CHAPTER 1

SCREENING FOR POSSIBLE DETERRENT OR ARRESTANT PROPERTIES OF FRESH AND DRIED LEAVES OF 52 LOCALLY AVAILABLE PLANT SPECIES ON ADULT CIGARETTE BEETLE, LASIODERMA SERRICORNE F. (COLEOPTERA : ANOBIIDAE)

Many of the insects are responsible for incalculable harm to human beings in several ways. Every year they have been destroying our agricultural products as well as stored commodities in different ways. The cigarette beetle, Lasioderma serricorne F. is one of the serious pests known to primarily infest cured tobacco leaves and not so infrequently spices, rice, atta, maida and other processed stored commodities. So, it is necessary to control tobacco beetle infestation right from the stage of preservation/storage, preferably without causing any health hazards to the human beings. Earlier, many chemical insecticides have been used to control the insect pests (Childs, 1966). Seriousness about the toxicity and residual activity of such insecticides sprayed over the stored products has been recognized only lately.

During the last decade, at least two comprehensive reviews (Chapman, 1974; Vigneron, 1978) and three extensive articles (Kubo and Nakanishi, 1977; Munakata, 1977; Norris, 1977) exclusively on the chemical inhibition of feeding in

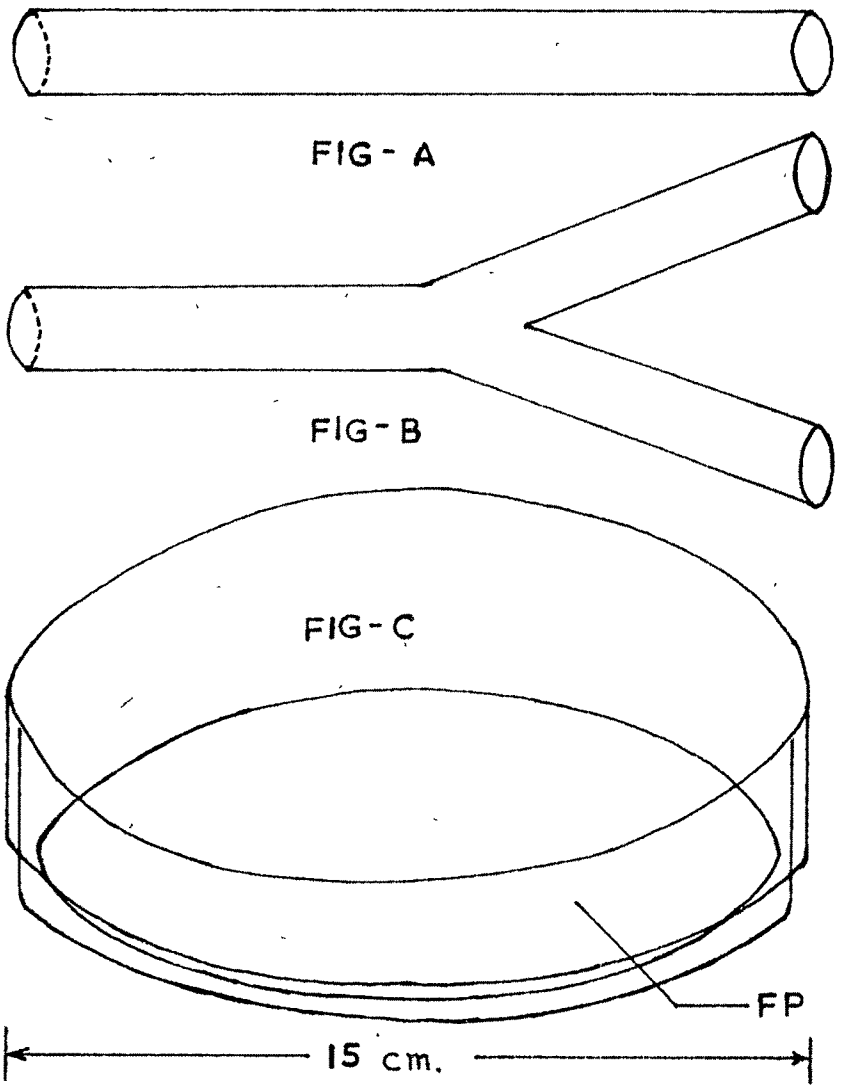
phytophagous insects have been published. Obviously, plants have evolved potent chemical defences which deter insects from feeding on them (Ehrlich and Raven, 1964). These compounds appear to be ideal weapons for insect control. Unfortunately, the track record for control of insect through the use of deterrents is not so satisfactory. Reasons cited for this lack include phytotoxicity (Schoonhoven, 1982), lack of persistence of action (Ladd, et al., 1978), and socioeconomic constraints (Berneys, 1983). Berneys (1983) opined that use of natural antifeedants (Schoonhoven, 1982), though apparently satisfying, is logistically a questionable tactic in crop-pest management. To facilitate the rate of progress in this field, several researchers (Barnes and Ratclif, 1967; Jermy, 1971; Chapman, 1974 and Munakata, 1977) have suggested sets of criteria that potential feeding deterrents must meet for successful use in the field. Among these properties are : adequate persistence for crop protection, systemic activity, low cost, and lack of phytotoxicity.

In the recent past, special efforts have been made to screen materials of plant origin for their deterrent activity (Munakata, 1970 & 1977, Reed, et al., 1981) so as to identify and locate safe and biodegradable alternatives to synthetic insecticides. The Indian neem tree (Azadirachta indica A. Juss.) of Meliaceae family, known since long to be a medicinal plant, appears to be the only plant

extensively used for its deterrent activity (Jacobson, 1958, 1975 & 1981; Kraus, et al., 1981; Reed, et al., 1982; and Warthen, 1979). In the light of above literature it was thought desirable to screen some more plants having possible potent deterrent or arrestant action on the adult cigarette beetle, L. serricorne F. (Coleoptera : Anobiidae), which could be utilized to protect the stored tobacco and its products as well as spices, food grains and other stored food products from the infestation by this species. This report deals with the results obtained during the initial screening of 52 plant species commonly found in India. Aim of this investigation was to identify such plants which would be promising for further intensive study. Although the primary goal was to identify plants having possible deterrent; the screening process revealed a few plants possessing arrestant qualities, which could also prove to be useful as baits for L. serricorne F.

MATERIAL AND METHODS

The cigarette beetle, L. serricorne F. (Coleoptera : Anobiidae) were reared in a BOD incubator at 28 ± 2°C and 65 ± 5 percent relative humidity. To raise healthy adult insects, wheat flour containing dried yeast powder (19 : 1) was employed as the culture medium. As many as 52 plant species belonging to 29 families were collected from



Legend to text Figure 1

Schematic diagrams of (A) straight glass tube, (B) Y-tube olfactometer and (C) Choice chamber (Glass petridish)

FP - Filter paper

different parts of Baroda city during January, 1987 to December, 1987. After collection the leaves of plants were washed carefully in running tap-water and then rinsed with distilled water. The leaves were kept in open at room temperature to drain of all water. In case of each of the plant species 20 leaf-discs of 10 mm diameter were punched out by using a stainless steel cork borer. To survey the response of adult L. serricorne F. beetles with fresh leaves, 10 discs were tested within half an hour of collection and the other 10 were tested after 24 hours of drying at room temperature. Testing was carried out with adult insects freshly collected from the stock culture; one adult individual at a time. The collected plant species were preserved in harbarium sheets for identification and further study.

During the course of present investigation straight glass tubes, Y-tube olfactometers and glass petridishes were used to study the responses of adult L. serricorne F. with fresh as well as 24-hour dried leaf-discs. In case of straight glass tube (Fig.1,A), 10 leaf-discs (fresh as well as dried) were placed at one end of the tube and a fan was placed one meter away from the sample containing tube through which air was blown (same velocity) uniformly through the straight glass tube. One adult insect, freshly collected from stock culture, was released at the opposite end of the glass tube. The responses were recorded after

5 minutes. On the other hand, in case of Y-tube olfactometer (Fig.1,B), 10 leaf-discs (fresh and dried) were placed at one end of single arm of the Y-tube and a small piece of cotton was placed at the another end of the tube. A fan also was fixed like previous tests. One adult insect was released every time at the open end of the Y-tube olfactometer and response was recorded after 5 minutes. Among the three bioassay test methods, the most suitable and easy method was found to be the one employing the glass petridish. Glass petridishes of 15 cm diameter (Fig.1,C) were used for standard "choice chamber" technique to study the responses of L. serricorne F. with each plant species. Filter paper (Whatman No 1) was used to cover the floor of the "choice chamber" to facilitate easy movement of the test insects. The leaf-discs were kept in the centre of chamber and the test insects were released at the periphery of the chamber; the distance being approximately 7 cm from the periphery to the centre. The choice chamber was kept undisturbed to observe minutely the activity of the released test insect. The responses were noted for 5 minutes and the experiments for each plant species were replicated 50 times. The total number of insects found on or near the test samples (Leaf-discs fresh and dried) were considered to be showing positive response ----- arrestants, and those moving around the periphery of the "choice chamber" were considered to exhibit a negative responses _____ deterrents. Same procedure

was adopted for fresh as well as dried leaf-discs. Recorded responses are calculated in terms of percentages.

RESULTS

Of the total of 52 plant species (Table 1) screened, 10 were found to be potential deterrents and 3 arrestant in action. Only one species, Datura matel, did not fit within the frame of reference, but was found to exhibit a narcotic action on those individuals which happened to come in contact with fresh leaf-discs, otherwise arrestant-like action was noted in case of both fresh and dried leaf-discs.

Out of the 10 deterrent plants 6 were found to exert almost 100% deterrent influence with fresh as well as dried leaf-discs viz.— Rauwolfia canescens, Vinca rosea, Tamarindus indica, Euphorbia neriifolia, Acalypha hispida, and Coleus barbatus. The other 4 species were observed to possess similar deterrent action but only in fresh condition and less so in dried condition viz.— Achyranthes aspera, Alangium lamarkii, Tecoma stans, and Vitex negundo (Table 2). Incidentally, during the course of present investigation, 3 plant species viz.— Duranta plumieri, Ocimum sanctum and Ipomoea fistulosa were

Table 1. Response of adult L. serricorne F. to the presence of leaf-discs of different plant species

Plant species	Fresh leaf-discs		Dried leaf-discs	
	Number of insects		Number of insects	
	Arrested	Deterred	Arrested	Deterred
Alangiaceae				
Alangium lamarkii	00	50	10	40
Apocynaceae				
Vinca alba	25	25	40	10
Tabernumontana coronaria	20	30	15	35
Nerium caudatum	30	20	25	25
Thevetia neriifolia	30	20	30	20
Rauwolfia canescens	00	50	00	50
Vinca rosea	00	50	00	50
Tabernumontana dichotoma	30	20	30	20
Anonaceae				
Polyalthia longifolia	20	30	20	30
Anona squamosa	25	25	35	15
Asclepiadaceae				
Calotropis gigantea	25	25	20	30
Amarantaceae				
Achyranthes aspera	00	50	10	40
Acanthaceae				
Adhatoda vasica	15	35	25	25

Table 1 (continued)

Bignoniaceae				
Tecoma stans	00	50	10	40
Heterophragma roxburghii	35	15	25	25
Begoniaceae				
Begonia minima	15	35	15	35
Caesalpiniaceae				
Caesalpinia pulcherima	20	30	35	15
Cassia siamea	25	25	25	25
Tamarindus indica	00	50	00	50
Cassia occidentalis	35	15	35	15
Convolvulaceae				
Ipomoea digitata	15	35	15	35
Ipomoea fistulosa	40	10	35	15
Combretaceae				
Terminalia catappa	30	20	35	15
Compositae				
Solidago canadensis	35	15	30	20
Euphorbiaceae				
Euphorbia neriifolia	00	50	00	50
Acalypha hispida	00	50	00	50
Labiatae				
Ocimum sanctum	40	10	35	15
Coleus barbatus	00	50	00	50

Table 1 (continued)

Lythraceae				
Lawsonia inermis	30	20	30	20
Punica granatum	30	20	25	25
Lagerstroemia indica	35	15	30	20
Meliaceae				
Azadirachta indica	05	45	10	40
Mimosae				
Acacia latronum	30	20	25	25
Myrtaceae				
Couroupitia guainensis	35	15	25	25
Malvaceae				
Hibiscus rosasinensis	30	20	35	15
Nyctaginaceae				
Bougainvellaea glabra	10	40	20	30
Papilionaceae				
Desmodium latifolium	25	25	30	20
Dalbergia sisso	20	30	30	20
Punicaceae				
Punica serpenta	30	20	35	15
Rubiaceae				
Ixora coccinea	15	35	15	35
Homalium zeylanicum	30	20	30	20
Rutaceae				
Murraya koenigii	10	40	20	30
Citrus limonia	15	35	20	30

Table 1 (continued)

Sapotaceae				
Achrus sapota	10	40	20	30
Solanaceae				
Datura matel	40	10	40	10
Tiliaceae				
Corchorus oletorius	30	20	20	30
Urticaceae				
Ficus benghalensis	30	20	20	30
Streptium asperum	30	20	35	15
Verbenaceae				
Chlerodendron inermae	25	25	30	20
Lantana camara	30	20	35	15
Vitex negundo	00	50	10	40
Duranta plumieri	40	10	35	15

(Identification of all plant species by courtsey of Dr. S. N. Padate Department of Botany, M. S. University of Baroda, Baroda, India)

Table 2. Summary of highly significant responses of adult L. serricorne F. to 14 plant species ——— in percentage

Plant species	Fresh leaves		Dried leaves	
	Arrested	Deterred	Arrested	Deterred
Rauwolfia canescens	00	100	00	100
Vinca rosea	00	100	00	100
Tamarindus indica	00	100	00	100
Euphorbia neriifolia	00	100	00	100
Acalypha hispida	00	100	00	100
Coleus barbatus	00	100	00	100
Achyranthes aspera	00	100	20	80
Tecoma stans	00	100	20	80
Vitex negundo	00	100	20	80
Alangium lamarkii	00	100	20	80
Datura matel	80	20	80	20
Ipomoea fistulosa	80	20	70	30
Ocimum sanctum	80	20	70	30
Duranta plumieri	80	20	70	30

observed to elicite 80% arrestant action with fresh leaves and 70% with dried leaves. In case of Datura matel 80% arrestant action was observed with both fresh and dried leaves. Another interesting point relates to the action of D. matel. After releasing the adult test insects into the choice chamber, it was seen that the insects directly go to the test sample and remain there upto 2 minutes but later when they move about it has been observed that during the test period (5 minutes) the test insects gradually slowed down and by about 15 minutes stoped moving completely, possibly due to a sort of narcotic action. Such knock down insects were observed to come back after about an hour.

DISCUSSION

Antonious and Tetsuo (1982) assayed 4 natural anti-feedants and chlordimeform by leaf-discs method for antifeeding activity on starved and nonstarved 5th instar larvae of tobacco cutworm, Spodoptera litura. The larvae exhibited varying antifeedant symptoms in their feeding behaviour against treated leaf-discs. Present work deals only with fresh and dried leaf-discs, particularly in relation to the action of vapour phase on adult L. serricorne F.; instead

of on larval feeding behaviour reported by Antonious and Tetsuo (1982). Mia et al., (1986) were found dried powdered leaves of Nishinda (Vitex negundo), Biskatali (Polygonum serrulatum), Neem (Azadirachta indica A. Juss.), and Tobacco (Nicotiana tabacum) to be the most effective repellents for Sitophilus oryzae. According to them, use of these leaf powders reduced the loss of stored grain significantly. During the course of present study 10 plants species were found to act as deterrents against adult L. serricorne F. Villani and Gould (1985a) have screened the effect of different parts including leaves of 78 plant species (24 families) against the late instar larvae of corn wireworm, Melanotus communis Gyl. (Coleoptera : Elateridae) and reported about the possible repellent/ deterrent action in case of 14 species and attractant/ arrestant effect with respect to 7 other species. All of the plant species screened by these authors were completely different from those employed in the present investigation. Present work, however, was restricted to only the leaf-discs as the test substances and that too, on adult L. serricorne F. Sighamony et al., (1984) studied the effect of natural products such as oils of clove, ceder wood, and karanja as well as the acetone extract of black pepper seeds by employing "choice chamber" method for repellency in adult Tribolium castaneum. The ceder wood and karanja oils and the pepper extracts were reported to be more potent than the standard

synthetic chemical repellent - dimethylphthalate. Belles et al.,(1985) have studied antifeedant activities of nine clerodane diterpenoids isolated from different species of Ajuga plants against phytophagous larvae of Egyptian cotton leafworm, Spodoptera littoralis (Boisd.; by application of leaf-discs method. Most of the compounds exhibited anti-feedant activity against the test larvae. Present author has employed the leaf-discs test method and found that this kept adult test insects away from these sources.

From the above discription it is clear that several plant parts possess some very promising constituents that possibly could be made use of either to keep the pests away from or lure them off by baiting with attractants/ arrestants in order to protect the stored products. The present screening process with regard to this aspect was successful to an extent in identifying the efforts to leaves of 10 plant species possessing deterrent effect and other 4 spiecies with arrestant qualities. It is, therefore, necessary to find out wheather or not it would be economi-cally, ecologically, and socioculturally acceptable to use such simple naturally occuring plant parts for protecting the stored products from insect pests. One of the chief purposes behind the present work was to locate such plant species which grow around wildly and in abundance and that can be easily identified by common man. Secondly, collection of leaves of such plant species should not pose any difficulty

and also should not lead to ecological disturbances of any significant nature. Thirdly, the plant leaves or extracts thereof, by simple house-hold methods, should be non-toxic as far as possible, longer acting and easily biodegradable. A perusal of the second table will easily indicate that the first two purposes are adequately met with by most of the plant species listed therein. With respect to the third point; it could be pointed out that the next phase of the present work was planned to find out suitable extraction methods for active principles from fresh as well as dried leaves of those plant species found to be significantly effective.

SUMMARY

52 locally available common plant species (29 families) found in and around Baroda city have been screened for deterrent or arrestant action against a stored-product pest insect, the cigarette beetle, L. serricorne F. (Coleoptera : Anobiidae). The"choice chamber"test employing leaf discs differentiated 10 plant species as potent deterrents of which 6 species (5 families) were found to exhibit almost 100% deterrent action both in fresh and dried condition of leaves. Other 4 species showed 100% deterrency with fresh leaves but 80% with dried leaves. Incidentally, other 4 plants D. plumieri, O. sanctum, I. fistulosa, and D. matel were noted to show significant arrestant qualities with fresh as well as dried leaves. Only D.matel was noted to show narcotic effect on L. serricorne F.

CHAPTER 2.1

OBSERVATIONS ON THE INFLUENCE OF CRUDE LEAF EXTRACTS OF 5 PLANT SPECIES ON ADULT AND LARVAL FORMS OF CIGARETTE BEETLE, LASIODERMA SERRICORNE F.

The infestation by cigarette beetle, Lasioderma serricorne F. (Coleoptera: Anobiidae) of a wide range of commodities has been critically reviewed by Howe (1957); it being known to infest rice, atta, maida, processed tobacco, dried vegetables, dried fishes, other processed foods, etc. The insects were also observed to be serious pests in flour mills, warehouse, military supply depots and food stores. The beetles thrive well in tropical to temperate climate. The damaging capacity of this beetle was found to be maximum at higher temperatures upto 35°C and relative humidities upto 70 percent, as compared to other stored product pests (Mallikarjuna Rao, Jacob and Mohan, 1972). Additionally, this insect has been also reported to feed on stored mustard seeds (Chatterji, Sarup and Bose, 1963); dry ginger (Tirumala Rao and Nagaraja Rao, 1955); as well as castor seeds (Hussain and Khan, 1966). It is difficult to detect the infestation of this pest untill its population has already increased beyond economically controlable threshold; essentially due to their clandestine habit (Chuman, et al., 1985).

Earlier many scientists have tried to control the insect pests with the help of chemical insecticides. Some workers (Reed and Vinzant, 1942; Tinhet, et al., 1957 & 1958; Childs, 1966 & 1967; and Childs, et al., 1966) have tested many inorganic insecticides such as HCN, DDVP, etc. against the cigarette beetle, L. serricorne F. Yadav, 1980; Yadav, et al., 1980; Kirpatric and Gillenwater, 1981 and Chouhan and Yadav, 1984 have studied toxicity of many insecticides against varieties of stored product insect pests including L. serricorne F. People were unaware of the toxicity and residual activity of such inorganic insecticides sprayed over the stored commodities. After realizing such hazards of chemical pesticides several researchers (Pandey, et al., 1981; Bestmann, et al., 1984; Kang and Chang, 1984; Barreto, et al., 1984; Samuel, et al., 1984, and Su, Helen, 1985) tried to introduce plant products having insecticidal properties for the management of insect pests of stored products. Chavan, et al., 1979; Chavan and Nikam, 1983; Nash, et al., 1986, and Qureshi, et al., 1986 have reported on the larvicidal properties of different plant products against different larval instars of mosquitoes and stored grain insect pests. Neem leaf and seed effectively reduced the productivity of the all beetles screened, Sitophilus oryzae L., S. zeamais M., Cryptotestes ferrugineus S. and Rhizopertha dominica F. and the adult emergence of

Sitotroga cerealella OL. and Ephestia cantella W., (Pereira and Wohlgemuth, 1982).

Another commendable area of insect pests control relates to use of antifeedants. To accelerate progress in this area, several researchers (Jermy, 1971; Chapman, 1974; Munakata, 1977) have recommended a certain set of criteria that potential feeding deterrents must meet for successful use in the field. Among this properties are; adequate persistance for crop protection, systemic activity, low cost and lack of phytotoxicity.

Neem seed cake and its powder has been used against insect-pests of stored products as well as standing crops and both have been proved to be very good protectants (Pradhan, Jotwani and Rai, 1963; Goyal, et al., 1971 and Ketkar, 1976).

In the light of above cited literature and on basis of observations reported in chapter 1 it was thought desirable to screen crude extracts of plant leaves which were earlier noted to possess deterrent properties against cigarette beetle, L. serricorne F., for both adult and larval stages. The results reported here concern with screening of crude extracts of 5 of the arbitrarily chosen plant species out of 10 species found effective deterrents in chapter 1. This was done only for convenience, instead dealing with all 10 species together. The aim of this phase of work was to see whether such extracts

would prove to be potentially better deterrents than using merely fresh or dried leaves. Moreover, another point of convenience was to facilitate further investigation pertaining to dosimetric studies and, if possible, to attempt chemical analysis as well as possible isolation of the potent factors of such extracts. A set of another five plant species will be dealt with in next sub-section of this chapter. The remaining four plants are described separately in the third sub-section of chapter 2, wherein dried seed extract of Ocimum sanctum and dried fruits of Duranta plumieri are also taken into consideration.

MATERIAL AND METHODS

The cigarette beetle, L. serricorne F. were reared in the laboratory incubator at a temperature range of 27°C to 32°C and at 65% to 75% R.H. The culture medium consisted of fresh wheat flour containing 5 percent brewers yeast powder. The plant leaves to be used for tests were extracted by following different procedures:-

(i) Aqueous extraction: 10 gm of fresh prewashed leaves of a particular plant species were ground with the help of mortar and pestle; within an hour of collection.

Finely ground leaves were then triturated throughly with 100 ml of distilled water. The homogenate was filtered through muslin cloth and the filtrate was centrifuged upto 40 minutes at 3500 r.p.m. The supernatant was filtered again though filter paper. The extract was collected and labelled as cold-water-extract. To get the second extract, the residue remaining on the muslin cloth was resuspended in 100 ml of distilled water in a beaker. This was boiled upto one hour continuously, adding adequate quantities of water periodically to maintain the volume close to 100 ml. The boiled solution was made upto 100 ml by adding distilled water and was kept for cooling upto 24 hours. After cooling the extract was centrifuged for 40 minutes at 3500 r.p.m. and filtered and labelled as II extract (hot-water-extract). These aqueous extracts were utilized for testing the response of adult beetles by paper-disc method. In case of larvae; the respective extracts were boiled to evaporate total water content. The dried powder was ground in mortars with 10 gm of wheat flour in each case to obtain treated samples.

(ii) Alkaline extraction: As in the previous case, 10 gm of prewashed leaves were macerated thoroughly in a mortar. Gradually 10% NaoH solution was added to ground leaves and the mixture was homogenized and allowed to stand for one hour. The supernatant was collected and boiled to

dryness. The dried residue, being hygroscopic, was pre - served in dessicators over calcium chloride. The dried extracts obtained from 10 gm of fresh leaves were ground with 10 gm of wheat flour in each case to serve as baits for larvae. Alternatively, the alkaline homogenate was reduced upto 75% of original volume by further boiling. After 24 hours of cooling the concentrated extracts were preserved in refrigerator. Filter paper discs (10 mm diameter) were dipped into the concentrated extracts. Such treated paper discs were employed as baits in case of adult insects.

(iii) Acetone extraction: Finely ground leaves were mixed with 100 ml of acetone and allowed to stand in the refrigerator at 5°C for one hour. Acetone soluble matter was then separated from the residue by decantation process, filtered through filter papers. The flask containing extracts were kept in open at room temperature till 75% of acetone was evaporated. 10 mm paper discs (Whatman No.1) were dipped in this concentrated extract, dried and then utilized as samples in testing the response of adult beetles. The extract was mixed in each case with 10 gm of fresh wheat flour and spread evenly in open at room temperature till all the acetone evaporated. The dried wheat flour clumps were ground to obtain fine powder and used as treated samples/baits for larval tests.

(iv) Alcoholic extraction: The finely crushed leaves were mixed with 100 ml of methyl alcohol and triturated thoroughly for about 10 minutes. After separation of insoluble parts the extract was concentrated by boiling to 75% and then utilized by paper disc method as described above in case of adult insects. Rest of the totally dried extract was placed into dessicator containing calcium chloride for 24 hours. In each case 10 gm of wheat flour was added to the dried extract and ground together for adequate dispersion and then employed as baits for larval tests.

Test procedure for adult insects:- Plastic* pertridishes (9 cm diameter) were used as the "Choice chamber". The floor of the chamber was roughened by placing a filter paper (Whatman No.1) so as to facilitate easy movement of insects. 10 mm diameter paper discs were punched out and in each experiment 10 paper discs were soaked in the concentrated extracts (treated discs) and the other 10 were soaked in the respective extracting media/solvents (control discs). Treated and control paper discs were placed at a distance of 1.9 cm from the centre of the choice chamber in opposite directions along the diameter. One adult insect was released each time at the centre of the chamber. The influence of the extract as far as

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* Special grade of plastic material that was odourless as well as colourless.

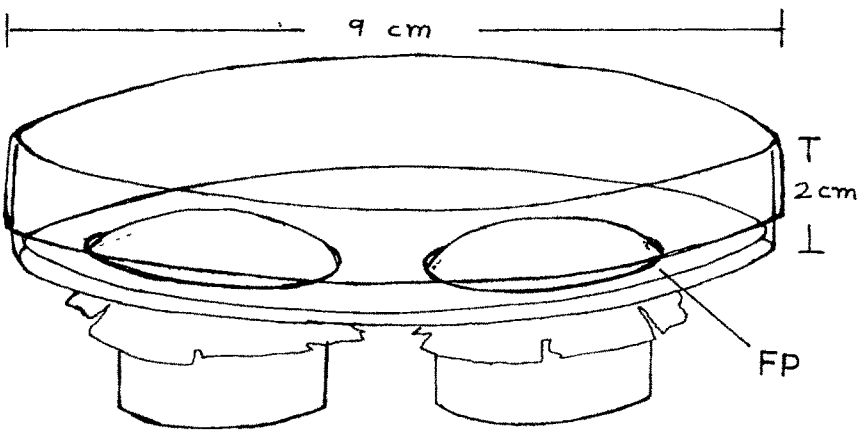

FIG. 2

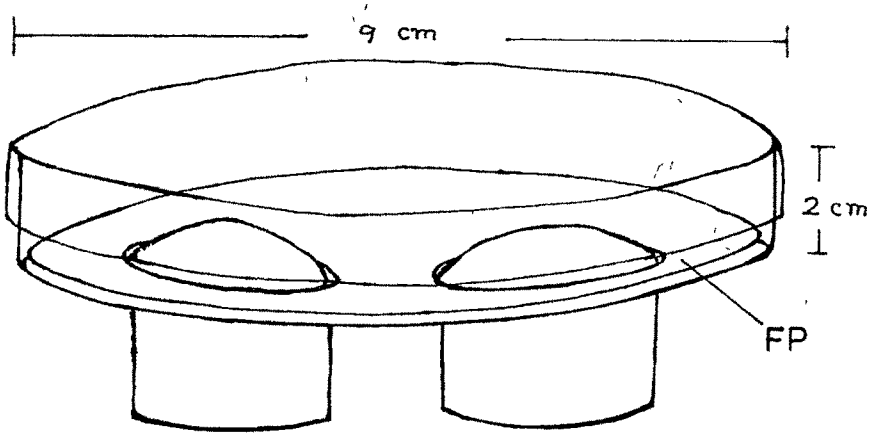

FIG. 3

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Fig. 2 Schematic diagram of covered thimble
" Choice Chamber "

Fig. 3 Schematic diagram of open thimole
" Choice Chamber "

FP - Filter paper

attractant/deterrent action is concerned was recorded at the end of 5 minutes. Tests with different extracts were replicated 50 times in each case using fresh individual insect every time. The test insects were collected from the stock culture 6 hours before testing.

The number of adult individuals found over the paper discs were regarded as showing a positive response to either treated or untreated samples and those found else-where were assumed to exhibit indifference. Statistical analyses were carried out as per methods of Mead and Curnow (1983).

Procedure for larval tests:- Similar petri dishes were used as "Choice chamber" for larval tests. Two holes (2.2 cm diameter) were bored at a distance of 1.7 cm from the centre of the chamber in opposite directions along the diameter. The floor of the chamber was roughened by placing filter paper (Whatman No.1) so as to facilitate easy movement of the larvae. For each experiment two glass thimbles (2 cm deep and 2.2 cm wide), each filled with treated and untreated flour samples respectively and covered with fine muslin cloth, were inserted through (Fig. 2) the two holes of a choice chamber. Tests were also carried out without the muslin cloth cover over the thimbles (Fig. 3).

In the experiment with larval instars, 5 larvae were released at each time in the centre of the

choice chamber. The chamber was kept in dark and responses were recorded after 15 minutes every time. The experiments were replicated 15 times for each instar. The tests larvae were collected two hours before testing. The muslin cloth and filter paper were changed every time before starting a new test to minimise any contaminating or disturbing factors left by larval forms. Number of larvae found in/over open/covered thimbles were regarded as showing a positive response to either treated or untreated samples as the case may be. Those found elsewhere were assumed to exhibit indifference. Number of larvae found over the two thimbles and else-where were taken into account. Statistical analyses were carried out as mentioned earlier.

During the course of present investigation, in case of 1st and 2nd instar larvae treated food was presented in covered thimbles only for testing, since their size was too small to be isolated later from the mass of food, whereas in case of 3rd and 4th instar larvae open thimble tests were carried out. The percentage distribution of larvae of L. serricorne F. with open as well as covered thimbles tests were calculated. The repellency or attractiveness of different leaf extracts was tested employing chi-square distribution (Mead and Curnow, 1983) based on expected distribution of 50 : 50 in respect of responses towards treated versus untreated food, treated food versus indifferent response.

RESULTS

The recorded responses of adult L. serricorne F. with different extracts of leaves of 5 plants species are represented in Table - 1. It could be seen that alkaline extracts of all the plant leaves elicited more or less significant avoidance response from the beetles except that of Vitex negundo. With cold-water-extracts, only that of Tecoma stans showed significant ($P < 0.01$) deterrent influence. Hot aqueous extracts of T. stans as well as Vinca rosea proved effective deterrents ($P < 0.01$) in this respect. Obviously T. stans leaves possess some factors, soluble in water, either cool or hot, that exhibited deterrent influence. None of the extracts of V. negundo could be seen to possess any potential deterrents.

From Table - 1, it can be noticed that T. stans and V. rosea act as deterrents to a better extent when employed as hot water and alkaline extracts, respectively. Alkaline extracts of Coleus barbatus leaves revealed highly significant ($P < 0.01$) deterrent action on adult beetles. It could be clearly assumed that there might be some chemicals which repelled the test insects from treated paper discs.

Table 1. Responses of adults Lasioderma serricorne F. to the cold and hot aqueous and alkaline extracts of the leaves of 5 plant species

Plant species	Distribution of adult insects								
	Cold water extract			Hot water extract			Alkaline extract		
	T	UT	IND	χ^2	T	UT	IND	χ^2	T	UT	IND	χ^2
Coleus barbatus	17	18	15	a 00.028 NS	19	14	17	00.756 NS	06	15	29	03.850 *
				b 00.125 NS				00.111 NS				15.114 **
Vitex negundo	21	18	11	a 00.230 NS	19	20	11	00.025 NS	21	12	17	02.454 NS
				b 03.124 NS				02.132 NS				00.421 NS
Tecoma stans	23	25	02	a 00.083 NS	07	38	05	21.354 **	10	18	22	02.284 NS
				b 17.640 **				00.333 NS				04.500 *
Vinca rosea	25	14	11	a 03.102 NS	23	08	19	07.258 **	09	23	18	06.125 *
				b 05.444 *				00.380 NS				03.000 NS
Datura matel	19	14	17	a 00.757 NS	11	16	23	00.925 NS	15	19	16	00.470 NS
				b 00.111 NS				04.234 *				00.032 NS

T: Treated, UT: Untreated, IND: Indifference.

* Level of significance ($P < 0.05$), ** Level of significance ($P < 0.01$), NS Not significant($P > 0.05$).

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 2. Responses of 1st and 2nd instar larvae of L. serricorne F. to wheat flour mixed with different leaf extracts of 5 plants (covered thimbles)

Plant species	Instars	Distribution of 1st and 2nd instar larvae				
		T	UT	IND		χ^2
Alkaline extract						
Tecoma stans	1st	13	10	52	a	00.391 NS
					b	23.400 **
	2nd	27	24	24	a	00.176 NS
					b	00.176 NS
Datura matel	1st	27	10	38	a	07.810 **
					b	03.270 NS
	2nd	24	08	43	a	08.000 **
					b	05.388 *
Vinca rosea	1st	13	06	56	a	02.578 NS
					b	26.796 **
	2nd	24	16	35	a	01.600 NS
					b	02.050 NS
Acetone extract						
Vitex negundo	1st	14	13	48	a	00.037 NS
					b	18.644 **
	2nd	13	06	56	a	02.578 NS
					b	26.796 **
Coleus barbatus	1st	13	06	56	a	02.578 NS
					b	26.796 **
	2nd	13	04	58	a	04.764 *
					b	28.520 **
Alcoholic extract						
Coleus barbatus	1st	18	12	45	a	01.200 NS
					b	11.570 **
	2nd	09	25	41	a	07.528 **
					b	20.480 **

T : Treated
UT: Untreated
IND: Indifference
Categories of comparison:- a : T vs UT, b : T vs IND.

* Level of significance ($P < 0.05$)
** Level of significance ($P < 0.01$)
NS Not significant ($P > 0.05$)

Table 3. Responses of 3rd and 4th instar larvae of L. serricorne F. to different extracts of Coleus barbatus leaves (open as well as covered thimbles)

Instars	Tests	Distribution of 3rd and 4th instar larvae											
		Aqueous extract				Acetone extract				Alcoholic extract			
		T	UT	IND	χ^2	T	UT	IND	χ^2	T	UT	IND	χ^2
3rd	Open	16	45	14	a 13.786 **	18	09	48	03.000 NS	16	17	42	00.030 NS
					b 00.132 NS				13.636 **				11.654 **
	Covered	14	49	12	a 19.444 **	30	10	35	10.000 **	18	15	42	00.272 NS
					b 00.152 NS				00.384 NS				09.600 **
4th	Open	23	31	21	a 01.184 NS	24	14	37	02.630 NS	33	11	31	11.000 **
					b 00.090 NS				02.770 NS				00.620 NS
	Covered	20	34	21	a 03.628 NS	26	12	37	05.156 *	13	29	33	06.094 *
					b 00.024 NS				01.920 NS				08.695 **

T: Treated

UT: Untreated

IND: Indifference

* Level of significance ($P < 0.05$)

** Level of significance ($P < 0.01$)

NS Not significant ($P > 0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 4. Responses of 3rd and 4th instar larvae of L. serricorne F. to different extracts of Vitex negundo leaves (Open and covered thimbles)

Distribution of 3rd and 4th instar larvae

Instars	Tests	Aqueous extract T	UT	IND		χ^2	Alkaline extract T	UT	IND		χ^2
3rd	Open	30	24	21	a	00.999 NS	18	17	40		00.028 NS
					b	01.588 NS					08.344 **
	Covered	25	28	22	a	00.169 NS	32	08	35		14.400 **
					b	00.194 NS					00.134 NS
4th	Open	10	53	12	a	29.348 **	11	29	35		08.100 **
					b	00.180 NS					12.520 **
	Covered	08	52	15	a	32.266 **	23	12	40		03.457 NS
					b	02.130 NS					04.587 *

T: Treated
UT: Untreated
IND: Indifference

* Level of significance ($P<0.05$)
** Level of significance ($P<0.01$)
NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 5. Responses of 3rd and 4th instar larvae of L. serricorne F. to different extracts of Tecoma stans leaves (Open and covered thimbles)

Instars	Tests	Distribution of 3rd and 4th instar larvae: Aqueous extract T	UT	IND		χ^2	Alkaline extract T	UT	IND		χ^2
3rd	Open	19	37	19	a	05.784 *	26	14	35		03.600 NS
					b	00.000 NS					01.327 NS
	Covered	16	40	19	a	10.285 **	16	18	41		00.117 NS
					b	00.257 NS					10.964 **
4th	Open	20	32	23	a	02.769 NS	18	12	45		01.200 NS
					b	00.209 NS					11.570 **
	Covered	19	31	35	a	02.880 NS	28	22	25		00.720 NS
					b	04.740 *					00.169 NS

T: Treated
UT: Untreated
IND: Indifference

* Level of significance ($P < 0.05$)
** Level of significance ($P < 0.01$)
NS Not significant ($P > 0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 6. Responses of 3rd and 4th instar larvae of L. serricorne F. to different extracts of Datura matel leaves (Open and covered thimbles)

Instars	Tests	Distribution of 3rd and 4th instar larvae								
		Aqueous extract				χ^2	Alkaline extract			χ^2
		T	UT	IND			T	UT	IND	
3rd	Open	31	20	24	a	03.048 NS	13	19	43	01.125 NS
					b	00.890 NS				16.071 **
	Covered	16	30	29	a	04.260 *	22	10	43	04.500 *
					b	03.750 NS				06.784 **
4th	Open	20	36	19	a	04.570 *	23	18	34	00.609 NS
					b	00.025 NS				02.122 NS
	Covered	15	36	24	a	08.647 **	15	21	39	01.000 NS
					b	02.076 NS				10.666 **

T: Treated
UT: Untreated
IND: Indifference

* Level of significance ($P<0.05$)
** Level of significance ($P<0.01$)
NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 7. Responses of 3rd and 4th instar larvae of L. serricorne F. to different extracts of Vinca rosea leaves (Open and covered thimbles)

Instars	Tests	Distribution of 3rd and 4th instar larvae: Aqueous extract T	UT	IND		χ^2	Alkaline extract T	UT	IND		χ^2
3rd	Open	16	22	37	a	00.947 NS	20	13	42		01.484 NS
					b	08.320 **					07.860 **
	Covered	10	26	39	a	07.110 **	18	18	39		00.000 NS
					b	17.163 **					07.736 **
4th	Open	24	34	17	a	01.724 NS	08	25	42		08.756 **
					b	01.195 NS					23.120 **
	Covered	20	34	21	a	03.629 NS	30	16	29		05.000 *
					b	00.024 NS					00.016 NS

T: Treated * Level of significance ($P<0.05$)

UT: Untreated ** Level of significance ($P<0.01$)

IND: Indifference NS Not significant ($P>0.05$)

Categories of comparison :- a : Tvs UT, b : T vs IND.

The results depicted in Table - 2, represent the responses of Ist and 2nd instar larvae. It can be observed that acetone and alcoholic extracts of C. barbatus exhibited statistically significant ($P < 0.01$) deterrent action on the Ist and 2nd instars. Alkaline extracts of two plant species viz.- V. rosea and T. stans imparted more or less similar feeding deterrent properties as far as the Ist instar larvae are concerned. It could also be seen that alcoholic as well as acetone extracts of C. barbatus leaves contained some substances that repelled both the Ist and 2nd instar larvae from feeding on treated flour samples.

Responses of 3rd and 4th instar larvae in case of open and covered thimble tests are recorded with respect to different extracts of the 5 plant species (Table - 3 to 7). During the course of present investigation, it was observed that among the three different extracts of C. barbatus (Table - 3) the aqueous one possessed comparatively more deterrent ($P < 0.01$) action than the alcoholic or acetone extracts, against both the 3rd and 4th instar larvae with open as well as covered-thimble tests. So the test larvae always kept themselves away from the treated flour samples due to the repulsive properties of the tested plant leaves.

From Table - 4, it is clear that with aqueous extracts of V. negundo there was no deterrent action on

influence on (Table - 7) only the 4th instar larvae ($P < 0.01$). From this it could be seen that some non-volatile substance(s) present in alkaline extract imparts to the flour a deterrent influence and that too, with respect to 4th instar larvae. As far as 3rd instar larvae are concerned, it is obvious that, whether it is aqueous extract or alkaline extract, they are not influenced by these components of V. rosea leaves, and that is why they do not avoid the treated baits.

DISCUSSION

This report is of preliminary nature and deals with only pilot screening tests in search of some easily as well as locally available plant products that may provide possible agents for protecting stored food products, particularly spices, tobacco and perhaps cereals. If one surveys the literature on influence of plants/their extracts on feeding behaviour of insect pests one finds that there could be deterrents/inhibitants as well as attractants/stimulants. Recently, a case in this regard has been reported by Abivardi and Benz (1984). They have tested aqueous extracts of 21 plants on the 3rd instar

larvae of the insect Pieris brassicae. These authors have shown that the extracts of the bulbs of Allium cepa and the leaves of Junglans regia stimulated the feeding activity significantly, whereas the extracts of Angelica archangelica, Eucalyptus sp. and Mentha piperita were 100% inhibitory. These authors used only the aqueous extracts of leaves and bulbs. Present work however, dealt with cold and hot aqueous, alkaline, acetonic and alcoholic extracts of five different plants.

Speaking of various plant extracts as stored food-protectants; it is worthwhile to cite the work of Hongo and Karel (1986) who reported on the effects of extracts of neem (Azadirachta indica A. Juss.) seed kernels, neem leaves, tomato leaves (Lycopersicon esculentum) and hot pepper fruits (Capsicum annum) on the incidence and damage by insect pests of stored common beans. Aqueous extracts from neem seed kernels and hot pepper fruits were shown by them to exhibit general deterrent effects on foliar beetle — Ootheca benniggeni, larvae of podborers Maruca testulalis and Heliothis armigera and other important insect pests of beans. The results obtained by them indicated that extracts of both the neem seed kernels and hot pepper fruits are potentially good protectants of beans. The purpose of present study was similar to that of Hongo and Karel (1986), but the plant species and test insects are different. The plant leaf extracts reported

upon in the present work are possible candidate for further work in this direction.

During the course of the present investigation it was observed that except the plants V. negundo and D. matel other 3 were more or less effective deterrents against the adult L. serricorne F.—alkaline extracts of C. barbatus and V. rosea and hot water extract T.stans leaves had highly significant deterrent properties. It is, therefore, of practical interest that such simple ways of extracting the leaves of latter 3 common plants species provide very promising agents worthy of further studies.

In the second part of the present investigation different leaf extracts of these 5 plant species were tested for possible antifeedant/deterrent action on the 3rd and 4th instar larvae of L. serricorne F. The observations presented here primarily prove that certain plant leaf extracts could be effectively used as stored grain protectants in case of L. serricorne F., particularly against the actively feeding stages like the 3rd and 4th instars. It is, therefore, necessary to follow this up with appropriate methods of isolating the volatile/nonvolatile substance(s) from the leaves of V. rosea and V. negundo to look for possible antifeedant/deterrent agents.

During course of the present investigation it became clear that extending this work further through application of better isolation procedures and intensive as well as extensive testing techniques may lead to potent stored product protectants. In the light of observations reported here it is suggested that future investigation can be centered around the following three species of plants viz. - T. stans, V. rosea and C. barbatus.

SUMMARY

Responses of adult and larval forms of the cigarette beetle, Lasioderma serricorne F. to crude leaf extracts of 5 common plant species, prepared in different ways, were tested by employing paper discs and wheat flour treated with extracts as samples by "Choice chamber" method. In case of adults it was observed that hot aqueous extract of Tecoma stans and alkaline extracts of Coleus barbatus and Vinca rosea repell the insects significantly ($P < 0.01$). D. matel showed mixed type action on adult beetles and probably it possesses arrestant/narcotic property. In larval tests, the 2nd instar larvae only were repelled by the alcoholic extracts of

the plant C. barbatus. More detailed tests were conducted on 3rd and 4th instars. The results indicated that aqueous extracts of V. negundo and alkaline extract of V. rosea were positive deterrents on the 4th instar stage. On the basis of these observations it could be suggested that V. negundo, T. stans, V. rosea and C. barbatus are the plants for further investigation.

CHAPTER 2.2

FURTHER OBSERVATIONS ON ANOTHER 5 EFFECTIVE PLANT SPECIES BUT WITH ONLY OPEN THIMBLES AND 3RD AND 4TH INSTAR LARVAE AND ON ADULTS OF *LASIODERMA SERRICORNE* F.

This chapter is essentially a continuation of the work reported in chapter 2.1 on the cigarette beetle, *L. serricorne* F. (Coleoptera: Anobiidae) which is almost a universal pest of cured tobacco leaves, and also various other stored food materials (Powell, 1931; Dick, 1937). According to Pradhan (1968) the safety of stored grains from the damage caused by insect pests largely depends on the proper management of three factors *viz.*— i) the moisture content of the grain, ii) the availability of oxygen, and iii) the development of temperature gradient within the stored grain. Now it has been amply realized by scientists that there are several harmful effects due to residual activity of the insecticides used against the stored product pests. Later several researchers have voiced the need for establishing the idea of pest control without causing health hazards. This possibly could be achived by using extracts of certain plants having pesticidal properties. Such extracts are comparatively more economical, safer, less toxic, and biodegradable.

Certain plants have already been reported to possess insecticidal properties. Excellent reviews on repellent/ antifeedant properties of plant have been written by Patel et al., (1968); Swarup and Srivastava (1971); Sandhu and Singh (1975); Pandey et al., (1977); Rao and Mehrotra (1978); Vigneron (1979); Gillenwater et al., (1980); Khan (1981); Abivardi and Benz (1984) and Hongo and Karel (1986).

In this chapter observations on five more species of plants are being reported with respect to deterrent effect of crude leaf extracts on the adults as well as 3rd and 4th instar larvae, the diffence being omission of 1st and 2nd larval stages for practical reasons. It was noted in the previous chapter 2.1 that the 1st and 2nd instar larvae are comparatively difficult to handle and were very slow in their movements. Low mobility gave rise to experimental errors as that is not condusive to adjudging behevioural preferences to desirable degree of acceptability. Hence, in further work only 3rd and 4th instar larvae were considered. Another departure from previous methodology was using only open thimble tests. This was done to facilitate behavioural response of the test organism, as it is well known that in many cases apart from just olfactory stimuli direct contact with the material is essential for eliciting proper preference or deterrence. Utilizing aqueous and ether extracts of leaves of these 5 plant species, an attempt was made to identify any potentially useful material for further study in the laboratory.

MATERIAL AND METHODS

All the methodological details were as given in the immediately preceding chapter. For present work the following five species of plants were selected:- i) Ipomoea fistulosa, ii) Acalypha hispida, iii) Alangium lamarkii, iv) Tamarindus indica, and v) Rauwolfia canescens. Freshly plucked healthy leaves were utilized after repeated tap-water washings so as to remove dust particles and then draining the leaves thoroughly.

Leaf extraction procedure:-

(i) Cold water extraction & (ii) Hot water extraction:- This would carried out as described in Chapter 2.1.

(iii) Ether extraction:- 10 gm of leaves were ground very finely in a mortar with 100 ml of ether and allowed to stand in the refrigerator at 5°C for 6 hours. Ether soluble matter was then separated by decantation and filtered through Whatman No.1 filter paper. The flask containing extract was kept in open at room temperature till 75% of the ether evaporated. Then the extracts were utilized for testing by paper discs method for adult insects. For larval tests the concentrated extracts were mixed with 10 gm of fresh wheat flour and spread in open

at room temperature till all the ether evaporated. The dried wheat flour clumps were ground again to obtain fine powder which was used as baits for larval test.

Test procedure for adults and larvae:- This was as described in previous chapter. Only open thimble tests were conducted in this experimental series.

RESULTS

Results are presented in Tables 1 to 4 depicting the responses of adults and 3rd and 4th instar larvae of L. serricorne F. with respect to cold or hot aqueous extracts and ether extracts of 5 different plant leaves. Data presented in Table 1 in respect of adult insects showed that both cold and hot water extracts of leaves of Alangium lamarkii were found to exhibit significant ($P < 0.01$) deterrent action, however, the latter extract was extremely effective. The rest of the four plants viz. R. canescens., T. indica, I. fistulosa, and A. hispida did not show comparable property. It is evident that there might be some agents in A. lamarkii leaves which repelled the test insects from the treated paper discs.

Table 1. Responses of adult L. serricorne F. to cold and hot water extracts of plant leaves

Plant species	Distribution of adult insects					Cold water extract			Hot water extract			
	Cold water extract			Hot water extract		T	UT	IND	χ^2	T	UT	IND	χ^2
Ipomoea fistulosa	16	20	14	12	14	24	a	00.444 NS	00.152 NS				
							b	00.132 NS	04.000 *				
Acalypha hispida	24	16	10	16	16	18	a	01.600 NS	00.000 NS				
							b	05.764 *	00.116 NS				
Alangium lamarkii	12	25	13	04	23	23	a	04.566 *	13.370 **				
							b	00.040 NS	13.370 **				
Tamarindus indica	17	21	12	15	21	14	a	00.420 NS	01.000 NS				
							b	00.862 NS	00.034 NS				
Rauwolfia canescens	16	22	12	18	19	13	a	00.946 NS	00.026 NS				
							b	00.570 NS	00.806 NS				

T: Treated, * Level of significance ($P<0.05$)

UT: Untreated, ** Level of significance ($P<0.01$)

IND: Indifference. NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 2. Responses to cold water extract of plant leaves by 3rd and 4th instar larvae of L. serricorne F.

Plant species	Larval instar	Distribution of 3rd and 4th instar larvae: Cold water extract T	UT	IND		χ^2
Ipomoea fistulosa	3rd	14	16	45	a	00.132 NS
					b	16.288 **
	4th	17	33	25	a	05.120 *
					b	01.522 NS
Acalypha hispida	3rd	15	09	51	a	01.500 NS
					b	19.636 **
	4th	15	39	21	a	10.666 **
					b	01.000 NS
Alangium lamarkii	3rd	16	18	41	a	00.114 NS
					b	10.964 **
	4th	24	32	19	a	01.142 NS
					b	00.580 NS
Tamarindus indica	3rd	30	11	34	a	08.804 **
					b	00.250 NS
	4th	23	28	24	a	00.490 NS
					b	00.020 NS
Rauwolfia canescens	3rd	12	13	50	a	00.040 NS
					b	23.290 **
	4th	08	25	42	a	08.756 **
					b	23.120 **

T: Treated, * Level of significance ($P<0.05$)

UT: Untreated, ** Level of significance ($P<0.01$)

IND: Indifference. NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 3. Responses to hot water extracts of plant leaves by 3rd and 4th instar larvae of L. serricorne F.

Plant species	Larval instar	Distribution of 3rd and 4th instar larvae: Hot water extract T	UT	IND		χ^2
Ipomoea fistulosa	3rd	39	14	22	a	11.792 **
					b	04.736 *
	4th	35	12	28	a	11.254 **
					b	00.776 NS
Acalypha hispida	3rd	25	14	36	a	03.102 NS
					b	01.982 NS
	4th	15	21	39	a	01.000 NS
					b	10.666 **
Alangium lamarkii	3rd	12	24	39	a	04.000 *
					b	14.294 **
	4th	15	22	38	a	01.324 NS
					b	09.980 **
Tamarindus indica	3rd	16	17	42	a	00.030 NS
					b	11.654 **
	4th	14	21	40	a	01.400 NS
					b	12.518 **
Rauwolfia canescens	3rd	09	24	42	a	06.818 **
					b	21.352 **
	4th	15	23	37	a	01.684 NS
					b	09.306 **

T: Treated, * Level of significance ($P<0.05$)

UT: Untreated, ** Level of significance ($P<0.01$)

IND: Indifference. NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 4. Responses to ether-extract of Rauwolfia canescens leaves shown by adults as well as 3rd and 4th instar larvae of L. serricorne F.

Test insects		Distribution of adults as well as 3rd and 4th instar larvae T	UT	IND		χ^2
Adults		11	20	19	a	02.612 NS
					b	02.132 NS
Larvae	3rd instar	14	13	48	a	00.036 NS
					b	18.644 **
	4th instar	08	27	40	a	10.314 **
					b	21.332 **

T: Treated, * Level of significance ($P<0.05$)

UT: Untreated, ** Level of significance ($P<0.01$)

IND: Indifference. NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

The observations with cold and hot water extracts of the leaves of all five plants on 3rd and 4th instar larvae are presented in the Tables 2 & 3, respectively. It was clear that out the 5 different cold water extracts, only that of R. canescens showed highly significant deterrent action on 4th instar. The 3rd instar larvae did not exhibit any significant difference in their choice as far as treated and untreated habits were concerned. From these observations it can be suggested that the 3rd instar larvae are probably not sensitive to the five leaf extracts to any significant extent, whereas the 4th instar larvae were highly sensitive to the extracts of R. canescens leaves and was positively deterred by treated bait. Larval forms are usually known to be voracious feeders and what could be surmised from the present observations is that a simple aqueous extract of the leaves of R. canescens decidedly deters the 4th instar larvae of L. serricorne F. from feeding.

Observations presented in Table 3 are on hot water leaf extracts. Among the 5 plant species, A. lamarkii and R. canescens leaves proved significant in reducing the feeding by larvae on treated baits. The deterrence was maximum in case of 3rd instar larvae with respect to hot water extract of R. canescens leaves. Another interesting observation was that both the instars were attracted significantly ($P < 0.01$) to the baits

treated with hot water extract of I. fistulosa.

Data presented in Table 4 are concerned with the responses of both adult and larval forms toward the ether extract of the leaves of the plant R. canescens. It is evident in case of adults that there was no significant change in response. As against this, the 4th larval instars were strongly deterred ($P < 0.01$).

DISCUSSION

As was mentioned earlier, the present report is in continuation of previous chapter (2.1) attempting to find out deterrent/attractant influence of leaf extracts of another five commonly available local plants on the behaviour of adult and last two larval instars of L. serricorne.

It is well recognized fact that only larval forms of L. serricorne F. represent active feeding stadia. The observation on arrestant/deterrent influence of 3rd and 4th larval forms was a very good pointer to a containing agent as far as feeding behavioural responses would permit in cases of L. serricorne F. infestation. Obviously control at larval stages of a pest could be

effected either through arresting them away from stored products or providing feed deterrents. In this context the work of Villani et al.,(1985) is worth noting. These authors tested crude extracts of 5 plant species viz.— Asclepias tuberosa, Hedera helix, Santolia virens, Salvia sclearea, and Pycnanthemum incanum as feeding deterrents on the southern corn rootworm, Diabrotica undecimpunctata B. They found a significantly lower frequency of damage due to treatment of stored corn with such extracts. The present finding has revealed that as far as different R. canescens extracts are concerned the 3rd and 4th larval instars could significantly be deterred from feeding. The cold water extract preferentially deterred the 3rd instar, whereas the hot water extract repelled the 4th instar larvae from feeding on respective treated food materials. Another interesting response that came to light was that the hot water extract of Ipomoea fistulosa exerted a significant arrestant influence on both the larval forms. A degree of mild arrestant action of deterrence was obvious in case of 4th instar to the cold extract of I. fistulosa. Though it can be a useful piece of information, no suggestion can be made at this stage about the practical utility of this finding unless the toxicity or otherwise of these extracts has been tried out.

As far as the influence of these leaf extracts on the response of the adult insect is concerned; it could be seen that hot aqueous extract of A. lamarkii is effective in deterring the adult insects. It is a known fact that adult L. serricorne F. do not feed, nevertheless, deterring them from the stored products would be of use.

The leaves of only R. canescens were extracted in ether, during the course of present investigation, under the assumption of detecting some ether soluble materials. The result did bear out that it probably contains a ether soluble substances that is active as a deterrent only in case of 4th instar of L. serricorne F.

In the light of present observations it could be suggested that plant leaf extracts may prove to be very handy and economical agents for the possible control of infestation of stored products by several insect pests, including L. serricorne F. However, it needs to be said that more work has to be done in arriving at suitable practical applications for control of this pest in an economical way.

SUMMARY

Influence of different aqueous and ether extracts of leaves of 5 more common plant species on behaviour of

adult and larval (3rd & 4th) forms of Lasioderma serricorne F. was studied by employing "Choice Chamber" technique. The hot water extract of Alangium lamarkii leaves significantly kept away adults as well as larval forms. In case of leaf extract of R. canescens, the three different extracts were found to effectively but differentially deter the 3rd and 4th instars. The practical significance of the observations is discussed.

CHAPTER 2.3

OBSERVATIONS ON THE INFLUENCE OF LEAF, SEED AND DRIED FRUIT EXTRACTS OF THE REMAINING FOUR PLANT SPECIES ON ADULTS AND 3RD AND 4TH INSTAR LARVAE OF LASIODERMA SERRICORNE F.

There are a number of plant materials which have been used in the past for keeping insects away from human beings, food materials and woollens (Golob and Webley, 1980). Insects have been prevented from feeding on valuable plant materials by using extracts of different plant parts as deterring agents (McIndoo, 1945; Jacobson, 1958; Mangilitz and Gorz, 1964 and McMillian, et al., 1969). The use of insect repellents/antifeedants offers hope for protecting stored grains from insect attacks, as they are quite specific and may also possess low toxicity to mammals. More than 1400 compounds obtained from plants have been tested as repellents against insect pests (Anon, 1959; Anon, 1966; Jacobson, 1976). Malik and Naqvi (1984) working on some indigenous plants of Pakistan have reported that Saussurea lappa Clarke. exhibited repellent activity against the red flour beetle, Tribolium castaneum Herbst. In respect of compounds of plant origins several chemical factors have been detected (Akeson, et al., 1967, 1968a, 1968b and 1969) which inhibit feeding activity of the insect pests. Various products of neem, Azadirachta indica A. Juss. have been used since long for the control of

various pests of stored grains as reported by Pandey et al., (1976); Roomi and Ariquddin (1977); Atri and Prasad (1979) and Ali et al., (1983). Work has also been done to study efficacy of some other plant extracts viz. Lantana (Atri and Singh, 1977), Yellow oleander (Deshmukh and Borle 1975; Pandey et al., 1976) and Sadabahar (Pandey, et al., 1976) in relation to the insect pests. The aqueous extracts of indigenous plant foliage deterred oviposition by Heliothis virescens (Tingle and Mitchel, 1984). On the other hand, three different plant leaf extracts were tested against leaf cutter ant, Atta cephalotes, exhibiting significant deterrent action (Chen, et al., 1983; Hubert and Wiemer, 1985; and Okunde and Wiemer, 1985).

Another commendable area of insect pests control relates to use arrestants/stimulants, contained in plant products, which cause insects to stop moving and thereby elicite feeding or oviposition (Wood, et al., 1970). In this context, many reports have been published by various scientists (Derr, et al., 1964; Stark, et al., 1965; Guerra and Shaver, 1969; Taylor and Agbaja, 1974; Yoshida, 1976; Ladd and McGovern, 1980 and Tipping, et al., 1986). These dealt with plants that were noted to produce arrestant/attractant qualities, which could effectively used to protect the stored products from infestations by insect pests. So, to aid in protecting the stored products from insect attacks there is a continuing need for finding

out newer arrestants, deterrents and antifeedants that would be still better in efficacy and possess longer persistency of action and also be more economical than the existing ones. Therefore, an attempt was made to investigate efficacy of various extracts of leaves of different plants. In Chapter 2.1 and 2.2 the results dealt with such leaf extracts, 10 plant species noticed to potential candidates for further study. The present chapter not only deals with leaf extracts but also with extract of seeds of O. sanctum and that of whole, dried fruits of D. plumieri. (These extracts were tested for arrestant/attractant or deterrant/repellent influences on the behaviour adult beetles as well as 3rd and 4th larval instars of L. serricorne F.).

MATERIAL AND METHODS

Test insects, both adults and larvae, were collected from the stock culture raised under the previously described methods. Only those methods of extraction are described here which were not mentioned earlier. The preference tests were carried out by Choice Chamber technique, as described earlier (chapter 2.1).

Extraction procedure in short were as described below:-

(i) Petroleum ether extraction: - 10 gm of finely crushed leaves were mixed with 100 ml of petroleum ether (boiling range 40°C - 60°C). The mixture was triturated thoroughly, homogenate was allowed to stand overnight in refrigerator at 5°C. After 24 hours the mixture was decanted and then filtered through filter paper (Whatman No.1). The filtrate was kept in open at room temperature till the volume got reduced to 25% due to evaporation of solvent. The concentrated extract was used for adult and larval experiments. For adult tests 10 paper discs were soaked in concentrated extract and called as "treated" paper discs. For larval tests concentrated extract was mixed with 10 gm of wheat flour. Mixture was dried in open at room temperature and then it was thoroughly ground in a mortar getting fine powder and labelled as "treated" flour sample. "Control" flour sample was prepared in similar after mixing 10 gm of wheat flour with 25 ml of solvent.

(ii) Extraction by steam-distillation:- 10 gm of pre-washed finely crushed leaves were mixed with 100 ml of distilled water and triturated thoroughly. The homogenate was then subjected to steam-distillation. Distillate was collected as the sample and processed as in previous case for testing.

(iii) Hot petroleum ether extraction:- Leaves of plant

species were washed and drained completely of extraneous water. 10 gm of processed leaves were ground into the mortar in 100 ml of petroleum ether (boiling range 40°C - 60°C) with frequent stirring. Then the mixture was refluxed for ___ hours, cooled and stored at 5°C temperature overnight. The residue was separated by decantation. Supernatant was filtered through filter paper (Whatman No.1) and filtrate was collected into conical flask. Later, the conical flask was held at 50°C untill the volume of extract was reduced to ¼ volume. This concentrated extract was labelled for the particular plant species.

(iv) Chloroform-methanolic extraction:- 10 gm of crushed leaves were mixed with 100 ml of a 2:1 chloroform : methanol mixture. The mixture was triturated thoroughly, allowed to stand overnight in the refrigerator, filtered through muslin cloth and the filtrate was centrifuged at 3500 r.p.m. The supernatant was filtered through filter paper (Whatman No.1). The final extract was allowed to stand at room temperature till the volume was reduced to 25% of original volume. The concentrated extract was treated as in previous cases for preparing samples for testing.

(v) Methanolic extracts of dried D. plumieri fruits:- Ripe fruits were collected, dried in open sun light daily for one week. 10 gm of clean dry fruits were crushed in

a mortar and mixed with 100 ml of methanol by trituration. The homogenate was allowed to stand overnight in the refrigerator at 5°C and then decanted to separate the residue. Supernatant was centrifuged at 3500 r.p.m. and filtered through filter paper (Whatman No.1). This extract was evaporated to ¼ volume. The concentrated methanolic extract was processed, as described earlier, for testing.

(vi) Petroleum ether extract of Ocimum sanctum seeds:-
Fully mature Ocimum sanctum seeds were collected during the month of October and November, 1987 and dried at room temperature for one week. 10 gm of dried seeds were ground very finely and then triturated thoroughly in 100 ml of petroleum ether (boiling range 40°C to 60°C). The homogenate was allowed to stand for 24 hours in the refrigerator, decanted, filtered through filter paper (Whatman No.1). The extract was reduced to 25% of its original volume. The concentrated extract was handled as described previously for testing. Testing procedure for adults and larval forms has been as described earlier. Statistical analyses were carried out as per methods of Mead and Curnow (1983).

RESULTS

Data presented in Table 1 depict responses of adult insects to the different solvent-extracs of the leaves of E. neriifolia. Among the 4 different extracts, only steam-distillate exhibited highest degree of deterrent action. Chloroform:methanol (2:1) extract also showed very good deterrent action on adult insects. Petroleum-ether extract did not elicit any significant response, however, the solvent itself was a noticeable deterrent. The aqueous extract was found to be of no interest. There might be some substances in steam-distillate and chloroform-methanol leaf-extracts, which warded off the adult cigarette beetles very effectively.

From perusal of table 2 it would become apparant that the leaf extract with chloroform : methanol was an attractive bait for the 3rd and 4th larval instars. The aqueous extract too, elicited an attractive influence on both of the larval stages, though it was less effective than the previous. Other extracts were found to exert no noticeable influence on the behaviour of larvae.

Aqueous extract of Achyranthes aspera (Table 3) was found to effectively deter the adult insects. Strangely enough the same was noted to exert a good

Table 1. Responses of adult Lasioderma serricorne F. to different leaf extracts of the plant Euphorbia neriifolia

Extractions	Distribution of adult insects T	UT	IND		χ^2	
Chloroform-methanol	05	12	33	a	02.882	NS
				b	20.630	**
Steam-distillation	02	07	41	a	02.776	NS
				b	35.372	**
Petroleum-ether	11	02	37	a	06.230	*
				b	14.082	**
Aqueous	16	26	08	a	02.380	NS
				b	02.666	NS

T: Treated, * Level of significance ($P < 0.05$)

UT: Untreated, ** Level of significance ($P < 0.01$)

IND: Indifference, NS Not significant ($P > 0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 2. Responses of 3rd and 4th instar larvae of L.serricorne F. to differemt leaf extracts of the plant E. neriifolia

Extractions	Instars	T	UT	IND		χ^2	
		Distribution of 3rd and 4th instar larvae					
Chloroform-methanol	3rd	35	26	14	a	01.326	NS
					b	09.000	**
	4th	38	16	21	a	08.962	**
					b	04.898	*
Steam-distillation	3rd	18	16	41	a	00.116	NS
					b	08.966	**
	4th	11	21	43	a	03.124	NS
					b	04.234	*
Petroleum-ether	3rd	24	18	33	a	00.856	NS
					b	01.420	NS
	4th	26	20	29	a	01.691	NS
					b	00.162	NS
Aqueous	3rd	26	15	34	a	02.950	NS
					b	01.066	NS
	4th	26	13	36	a	04.332	*
					b	01.612	NS

T: Treated, * Level of significance ($P < 0.05$)

UT: Untreated, ** Level of significance ($P < 0.01$)

IND: Indifference, NS Not significant ($P > 0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 3. Responses of adult and 3rd and 4th instar larvae of L. serricorne F. to different leaf extracts of plant Achyranthes aspera

Distribution of adult insects

Extractions	T	UT	IND		χ^2	
Hot petroleum ether	10	11	29	a	00.046	NS
				b	09.256	**
Aqueous	02	15	33	a	09.940	**
				b	27.456	**

Distribution of 3rd and 4th instar larvae

Extractions	Instars	T	UT	IND		χ^2	
Hot petroleum ether	3rd	26	05	44	a	14.224	**
					b	04.628	*
	4th	33	06	36	a	18.692	**
					b	00.130	NS
Aqueous	3rd	36	06	33	a	21.428	**
					b	00.130	NS
	4th	21	19	35	a	00.100	NS
					b	03.500	NS

T: Treated, * Level of significance ($P < 0.05$)
UT: Untreated, ** Level of significance ($P < 0.01$)
IND: Indifference, NS Not significant ($P > 0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 4. Responses of adult and 3rd and 4th instar larvae of L. serricorne F. to the chloroform-methanolic leaf extracts of the plant Ocimum sanctum

Distribution of adult insects

Plant species	T	UT	IND		χ^2	
Ocimum sanctum	44	05	01	a	31.040	**
				b	41.088	**

Distribution of 3rd and 4th instar larvae

Plant species	Instars	T	UT	IND		χ^2	
	3rd	50	15	10	a	18.846	**
Ocimum sanctum					b	26.666	**
	4th	55	12	08	a	27.596	**
					b	35.062	**

T: Treated, * Level of significance ($P < 0.05$)

UT: Untreated, ** Level of significance ($P < 0.01$)

IND: Indifference, NS Not significant ($P > 0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 5. Responses of adult and 3rd and 4th instar larvae of L. serricorne F. to the methanolic dried fruit extracts of the plant Duranta plumieri

Distribution of adult insects

Plant species	T	UT	IND	χ^2	
Duranta plumieri	47	00	03	a 47.000	**
				b 38.720	**

Distribution of 3rd and 4th instar larvae

Plant species	Instars	T	UT	IND	χ^2	
Duranta plumieri	3rd	62	07	06	a 43.840	**
					b 46.116	**
	4th	67	05	03	a 53.388	**
					b 58.524	**

T: Treated, * Level of significance ($P<0.05$)

UT: Untreated, ** Level of significance ($P<0.01$)

IND: Indifference, NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

Table 6. Responses of adult and 3rd and 4th instar larvae of L. serricorne F. to petroleum-ether extract of the seeds of the plant Ocimum sanctum

Distribution of adult insects

Plant species	T	UT	IND		χ^2	
Ocimum sanctum	25	13	12	a	03.788	*
				b	04.567	*

Distribution of 3rd and 4th instar larvae

Plant species	Instars	T	UT	IND		χ^2	
Ocimum sanctum	3rd	27	22	26	a	00.510	NS
					b	00.018	NS
	4th	26	25	24	a	00.018	NS
					b	00.080	NS

T: Treated, * Level of significance ($P<0.05$)

UT: Untreated, ** Level of significance ($P<0.01$)

IND: Indifference, NS Not significant ($P>0.05$)

Categories of comparison :- a : T vs UT, b : T vs IND.

degree attractive influence on the 3rd instar only. Other extracts were inconsequent in this context.

Table 4 presents the data of adult and 3rd and 4th instar larval responses against the chloroform-methanolic leaf extracts of the plant Ocimum sanctum. From the Table 4 it was very clear that there are some strong arrestant constituents in the leaf extracts of O. sanctum. Maximum attraction was exerted in case of adult beetles. The larval forms also exhibited highly significant positive response, though to a little lesser extent than adults. It can be suggested, therefore, that by isolating the attracting constituents from this whole leaf extract it could positively be used as a bait to trap beetles and their late larval instars from the already infested stores of commodities to minimize further damage.

The results depicted in Table 5 represent the responses of adult and larval forms to methanolic extract of dried fruits of the plant D. plumieri. It is very clear from these tests that the adults as well as 3rd and 4th larval instars were positively attracted towards the treated baits. The level of significant were highest ($P < 0.01$) in respect of the adult beetles. It is, therefore, a highly fortunate finding that the dried fruits of D. plumieri would prove to be an excellent agent for effective management of L. serricorne infestations. Another remarkable feature is that this plant is so

commonly grown as a hedge-plant in several parts that it would be easy to collect the ripe fruits (berries) of this plant quite easily. These berries are normally not used for any useful purpose by people at large. Ripe fruits usually are available during October to December every year. Petroleum ether extract of dried seeds of Ocimum sanctum were tested against the adult as well as 3rd and 4th larvae of L. serricorne F. (Table 6). Adults exhibited a positive response towards treated paper discs to good extent but the 3rd and 4th instar larvae did not. So, it seems that leaves of this plant contain arrestant factor(s) but the seeds do not possess such component, at least when the latter were extracted in petroleum ether.

In the light of the above description, it is obvious that of the 4 plant materials tested the leaf extracts of E. neriifolia (steam-distillate) and A. aspera (plain aqueous extract) possessed strongly deterrent substances. On the other hand the chloroform-methanol extract of the leaves of O. sanctum elicited a highly significant attraction on the adults as well as larvae. Similarly, the methanolic extract of sun-dried whole berries of D. plumieri demonstrably contained a highly attractive component. Both of these findings really need further intensive work in order to have highly effective methods of management of the insect pest under investigation.

DISCUSSION

As far as the present author is aware of there are no previous report on the four species of plants investigated during the course of present work, on behavioural responses of the cigarette beetle, L. serricorne F. Neeta et al., (1987) extracted 5 indigenous plants viz.— Ipomoea cornea, Adhatoda vasica, Parthenium hysterophorus, Tridax procumbens and Embelia ribes employing different solvents viz.— petroleum ether, benzene and alcohol. These extracts were applied at a rate of 4 parts/100 parts of cowpea (Vigna unguiculata L.) seeds for testing their repellent properties, against the pulse beetle, Callosobruchus maculatus F. All the plant extracts protected cowpea seeds from the infestation upto 60 days after treatment. However, the extract of T. procumbens in petroleum ether extracts was most effective. For the present investigation four plant species viz.— E. neriifolia, A. aspera, O. sanctum and D. plumieri were chosen. E. neriifolia leaves were extracted by plain aqueous extraction, steam-distillation, and using solvents like chloroform : methanol (2 : 1) and petroleum-ether. Of these different extracts of leaves of E. neriifolia , the steam-distillate exhibited excellent deterrent action on adults as well as larval forms of L. serricorne F. The plain aqueous extract was also effective.

Present finding also agree with the work done by Villani and Gould (1985b) who screened whole extract from 74 plant species for deterrent/ antifeedant activity against the late instar larvae of corn wireworm, Melanotus communis Gyll. (Coleoptera : Elateridae). These authors found that only 5 plant species showed statistical significant deterrent/ antifeedant properties. Extracts of Asclepias tuberosa and Hedera helix were noted by them to exhibit exceptional level of feeding deterrency.

Su,Helen (1987b) studied persistency of repellent effect of dillseed extract on Tribolium confusum (J & V.) and reported that even after two years over 50% of the original repellency remained. Bowry et al.,(1984) working on Sitophilus oryzae have shown that neem cake powder rubbed onto maize protected the latter from weevil attack in an effective manner. These authors also reported that seed cake-powders of linseed, mohua, mustard, and castor were equally effective in protecting the stored grains from insect infestation.

It was observed here that the aqueous extract of the plant A. aspera leaves showed most significant deterrent action on adult beetles but not as larval stages. Chandravadana (1987) isolated triterpenoid from the leaves of Momordica charantia Linn. (bitter gourd), which were found to exert a deterrent activity against red pumpkin beetle, Aulacophora foveicoolis Lucas. This work was similar

to the present one in the only respect that plant leaves were utilized, however, the present author has not made an attempt to isolate any particular constituents of leaves. Harish and Ahmed (1987) reported on the effectiveness of natural embelin isolated from berris of Embelin ribes as a grain protectant for stored wheat against infestation by larvae of Corcyra cephalonica (Stainton) Ephestia cantella (Walker) and Trogoderma granarium (Everts), at a concentration of 0.0125%. During the course of present work berries of D. plumieri were examined for influence on behaviour of cigarette beetle, after extracting sun-dried fruits in methanol. This methanolic extract, quite contrary to the deterrent property of Embelin ribes, was found to possess highly arrestant effect on L. serricorne F. In addition to this the present author has found that chloroform:methanolic extract of the leaves of O. sanctum was also capable of exerting strong arrestant action on L. serricorne F., adults and larval forms. It is, therefore, suggested that O. sanctum leaves and D. plumieri fruits could be used as a bait to trap the adult and larval forms of L. serricorne F.

It is very clear that plant products can protect the stored commodities from infestation by insect pests. Such plant products are easily biodegradable and hence preferable. These should be tested for toxicity. Dosimetric studies are also very essential before the products obtained

could be passed for use as repellents/deterrents. More easy and suitable extraction methods should be found out so as to make it possible for common man to handle them effectively either on small or large scale against the L. serricorne F.

SUMMARY

The investigated four species of plant leaf, seed and fruit$ extracts in different solvents, exhibited excellent deterrent/arrestant activities against the test insect Lasioderma serricorne F. Steam-distillate of Euphorbia neriifolia leaves and aqueous leaf extract of Achyranthes aspera showed very promising deterrent actions, whereas chloroform-methanolic leaf extract of Ocimum sanctum and methanolic fruit extract of the plant Duranta plumieri exhibited very high arrestant influence on the test insects. Possible practical utility is mentioned.