under investigation, the corresponding bands on PTLC were eluted with respective 50 ml of solvent mixtures. The volume of eluate was reduced to one-fourth by evaporation at room temperature. Such concentrated eluates were then utilized for assessing their influence on behaviour. The bioassay test procedure and the counting of percentage of deterrent influence were similar to those described earlier (Chapter 3.1).

RESULTS

The hot water leaf extract of A. lamarkii showed the presence of two alkaloids namely Alangimarkine (F-1) and Alangicine (F-2), the sugars - glucose and rhamnose, aminoacids - Serine, Cystine, Histidine and Arginine and a flavonoid namely Quercetin. Among these components only alkaloidal components were found effective deterrent agents against both adults as well as larval forms of L. serricorne F. Each of these isolated alkaloid fractions were tested separately for deterrent action (Table 2). Fraction 1 (Alángimarkine)deterred 90% of the adults insect from the treated baits. In case of fraction 2(Alangicine), 50% dèterrence was recorded in case of adults. In larval tests, F-1 exhibited 82.66% deterrence of 3rd instars and 77.33% deterrence of 4th instar larvae. Thus, both the instars were positively deterred by F-1. On the other hand, F-2 was not so effective. From the above finding only F-1 (Alangimarkine) showed promising deterrent action against adult as well as 3rd and 4th instar larvae of the test insect.

T. stans hot water leaf extract contained the alkaloids - Tecomine (F-1) and Tecostamine (F-2), an unidentified carboxylic acid, sugars like glucose and rhamnose and one unkown, aminoacids - Serine, Cystine and

Table 1. Representing the obtained results of chemical analysis of three different plant species (*Alangium lamarkii*, *Tecoma stans* and *Vinca rosea*)

Leaves of	Mode of extractions	Sugars	Amino acids	Alkaloids	Flavonoids	Carboxylic acids
A. lamarkii	Hot water extract	Glucose Rhamnose	Serine Cystine Histidine Arginine	Alangimarkine (F-1) Alangine (F-2)	Quercetin	Absent
T. stans	Hot water extract	Glucose Rhamnose one unidentified	Serine Cystine Methionine	Tecomine (F-1) Tecostamine (F-2)	Celycoflavone Orientin	One un-identified
V. rosea	Alkaline extract	Glucose	Serine Cystine Valine & Number of peptides	Ajmaticine (F-1) Serpentine (F-2)	Quercetin Kaempferol	Absent

Table 2. Responses of adults and larvae of *L. serricorne* F. to the F-1 and F-2 alkaloid fractions isolated from the hot water leaf extract of *Alangium lamarkii*

Adults

Fractions	Responses			PPR	Deterrence % due to "treated" sample
	TPD	UTPD	IND		
F - 1	05	19	26	10.00 %	90.00 %
F - 2	25	16	09	50.00 %	50.00 %

Larvae

Fractions	Instars	Responses			PPR	Deterrence % due to "treated" sample
		TFS	UTFS	IND		
F - 1	3rd	13	24	38	17.34 %	82.66 %
	4th	17	26	32	22.67 %	77.33 %
F - 2	3rd	22	18	35	29.34 %	70.66 %
	4th	31	22	22	41.34 %	58.66 %

TPD / TFS = Treated paper discs/ Treated flour samples
UTPD / UTFS = Untreated paper discs/ Untreated flour samples
IND = Column showing indifferent behaviour
PPR = Percentage of positive response to "treated" sample

Table 3. Responses of adults and larvae of L. serricorne F. to the carboxylic acid component (C-1) separated from the hot water leaf extract of Tecoma stans

Adults

Fraction	Responses			PPR	Deterrence % due to "treated" sample
	TPD	UTPD	IND		
C - 1	15	16	19	30.00 %	70.00 %

Larvae

Fraction	Instars	Responses			PPR	Deterrence % due to "treated" sample
		TFS	UTFS	IND		
C - 1	3rd	18	41	16	24.00 %	76.00 %
	4th	26	18	31	34.66 %	65.34 %

TPD / TFS = Treated paper discs/ Treated flour samples
UTPD / UTFS = Untreated paper discs/ Untreated flour samples
IND = Column showing indifferent behaviour
PPR = Percentage of positive response to "treated" sample

Table 4. Responses of adults and larvae of L. serricorne F. to the F-1 and F-2 alkaloid fractions isolated from the hot water leaf extract of *Tecoma stans*

Adults

Fractions	Responses			PPR	Deterrence % due to "treated" sample
	TPD	UTPD	IND		
F - 1	03	26	21	06.00 %	94.00 %
F - 2	25	17	08	50.00 %	50.00 %

Larvae

Fractions	Instars	Responses			PPR	Deterrence % due to "treated" sample
		TFS	UTFS	IND		
F - 1	3rd	06	35	34	08.00 %	92.00 %
	4th	20	28	27	26.67 %	73.33 %
F - 2	3rd	24	19	32	32.00 %	68.00 %
	4th	33	19	23	44.00 %	56.00 %

TPD / TFS = Treated paper discs/ Treated flour samples
UTPD / UTFS = Untreated paper discs/ Untreated flour samples
IND = Column showing indifferent behaviour
PPR = Percentage of positive response to "treated" sample

Table 5. Responses of adults and larvae of L. serricorne F. to the F-1 and F-2 alkaloid fractions isolated from the alkaline leaf extract of *Vinca rosea*

Adults

Fractions	Responses			PPR	Deterrence % due to "treated" sample
	TPD	UTPD	IND		
F - 1	22	18	10	44.00 %	56.00 %
F - 2	04	18	28	08.00 %	92.00 %

Larvae

Fractions	Instars	Responses			PPR	Deterrence % due to "treated" sample
		TFS	UTFS	IND		
F - 1	3rd	28	21	26	37.34 %	62.66 %
	4th	31	18	26	41.34 %	58.66 %
F - 2	3rd	12	26	37	16.00 %	84.00 %
	4th	15	26	34	20.00 %	80.00 %

TPD / TFS = Treated paper discs/ Treated flour samples
UTPD / UTFS = Untreated paper discs/ Untreated flour samples
IND = Column showing indifferent behaviour
PPR = Percentage of positive response to "treated" sample

Methionine, the flavonoids Celycoflavone and Orientin. Among the isolated components the unidentified carboxylic acid (C-1) and the alkaloids (F-1 and F-2) were found to be effective deterrents for the test insect. Adult and larval responses to the carboxylic acid fraction C-1 are presented in Table 3. Adults were deterred to 70% extent and the 3rd and 4th instars larvae to 76% and 65.34%. Thus, the carboxylic acid component may not be considered as a highly potential deterrent for the test insect.

Deterrent influence on adults and larvae of the two alkaloidal fractions (F-1 and F-2) of the hot water leaf extract of T. stans are depicted in Table 4. F-1 — Tecomine fraction was found to possess a very high deterring influence —94% on the adult insects. On the other hand, F-2 (Tecostamine) exhibited only 50% deterrent influence. As far as the 3rd and 4th instar larvae are concerned, F-1 could deter them to 92% and 73.33% levels, respectively. F-2 exerted deterrent action upto 68% and 56% on the 3rd and 4th instar larvae, respectively. From the data presented in Table 4 it was clear that F-1 — Tecomine —exhibited a highly significant deterrent influence on the test insect.

V. rosea alkaline leaf extract contained 2-alkaloids —Ajmaticine (F-1) and Serpentine (F-2), sugar-glucose, aminoacids - Serine, Cystine and Valine and a number of peptides bonds, flavonoids - Quercetin and

Kaempferol. Among the isolated components only alkaloid components were found to possess deterrent property against the test insect. Each of these alkaloid fractions viz.— F-1 and F-2 were tested individually against the L. serricorne F. both adult and larval forms (Table 5). 92% of the adult insects were deterred by the F-2 — Serpentine and 56% by the fraction 1 (F-1)— Ajmaticine. In case of larval tests 3rd and 4th instar larvae, 62.66% and 58.66% deterrent activity was evident with F-1, and in case of F-2 84% and 80% on the 3rd and 4th instar larvae. It could be clearly seen (Table 5) that fraction 2 —Serpentine —was very effective deterrent for the adult as well as 3rd and 4th instar larvae of the L. serricorne F.

DISCUSSION

Choice of the three species of plants viz.— A. lamarkii, T. stans, and V. rosea was based on the observations described previously (Chapter 2.1) and dosimetric studies (Chapter 3.1) in this work, as they were found to be potential deterrents. Each of the isolated fractions was tested individually against both the larval and adult L. serricorne F. for the particular chemical components, exclusively responsible for the deterrent action. In this context, the work of Su, Helen and Horvat (1988) is worth noting. These authors observed that acetone extract of dill seeds (Anethum graveolens Linn.) exhibited a long-lasting repellency against several species of stored product insects; including the confused flour beetle, Tribolium confusum J and V. The effective repellent components were isolated, purfied and identified as 2-methyl-5-(1-methylethyl) - 2 - cyclohexene-1-one (d-carbone) and 4,5-dimethoxy-6-(2-propenyl) - 1, 3-benzodioxole (dillapiol). The present finding has revealed that alkaloids —Alangimarkine, Tecomine and Serpentine were the most potent deterrents for L. serricorne F. These fractions elicited 90% to 94% deterrence of adults, 82% to 92% deterrence in case of 3rd and 4th instar larvae of L. serricorne F.

Alfaro et al., (1981) were studied the feeding deterrent activity of fractions from the foliage of western red ceder, Thuja plicata Donn. in the case of white pine weevile, Pissodes strobi Peck. The most active fractions were the monoterpene hydrocarbon, thujone and terpene alcohol fractions.

De Boer and Hanson (1987) tested six solanaceous alkaloides against the larvae of tobacco horn worm, Manduca sexta. Among the tested alkaloids solanocapsine deterred feeding by the larvae of horn worm. Dreyer et al., (1985) have measured the feeding deterrency of a series of pyrrolizidine, indolizidine, and quinolizidine alkaloids against the pea-aphid (Acyrthosiphon pisum Harris.). During the course of present investigation also leaf alkaloids namely Alangimarkine, Tecomine and Serpentine exhibited excellent deterrent activities with respect to a stored product pest, L. serricorne F. The salient feature of the present work is the case with which a strong feeding deterrent can be prepared even by common persons for practical application. Further, this also suggests that isolation of deterrent alkaloids might be used to protect the stored commodities against the infestation by L. serricorne F. Moreover, these plants are very common and wide spread. Collection of only leaves, when needed, would pose no hardship nor it would cause any serious damage to these plants. Preparing an extract only by boiling the washed leaves would be an easy task for providing protection to stored products from L. serricorne F. infestation under endemic occurrence.

In case of hot water leaf extract of T. stans a fraction containing carboxylic acid (unknown) also deterred the test insect (70% for adult and 65% to 76% for larvae). The percentage of deterrence was lesser than the alkaloid compounds with respect to both adult and larval forms. In this context, the work of Halliday and McGovern (1987) may be cited wherein they report on the repellency of 123 closely related carboxyamides against adult confused flour beetle, Tribolium confusum J and V. Sixteen of these showed significant high repellency. The best repellent contained a 1-(2-cyclohexyl-1-oxoethyl) or a 1 - (3-cyclohexyl-1-oxopropyl) group as their acid moiety. Halliday and McGovern (1987) have mentioned that repellency was due to acid moieties. Faustini et al.,(1987) and Blaney et al.,(1988) have reported on use of synthetic and natural chemicals as deterrents/repellents against different insect pests, including L. serricorne F. The present author has used plant products containing organic chemicals which are easily biodegradable. However, it should be mentioned here that, unless and untill the toxicity tests are conducted the three alkaloids tested simply can not be recommended for safe application. The author intends to carry out such toxicological work in immediate future, if proper facilities permit, so that some benificial tool of practical use can be made available.

SUMMARY

Present observations indicated the deterrence of adults and 3rd and 4th instar larvae of L. serricorne F., due to the different alkaloid fractions and a carboxylic

acid contained compounds isolated by TLC from different leaf extracts of the three plants species *viz.*— *A. lamarkii*, *T. stans*, and *V. rosea*. It was observed that three alkaloids namely, Alangimarkine, Tecomine and Serpentine were the most potent deterrents for both the adult and larval forms of *L. serricorne* F. These three alkaloids have to be tested toxicologically before they can be recommended for safer practical application with respect to management of *L. serricorne* F., a serious pest of not only the cured tobacco stores but also of spices and other food products.

CHAPTER 4.2

SEPARATION OF ARRESTANT COMPONENTS FROM THE METHANOLIC EXTRACT OF FRUITS (DRIED) OF *DURANTA PLUMIERI* AND THEIR INFLUENCE ON BEHAVIOUR OF CIGARETTE BEETLE, *LASIODERMA SERRICORNE* F.

In the recent past a lot of information has become available on various natural and synthetic chemical compounds that attract several different species of insects. Such insect attractants could be extremely useful in monitoring agricultural field pests, as well as those of stored products. Chemical attractants isolated from plant extracts are employed as baits in traps for the control of infestation of stored commodities by several pests; including *L. serricorne* F. Butyl acetate, a chemical component of many fruits and vegetables has been found to be attractive to the picnic beetle, *Glischrochilus quadrisignatus* (Foott and Hybsky, 1976; Attwater and Busch, 1983 and Alm, *et al.*, 1985). Recently, effective chemical attractants have been developed for the Japaneses beetle, *Popillia japonica* Newman, such as phenethylpropionate (PEP) + eugenol, 7 : 3 (McGovern, *et al.*, 1970, and Ladd, *et al.*, 1973); PEP + eugenol, 3 : 7 (Ladd, *et al.*, 1976) and PEP +

eugenol + geraniol, 3 : 7 : 3, (Ladd and McGovern, 1980). Later it was reported that 2-methoxy-4-propylphenol in combination with phenethyl propionate (7 : 3 or 3 : 7) was as effective as eugenol containing mixtures for *P. japonica* (McGovern and Ladd, 1984). In another report Ladd (1984) described two compounds, isoeugenol and 2-methoxy-4-propylphenol as attractants and compared them for attractancy with eugenol in respect of the Northern corn rootworm, *Diabrotica barberi* Smith and Lawrence (Coleoptera : Chrysomelidae).

Another report on the boll weevil, *Anthonomus grandis grandis* B., described the attractancy of volatile oil produced by cotton seeds (Minyard, *et al.*, 1969 and McKibben, *et al.*, 1977). It has also been demonstrated by Freedman, *et al.*, (1982) and Mikolajcak, *et al.*, (1983) that oat triglycerides, composed primarily of palmitic, oleic, and linoleic acids, stimulate aggregation of the sawtoothed grain beetle, *Oryzaephilus surinamensis* L.; when bioassayed in a two choice, openarena petridish chambers.

There were many reports on several chemicals obtained from various plant species which are effective against various field and stored product pests. However, almost no reports are available on the cigarette beetle, *L. serricorne* F. So, the objective of the present work was to isolate, identify and bioassay the attractant chemical compounds/ components of *D. plumieri* dried fruits extract, that was shown (Chapter 2.3) to arrest the L. serricorne F. adults as well as late larval instars.

MATERIAL AND METHODS

The TLC methods employed for separation of various alkaloids and essential oils of dried fruit extract of D. plumieri were as outlined in chapter 4.1. Percentage arrestant influence was calculated as per method described in chapter 3.2.

RESULTS

On the basis of a highly significant arrestant action of the extract of dried fruits of D. plumieri, it was selected for further gross chemical analysis. An attempt was made to separate and isolate effective components of this extract by applying TLC and PC techniques. It was found that D. plumieri dried fruit methanolic extract contained four unknown alkaloids, two sugars (Glucose and Rhamnose), four aminoacids (Arginine, Serine, Leucine and Asparagine), and four unknown essential oils. Among the isolated chemical

Table 1. Responses of adult L. serricorne F. to the different alkaloid fractions of the methanolic fruit (dried) extract of *Duranta plumieri*

Fractions	Responses			Arrestant action % due to "treated" sample
	TPD	UTPD	IND	
F - 1	15	17	18	30.00 %
F - 2	45	04	01	90.00 %
F - 3	18	13	19	36.00 %
F - 4	41	06	03	82.00 %

TPD = Treated paper discs
UTPD = Untreated paper discs
IND = Column showing indifferent behaviour

Table 2. Responses of larvae of *L. serricorne* F. to the different alkaloid fractions of the methanolic fruit (dried) extract of *Duranta plumieri*

Fractions	Instars	Responses			Arrestant action % due to "treated" sample
		TFS	UTFS	IND	
F - 1	3rd	28	25	22	37.33 %
	4th	19	40	16	25.33 %
F - 2	3rd	51	14	10	68.00 %
	4th	52	16	07	69.33 %
F - 3	3rd	23	19	33	30.66 %
	4th	29	26	20	38.66 %
F - 4	3rd	37	12	25	49.33 %
	4th	47	13	15	62.66 %

TFS = Treated flour samples
UTFS = Untreated flour samples
IND = Column showing indifferent behaviour

Table 3. Responses of adults and larvae of L. serricorne F. to the combined fractions (F-2 and F-4)

Adults

Fractions	Responses			Arrestant action % due to "treated" sample
	TPD	UTPD	IND	
F 2 + 4	41	05	04	82.00 %

Larvae

Fractions	Instars	Responses			Arrestant action % due to " treated " sample
		TFS	UTFS	IND	
F 2 + 4	3rd	54	07	14	72.00 %
	4th	57	08	10	76.00 %

TPD / TFS = Treated paper discs/ Treated flour samples
UTPD / UTFS = Untreated paper discs/ Untreated flour samples.
IND = Column showing indifferent behaviour

Table 4. Responses of adult L. serricorne F. to the different essential oil fractions of the methanolic fruit (dried) extract of *Duranta plumieri*

Fractions	Responses			Arrestant action % due to "treated" sample
	TPD	UTPD	IND	
E - 1	18	17	15	36.00 %
E - 2	19	17	14	38.00 %
E - 3	26	16	08	52.00 %
E - 4	16	20	14	32.00 %

TPD = Treated paper discs
UTPD = Untreated paper discs
IND = Column showing indifferent behaviour

Table 5. Responses of larvae of L. serricorne F. to the different essential oil fractions of the methanolic fruit (dried) extract of *Duranta plumieri*

Fractions	Instars	Responses			Arrestant action % due to " treated " sample
		TFS	UTFS	IND	
E - 1	3rd	30	19	26	40.00 %
	4th	23	26	26	30.66 %
E - 2	3rd	28	21	26	37.33 %
	4th	32	20	23	42.66 %
E - 3	3rd	21	17	37	28.00 %
	4th	30	21	24	40.00 %
E - 4	3rd	33	17	24	44.00 %
	4th	44	15	16	58.66 %

TFS = Treated flour samples
UTFS = Untreated flour samples
IND = Column showing indifferent behaviour

components only alkaloidal compounds exhibited arrestant influence on the test insect. All the four essential oil fractions were found to possess no particular attractive properties.

Responses of adult as well as 3rd and 4th instar larvae to the isolated four different fractions of unidentified alkaloid components are tabulated in the Tables 1 and 2. The adult insects (Table 1) were arrested to the extent of 30% by fraction 1, 90% by fraction 2, 30% by fraction 3 and 82% by fraction 4. Fraction 2 and 4 were found to be the most potent arrestants for adult L. serricorne F. In larval tests (Table 2), 3rd and 4th instar larvae were attracted to the extent of 37.33% and 25.33% towards F - 1, 68.00% and 69.33% towards F-2, 30.66% and 38.66% towards F-3 and 49.33% and 62.66% towards F-4. It was clear from the Table 2 that fractions 2 and 4 contained the alkaloids which arrested the adults as well as larvae to a significant extent.

As noted above, F-2 and F-4 were capable of eliciting highly significant attractant behaviour of the larval and adult forms. Hence, it was thought desirable to test the efficacy of a combination of these two fractions with respect to attraction of beetles and larvae. Another set of bioassay tests was conducted by combining fractions 2 and 4. Responses to combined fractions are recorded in the Table 3. The adult attraction was 82% and the 3rd and 4th instar larval attraction 72.00% and 76.00%, respectively. It was, therefore, apparant that combination of F-2 and F-4 led to no advantage as far as the adult insects are concerned. However, in the case of 3rd and 4th larval instars, there was a slight improvement of attractant property. It became evident that recombination of fractions, as it occurs in nature, could not better the action of individual factors.

Responses of adult and larval forms of L. serricorne F. to the four different essential oil fractions of fruit extract of D. plumieri are recorded in the Table 4 and 5. Both the adults and larvae showed insignificant results. Adults exhibited (Table 4) 36%, 38%, 52% and 32% positive responses towards fractions E-1 to E-4, respectively. In larval tests (Table 5) 3rd and 4th instar larvae exhibited respectively, 40% and 30.66% positive responses to E-1, 37.33% and 42.66% to E-2, 28% and 40% to E-3 and 44% and 58% to E-4. It was clear that samples containing essential oils were not the attracting agents for both the adult and 3rd and 4th instar larvae of L. serricorne F.

DISCUSSION

Present observations dealt with the D. plumieri fruit (dried) extract fractions separated by TLC and PC techniques using appropriate solvent systems as mobile phase and silica gel G as the stationary phase. Four different alkaloid fractions (F-1 to F-4) of unidentified nature could easily separated. Each fraction was tested individually for arrestant activity on L. serricorne F. It was seen that F-2 and F-4 fractions exerted more than 80% attractive influence on adult beetles and about 70% on late larval instars. Further it was observed that recombination of F-2 and F-4 did not lead to any improvement of attractiveness for adults and only to a slight betterment in the case of larval instars. It is, therefore, possible to suggest that F-2 is the most effective attractive component (90%) of the methanolic extract of the dried fruits of D. plumieri. Its close ally was F-4 (82%). If these two fractions or even only F-2 could be isolated in large quantities, the same would serve as an effective bait for trapping the larvae as well as adult insects, L. serricorne F., for effective pest management practice. What essentially remains to be established is the proper chemical identity and the safety of handling this alkaloid fraction.

A great deal of information is available on volatile, natural and synthetic chemicals that attract different species of important insect pests (Pomonis, et al., 1980; Conn, et al., 1983; Buttery and Louisa, 1985; Alm, et al., 1986; McGovern, et al., 1986 and 1987 and McGovern and

Cunningham, 1988). The present investigation, however, drew a nought with essential oil fractions E-1 to E-4 of the methanolic fruit (dried) extract of *D. plumieri*.

It was an unfortunate thing that this author could not locate any reference regarding alkaloids being attractants/arrestants in case of any insect pests. Perhaps this is the first report on alkaloid fractions that were attractants to *L. serricorne* F. The present finding holds a good promise for effective management of *L. serricorne* F., infestations in godowns of cured tobacco leaves, spices and perhaps occasionally of other food product stores. The author wishes to put on record that before this finding could be recommended for practical application, proper chemical identity of alkaloids of F-2 and F-4 should be established and safety factors in respect of handling of these alkaloids should be examined thoroughly. Within the limitations of doctoral work and time limits, it was unfortunately not possible for this author to attempt these investigations.

SUMMARY

Attraction of adults as well as 3rd and 4th instar larvae of *L. serricorne* F. towards four different alkaloid fractions (unidentified) and four essential oil fractions isolated from the methanolic dried fruit extract

of the plant D. plumieri by applying TLC technique was studied. Fractions 2 and 4 exhibited 90% and 82% arrestant influence on adult insects. In case of larvae, fraction 2 arrested 68% and 69.33% of the 3rd and 4th instar larvae and fraction 4 arrested 49.33% and 62.66% of same instar larvae, respectively. Combination of fractions 2 and 4 exerted 82% arrestant action on adult and 72% and 76% on 3rd and 4th instar larvae, respectively. For essential oil fractions were not showing so effective attractants for both the adult and larvae. Other isolated components were sugars and aminoacids, which had no arrestant activity on the adults and larval forms. It has been suggested that this is, perhaps, the first report on alkaloids being attractive to L. serricorne F., and that fractions 2 and 4 were effective in attracting over 80% of individuals. These fractions need to be chemically characterized and tested toxicologically before being cleared for practical application of management of this insect pest.