ISOLATION OF THE BIOACTIVE PRINCIPLES OF *Thevetia neriifolia* JUSS. (APOCYNACEAE) AND DETERMINATION OF THEIR BIOLOGICAL ACTIVITIES

BY

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DECLARATION

I hereby declare that this thesis entitled **"Isolation of the bioactive principles of *Thevetia neriifolia* Juss. (Apocynaceae) and determination of their biological activities** " is a bonafide record of research work done by me during the course of research and that the thesis had not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other university or society.

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CERTIFICATE

Certified that this thesis entitled "**Isolation of the bioactive principles of *Thevetia neriifolia* Juss. (Apocynaceae) and determination of their biological activities**" is a record of research work done independently by **Smt.Hebsy Bai** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Pest problems and actions to alleviate them are as old as human attempt to grow crops for meeting his food needs. Since the earliest recorded use of sulphur for pest control by ancient Summerians in about 2500 BC and insecticides formulated from herbs and oils by Egyptians and Chinese, till now man has toiled to contain these herbivores, but with limited success. Pest problems still loom large in the horizon.

Throughout their evolutionary history, insects had to deal with a plethora of naturally occurring environmental toxicants. To survive, insect species evolved a variety of mechanisms to make the toxic materials innocuous to them. Just as these mechanisms reduced the toxicity of neutral substances, they also reduced the toxicity of human-contrived chemicals. Evidently the insecticidal approach, which entailed identifying the pest and finding the most "effective" chemical to control them with little concern for other factors, got proved as a wrong move. Evolution of viable alternate technologies became inevitable for sustainable dissolution of pest problems.

In this context, ecological pest management employing 'reduce number strategy' became a feasible proposition. Plant protection technologists turned to tactics that could be utilized for increasing mortality in pest population by introducing less hazardous components in the agroecosystem like resistant cultivars, natural enemies, behaviour modulators and insect growth regulators. Such chemicals offer several advantages to environmental and human safety.

"Nature's chemical factories", the plants, offer an excellent source of biologically active natural products which can limit insect populations. The higher plants produce a vast number of diverse metabolites ranging in chemical complexity and biological activity. The dependency of insects on plants for food, leads to the production of secondary chemicals which are capable of producing adverse effects on insects like growth regulation, feeding inhibition and reproductive regulation, as a defensive mechanism. These phytochemicals are being recently explored extensively as desirable components of pest management systems.

Though more than 2000 plant species have been reported to contain bioactive principles effective against insects, presently the only plant exploited for practical pest control programmes is the neem tree. Over the past three decades this plant has been researched and extensively deliberated by scientists all over the world. However this single plant species, is not likely to meet the global requirement of pesticides. Other indigenous sources of botanical pesticides have to be identified and exploited for the effective substitution of the huge quantities of synthetic insecticides now being used.

Kerala is well reputed for its diverse tropical flora . Unfortunately this floral diversity remains virtually untapped for pest control purposes and many potential plants await discovery. Saradamma (1989) screened 20 commonly available plants and ranked *Clerodendron infortunatum* Linn., *Nerium oleander* Linn., *Thevetia neriifolia* Juss, and *Eupatorium odoratum* Linn. as highly promising for pest management in Kerala. Among these, *T. neriifolia* commonly known as yellow oleander is grown for its elegant and shady foliage and scented

handsome flowers or as border plant. It requires little attention except sunshine and well drained soil. It can be raised from seeds and cuttings and can endure drought and frost. All parts of the plant are toxic.

Antifeedant activity of seeds of *T.neriifolia* to *Athalia proxima* Klug (Pandey *et al.*, 1977), leaves to *Henosepilachna vigintioctopunctata* F. (Saradamma, 1989), juvenomimetic activity of leaves to *Dysdercus cingulatus* F. (Saradamma, 1989), stomach toxicity of seeds to *Ostrinia nubilalis* (Hubner) (Freedman *et al.*, 1979) and insecticidal activity of seeds to *H.vigintioctopunctata* (Sathpathi and Ghathak, 1990) have been reported. The bioactivity of the plant was attributed to the cardiac glycosides, thevetin (Gattefosse, 1949) and neriifolin (McLaughlin *et al.*, 1980). While thevetin was reported to act as a stomach poison to *A.proxima* (Johri *et al.*,1994) neriifolin was observed to be a stomach poison for *O.nubilalis* (McLaughlin *et al.*, 1980) and antifeedant for *Popillia japonica* Newman (Reed *et al.*, 1982). Saradamma (1989) reported that water extract of dried leaves of *T.neriifolia* checked the pests of brinjal (*H.vigintioctopunctata* , *Centrococcus insolitus* Gr. and *Aphis gossypii* Glover) and *H.vigintioctopunctata* of bittergourd in microplot trials. Reduction in the population of the leaf webber of amaranthus was seen in the field when sprayed with four per cent water extract of dried leaves (Srinath, 1990).

Detailed information on the bioefficacy of the plant extracts on different insects under the laboratory and field condition, mode of action, safety to non-target organisms in field are yet to be gathered for the exploration of the plant at field level and the present investigations cover the following aspects:

1. Assessment of the bioactivity of seed and leaf of *T.neriifolia* on different insect pests.
2. Identification of a suitable solvent and method for extracting the bioactive components from different parts of *T.neriifolia*.
3. Isolation and determination of bioefficacy of the components in plant parts.
4. Assessment of the biochemical changes in the insects treated with the plant extracts.
5. Assessment of the effect of the extracts on the survival and development of the pupal parasitoid, *Chrysocharis johnsoni* S.
6. Evaluation of the acute and chronic toxicity of the extracts to rats.
7. Evaluation of the treatments for the control of pests of bittergourd and amaranthus in the field and safety of the treatments to natural enemies of the pests.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The antifeedant, insecticidal, juvenomimetic / sterilant and physiological activities of extracts of *Thevetia neriifolia*, their field performance and effects on non target organisms were studied in the present investigation. The literature relevant to the different aspects covered in the studies have been briefly reviewed in this chapter.

2.1 Antifeedant activity

Antifeedant activity of different parts of a variety of plants has already been reported .

2.1.1. Crude extracts of plant parts

2.1.1.1. Root

Crude extracts of roots of *Medicago sativa* L. showed strong feeding deterrent activity against the larvae of *Costelytra zealandica* (White) (Sutherland *et al.*, 1975). Nakatani *et al.* (1981) reported the antifeedant activity of root barks of *Trichilia roka* to *Spodoptera eridania* Cramer and *Epilachna varivestis* Muls. The feeding deterrency of extracts of root bark of *Calotropis gigantea* L. to *Schistocerca gregaria* Forskal (Rao, 1982) and *Tripterygium wilfordii* Hook G. and *T.hypoglaucum* to *Pieris rapae* L. were observed by Tong and Chiu(1988) . Antifeedant activity of roots of *Catharanthus* sp., *Lantana camara* L., *Nerium oleander* (Kumuda Sukumar,

1988), *Psoralea corylifolia* L. (Chintalwar *et al.*, 1992)to fourth instar larvae of *Spodoptera litura* were also reported.

2.1.1.2. Stem

Rhizomes of *Acorus calamus* L. showed antifeedant activity to *Henosepilachna vigintioctopunctata* (Tewari and Moorthi, 1985). Arivudainambi and Nachiappan (1993) reported the antifeedant activity of extracts of stem of *Ipomoea carnea* Jacquin to *Achoea janata* Linn.

2.1.1.3.Bark

Extracts of bark of *Carya ovata* Koch.acted as feeding deterrent for scolytid bark beetles (Gilbert *et al.*, 1967). Kubo *et al.* (1977) reported the antifeedant activity of extract of bark of *Warburgia ugandensis* Sprague to *Spodoptera littoralis* F. and *S. exempta*. The extracts of bark of *Phellodendron amurense* Rupr. also had strong antifeedant activity against the termite *Reticulitermes separatus*, a serious pest of paper and wood (Kawaguchi *et al.*, 1989).

2.1.1.4. Leaf

The feeding deterrency of extracts of leaves of several plants like *Aloe vera* Tourn. and *L. camara* to *Athalia proxima* Klug. (Pandey *et al*, 1977), *C.gigantea* to *S.gregaria* (Rao and Mehrotra, 1977), *Annona reticulata* L. to lady bird beetle and adults of pulse beetle (Islam, 1984), *Clerodendron inerme* (L.) Gaertn. to *Diacrisia obliqua* W. (Tripathi and Rizvi, 1985), *Azadirachta indica* A. Juss to second and third instar larvae of *Pieris brassicae* L. (Kirpal Singh and Sharma, 1987), *Chloroxylon swietenia* DC. to *S. litura* (Srimannarayana and Rao, 1989), *Tanacetum vulgare* L.,

Salvia officinalis L., *Ocimum basilicum* L. and *Anethum graveolens* L. to *Leptinotarsa decemlineata* L .(Hough Goldstein, 1989), *N. oleander, T. neriifolia, Clerodendron infortunatum, Eupatorium odoratum* and *A. indica* to *H. vigintioctopunctata* and *S. litura* (Saradamma, 1989), *Annona squamosa* L., *Argemone mexicana* L., *Cacopris gigantea* L. and *Ricinus communis* L. to grubs of *H. vigintioctopunctata* (Rao and Chitra, 1990), *Ipomoea fistulosa* Mart. to *Periplaneta americana* L. (Grover and Hiradhar, 1992), *Vitex negundo* L. to several insects (Kalavathi *et al.*, 1992), *Bougainvillea spectabilis* Willd. to *H. vigintioctopunctata* (Rao *et al.*, 1992; Janardhan *et al.*, 1992) and *Sida acuta* Burm. f. *to Earias vitella* F. (Dongre and Rahalkar, 1993) have been reported.

2.1.1.5. Flower

Flowers of *Eupatorium maculatum* were reported as effective antifeedants to *Popillia japonica* (Metzger and Grant, 1982). Extracts of *Hibiscus sabdariffa* L. calyxes exhibited antifeedant activity against *E.vitella* (Dongre and Rahalkar, 1992). Grover and Hiradhar (1992) and Shin Foon and Yu Tong (1993) reported the antifeedant activity of flowers of *Tagetes erecta* L. to *P. americana* and *Rhododendron molle* G.Don to larvae of *Plutella xylostella* L. respectively. Lily (1995) observed the high antifeedant activity of flowers of *C. infortunatum* to *H.vigintioctopunctata* .

2.1.1.6. Fruits

Unripened fruits of *Xylocarpus molluccensis* (Lamk.) Roem. acted as an antifeedant against *S.exempta* (Kubo and Nakanishi, 1977). The skin scrappings of fruits of *Momordica charantia* Linn . showed antifeedant activity to *P. americana* (Grover and Hiradhar, 1992). The presence of antifeedant

components in the mesocarp of ripened neem fruits was reported by Ponnuswamy *et al.* (1993).

2.1.1.7. Seeds

Antifeedant activity of seeds of neem was reported against *P. japonica* (Ladd *et al.*, 1978), *S. littoralis* (Meisner *et al.*, 1981), *Spodoptera frugiperda* Smith (Jacobson *et al.*, 1983), *P. brassicae* (Kirpal Singh and Sharma, 1987), *Pygaera cupreata* Butler (Bhandari *et al.*, 1988), Southern corn root worm (Landis and Gould, 1989), *Heliocoverpa armigera* Hubn. (Sinha, 1993), *P. xylostella, S. litura* and *Hellula undalis* Fb. (Sombatsiri, 1993).

Besides neem, feeding inhibition of seeds of *A. reticulata* to lady bird beetle (Islam, 1983), *A. calamus* to *H. vigintioctopunctata* (Tewari and Moorthi, 1985), *Vateria indica* L., *Butea frondosa* Koenig., *A. squamosa, Bassia latifolia* Roxb. and mango to *S. litura* (Kumar and Takur, 1988), *Carpora procera, Lansium domesticum* Jack and *Suretenca macrophylla* to corn root worm (Landis and Gould, 1989) and *M. charantia* to *P. americana* (Grover and Hiradhar, 1992) have been reported.

2.1.1.8. Relative efficacy of different parts of plants

Biocides present in different parts of plants vary in identity and concentrations and hence the activity of the different plant parts also vary.

The leaf and flower extracts of *C. gigantea* contained phagostimulants, while the extracts of fruits and root barks of the same plant were phagodeterrents (Rao, 1982). Kirpal and Sharma (1987) evaluated the relative efficacy of neem seed kernel suspension (0.1,0.2 and 0.4 per cent) and water extract of leaves (0.5,1.0 and 2 per cent) and found that all three

concentrations of neem seed kernel were as effective as two per cent leaf extract in antifeedant activity against *P. brassicae*.

Extracts of seed coat and root bark of *Melia volkensii* L. had high antifeedant activity against *Aedes aegypti* L. and *Locusta migratoria* L. while whole fruit had only moderate activity (Mwangi and Kabanu, 1993). Similarly, leaf and flower extracts of *C.infortunatum* exhibited higher antifeedant activity against *H.vigintioctopunctata* than the root and stem extracts of the plant (Lily, 1995).

2.1.2 Active components isolated from plant extracts

More than 35 constituents of neem, classified under terpenoids and flavanoids, have been isolated from seeds and tested against different insect pests. Azadirachtin, the main bioactive component of neem had been identified as a potent antifeedant for different insect pests viz., *L. migratoria* (Butterworth and Morgan, 1971), *Oncopeltus fasciatus* Dallas and *S. frugiperda* (Redfern *et al.*, 1981), *Diaphania indica* (Chitra and Kandasamy, 1988), *Eyprepocnemis plorans* (Ascher *et al.*,1989) and *S. littoralis, S. frugiperda, Heliothis virescens* Fabr. and *H.armigera* (Blaney *et al.*, 1990).

Besides azadirachtin, meliantriol (Lavie *et al.*, 1967) also deterred insect feeding. Salannin, a substance structurally related to azadirachtin was reported to be effective at 0.005 per cent against *S.littoralis* and at 0.01 per cent against *Earias insulana* Boisd. (Meisner *et al.*, 1978). Nine compounds from the seeds of *A. indica* namely, nimbolinin-B, nimbolidin A and B, Ohchinolid A and B, diacetyl vilasinin, 3 deacetyl salannin, salannol and nimbondiol compounds exhibited antifeedant activity against *Epilachna varivestis*

Muls.(Kraus *et al.*, 1980). Deacetyl azadirachtinol isolated from fresh fruits (Jacobson, 1987), vepaol , isovepaol and nimidin from seeds (Sankaram *et al.*, 1987) also were potent feeding deterrents.Rojatkar *et al.* (1993) reported Ochinoloid-B as a good antifeedant for *S.litura.*

Several components isolated from *Clerodendron* spp. like myrcoside (Cooper *et al.*,1980), clerodendrin A and B and clerodin (Antonious and Saito, 1981) and clerodin (Van Beek and de Groot, 1986) showed antifeedant activity. Kubo and Nakanishi (1977) reported the antifeedant activity of xylomolin isolated from the ripened fruits of *X. molluccensis.* Warburganal and muzidadial extracted from the bark of *W. ugandensis* acted as feeding deterrents to *S.littoralis* and *S.exempta* (Kubo *et al.*, 1977) and plumbagin from *Plumbago capensis* Thumb. to *S. exempta* (Kubo *et al.*, 1980). Reed *et al.* (1982) reported the antifeedant activity of neriifolin isolated from *T. neriifolia* against codling moth, striped cucumber beetle and japanese beetle. Trilobolide isolated from *Lasepitilam* spp. showed high feeding deterrent activity (Nawrot *et al.*, 1983). The limonoids, cedrelone and limonin from *Cedrella toona* R. deterred the feeding of larvae of *S.litura* (Koul, 1983).

The alkaloids vasicine, vasicinol, deoxyvasicine, vasicinone and deoxyvasicinone extracted from *Adhathoda vasica* Nees. deterred the feeding of epilachna and pumpkin beetles (Saxena and Khan , 1986). Pedonis, a spirotetranortriterpenoid showed antifeedant activity against *Eldana saccharina* and *Maruca testulalis* Geyer isolated from *Harrisonia abyssinica* (Hassanali *et al.*, 1987).

High antifeedant activity was exhibited by Ajugarin I from *Ajuga remota* and (-) polygodial from *Polygonum hydropiper* L. against larvae of *P.xylostella* and *Myzus persicae* Shulz. (Pickett *et al.*, 1989), karanjin from *Pongamia glabra* Vent.,maxima substance - C from *Tephrosia purpurea* Pers. and lonchocarpic acid from *Derris scandens*(Roxb.) Benth.against *S. litura* (Srimannarayana and Rao, 1989), coumarin derivatives like methoxsalen from *Atalantia racemosa* DC. against *S. litura* (Lutharia *et al.*, 1989) and a steroidal ketone, petuniasterone from *Petunia parodii* against *H.zea* (Elliger *et al.*, 1989) and glyceollin isolated from *Glycine max* Mer. against *E. varivestis* (Fisher *et al.*, 1990). Other feeding deterrent principles isolated from plants include dithyreanitrile from seeds of *Dithyrea wislizenii* against larvae of *S. frugiperda* (Powell *et al.*, 1991), psoralen and isopsoralen from *P. corylifolia* (Chintalwar *et al.*, 1992), angulateuoid G. from seeds of *Celastrus angulatus* Willd. against *A. femoralis* and *P. xylostella* (Wu *et al.*, 1992), cardenolide diglycoside from *Erysimum cheiranthoides* against *P. rapae* (Sachdev Gupta *et al.*, 1993), six sesquiterpene lactones like 4,5 - dihydroniveusion , agrophyllin B, agrophyllin A, 15-hydroxy-3-dehydrodesoxytifruition, niveusin B, anhydrodoniveusin A from sunflower against *Diabrotica virgifera virgifera* (Le Conte) (Chou and Mullin, 1993), wilforine from *T. wilfordii* against *P. xylostella* (Shin-Foon and Yu-Tong, 1993), 'Tagitinin C' isolated from *Tithonia diversifolia* (Hemsl.) Gray against *D. obliqua* (Dutta *et al.*, 1993) and ajugarin, ajugareptansin, ajugareptansone, ivains, ajugapitins and ajugamarins from *Ajuga* spp. (Bela Darvas *et al.*, 1993).

2.2 Juvenomimetic / Sterilant activity

The occurrence of insect growth regulatory substances in plants was first detected by Slama and Williams (1966). Subsequent investigations revealed that several plants were potent sources of insect growth regulators and sterilants. Exogenous application of these allelochemicals cause several growth and reproductive aberrations in insects. The important literature available on aspects like duration and mortality of life stages, malformations, fecundity and egg hatchability and physiological changes is reviewed briefly.

2.2.1 Effect on the duration of different instars.

2.2.1.1 Crude extracts

The duration of fifth instar larvae of *S. litura* was prolonged by two days when fed on castor leaves treated with extract of *Eucalyptus* spp. at 300 and 400 ppm (Chockalingam *et al.*, 1986). An active fraction of the aquatic weed, *Eichorhnia crassipes* when applied on freshly moulted fifth instar larvae of *Corcyra cephalonica* St. at 0.25 and 0.5 µg prolonged the larval period (Jamil *et al.*, 1988). Saradamma (1989) reported that extracts of *C. infortunatum*, *A. indica*, *T.neriifolia*, *N.oleander* and *E.odoratum* caused prolongation of the post treatment nymphal duration of *Dysdercus cingulatus* . Incorporation of five per cent foliage extract of *Liriope muscari* adversely affected larval period of *H.zea* (Adeyeye and Blum, 1989). But treatment of grubs of *H. vigintioctopunctata* with extracts of *C. infortunatum* did not significantly influence the nymphal duration (Lily, 1995).

Pupal duration of *S.litura* was significantly prolonged when treated with acetone extracts of *A.indica*, *Ageratum conyzoides* L. and *E.odoratum*

(Saradamma, 1989). and *S.littoralis* and *Agrotis ipsilon* Rott. when treated with chloroformic and methanolic extracts of *Tephrosia nubica* (Sharaby and Ammar, 1993).

2.2.1.2. Active principles

Azadirachtin treatment at 0.2 to 0.5 µg/larva of *Ephestia kuhniella* Walker caused delayed development (Rembold *et al.*, 1981). Application of azadirachtin on early larval stage of *L. migratoria* extended duration of larval stage to several weeks (Sieber and Rembold, 1983). Topical application of the compound to larvae of *P. japonica* completely disrupted subsequent development to adult stage. Larvae treated with low doses had significantly longer larval, prepupal and pupal period (Ladd *et al.*, 1984). Similar prolongation of larval period was observed in *H.virescens* orally injected with azadirachtin (Barnby and Klocke, 1987). But, Rao and Subrahmanyam(1987) found that the larval and pupal periods of *S.litura* treated with azadirachtin were unaffected.

Three active fractions isolated from *E.crassipes* at 0.25 and 0.5 µg when applied on freshly moulted fifth instar larvae of *C.cephalonica* prolonged the larval period(Jamil *et al.*, 1988).

2.2.2 Mortality in different instars

2.2.2.1.Crude extracts

Feeding of *Trogoderma granarium* Everts. with neem extracts caused significant larval mortality (Siddigi, 1981). Newly hatched to 12 day old larvae of *Tribolium castaneum* Herbst. exposed to volatile substances from crushed seeds of neem failed to develop further (Maheswaran and Ganesalingam, 1988).

Acetone extract of *Catharanthus roseus* (L.) G.Don at 5000 ppm caused high larval mortality in *H.armigera* (Deshpande *et al.*, 1988). Petroleum ether extracts of water hyacinth when mixed with larval diet of *T. castaneum* and *C.cephalonica* caused mortality in the fourth larval instar (Rani and Jamil, 1989). Water extract of leaves of *N.oleander*, *A.indica*,*T.neriifolia* and *C.infortunatum* produced nymphal mortality in *D.cingulatus* (Saradamma, 1989). Treatment of second and third instar larvae of *E.narcissus* with extract of neem kernel resulted in a high mortality and it was higher when applied on second instar larvae (Sara, 1993). Lily (1995) reported low larval mortality (20 per cent) in *H. vigintioctopunctata* grubs treated with four per cent benzene extract of leaves of *C.infortunatum*

Treatment of larvae of *E.varivestis* with extracts of leaves and rhizomes of *Asarum europaeum* L.increased mortality during pupal moult (Schmutterer and Kleffner, 1988). Saradamma (1989) reported significant pupal mortality of *S.litura* larvae treated with acetone extracts of *E.odoratum* (60 per cent), *Ocimum sanctum* L.(45 per cent) and *Solanum indicum* L.(40 per cent), petroleum ether extracts of *Plumeria rubra* Ait. (100 per cent) and *Codiaeum variegatum* Blume (40 per cent) and water extract of *S.indicum* and *Manihot esculenta* Crantz. Thirty per cent pupal mortality was observed in *H. vigintioctopunctata*, the grubs of which were treated with acetone extract of fresh leaf, benzene extract of fresh leaf and flower and petroleum ether extract of fresh leaf of *E. odoratum* (Lily, 1995). Ethanol extract of shade dried flowers and leaves, benzene extract of shade dried flowers and root and water extract of stems also resulted in significant pupal mortality.

2.2.2.2. Active principles

Treatment of *Apis mellifera* L. larvae with azadirachtin (0.5µg per larvae) resulted in high mortality (Rembold *et al.*, 1981). When incorporated into the diet of third instar larvae of *Ostrinia nubilalis,* it caused mortality at 10 ppm (Arnason *et al.*, 1985) and treatment of larvae of *S.exempta* at 10 µg/larva resulted in larval mortality (Tanzubil and McCaffery, 1990) .Similarly application of the compound on *E.narcissus* (10 to 0.1 µg/µl) resulted in high mortality in second and third instars. Jeyabalan and Murugan (1995) reported significant inhibition on the growth of different larval instars of *H.armigera* when treated with deacetyl nimbin, M.hydroxyazadirone, gedunin, salannin and deacetyl gedunin.

Three active fractions isolated from *E.crassipes* when applied at 0.75 and 1µg on fifth instar larvae of *C.cephalonica* resulted in significant larval mortality (Jamil *et al.*,1988). Petuniasterone N, isolated from *P. parodii* inhibited larval growth of *H. zea* (Elliger *et al.*, 1989). Similarly mimosine from *Leucaena leucocephala* (Lamk.) deWit (Ishaaya *et al.*, 1991) inhibited growth and development of first and fourth instar larvae of *T.castaneum.*

2.2.3. Inhibition of moulting

2.2.3.1 Crude extracts

Non emergence of adults from larvae treated with leaf and seed extracts of neem was reported on *D. cingulatus* (Abraham and Ambika, 1979; Saradamma, 1989) and *P. japonica* (Jacobson *et al.*, 1983).

Klaus Richter and Heiner Birkenbeil (1987) observed a delay in the onset of imaginal moult in *P.americana* treated with petroleum ether and

ethanol extracts of *Ajuga reptans.* Treatment of larvae of *E.varivestis* with extracts of leaves and rhizomes of *A. europaeum* increased mortality during pupal moult (Schmutterer and Kleffner, 1988).

Powdered leaves of *C.inerme, Tylophora asthmatica* Wright and Arn. and *Justicia betonica* L. reduced adult emergence of *C.cephalonica* (Chander and Ahmed, 1986). Besides, larvae of *H.zea* fed with artificial diets fortified with five per cent foliage of *L.muscari* (Adeyeye and Blum, 1989), fifth instar nymphs of *D.cingulatus* treated with water and benzene extracts of *L.camara, N.oleander, T. neriifolia, M. esculenta* and *Pandanus odoratissimus* L.f. . (Saradamma, 1989), cabbage worm treated with 0.5 per cent extract of *Ajuga nipponensis* (Shin Foon, 1990), *Pericallia ricini* F. treated with flower extract of *Delonix regia* (Boj.) Raf. at 200 ppm (Chockalingam *et al.*, 1992) and *E.septima* treated with leaf extracts of *V.negundo* (Kalavathi *et al.*, 1992) and pupae of *H.vigintioctopunctata* with *A.calamus* oil (Gupta and Dogra, 1993) failed to emerge.

Dass *et al.* (1989) observed that presence of flowers of *T.peruviana* in the rearing environment of *E.vitella* increased the number of adults emerging.

2.2.3.2 Active principles

Moulting inhibitory effects of azadirachtin on large milk weed bug was reported by Redfern *et al.*(1980). Barnby and Klocke (1987) reported that orally injected azadirachtin inhibited development to the adult stage in *H.virescens.* Low doses of the compound inhibited and disrupted moulting of *S.exempta* (Tanzubil and McCaffery, 1990) . However, treatment of *Ceratitis capitata* Wiedemann and *Dacus dorsalis* Hendel did not affect

formation of puparia though it inhibited adult emergence (Stark *et al.*, 1990). Garcia *et al.* (1990) reported that azadirachtin decreases or even abolishes moulting of *Rhodnius prolixus* Stal. even 120 days after its application. Mimosine when incorporated into artificial diet retarded pupation (Ishaaya *et al.*, 1991).

2.2.4 Malformation in development

2.2.4.1. Crude extracts

Prabhu and John (1975) reported the induction of a sixth instar larvae retaining varying degrees of nymphal character in *D.cingulatus* treated with acetone extracts of *Anthocephala cadamba* Miq. *L.camara*, *Tectona grandis* L., *Callophyllum* sp. and *Phyllanthus emblica* L. Schmutterer and Rembold (1980) reported blackish legs in fourth instar larvae of *E.varivestis* when fed on leaves treated with neem seed extracts. They also caused reddish brown spots on the thorax.

Pronounced malformations were observed in larval, pupal and adult stages in *E.varivestis* treated with *A.indica* (Rembold *et al.*, 1980), *S.litura* with extracts of *Aristolochia* sp.(Merdelyn Caasi-Lit and Belen Morallo Rejesus, 1990), and *T. granarium* with need seed kernel extract (Chellayan and Karnavar, 1990; Sara, 1993). Treatment of larvae of *S.littoralis* with extracts of *Dieffenbachia maculata* L. resulted in pupae retaining larval thoracic legs (Antonious *et al.*,1992).

Morphogenetic abnormalities like wing deformation, development of wingless adults and unplasticization of wing lobes were observed by the application of *A.europaeum* on *E. varivestis* (Schmutterer and Kleffner, 1988), *L.muscari* on *H.zea* (Adeyeye and Blum 1989), *E.odoratum*, *A.indica*,

C.infortunatum, *L.camara*, *N.oleander*,*O.sanctum* and *P.odoratissimums* on *D.cingulatus* (Saradamma, 1989), *Aristolochia* spp. (Merdelyn Caasi-Lit and Belen Morallo Rejesus, 1990), *Blumea laciniata* DC. on *D. cingulatus* (Jaiswal and Srivastava, 1992), *Mentha arvensis* L. on *S. gregaria* (Subrahmanyam and Rao, 1993) and *A. squamosa* on *D.koenigii* (Kumuda Sukumar *et al.*,1995).

Increased melanisation was detected on *D. cingulatus* treated with alkaloids of *C. roseus* (Kumuda Sukumar and Osmani, 1981), *Chilo partellus* Swinhoe larvae treated with methanol extract of *A. conyzoides* (Raju *et al.*,1987), *C. cephalonica* treated with petroleum ether extract of water hyacinth (Rani and Jamil, 1989) and *D. koenigii* treated with acetone extract of leaves of *A. squamosa* (Kumuda Sukumar *et al.*, 1995).

2.2.4.2 Active principles

Azadirachtin treatment produced defective pupae or adults or larval pupal intermediates in *H.virescens* (Barnby and Klocke, 1987) and pupal and adult malformations on *A. mellifera* (Rembold *et al.*, 1981), larval pupal intermediates in *S.litura* (Gujar and Mehrotra ,1983), and *S.exempta* (Tanzubil and McCaffery, 1990) and death during ecdysis in *E.narcissus* (Sara, 1993).

The active fractions isolated from *E.crassipes* showed metamorphic abnormalities in *C.cephalonica* (Jamil *et al.*, 1988).Terpenic fractions of *M.arvensis* resulted in deformities at nymphal adult moult (Subrahmanyam and Rao, 1993). A thin layer chromatography (TLC) purified fraction of *B.laciniata* also resulted in supernummerary instars and abnormal adults in *D.cingulatus* (Jaiswal and Srivastava, 1992). Fifth instar nymphs of *D.koenigii* treated with plumbagin isolated from *P.zeylanica* showed deformed wings and legs in adults (Smita Banerjee and Sujatha Magdum, 1995).

2.2.5. Fecundity

2.2.5.1. Crude extracts

The oil and vapour of *A. calamus* was found to sterilize females of *D.koenigii* (Saxena and Mathur, 1976). The leaf alkaloid of *C. roseus* gave better sterilant action to male *D.cingulatus* and root alkaloid to females (Kumuda Sukumar and Osmani, 1981). Sterility was also reported in *E.varivestis* exposed to food treated with extracts of *A. europaeum* (Schmutterer and Kleffner, 1988) *L. migratoria* fed on *A. conyzoides* (Polivanova and Triseleva, 1989), males of *D.similis* treated with flower extracts of *T. neriifolia* (Raju *et al.*, 1990) and *D.cingulatus* treated with ethanol extract of neem seeds (Akhila Thomas and Hiradhar, 1993).

Fecundity was reduced by plant juvenoids from *Polyscius quilfoytei* Bailey in *D.cingulatus* (Rajendran and Gopalan, 1985), *A.vasica* in *D. koenigii* and *T. castaneum* (Saxena and Khan, 1986), *O.basilicum* and *Eucalyptus rostrata* Schlecht. in *E. vitella* (Pathak and Krishnan, 1987), *Artiplex* spp., *N. oleander*, *C. infortunatum* and *Ipomoea palmata* Forsk. in *Callosobruchus chinensis* L. (El Ghar and El Sheikh, 1987), *L. muscari* in *H. zea* (Adeyeye and Blum, 1989), *T.neriifolia*, *N.oleander*, *C.infortunatum*, *E.odoratum* and *A.indica* in *D. cingulatus* and *S. litura* (Saradamma, 1989), neem extracts in *T. castaneum* (Mukherjee and Ramachandran, 1989), *T. granarium* (Chellayan, 1989), *C. partellus* (Banerji and Sharma, 1993) and *C.cephalonica* (Chandraletha, 1994).

2.2.5.2 Active principles

The adverse effect of azadirachtin on ovarian development, fecundity, oviposition and egg viability in insects was reported by Redfern *et al.*(1981), Schluter *et al.* (1985), Dorn (1986) and Babu and Murugan (1995). Koul (1984) found that on topical application of azadirachtin a significant sterility effect was exhibited by the females of *D.koenigii*. Pronounced reduction in egg output and egg viability was reported when both sexes of adult moths of *E.vitella* (Pathak and Krishna, 1987) and *D.koenigii* (Krishna, 1990) were exposed to neem oil vapours. Chellayan (1989) too had shown disruption of oocyte development and reduction of vitellogenic oocytes following treatment with azadirachtin. The limnoid compounds deacetyl nimbin, 17-hydroxyazadiradione, gedunin, salannin and deacetyl gedunin reduced fecundity in *H.zea* (Jeyabalan and Murugan, 1995).

Saxena *et al.* (1979) reported that administration of aristolochic acid isolated from *Aristolochia bracteata* Retz.on *D.koenigii* and *T.castaneum* resulted in degeneration of oocyte. Terpenic fractions of *M.arvensis* also reduced fecundity in *D.cingulatus* (Subrahmanyam and Rao, 1993). β asarone isolated from *A.calamus* resulted in sterility in *Prostephanus truncatus* Horn. (Schmidt, 1993).

2.2.6. Hatchability

2.2.6.1. Crude extracts

Reduction in hatchability of eggs laid by the adultoids produced after the application of plant extracts was reported in *D. cingulatus* (Prabhu and John, 1975), *S. littoralis* (El Sayed, 1985), *O. fasciatus* (Dorn, 1986) and on

S. gregaria and *Pthorimoea operculella* Zeller (Singh and Singh, 1987; Shelke *et al.*, 1987). The rate of egg hatch in *E. vitella* declined sharply when mating pairs were continuously exposed to the odours of *A. indica*, *O.basilicum* or *E. rostrata* (Pathak and Krishna, 1987).

Hatchability of eggs laid by *D.cingulatus* was completely suppressed when the fifth instar nymphs were treated with leaf extracts of *C. infortunatum*, *E. odoratum, P. rubra, M. esculenta, N. oleander*, and *L. camara*. In *C. gigantea* only 38.21 per cent eggs hatched (Saradamma, 1989). Raju *et al.*(1990) observed that only 10 per cent of the eggs laid by females of *D. similis* mated with males emerging from fifth instar nymphs treated with methanol extract of *T. neriifolia* flowers hatched. Significant reduction in hatching of eggs laid by *D. cingulatus* treated with ethanol extract of neem seeds (Akhila Thomas and Hiradhar, 1993a)and *S.littoralis* and *A.ipsilon* treated with chloroformic and methanolic extract of *T.nubica* (Sharaby and Ammar, 1993) were reported.

2.2.7. Effect on physiological parameters

2.2.7.1. Crude extracts

The diverse effects of neem on insect systems have been well investigated. Schluter and Schulz (1984) found a decrease in protein concentration in the oocytes and haemolymph of neem treated *E.varivestis*. A similar decrease of protein concentration in haemolymph when treated with neem seed kernel extract was reported in *S.gregaria* (Parmar, 1987), *Teleogryllus mitratus* (Suresh, 1994) and *D. cingulatus* (Shilu Varghese, 1995). Chellayan (1989) stated that neem kernel extract application decreased the protein level of pupae of *T. granarium*. Neem seed kernel extract and neem oil

interfered with the food utilization, biochemical and enzymic profiles related to nutritive and reproductive physiology of *H. armigera* (Murugan *et al.*, 1993). The amount of food consumed, and the Efficiency of Consumption Index (ECI) were markedly inhibited. Protein levels and fat body development decreased in the larvae, oocytes failed to mature and enzymes involved in lipogenesis were suppressed in both sexes.

Selvisabhanayagam *et al.*(1993) found that neem oil induced derangement in physiology of sperm activation and motility and trehalase activity in *Odontopus varicornis* Dist. In *T. mitratus*, neem kernel extract induced a dose dependent decrease in the protein levels in ovary and fat body and triglyceride levels in ovary, fat body and leg muscles (Suresh, 1994).

Ecdysteroid production in *P.americana* stayed at the low levels of untreated intermoult animals when treated with ethanol extract of *A. reptans* after the last larval moult (Klaus Richter and Heiner Birkenbeil, 1987). Five per cent extract of leaves of *I. fistulosa* reduced the amylase, invertase and protease activity in *P.americana* (Grover and Hiradhar, 1992).

The activity of amylase and invertase was reduced in cockroaches treated with aqueous extract of leaves of *Cestrum nocturnam* L.while the activity of protease was increased in treated insects exposed to sublethal dose (Jawale and Mahajan, 1995). Petroleum ether-methanol extract of seeds of *A. squamosa* and acetone extract of leaves of *A. excelsa* when topically applied to fresh fifth instar nymphs of *D. koenigii* showed substantial depletion of protein bands. Development retardation appeared to have a direct correlation with protein reduction (Kumuda Sukumar *et al.*, 1995)

Kavitha and Murugan (1995) found that extract of *V. negundo* decreased lipid content of ovary, oviduct and spermatheca of *Attractomorpha crenulata* Fab. Methanolic extract of seeds of *Pongamia* sp. decreased total concentration of protein in various reproductive organs of *A. crenulata* (Sivaramakrishnan and Murugan, 1995).

2.2.7.2.Active principles

Azadirachtin was reported to inhibit ecdysteroid titre in *O.fasciatus* (Redfern *et al.*, 1982) *L.migratoria* (Sieber and Rembold, 1983), *Manduca sexta* L. (Schluter *et al.*, 1985), *S.litura* (Rao and Subrahmanyam, 1987) *Galleria* spp. (Malczewska *et al.*, 1988), and *T.mitratus* (Suresh, 1994). Azadirachtin has been proposed to interfere with the synthesis and release of prothoracico tropic hormone in *L.migratoria* (Subrahmanyam and Rembold, 1989), *H.virescens* (Barnby and Klocke, 1987)and in *R. prolixus* (Garcia *et al.*, 1990)

Topical application of azadirachtin on *S.exempta* was found to decrease protein levels and fat body development in females (Tanzubil and McCaffery, 1990). Injection of azadirachtin (1µg/g body weight) into the final instar larvae of *S.litura* decreased haemolymph protein and the electrophoretic protein profile showed the appearance of the storage protein SP 3 a day earlier in the treated larvae (Rao *et al.*, 1993). Mukherjee and Sharma (1993) reported significantly increased midgut estrase activity in sixth instar larvae of *S.litura* fed with azadirachtin treated castor leaves at 30.and 50 ppm levels. A significant lowering of aspartate amino transferase (AST) and alanine amino transferase (ALT) activity was also reported when *T.mitratus* were treated with azadirachtin. Calcium level was not affected (Suresh, 1994). Protein ,carbohydrate and lipids

were significantly reduced in the ovary, oviduct and spermatheca and accessory reproductive glands of *A.crenulata* treated with azadirachtin (Babu and Murugan, 1995)

Ishaaya *et al* .(1991) observed that activity of trehalase, invertase and amylase in the fourth instar larvae of *T.castaneum* treated with mimosine decreased while protease and carbohydrase activities were not affected.

Topical application of plumbagin (115µg/g body weight) on final instar of *H.armigera* decreased significantly haemolymph protein and magnesium and cuticular protein and chitin but increased significantly sodium and amino acid concentrations (Krishnayya and Rao, 1995).

2.3. Insecticidal activity

Several plants have been reported to show insecticidal activity on crop pests. However, available information suggests that with the exception of pyrethrin, nicotine and rotenone, the use of botanicals as insecticide on a wide scale is limited. Even neem, a widely exploited plant for pest control showed better antifeedant and juvenile hormone /sterilant activities than insecticidal action.

2.3.1 Crude extracts

2.3.1.1 On Lepidopteran pests.

Extracts of *A. indica* showed toxicity to several pests like *E.insulana* (Meisner *et al*., 1978), *B. brassicae* (Singh and Sharma, 1986), *H.armigera* (Sinha,1993) and *P. xylostella* (Gomez *et al*., 1993).

Other plants reported to possess insecticidal properties to lepidopterans include *Thevetia* spp. which was toxic to *O. nubilalis,* (Gattefosse 1949; Freedman *et al.*, 1979) and *Cydia critica* and *P. xylostella* (Sathpathi and Ghathak, 1990). Leaf extract of *A. conyzoides,* rhizome extract of *A. calamus* and seed extract of *O.basilicum* were toxic to *P. operculella* (Pandey *et al.*, 1982). Acetone extracts of root barks of *T. wilfordii* has systemic action against newly hatched larvae of the rice yellow stem borer, *Scirpophaga incertulas Walker* (Chiu and Zhang, 1982) and *P.rapae* (Shin Foon, 1990). The steam extract from the seeds of *Heracleum sosnowsitii* was toxic to the fourth instar larvae of *Spilosoma rhodophila* (Verma *et al.*, 1991). Acetone leaf extract of *V.negundo* caused 100 per cent mortality to *E. vitella* (Kalavathi *et al.*, 1992). Petroleum ether extract of *Scenedesmus acutus* showed insecticidal activity against *S.littoralis* (Sharaby *et al.*, 1993) .

2.3.1.2 On Coleopteran pests

Plants reported to have insecticidal activity on Coleopteran pests include neem on *E.varivestis* (Streets,1975), *T. thevetioides* on striped cucumber beetle (Reed *et al.*, 1982), seed oil of *P.glabra* on *Aulacophora stevensi* (Tare and Sharma, 1984), rhizome extract of *A. calamus* against *Oryzaephilus surinamensis* Linn. and *T.castaneum* (Prakash and Rao, 1985), chloroform extracts of leaves of *A. vulgaris* to *T. castaneum* (Ferolino and Prodolino, 1985), seed extract of *G. gyandra* to epilachna beetle (Chandel *et al.*, 1987), petroleum ether extract of *C. inerme, I. palmata* and *N.oleander* to *C.chinensis* (El Ghar and El

Sheikh,1987), extract of root bark of *T.wilfordii* to *A. foveicollis* (Shin Foon, 1990) and acetone leaf extract of *V.negundo* to *E. septima* (Kalavathi *et al.*, 1992.

2.3.2 Active principles.

Wada and Munakata (1968) isolated an insecticidal alkaloid 'cocculolidine from *Cocculus trilobus* DC. Nagilactone and hallactone isolated from leaves of *Podocarpus* spp. had insecticidal action (Russel *et al.*, 1972 ; Russel *et al.*, 1973). Two toxic principles isolated from the seeds of *Tagetes* spp. were found toxic to green leafhopper and brown plant hopper of rice (Morallo Rejesus and Eroles, 1978). 'Allitin' isolated from garlic was reported to be an insecticide of low persistence (Banerji *et al.*, 1979)

Mc Laughlin *et al.* (1980) crystallized the active insecticidal principles neriifolin and 2-acetyl neriifolin from the seeds of *T. thevetioides* and these were found toxic to *O.nubilalis.* Reed *et al.* (1982) reported the toxicity of neriifolin to codling moth. 2- tridecanone isolated from the wild tomato *Lycopersicon hirsutum* Humb. was toxic to *M. sexta*, *H. zea* and *A. gossypii* (William *et al.*, 1980). Methyl cinnamate and methyl chevicol, obtained from *O. basilicum* were toxic to *T. castaneum*, *S. oryzae* and *B. chinensis* (Pandey *et al.*, 1982).

Kole *et al.* (1992) found that amorpholone, a rotenoid insecticide isolated from leaves of *Tephrosia candida* (Roxb.) DC.was effective against *S. litura*. A compound isolated and identified as rocaglamid from the twigs of *Aglaia odorata* (Lour.) showed significant insecticidal activity against *S. litura* (Janprasert *et al.*, 1993). Terpenic fractions of *M. arvensis* was toxic to *D. obliqua* (Subrahmanyam and Rao, 1993). Melannein and dalbergiphenol

isolated from the alcohol extract of heartwood of *Dalbergia sissoo* Roxb.showed high toxicity to *D.koenigii*and *D. similis* (Venkatkumar *et al.*, 1993).

2.4 Performance of solvents used for the extraction of bioactive principles

Different solvents have been effectively used for extraction of bioactive components from plants.

2.4.1 Acetone

Antifeedant activity of acetone extracts of *Ailanthus excelsa* L. (Tripathi and Rizvi, 1985), *A.reticulata* (Islam, 1984), *G.pentaphyllum* (Deshpande *et al.*, 1988) and *C.infortunatum* (Lily, 1995) have been reported.

Similarly, juvenile hormone/sterilant activity of acetone extracts of *T.grandis, Pteropus marsupiam* Roth ., *Vertomia indicum* Less., *A.cadamba, L.camara, P.emblica* and *Calophyllum* spp. (Prabhu and and John, 1975) have also been reported.

Acetone extracts of *Urtica parviflora* Roxb. (Lal, 1976), *T.wilfordii* (Chiu and Zhang, 1982) , *A.indica* (Singh and Singh, 1985) and garlic (Barakat *et al.*, 1986) showed insecticidal activity.

2.4.2. Benzene

Benzene extract of several plants viz., *Ficus carica* L., *Caryopteris divaricata;* M. and *Cellicarpa japonica* (Hosozawa *et al.*, 1974), *C.infortunatum, A.indica, T.neriifolia , E.odoratum* and *N.oleander* (Saradamma, 1989) and *C.infortunatum* (Lily, 1995) showed high antifeedant activity.

2.4.3. Ethanol

Ethanol extract of different plants like *A.reticulata* (Islam, 1984), *A.indica, C.procera, L.domesticum* and *S.macrophylla* (Landis and Gould , 1989), *Euphorbia* spp. and *I.carnea* (More *et al.*, 1989), *H.sabdariffa* and *S.acuta* (Dongre and Rhalkar, 1992) showed antifeedant activity.

Growth regulatory activity of ethanol extracts of plants like *A.reptans* (Klaus Richters and Heiner Birkenbeil, 1987), *A.indica* (Singh, 1987; Akhila Thomas and Hiradhar, 1993a) and *Annona* spp. and *Ipomoea* spp. (Akhila Thomas and Hiradhar, 1993b) were reported.

Extracts of *A.squamosa* and *Croton tiglum* L. (Deshmukh and Borle, 1975), *P.glabara* (Vimal and Naphade, 1980), *Schinus terebinthifolius* Raddi . (Abbassy, 1982) and *T.thevetioides* (Freedman *et al.*, 1979) showed insecticidal activity.

2.4.4. Hexane

Hexane was reported as an efficient extractant of antifeedant principles of neem (Jacobson *et al.*, 1978). While extracts of *C.procera, L.domesticum* and *S.macrophylla* were inactive (Landis and Gould, 1989), extract of *Jatropha curcas* L.gave high antifeedant activity (Cobbinah, 1993).

2.4.5. Methanol

Ascher(1981) and Bhandari *et al.*(1988) found that methanol was a good solvent for preparing neem seed kernel extract which showed high antifeedant activity. Apart from neem, the feeding deterrent activity of the solvent extract of *C.gigantea* (Rao, 1982), *C.swietenia* and *P.glabra* (Srimannarayana and Rao,1989), *P.amurense* (Kawaguchi *et al.*, 1989) *O.sanctum*

(Mallick and Banerji, 1989) and *C.siphonanthus*, *C.inerme*, *C.fragrans* and *C.scendens* (Roy Choudhury, 1994) have been reported.

Methanol extract of several plants showed significant juvenile hormone and sterilant activities. They include *A.balsamea*, *Thuja plicata* D. and *Picea sitchensis* Carr. (Man Singh *et al.*, 1970), *A.indica* (Leuschner, 1972; Ascher, 1981; Hellpap, 1984; Umeh, 1988; Schmutterer, 1989; Osman 1993), *A.conyzoides* (Raju *et al.*, 1987) *T.neriifolia* (Raju *et al.*, 1990) and *P.pinnata* (Jeyabalan and Murugan, 1995).

Insecticidal activity of the methanol extract of neem seed kernel was reported by Bandara and Kudagamage (1993).

2.4.6. Petroleum ether

Several reports are available on the efficacy of petroleum ether for extracting antifeedant principles from plants. The plants include *C. fragrans*, *C. calamilosum* and *C. cryptophyllum* (Hosozava *et al.*, 1974), *Melia azedarach* L. and *A. calamus* (Tewari and Moorthi, 1985), *V.indica*, *B. frondosa*, *A. squamosa*, *B. latifolia* and *Polyathia latifolia* (Sonner) (Kumar and Takur, 1988), *C. swietenia* (Srimannarayana and Rao, 1989), *A. squamosa*, *A. mexicana*, *C. gigantea* and *R. communis* (Rao and Chitra, 1990), *P. corylifolia* (Chintalwar *et al.*, 1992), *Jatropha gossypifolia* L. (Chockalingam *et al.*, 1992), *B. spectabilis* (Rao *et al.*, 1992; Janardhan *et al.*, 1992), *H. sabdariffa* (Dongre and Rahalkar, 1992) and *S. acuta* (Dongre and Rahalkar, 1993).

Reports on juvenile hormone / sterilant activity of petroleum ether extracts of plants like *Pseudotsuga menziesii* (Mirb.) Franco (Wellington, 1969), *C.infortunatum*, *N.oleander*, *T.palmata* and *Artiplex* spp. (El Ghar and El Sheikh

1987), *A.reptans* (Klaus Richter and Heiner Birkenbeil, 1987), *E.crassipes* (Rani and Jamil, 1989), *Strychnos nux-vomica* L. and *Butea monosperma* (Lamk.) Taudert (Nagaraja Rao and Pradeep Kumar, 1994) are also available.

Extracts of *A.calamus* (Pandey *et al.*, 1977 ; Sudhakar *et al.*, 1978; *C.inerme* and *I. palmata* (El Ghar and El Sheikh, 1987), *A. indica* (Chavan and Nikam, 1988; Bandara and Kudagamage, 1993) and *Tabernaemontana coronaria* R.BR. (Guddewar *et al.*, 1993) showed insecticidal activity

2.4.7. Water

Pradhan *et al.* (1962) observed the antifeedant activity of water extract of neem seed kernel while Singh and Sharma (1986) observed strong antifeedant action of neem leaves at one and five per cent concentrations. Besides neem , the antifeedant activity of water extracts of *C. gigantea* (Rao and Mehrotra, 1977), *M.arvensis* (KAU, 1981), *T.vulgare, S. officinalis, O. basilicum, A. graveolens, N. cataria* and *R. graveolens* (Hough Goldstein, 1989), *Euphorbia* sp. and *I.carnea* (More *et al.*, 1989), *T. neriifolia, C.infortunatum* (Saradamma, 1989) and leaves of *A. squamosa, A. mexicana, C. gigantea* and *R. communis* (Rao and Chitra, 1990) were reported.

Growth regulatory /sterilant activity of water extract of *A.indica* (Singh, 1987; Tanzubil and McCaffery, 1990 ; Banerji and Sharma, 1993), *A.reptans* (Klaus Richter and Heiner Birkenbeil, 1987) and *T.neriifolia* (Saradamma, 1989) were reported.

Water extract of neem (Cherian and Menon, 1944; Meisner *et al.*, 1978, Saxena *et al.*,1984; Singh and Sharma, 1986; Bandara and Kudagamage,

1993; Hellpap, 1993; Ganesalingam, 1993), *A.calamus* (Trehan, 1956) and *L.camara* (Gopakumar *et al.*, 1993) were shown to have insecticidal activity.

2.4.8. Relative performance of different extractants

Acetone was found better than methanol in extracting antifeedants from neem seeds (Ascher, 1981; Schauer and Schmutterer, 1981). Among five solvents tested, petroleum ether, chloroform and methanol extract of leaves of *C. swietenia* showed high antifeedant activity (98-99 per cent) against *S. litura* (Srimannarayana and Rao, 1989). Saradamma (1989) evaluated solvents like water, acetone, ether and benzene for their relative efficacy and found that benzene and water were the best extractants of antifeedants .from *T. neriifolia, N. oleander, C. infortunatum, E. odoratum* and *A. indica* when used against *H. vigintioctopunctata*. Petroleum ether and water were equally good extractants of antifeedants from leaves of *A. squamosa, A. mexicana, C. gigantea* and *R. communis* (Rao and Chitra, 1990).

Methanol, petroleum ether and water when tried for extracting antifeedants from neem kernel and used against cabbage leaf eating caterpillar complex were found to be highly effective (Bandara and Kudagamage, 1993). Islam (1993) reported that water and alcohol were equally effective solvents for extracting antifeedants from *A. indica, M.azedarach, Annooka ruhituka* Wright and Arn., *A. reticulata* and *A. squamosa*. Various solvent extracts of *J. curcas* seeds, when evaluated for antifeedant activity against *Zonocerus variegatus* L. showed hexane as the best (Cobbinah, 1993). Among the five solvents tested , acetone and benzene proved superior to ethanol, water and petroleum ether for extracting antifeedant components of *C.infortunatum* (Lily, 1995).

2.5. Effect of plant extracts on natural enemies of insect pests

2.5.1. Parasitoid

Application of neem oil in rice fields was harmless to natural enemies of plant hoppers and leaf hoppers (Saxena *et al.*, 1981a). It also augmented parasitization of leaf folder larvae by the ichneumonid, encyrtid and braconid parasitoids since neem oil prevented the larvae from folding rice leaves and kept the larvae exposed for easy parasitization (Saxena *et al.*, 1981b).

Growth and development of endoparasitic hymenopterans on larvae of *Cnaphalocrocis medinalis* Guen. exposed to rice leaves treated with neem were unaffected (Schmutterer *et al.*, 1983). Grubs of *H. vigintioctopunctata* freshly treated with 0.5 and 0.1 per cent petroleum ether extracts of seeds of *M. azedarach* and rhizomes of *A. calamus* were significantly less parasitized by *Pediobius foveolatus* (Crawford). Exposure 24 hours after treatment did not adversely affect parasitization (Tewari and Moorthi, 1985).

Safety of neem seed oil to *A. cypris* of brown planthopper (Wu, 1986), ethanol extract from bark of *T. wilfordii* to *Tetrastichus israeli* (Mani and Kurian) (Tong and Chiu, 1988), NSKE and neem oil 50 EC to *Trichogramma japonicum*, *Bracon hebtor* (Say) and *Apanteles plutellae* Kurdjumor (TNAU, 1992) and *A. plutellae* (Bandara and Kudagamage, 1993) have been reported .

Patel and Yadav (1993) reported that nicotine sulphate, Repelin and Neemark were highly toxic to the adults of *Tetrastichus coccinellae*.

(Srinivasa Babu *et al.*, 1993) observed that Repelin and Neem guard were safe at lower concentrations to the parasitoids *Trichogramma australicum* (Giralt), *B. hebetor* and *T. israeli.*

2.5.2. Predators

Neem seed kernel suspension was reported to be safe to *Chrysopa scelestes* Banks (Joshi *et al.*, 1982). Topical application of neem oil, chinaberry oil and custard apple oil on *Lycosa pseudoannulata* B & S caused only low mortality even at a high dose of 50µg neem oil per spider (Saxena *et al.*, 1984). However neem oil was found toxic to the predatory mirid *Cyrtorhinus lividipennis* (Renter). Wu (1986) also reported the safety of neem seed oil to *L. pseudoannulata.* Effect of methanol, ethanol, acetone and pentane extracts of neem on the predatory phytoseid, *Phytoseilus persimilis* A. was studied by Mansour *et al.* (1987). Pentane extract was most toxic to *P. persimilis.*

Neem products did not affect the population of insect predators like *C. lividipennis* and *L. pseudoannulata* (Saxena, 1989) and *Oxyopes javanus* Thorell and *Chrysoperla carnea* (TNAU, 1992). *Eucalyptus* sp. and *C. roseus* had very low toxicity against *Lycosa* spp. and *Cyrtorhinus* sp. even at high concentrations (Shanthi and Janardhanan, 1991). The extracts of *E. terticornis* and *T. erecta* were toxic to *Microvelia atrolineata* Berqroth but were compartively safe to T. *maxillosa* spiderlings (Mahima Shanthi and Mohana Sundaram, 1992). Patel and Yadav (1993) reported that botanicals like nicotine sulphate, Repelin and Neemark were 100 per cent safe to *Menochilus sexmaculatus* F. an important aphid predator. However predatory mite population diminished in plots treated with Neemark (0.5 per cent), Repelin(0.5 per cent) and Margoside

(0.8 per cent) (Rai *et al.*, 1993). Mansour (1993) observed that while Margosan-O and Azatin had no toxic effect on the spider, *Chircanthium mildei* and the predacious mite *Typhlodromus athiasae*, Repelin was highly toxic to *T. athiasae*.

2.6. Effects of plant extracts on higher animals

2.6.1. Acute toxicity

The LD_{50} of usaramine, an alkaloid isolated from *Crotalaria brevifolia* was reported to be 300 mg / kg body weight in mice (Singh *et al.*, 1969). Bhakuni *et al.* (1969) observed that the LD_{50} of 50 per cent ethanol extract of seeds of *T. peruviana* in mice was 500 mg / kg body weight. Physalin isolated from *Physalis minima* L. showed an LD_{50} of 2g/kg body weight when administered orally and 1 g /kg body weight intraperitoneally (Mohana *et al.*, 1979). The minimum lethal dose of alcoholic extract of the aerial part of *Indigofera tinctoria* L. was greater than 100 mg /kg in mice (Anand *et al.*, 1979). Acute toxicity studies conducted by Bhavani *et al.* (1980) on different species of animals including non rodents revealed that alcoholic extract of turmeric was not toxic even at very high level (2.5g / kg body weight). Rats fed with a neem extract at a dose upto 600 mg / kg body weight increased body weight without overt toxicity (Quadri *et al* ., 1984).

2.6.2 Chronic toxicity

2.6.2.1. Effects on behaviour

Phyllemblin (100 mg/kg body weight) isolated from ethanolic extract of fruits of *Emblica officinalis* Gaertn. injected intraperitoneally in

mice showed depression of the motor activity (Rajarama Rao and Siddiqui, 1963). The animals became calm, drowsy and sluggish, preferred seclusion and remained in the darker areas of cages. White leghorn birds became sluggish after administration of water extract of neem berries (Singh *et al.*, 1985). Alcoholic extract of *Clitoria ternatea* L. at 230 and 460 mg / kg ip doses produced increased sedation, diminished alertness, inhibited conditioned avoidance response and hypothermia in rats and mice (Kulkarni *et al.*, 1988).

2.6.2.2. Effects on growth

Khanna *et al.* (1986) reported loss of body weight of rats fed with tulsi *(O.sanctum)* for three months at 400 and 200 mg / 100g body weight. Administration of vasicine isolated from *A. vasica* for six months in rats and monkeys did not show any apparent changes in the growth of the animals (Pahwa *et al.*, 1987). No loss in weight was observed in male mice fed with 10 per cent alcohol extract of pericarp of *Balanites roxburghii* Planch. at 250 mg/kg body weight per day for 30 days (Shah *et al.*, 1994).

2.6.2.3. Physiological effects

Chronic administration of methyl ester of aristolochic acid at 60 mg/kg / day for five days per week for four weeks increased liver alkaline phosphatase activity, depleted liver glycogen and decreased kidney alkaline phosphatase activity showing damage to kidney and liver (Pakrashi and Saha, 1979). White leghorn birds administered with water extract of neem berries showed fragile liver undergoing degenerative changes with focal cogestion, retention of bile in gall bladder and congested kidneys (Singh *et al.*, 1985).

Haematological and biochemical determinations of rats and monkeys treated with vasicine isolated from *A. vasica* for 6 months were within normal physiological range. Histopathological examination of major organs did not reveal any abnormality (Pahwa *et al.*, 1987). Macesar and Lim (1988) reported that decoctions from leaves of *T.peruviana* and expressions like methyl methane sulfonate, tetracycline and N-nitrospyrrolidine from kernels and seeds exhibited antimutagenic effects in bone marrow cells of treated mice . Crushed ground seeds of *Thevetia* spp. when fed at 20 and 30 per cent concentrations for 10 days resulted in hind limb paralysis , rolling of body on the long axis, circular flailing of tail, muscular twitch, tetanic convulsions, tremors and death of rats .

Significant reductions in red blood cell count, total leukocyte count and neutrophils and increased lymphocytes were also observed. Reductions in blood glucose and serum proteins and increased BUN, AST and LDH were significant. Histopathological studies showed inflammatory and degenerative changes in the liver and kidney (Pahwa and Chatterjee, 1990).

Singh and Agarwal (1992) found that 50 per cent ethanol extract of fresh leaves of *O.sanctum* , volatile oil from fresh leaves and fixed oil from the seeds significantly protected guinea pigs against histamine and acetylcholine induced pre-convulsive dyspnea. Extracts of *Saraca asoca* (Roxb.) de Wilde flowers and bark inhibited the growth of sarcoma-180 tumour in mice. SGPT and SAKP levels were maintained in the near normal range (Varghese *et al.*, 1992). Chronic administration of neem oil to adult albino rats for eight days showed microscopic lesions in liver and kidney. Haemoglobin, blood glucose,

serum proteins, transaminases and serum cholesterol also showed marked changes (Badri Srimannarayana *et al.*, 1993).

Non fasted rats which consumed baits containing 2700µg /kg body weight of ground *T. neriifolia* seeds showed serious neuromuscular and cardiac malfunctioning and died within 24 hours of injestion (Oji *et al.*,1993). Jaiswal *et al.* (1994) reported anxiolytic activity of fresh leaf extract of *A. indica* to rats. Low and moderate dose of *Clerodendron colebrookianum* (20 to 40 mg/kg) leaf extract did not affect haematological and biochemical parameters. High dose (80 mg/kg body weight) affected liver and kidney functions and metabolism (Gupta Malaya *et al.*, 1994). Crude root extract of *A. conyzoides* showed muscle relaxant and cardio-depressant activity in rabbit. (Achola *et al.*, 1994). Sharma and Mahanta (1995) reported that extracts of *Plumbago rosea* L. and *Shorea robusta* Gaertn. f. when administered to female albino rats significantly altered the total glycogen, protein and RNA content in uterus tissue. Structural degeneration of endometrial glands coupled with biochemical changes were seen in the uterus of treated rats.

2.6.2.4. Effects on reproduction

Mohana *et al.* (1979) reported the abortifacient activity of Physalin isolated from *P. minima* in albino rats at a dose of 100 mg/kg body weight. Subcutaneous administration of 15, 20 and 30 doses of extract of *Portulaca oleracea* L. (1dose=50 mg/ mouse /alternate day) produced mass atrophy of spermatogenic elements (Verma *et al.*, 1982). Chronic administration of garlic powder resulted in spermatogenic arrest in albino rats (Dixit and Suresh

Joshi, 1982). Bhargava (1984) observed that plumbagin from roots of *P. zeylanica* caused selective testicular lesions.

Khanna *et al.*(1986) reported that tulsi (400 and 200 mg/100g body weight) feeding upto three months decreased sperm count, motility and weight of reproductive organs. Crude alcoholic extract of *Solanum xanthocarpum* Schrad. and Wendl. seeds showed spermicidal activity in rats (Rao, 1988) and solasonine isolated from it showed antifertility effect (Arjmand *et al.*, 1992). A lipid terpenoid of *A. indica* seeds caused long term block of fertility in rodents and monkeys (Garg *et al.*, 1993). Antiimplantation and abortifacient activities of *A.excelsa* at 250 mg/kg body weight in albino rats was reported by Dhanasekaran *et al.* (1993).

Manoranjitham *et al.* (1993) and Sampathraj *et al.* (1993) reported adverse effect of neem oil on testicular function in albino rats. Oral feeding of alcoholic extract of *B. roxburghii* fruit at 250 mg/kg body weight per day for 30 days resulted in a loss of spermatogenic activity and declined fertility rate of rats (Shah *et al.*, 1994).

Reversible antifertility effect of alcohol extract of *C. papaya* seeds were reported in male rats (Priya Padman and Chinoy, 1994) and in female rats (Harsha Joshi and Chinoy, 1994) and alcohol extract of pericarp of *B.roxburghii* in male mice (Shah *et al.*, 1994) Sharma and Mahanta (1995) reported the antifertility properties of *P. rosea* and *S. robusta.*

2.7. Field evaluation of plant products

2.7.1. Neem

When one per cent seed suspension of neem, *Bassia* sp. and *Hydrocarpus* sp. and 0.5 per cent eupatorium oil were applied against pests of brinjal in the field, neem was effective in reducing the population of aphids and jassids. None of the deterrents was effective in checking the infestation by fruit borers and epilachna beetle (Asari and Nair, 1972).

Weekly sprayings of one, two and four per cent extracts of leaves and seeds of neem reduced infestation by *Podagrica* spp.and *Sylepta derogata* F. in okra and increased the yield (Adhikary, 1984). Two per cent neem seed kernel suspension was as good as insecticides in reducing *S. litura* damage in tobacco nurseries (Ramaprasad *et al.*, 1987). Similarly different concentrations of neem emulsions reduced the incidence of *Selepa docilis, Urentius* sp. and *Z.variegatus* in aubergine and *S. derogata* and *P. sjostedt* in the field (Cobbinah and Osci-Owusu, 1988).

Neem cake extract at one and three kg ai/ha controlled *H. armigera, Anaemerus fuscus* and *Brachycerus granulatus* of groundnut (Gahukar, 1988). Saradamma (1989) reported the superiority of benzene extract in controlling *H. vigintioctopunctata, Centrococcus insolitus* and *A. gossypii* of on brinjal in the field. Venkatarami Reddy *et al.* (1990) found that one per cent petroleum ether extract of *A. indica* reduced larval population of *H. vigintioctopunctata* in the field. Aqueous seed extract of neem proved effective against important pests of beans,cabbage, cucumber, egg plant, melon, okra and tomato (Hellpap, 1993). An IPM package including neem, tested in field for the control of

potato pests witnessed an outbreak of cut worms (*A. ipsilon* and *H. segetum*) and negligible infestation by other pests in the first season. However the damage was significantly reduced in the second season (Siddiqi and Khalafalla, 1993). Passerini and Hill (1993) reported the effectiveness of neem insecticide against the Sahelian grasshopper on millet in Mali. Oil emulsion of *A. indica* was very effective for the control of American serpentine leaf miner and pea aphid (Reghunath and Gokulapalan, 1994).

2.7.2. Other plants

Among the plant products like infusions of lemon grass, neem leaf, *V.negundo* and tobacco, infusion of *V.negundo* was found to be promising in the field (KAU Research Report, 1985). Significant control of *P.rapae* on cabbage was obtained in the field by spraying three per cent ethanol extract of the bark of *T. wilfordii* (Tong and Chiu, 1988). Saradamma (1989) reported the efficacy of benzene extracts of *E. odoratum*, *C. infortunatum*, *N. oleander* and *T. neriifolia* and water extract of *T. neriifolia* in controlling *H. vigintioctopunctata*, *C. insolitus* and *A. gossypii* of brinjal and *H. vigintioctopunctata* on bittergourd in microplot trials. In a field trial for the management of pests of amaranthus and bhindi; four and two per cent emulsions of leaf extracts of *A.indica*, *T.neriifolia* and *C. infortunatum* controlled the pests of amaranthus and bhindi with increased yield whereas tobacco decoction controlled only aphids on bhindi (Srinath, 1990). Petroleum ether extract of *A. squamosa* was effective in reducing *H. vigintioctopunctata* in the field (Venkatarami Reddy *et al.*, 1990). Water extracts of *Derris elliptica* (Wall.) Benth.

and *Eucalyptus antiquorum* were toxic to leaf folder in the rice field (Luong Minh Chan, 1993). Oil emulsion of *Samedara indica* Gaertn. effectively checked American serpentine leaf miner and pea aphid (Reghunath and Gokulapalan, 1994).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

Fresh and dried leaves and seeds of *Thevetia neriifolia* were tested for their bioactivities. Acetone, benzene, ethanol, hexane, methanol and water were the solvents evaluated for their comparative efficacy in extracting the bioactive components.

3.1. Processing of plant materials

3.1.1. Fresh leaf

Fresh twigs of *T. neriifolia* were collected from in and around the College farm. The tender top and older bottom leaves were discarded and the fresh middle leaves were washed thoroughly and chopped into small pieces.

3.1.2. Dried leaf

Fresh leaves collected and processed as described in 3.1.1 were dried thoroughly in a room under normal temperature. When they attained a constant weight, the leaves were ground finely in an electric grinder.

3.1.3. Seed

Fruits of *T.neriifolia* were collected and dried in the sun. The dried rind of the fruits was removed and the kernels were washed thoroughly and dried. They were then split-opened and the seeds inside were removed, dried in room temperature and then ground in an electric grinder.

3.2. Extraction of bioactive components

3.2.1. Crude extraction

Fresh chopped leaves(40g) were macerated in an electric grinder and kept at room temperature in 100 ml of the respective solvents for 48 hours. Twenty grams of finely powdered dried leaves and ten grams of seeds were shaken in 100 ml of the respective solvents in a reagent bottle for ten minutes and then kept undistured for 48 hours. These solutions were then filtered through cheese cloth and Whatman No.1 filter paper in succession. The volume was made upto 100 ml. This was treated as the stock extract. The dilutions used for the different experiments were made from this stock using distilled water containing one per cent teepol.

3.2.2. Soxhlet extraction

Powdered samples of dried leaf and seed were extracted with the respective solvents for six hours.The volume was made upto 100 ml. Seed samples were first extracted with hexane to remove oil. The dried marc was then extracted with the respective solvents and the volume made up as described in 3.2.1.

3.3. Rearing of test insects

The tobacco caterpillar, *Spodoptera litura* , the epilachna beetle, *Henosepilachna vigintioctopunctata* and the red cotton bug, *Dysdercus cingulatus* were the test insects used in the different studies.

3.3.1. *S.litura*

The eggs and larvae of the insect were collected from the host plant (castor) in the College farm. The adults obtained from rearing were confined in circular glass jars for egg laying. The eggs laid each day were collected separately and kept in glass troughs with castor leaves for hatching. The leaves were changed on alternate days. This procedure facilitated the availability of known life stages of the test insects for different experiments.

3.3.2. *H.vigintioctopunctata*

Egg masses of the beetle were collected from bittergourd culture plants grown in the College farm. The emerging grubs were collected daily and maintained in circular glass jars provided with leaves of bittergourd for getting life stages of the insects of known age for different experiments.

3.3.3. *D.cingulatus*

Nymphs collected from the field were fed on water-soaked cotton seeds and maintained in the laboratory. The adults emerging from the nymphs were transferred to cylindrical glass jars (30 x 15 cm) for mating and egg laying. Moist sand was placed at the bottom of the rearing jars to a height of 2 cm over which soaked cotton seeds were kept in wire meshes for the insects to feed. The jar was closed with a muslin cloth and kept in position with a rubber band. The moist sand prevented early drying of the soaked seeds and eggs laid by the insect. The eggs were removed daily and kept in separate containers for further development. The growth and moulting of the nymphs were observed daily to obtain the instars of correct age.

3.4. Evaluation of bioactivities

3.4.1. Antifeedant effect

Circular pieces of 5 cm diameter were cut from castor leaves of uniform age and dipped in the extracts and air dried. Each leaf disc was placed on a filter paper kept over wet padding of cotton in a petri dish. One pre - weighed fourth instar larva of *S. litura* was exposed to the leaf. The larvae were preconditioned without food for four hours. Five such replications were maintained for each treatment. Leaf discs treated with solvent alone served as control. The larvae were allowed to feed for 48 hours. The leaf area consumed was calculated. The larvae in different treatments were weighed at the end of 48 hours.

Third instar grubs of *H.vigintioctopunctata* were used for the experiment. Pre-weighed bittergourd leaves of uniform age and size were dipped in the extracts and dried. Five third instar grubs pre-conditioned without food for four hours were weighed and released to a leaf The uneaten portions of leaves after 48 hours were taken, cleaned and weighed. The difference between the pre-treatment and post-feeding weights gave the weight of leaf consumed. Pre-weighed leaves dipped in solvent alone and exposed to grubs served as control. The weight loss of leaf in a similar set, kept without exposure to larvae served to find the natural loss of leaf weight due to evaporation and allowed to make adjustments in the weight of leaves consumed by the grubs. Each treatment was replicated thrice. The grubs were taken out and weighed after 48 hours. The difference in weights gave the gain or loss in weight of grubs. The grubs kept without food served as starved larvae.

The percentage of leaf area/weight protected by the extracts was estimated as $= \frac{A - B}{A} \times 100$

where A = area/weight of leaf consumed in control and B = area/weight of leaf consumed in treatment. Percentage of larval starvation in treatments was calculated as $\frac{(C-E)}{(C-S)} \times 100$

where C = mean weight gain of control larvae in 48 hours, E = mean weight gain of experimental larvae in 48 hours and S = mean weight gain of starved control larvae in 48 hours (the figure is negative).

3.4.2. Juvenomimetic activity

The plant extracts were applied topically on the abdominal tergities of the newly moulted fifth instar nymphs of *D.cingulatus* using a Hamilton micro-applicator prepared from ten lamda microcapillaries. Groups of ten insects each treated with two µl of the extracts and confined in nine cm diameter petridishes served as one treatment. Two µl each of the solvent applied on 10 insects served as control. All the treatments were replicated five times. The treated insects were fed on fresh food material in petridishes over which chimneys were placed. The chimneys were closed with muslin cloth.

Nymphal mortality, nymphal duration, nature of adults emerged, longevity, fecundity and hatchability of eggs were observed.

3.4.3. Chemosterilant action (Reproductive inhibitory potency)

Varied concentrations required for the experiments were prepared and the method of application adopted was as described in para 3.4.2. Groups of

ten fifth instar female nymphs confined in nine cm diameter petri dishes, each treated with two µl of the varying concentrations of the ethanol extract of seed and leaf, served as one replication. Two µl of the solvent applied on 10 insects served as control. All the treatments were replicated five times. The treated nymphs kept with fresh food were observed daily till adult emergence.

Assessment of adult longevity, mating behaviour and fecundity

The emerging adults were transferred individually to separate glass chimneys into which one newly emerged male obtained from untreated nymphs was introduced and was supplied with soaked cotton seeds for feeding. Ten such pairs were maintained for each treatment. They were maintained till death allowing the adults to mate and lay eggs. The following observations were recorded.

3.4.3.1. Longevity

The dates on which the insects died were recorded and the mean longevity of females in each replication was computed.

3.4.3.2. Mating

The premating period and mating duration of each pair was separately recorded.

3.4.3.3.Fecundity

The number of eggs laid by each isolated insect was counted daily to determine the fecundity. The eggs were kept separately in petri dishes and the hatching of eggs was observed and recorded daily.

3.4.3.4. Assessment of development of ovary and oocyte and production of protein, lipid, and glycogen

Ethanol extract of seed (five per cent) and leaf (20 per cent) prepared as described in para 3.2.1 were topically applied on newly moulted fifth instar female nymphs of *D.cingulatus* as described in 3.4.2. Groups of twenty insects each treated with two µl of the extract and confined in glass troughs served as one replication. Five such replications were maintained for each extract. Two µl of the solvent each applied on 20 insects served as one replication of control and five replications were thus maintained. The treated insects were fed on fresh pre-soaked cotton seeds kept in petri dishes. Cotton wads, soaked in water were also provided. The upper end of the troughs were closed with muslin cloth.

The ovarian development and production of protein, glycogen and lipid in the emerging adults were studied by dissecting the adults daily from emergence to five days after moulting. Ten adult insects were selected at random daily and were immobilized by chilling for three to five minutes. Haemolymph was obtained by cutting the base of the antenna or the leg and by applying gentle pressure. The oozing haemolymph was collected into a calibrated capillary micro pipette. The insect was then dissected in ice-cold ringer solution to remove ovary and fat body. They were washed in insect ringer and the ringer solution was then blotted off with filter paper. The length of the ovary, number of oocytes, the length and breadth of basal, antepenultimate and penultimate oocytes were measured under a binocular microscope with a micrometer. Haemolymph, ovary and fat body were processed for determination of the total

protein, glycogen and lipid content. Ten replications were maintained for each assessment.

3.4.3.4.1. Total protein

The total protein content of the tissues was estimated by the method of Lowry *et al.* (1951) with some modifications. Known quantities of haemolymph (2.5 µl), ovary (two mg) and fat body (two mg) were homogenised with 0.5 ml of 0.85 per cent potassium chloride and 0.5 ml of insect ringer in a Potter- Elvehjen homogeniser immersed in crushed ice. The homogenate was then transferred into centrifuge tubes and the homogenizer was washed with 0.5 ml potassium chloride and 0.5 ml insect ringer mixture. To each sample, two ml of ten per cent trichloroacetic acid (TCA) was added. The mixture was then centrifuged at 10,000 g (2000 rpm) for 20 minutes in a refrigerated centrifuge. The supernatent was decanted and the residue was dissolved in one ml of 0.1N sodium hydroxide. Folin working solution (0.5 ml of one per cent copper sulphate + 0.5 ml of two per cent sodium potassium tartarate + 49 ml of two per cent anhydrous sodium carbonate) was added to the tubes at 3.5ml per tube and kept aside for ten minutes. Then 0.5 ml phenol reagent of Folin-Ciocalteu (one volume of commercial phenol reagent diluted with one volume of distilled water) was added, mixed well and kept aside for 30 minutes. A blank consisting of one ml sodium hydroxide + 3.5 ml. working solution + 0.5 ml phenol reagent was also maintained. The protein content of the test sample was read photometrically at 580 nm against a standard of bovine serum albumin.

3.4.3.4.2. Total glycogen

The total glycogen content of the tissues was determined by the anthrone method of Seifter *et al.* (1950) with some modifications. Fresh tissues were used for this purpose since glycogen concentration would be changed by storage. A known weight of the tissues, haemolymph (2.5 ul), ovary (two mg), and fat body (two mg), were digested with one ml of 30 per cent potassium hydroxide solution and boiled in a water bath for 20 minutes. After cooling, 50 µl of sodium sulphate was added followed by two ml of 95 per cent ethyl alchohol to precipitate glycogen. The solution was stirred well and set aside for ten minutes. It was then heated at 80°C for ten minutes, cooled and centrifuged for 15 minutes at 3000 rpm. The supernatent ethanol was decanted off and the sedimented glycogen was dissolved in 0.5 ml distilled water. To this, 2.5 ml freshly prepared 0.2 per cent anthrone reagent (ten mg anthrone in five ml concentrated sulphuric acid) was added. The sample was heated in a water bath for ten minutes, cooled and absorbance was measured at 620nm. It was compared with the standard, 'glucose. Distilled water (0.5 ml) + anthrone reagent (2.5 ml) served as the blank.

3.4.3.4.3. Total lipid

The total lipid content was estimated by the method of Bragdon (1951) with modification. Predetermined weights of tissues - haemolymph (2.5µl), ovary (two mg) and fat body (two mg) were homogenised in five ml of 2:1 chloroform- methanol mixture in a Potter - Elvehjen homogeniser. The homogenate was kept in an oven at 60°C for two hours to extract the lipid from the tissues. The sample was then centrifuged at 2,500 rpm for ten minutes. The

supernatent was transferred into a boiling tube and dried for 12 hours at 60°C. A known volume (2.5 ml) of dichromate reagent (prepared by dissolving 20 µI. potassium dichromate in ten ml concentrated sulphuric acid) was added to the boiling tube and heated in a waterbath for 20 minutes. The sample was then cooled and diluted with five ml of double distilled water. The optical density of the sample was read at 580 nm and compared with the standard, palmitic acid (eight mg palmitic acid in ten ml chloroform). A suitable blank (2.5 ml dichromate reagent + 5 ml distilled water) was also maintained.

3.4.4. Contact toxicity

Emulsions of the extracts were prepared as described in 3.2.1. Required grubs of uniform age and size of *H.vigintioctopunctata* were collected from the mass culture maintained in the laboratory. The extracts were sprayed directly on the grubs taken in clean petridishes under Potter's tower. One ml each of the extract was sprayed in one dish containing ten grubs which formed one replication. Five replications were taken for each treatment. Controls were sprayed with the respective solvents only. The sprayed fluids were evaporated under a fan. The sprayed grubs were then transferred to petridishes with fresh food material. Chimneys were placed over the petridish and the open end was closed with a muslin cloth. Mortality counts were taken at the end of 24 and 48 hours.

3.5. Isolation of the bioactive components

3.5.1. Leaf

Fresh leaves of *T.neriifolia* (500g) prepared as described in 3.1.1.

were macerated in an electric grinder and soaked in three litres of ethanol for seven days. The solvent was then removed by decantation and the leaves were further percolated with two litres of the solvent. The elutes were combined and concentrated *in vacuo* to 200 ml. Thin layer chromatography (TLC) was done to obtain an indication of the components present in the extract and the solvents to be selected for eluting the columns to obtain the different fractions. The concentrated leaf extract was then completely evaporated under vacuum in a rotary evaporator. Column chromatography was performed in appropriately - sized column using silica gel (100-200 mesh). The column was packed with silica gel in petroleum ether. The quantity of silica gel used was twenty times the weight of the product. The crude product (weight predetermined) was dissolved in minimum quantity of dichloromethane and transferred to the column. It was left as such for half an hour for adsorption. About 300 ml of petroleum ether wash was done to remove chlorophyll and β–carotene. Development was made with ethyl acetate at varying polarities. The column was eluted with ethyl acetate in petroleum ether mixtures containing 10,20,30,40,and 50 per cent ethyl acetate and finally with ethyl acetate alone. The various fractions were collected separately and were identified as fractions I to VI in different experiments. The solvent was then evaporated in a rotary vacuum evaporator.

3.5.2.Seed

Powdered air-dried seed (150g) prepared as described in 3.1.3 was soaked in sufficient quantity of ethanol for seven days. The solvent was removed by decantation and the seed powder was further percolated with the solvent. The

solutions were combined and concentrated in a rotary vacuum evaporator. Thin layer chromatography (TLC) was done to determine the components present in the extract and the solvents to be selected for eluting the columns to obtain the different fractions. The concentrated extract was further subjected to column chromatography.

Chromatographic columns were packed with silica gel (100-200 mesh) in petroleum ether. The silica gel was taken in the ratio 1:20. The residue was weighed and dissolved in minimum quantity of dichloromethane. It was carefully transferred into the column without disturbing the surface of silica gel. A plug of cotton was kept above this to keep the extract intact. Fifty ml of petroleum ether was added to the column and left undisturbed for 30 minutes for adsorption. The column was then eluted with petroleum ether alone till the non-polar fats were completely removed. The oil fractions were collected separately in conical flasks and concentrated using rotary evaporator. The column was then eluted with ethyl acetate - petroleum ether mixtures containing 10,20,and 50 per cent ethyl acetate and finally with 100 per cent ethyl acetate. Various fractions were collected separately and the solvent was completely evaporated in a rotary vacuum evaporator. The dried fractions obtained in 3.5.1 and 3.5.2 were dissolved in acetone and used as stock solution.

3.5.3. Bioassay

Using the stock solutions prepared as described in paras 3.5.2., 1 and 0.5 per cent concentrations were prepared by adding required quantities of water containing one per cent teepol. They were tested for their antifeedant activity against *H.vigintioctopunctata.*

3.6.Safety of extracts to beneficial organisms

3.6.1. Mass multiplication of *Chrysocharis johnsoni.*

Parasitized grubs and pupae of *C.johnsoni* were initially collected from bittergourd plants raised in the College farm. The emerging parasitoids were then transferred to culture tubes. Cotton swabs soaked in ten per cent sucrose solution were kept at the side of the glass tubes. Grubs of *H. vigintioctopunctata* were provided for oviposition. The blackened parasitized grubs were transferred to clean tubes for parasitoid emergence. The emerging parasitoids were used for the experiments.

3.6.2. Assessment of the effect of extracts on the parasitization of *H.vigintioctopunctata* by *C. johnsoni*

Stock ethanol and water extracts of fresh and dried leaves and seeds prepared as described in para 3.2.1. and two per cent emulsions of each were evaluated for their effect on the development of *C.johnsoni*. One ml of the extract was sprayed in a petridish containing five fourth instar grubs of *H. vigintioctopunctata*. Grubs sprayed with respective solvents alone served as control. The grubs were then transferred to fresh bittergourd leaves in petridishes. Three such sets were maintained. Three pairs of male and female parasitoids were released to each set immediately, 24 and 48 hours after spraying respectively. Five replications were maintained. Mortality of the parasitoids, general behaviour and infectivity (assessed in terms of the number of parasitoids emerging from the infected grubs) were recorded.

3.6.3. Assessment of the toxic effect of the extracts to *C.johnsoni*

Rimless test tubes (25x25cm) were treated with one ml each of the

solutions of different extracts of fresh and dried leaves and seeds of *T.neriifolia* at different concentrations. The tubes were rotated till the solvents had evaporated leaving a dry film of the residue deposited uniformly on the inner surface of the tubes. The tubes were then placed under a fan for one hour for clearing the tubes of the fumes of solvent. Tubes prepared in the same way using solvents alone served as control. Each treatment was replicated five times. Ten adult parasitoids were released in each tube and were exposed to the toxicants for one hour. They were then transferred to clean test tubes and mortality was observed after 24 and 48 hours. The data were corrected for mortality in control using Abbot's formula (Abbot, 1925).

3.7. Safety to higher animals

3.7.1. Acute toxicity studies

Healthy adult albino mice (Strain BALB) of both sexes weighing 15-30 g were used for the study. A group of eight mice (four males and four females) served as one treatment. Different doses of ethanol extract of leaves and seed of *T. neriifolia* (50, 100, 200, 400, 600, 1000mg /kg body weight) were orally administered to the test animals. One group served as a vehicle-treated control. They were maintained under standard husbandry and management conditions with 12:12 hour L.D. period and at room temperature. They were fed with pelleted feed and water *ad libitum*. Mortality counts, behaviour and appearance of the test animals were observed 24 and 48 hours after administration of the extracts.

3.7.2.Chronic toxicity studies

Healthy adult albino rats ,Wistar strain (SCTIMST) of both sexes weighing 160-205g were used in the experiment. Each treatment consisted of two male and two female rats . Five such groups were used for each treatment. The rats were orally administered daily with seed and leaf extracts @ 400 and 800 mg/kg body weight respectively for 14 days. One group served as a vehicle treated control. The animals were maintained on standard rat feed and water was given *ad libitum*. All the animals were sacrificed 24 hours after the administration of the last dose of the extract. The animals were dissected and liver, kidney and heart were weighed and subjected to histological examinations.

Blood samples taken from the animals were subjected to hematological examinations. Total counts of RBC, haemoglobin content and differential counts of leukocytes were estimated .

3.7.3. Isolated perfused heart preparation of frog

The frog (*Rana* sp.) was stunned by a blow to the head with a wooden hammer and then pithed. The heart was exposed and isolated using a perfusion cannula made from a 16 gauge hypodermic needle. The heart was mounted on a modified perfusion apparatus consisting of a reservoir of capacity 500 ml which was connected to the perfusion cannula by means of a non-toxic PVC tubing. The heart was perfused with Frog Ringer solution. The solution was constantly bubbled with air at room temperature. Under these conditions, the ringer solution showed a pH of about 7.8. The perfusate was delivered to the heart at seven cm water filling pressure. This pressure was achieved by maintaining the level of perfusate in the reservoir at exactly seven cm above the

tip of the perfusion cannula thereby creating seven cm water head. The level in the reservoir was kept constant by delivering the perfusate at an appropriate rate. The ventricular contraction was recorded on a smoked drum using a previously calibrated heart lever.

3.8. Field evaluation

Four field trials, two each on bittergourd and amaranthus were conducted in successive seasons to evaluate the efficacy of water and ethanol extracts of fresh and dried leaves and seeds of *T.neriifolia* in suppressing pests under field conditions. The effects were compared with an insecticide check.

3.8.1. Lay out of the experiment

3.8.1.1. Bittergourd

The experiment was laid out in randomised block design. Plots of 6.0 m x 2.0 m were taken and pits of 60 cm diameter and 30 cm depth were dug at a spacing of 2.0m x 2.0 m. Three seeds were sown in a pit. Unhealthy plants were removed two weeks after sowing and only one plant was retained per pit. There were eight treatments and four replications. The treatments included in the experiment were

T1 - Water extract of dried leaf (2%)

T2 - Water extract of fresh leaf (2 %)

T3 - Water extract of seed (2%)

T4 - Ethanol extract of dried leaf (2%)

T5 - Ethanol extract of fresh leaf (2%)

T6 - Ethanol extract of seed (2%)

T7 - Carbaryl (0.15%)

T8 - Control (water spray)

Application of fertilizer and other crop husbandry practices recommended in the package of practices of Kerala Agricultural University (KAU, 1989) were adopted excluding the plant protection measures.

3.8.1.2. Amaranthus

Nursery was raised in 1.0m x 1.0 m plots. Plots of 2.0 m x 2.6 m. were taken in the mainfield and trenches of 30 cm width were taken 30 cm apart in each plot. One month old seedlings were transplanted at a distance of 20 cm. The treatments included in the experiment were as described in para 3.8.1.1. Malathion (0.1 per cent) was used as the insecticide check in this trial. The plants were maintained as per the package of practices of Kerala Agricultural University (KAU, 1989).

3.8.2. Application of plant extracts

The plant extracts were applied in the respective plots with a pneumatic knapsack sprayer when the pest population caused injury at visible levels. Spraying of plant extracts was done at weekly and insecticide at fortnightly intervals ensuring a thorough and uniform coverage of the plants.

3.8.3. Assessment of results

3.8.3.1. Bittergourd

Pre-treatment and post treatment counts of population of

H.vigintioctopunctata(egg masses, grubs and pupae) were recorded. The extent of leaf damage was scored at weekly intervals. The score index adopted was as follows:

Per cent damage	Score
0-25	1
26-50	2
51-75	3
>75	4

At harvest the number and weight of marketable and consumable fruits were recorded. The number of fruits infested by fruit fly, *Dacus cucurbitae* Coq. was recorded separately. Counts of parasitized egg masses, grubs and pupae were taken at weekly intervals.

3.8.3.2. Amaranthus

The population of leaf webber and other pests prior to spraying and 48 hours after spraying were recorded. The extent of leaf damage was also recorded at weekly intervals. At harvest the damaged leaves were removed and weight of the healthy plantswas recorded.

The data were subjected to statistical analysis.

RESULTS

4. RESULTS

4.1. Antifeedant action of extracts of *Thevetia neriifolia* on different test insects

4.1.1. *Henosepilachna vigintioctopunctata*

Antifeedant activity of leaf and seed extracts of *T. neriifolia* assessed in terms of percentages of leaf protection and larval starvation are presented in Table 1.

4.1.1.1. Leaf protection

The mean percentage of leaf protection given by different plant parts showed that seed extract (61.73 per cent) was significantly superior to leaf extracts. No significant difference was observed between dried (49.69 per cent) and fresh leaf (45.24 per cent) extracts.

The solvents tested differed significantly in their performance. Mean percentage of leaf protection indicated that maximum leaf protection was obtained with ethanol (86.98 per cent). It was on par with methanol (81.63 per cent) and the latter was on par with water (70.57 per cent). Benzene (60.78 per cent), hexane (5.86 per cent) and acetone (38.19 per cent) extracts did not give any satisfactory leaf protection. The response of the solvents was the same with respect to the different plant parts. Ethanol, methanol and water proved equally effective in extracting the antifeedant principles from dried leaf, fresh leaf and seed. The extent of leaf protection given by these solvents were 80.09, 74.99 and 63.64 per cent respectively for dried leaf, 84.87, 80.31 and 69.41 per

Table 1 **Antifeedant action of leaf and seed extracts of *Thevetia neriifolia* on third instar grubs of *Henosepilachna vigintioctopunctata***

Solvents used	Plant part used: Dried Leaf (2%)	Fresh Leaf (4%)	Seed (1%)	Mean
Leaf protection (percentage)				
Acetone	44.28 (6.73)	21.62 (4.75)	52.36 (7.31)	38.19 (6.26)
Benzene	55.39 (7.51)	60.28 (7.83)	66.54 (8.22)	60.78 (7.86)
Ethanol	80.09 (9.01)	84.87 (9.27)	96.61 (9.88)	86.98 (9.38)
Hexane	6.38 (2.72)	1.39 (1.54)	12.06 (3.61)	5.86 (2.62)
Methanol	74.99 (8.72)	80.31 (9.02)	89.82 (9.53)	81.63 (9.09)
Water	63.64 (8.04)	69.41 (8.39)	79.05 (8.95)	70.57 (8.46)
Mean	49.69 (7.12)	45.24 (6.80)	61.73 (7.92)	
Larval starvation (percentage)				
Acetone	21.31 (4.72)	30.97 (5.65)	42.69 (6.61)	31.04 (5.66)
Benzene	66.60 (8.22)	80.92 (9.05)	88.70 (9.47)	78.57 (8.92)
Ethanol	77.71 (8.87)	90.30 (9.55)	100.00 (10.05)	89.06 (9.49)
Hexane	0.00 (1.00)	7.28 (2.88)	25.01 (5.10)	7.95 (2.99)
Methanol	84.44 (9.24)	92.09 (9.65)	100.00 (10.05)	92.12 (9.65)
Water	70.47 (8.45)	84.50 (9.25)	100.00 (10.05)	84.56 (9.25)
Mean	44.56 (6.75)	57.83 (7.67)	72.27 (8.56)	

Figures in parantheses are transformed values : $\sqrt{x+1}$

CD (0.05)	Leaf protection	Larval starvation
Plant part	0.627	0.352
Solvent	0.887	0.498

cent respectively for fresh leaf and 96.61, 89.82 and 79.05 per cent respectively for seed extracts.

4.1.1.2. Larval starvation

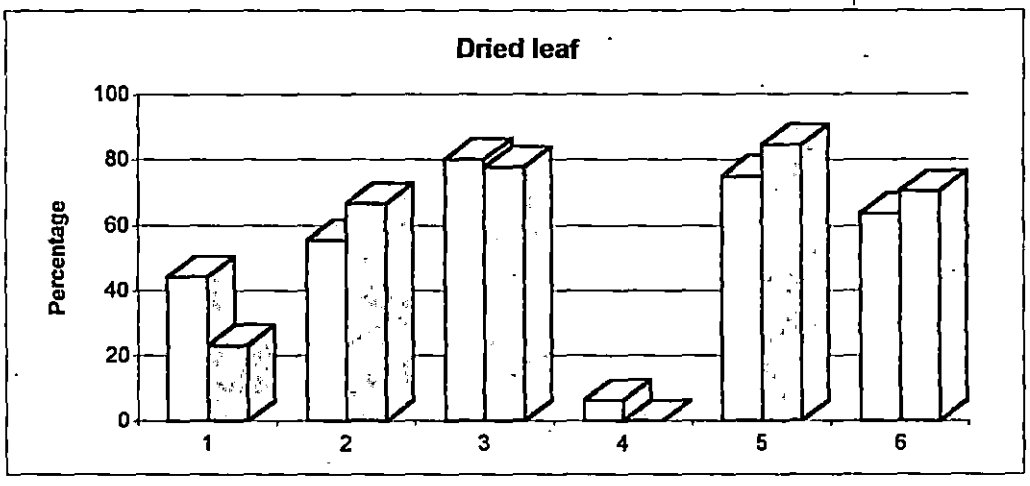
The mean larval starvation in different treatments showed that seed extract (72.27 per cent) was significantly superior to leaf extracts. Between the fresh and dried leaf extracts, fresh leaf extract (57.83 per cent) was found to be more effective and differed significantly from dried leaf extract (44.56 per cent)

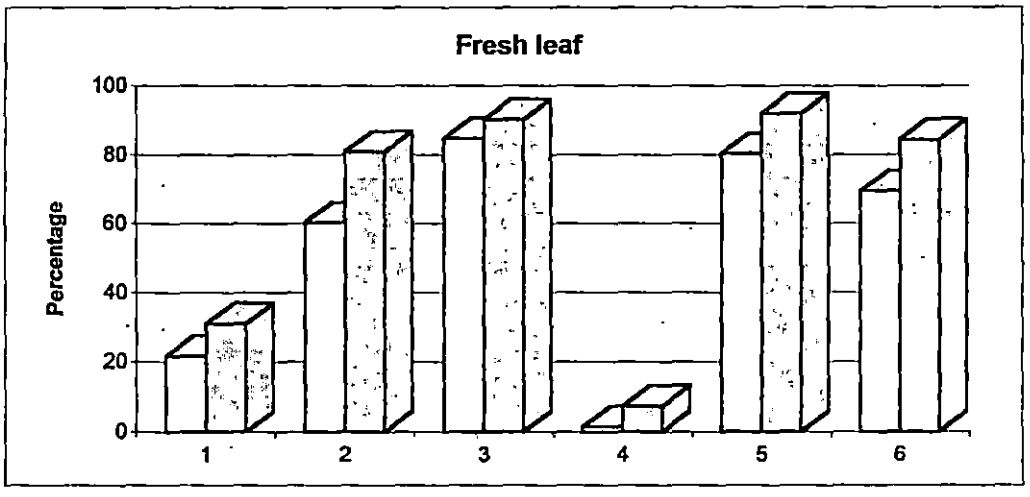
Mean larval starvation caused by different solvents used showed that methanol (92.12 per cent) had the highest activity and it was on par with ethanol (89.06 per cent) and water (84.56 per cent). These solvents were closely followed by benzene (78.57 per cent). Acetone and hexane extracts did not show any significant larval starvation (31.04 and 7.95 per cent only). The extent of larval starvation induced by ethanol, methanol and water extracts of the different plant parts were 77.71, 84.44 and 70.47 per cent respectively for dried leaf and 90.30, 92.09 and 84.50 per cent respectively for fresh leaf. Seed extract of all the three solvents caused 100 per cent larval starvation. Benzene extracts of dried leaf, fresh leaf and seed gave 66.60, 80.92 and 88.70 per cent larval starvation respectively.

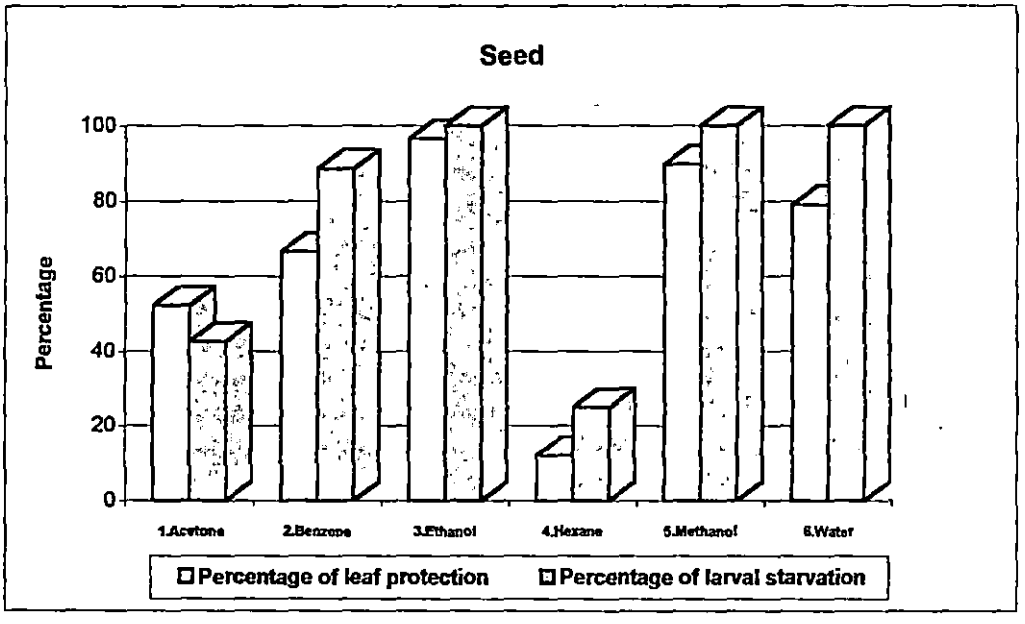
4.1.1.3. Leaf protection and larval starvation as indices of bioactivity of different extracts of *T.neriifolia*.

The comparative efficacy of leaf protection and larval starvation percentages as indicators of bioactivity of grubs of *H.vigintioctopunctata* are presented in Fig.1. With the exception of acetone and hexane, all the other

Fig.1.Comparative performance of different extracts of *Thevetia neriifolia* when used against *Henosepilachna vigintioctopunctata* based on leaf protection and larval starvation







solvent extracts (benzene, ethanol, methanol and water) of different plant parts (fresh leaf, dried leaf and seed) showed broadly the same comparative efficacy on both the criteria. Seed extract was superior to the leaf extracts and between the leaf extracts, fresh leaf extract was better. Among the solvents, ethanol and methanol were superior and on par based on both leaf protection and larval starvation indices. Water was significantly inferior to ethanol but was on par with methanol on the basis of leaf protection while all the three were on par and highly effective in causing larval starvation.

4.1.2. *Spodoptera litura*

Different solvent extracts of *T.neriifolia* leaves (fresh and dried) and seed did not show any significant antifeedant activity against *S.litura*

4.2. Efficacy of different methods of extraction in isolating the bioactive components of *T.neriifolia*

Data presented in Table 2 revealed that maceration of the plant parts in an electric grinder followed by soaking in the solvents for 48 hours(crude extraction) was the better method of extraction,compared to soxhlet extraction based on the antifeedant activity assessed in terms of percentage of leaf protection. The effect of acetone extract was significantly better in the crude extract, the percentage of leaf protected being 46.3 and 53.17 in leaf and seed extracts respectively compared to 12.97 and 36.53 in soxhlet-extracted acetone extracts of leaf and seed respectively. No significant difference could be observed when leaf and seed were extracted with benzene and methanol by the two methods. Though there was no significant difference in the activity when

Table 2 Efficacy of soxhlet and crude extraction techniques in the assessment of bioactivity of *Thevetia neriifolia* on *Henosepilachna vigintioctopunctata* (as percentage of leaf protection)

	Soxhlet extraction		Crude extraction	
Solvent	Leaf (2%)	Seed (1%)	Leaf (2%)	Seed (1%)
Leaf protection (percentage)				
Acetone	12.97	36.53	46.30	53.17
Benzene	54.70	67.43	55.57	66.67
Ethanol	71.87	84.83	80.10	96.67
Hexane	2.77	22.60	1.97	13.00
Methanol	75.93	87.50	75.10	90.00

CD(0.05) for method : 7.280
CD (0.05) for solvent : 4.511

leaves were extracted with hexane by the two methods, soxhlet-extracted seed gave a slightly better protection (22.6 per cent) than crude extract (13.0 per cent). However ethanol extract of seed and leaf obtained through crude extraction showed significantly better activity than soxhlet-extracted sample. The extent of leaf protections were 71.87 and 84.83 per cent for leaf and seed extracted in soxhlet using ethanol, while 80.1 and 96.67 per cent protections were observed in the corresponding crude extracts .

4.3. Juvenomimetic / Chemosterilant activity of ethanol extracts of *T.neriifolia*

Among the different solvents tested, ethanol extract was found to give higher leaf protection and greater larval starvation. Detailed studies were conducted on the juvenomimetic /chemosterilant potential of ethanol extract of *T. neriifolia* seeds and leaves on *D.cingulatus* and the results are presented in Tables 3 and 4.

4.3.1. Effect on nymphal mortality

Highest larval mortality was observed in 10 per cent seed extract treated insects (70 per cent) which was on par with 40 per cent leaf extract (48 per cent). Twenty per cent leaf extract resulted in 20 per cent mortality while 5 per cent seed extract resulted in 26 per cent mortality. Leaf extract (10 per cent) caused only 10 per cent nymphal mortality and seed extract (2.5 per cent) resulted in 14 per cent mortality. All the four treatments were on par (Table 3).

4.3.2. Effect on nymphal duration

It was seen that topical application of the extracts at different concentrations prolonged the duration of the last nymphal stage of *D. cingulatus*

Table 3 Effect of ethanol extracts of fresh leaf and seed of *Thevetia neriifolia* (2µl of emulsion/insect) topically applied on the last instar nymphs of *Dysdercus cingulatus*

		Nymphs			Adults			
Treatment	Dose (%)	Mortality (%)	Duration days	range	Normal (%)	Abnormal (%)	Mortality prior to oviposition (%)	Nature of mating
Leaf extract	40	48.00 (6.87)	9.06	8-11	38.00 (6.18)	14.00 (3.15)	51.32 (7.06)	N
Leaf extract	20	20.00 (4.53)	9.64	6-11	60.00 (7.77)	20.00 (4.53)	22.44 (4.46)	N
Leaf extract	10	10.00 (3.11)	7.42	6- 9	88.00 (9.42)	2.00 (1.46)	14.94 (3.61)	N
Seed extract	10	70.00 (8.37)	10.58	9-12	22.00 (4.64)	8.00 (2.38)	100.00 (10.00)	-
Seed extract	5	26.00 (4.95)	10.50	9-12	60.00 (7.79)	14.00 (3.77)	16.38 (4.16)	N
Seed extract	2.5	14.00 (3.82)	6.82	6-9	80.00 (8.99)	6.00 (2.18)	7.22 (2.16)	N
Control		6.00 (2.39)	6.76	6- 8	94.00 (9.74)	0.00 (1.00)	5.00 (1.47)	N
CD (0.05)		(1.51)	(0.70)		(1.01)	(1.68)	(2.63)	

Figures in parantheses are transformed values: $\sqrt{x}$ / $\sqrt{x+1}$

* Surviving adults did not mate.

significantly. Ten per cent leaf extract and 2.5 per cent seed extract did not cause significant variation in the nymphal duration, the duration being 7.42 (6-9 days) and 6.82 (6-9 days) as against 6.76 days (6-8 days) in the control. However, 40 and 20 per cent leaf extracts significantly prolonged the nymphal duration by 2-3 days, but there was no significant difference between the two doses. While the nymphal duration was 9.06 days (8-11 days) in 40 per cent leaf extract treated insects, it was 9.61 days (6-11 days) in 20 per cent leaf extract treated insects. The seed extracts 10 and 5 per cent significantly prolonged the nymphal duration; the prolongation being by three days. The nymphal duration in these treatments ranged from 9-12 days while in control it ranged from 6-8 days (Table 3).

4.3.3. Effect on adult emergence

Considering the emergence of normal adults, seed extract (10 per cent) resulted in significantly lower percentage and it was followed by leaf extract (40 per cent), the percentage of normal adults in these treatments being 22 and 38 respectively. Leaf extract (20 per cent) and seed extract (5 per cent) were on par in their inhibition of normal adult emergence. While leaf extract at 20 per cent resulted in 60 per cent normal adult emergence, at 10 per cent concentration it resulted in 88 per cent normal adult emergence and it was on par with control (94 per cent). Seed extract at 5 per cent resulted in 60 per cent normal adults while 2.5 per cent concentration resulted in 80 per cent normal adults (Table 3).

Emergence of malformed adults was not significant in leaf extract (10 per cent) and seed extracts(10 and 2.5 per cent) compared to control while leaf extracts (40 and 20 per cent) and seed extract (5 per cent) recorded significant increase in the percentage of abnormal adult emergence. The percentage of malformed adults produced in these treatments were 14, 20 and 14 respectively. The percentage of malformed adults observed in leaf extract (10 per cent) and seed extracts (10 and 2.5 per cents) were 2, 8 and 6 respectively and they were on par with control.

Generally, the number of malformed adults in all the treatments was low ranging from 2 to 20 per cent in leaf extract treatments and 6 to 14 per cent in seed extract treatments. The abnormal adults were short lived and survived only for one or two days after emergence.

4.3.4 Adult mortality before oviposition

A significant percentage of normal adults(51.32)which emerged in 40 per cent leaf extract treatment died prior to oviposition (Table 3). The adult mortality percentages were 22.44 and 14.94 in 20 per cent and 10 per cent leaf extract treatments respectively. In the seed extract treatments, the adult mortality varied from 7.22 per cent in 2.5 per cent seed extract and 16.38 per cent in 5 per cent to 100 per cent in 10 per cent seed extract treatments respectively. Leaf extract (10 per cent) and seed extract (2.5 per cent) were on par with control while leaf extract (20 per cent) and seed extract (5 per cent) were on par and better than these treatments.

4.3.5. Mating

Females emerging from treated fifth instar nymphs when released

with normal males, mated normally (Table 3). Leaf extract treated females mated on the second to fourth day after emergence. Seed extract treated females also mated normally, the mating being observed from second and third day after emergence.

4.3.6. Effect on day of oviposition

The data relating to the day of oviposition during the first, second and third gonadotropic cycles are presented in Table 4.

A significant delay was observed in the commencement of egg laying in 20 per cent leaf and 5 per cent seed extract treated insects. While insects in control oviposited on 6.56 day after adult emergence, the adults in the above treatments laid eggs only on 10.14 and 10.34 days after emergence. Forty per cent leaf extract treated insects were on par with these treatments. They laid eggs on 8.24 day after emergence. While egg laying ranged from 8 to 14 days after emergence in 20 per cent leaf extract treatment, it was observed to be between 9 and 12 days after emergence in 40 per cent leaf extract treatment. The other two treatments (leaf extract 10 per cent and seed extract 2.5 per cent) did not vary significantly from control on the day of egg laying.

Interoviposition period between first and second as well as second and third gonadotropic cycles did not exceed the normal range of 6 ± 1 days.

4.3.7. Effect on fecundity

The number of eggs laid per female recorded during the first, second and third gonadotropic cycles are presented in Table 4. The fecundity of the females emerging from treated nymphs varied significantly. During the first gonadotropic cycle, except those treated with 10 per cent leaf extract and 2.5 per

Table 4 **Effect of ethanol extracts of fresh leaf and seed of *Thevetia neriifolia* (2µl of emulsion /insect) topically applied on the last instar nymphs of *Dysdercus cingulatus***

Treatments	Day of oviposition DAE			No.of eggs laid per female				Incubation period			Hatching percentage			Longevity of adults	Total eggs obtained from adults of 50 nymphs	Decrease over control (%)
	I	II	III	I	II	III	Total	I	II	III	I	II	III	days		
Leaf (40%)	8.24	11.76	0.00*	32.84	25.60	= 0.00*	58.40	5.36 (8.71)	5.56 (8.01)*	0.00*	75.92	36.12	0.00*	13.10	584	93.27
Leaf (20%)	10.14	16.11	20.00	43.10	45.74	=18.86	107.70	5.65 (9.46)	5.52 (9.44)	5.54 (9.12)	89.45	89.11	82.21	20.20	2447	71.82
Leaf (10%)	6.28	12.05	18.12	86.76	75.98	37.32	200.06	5.73 (9.61)	5.86 (9.37)	5.80 (9.31)	92.30	87.80	85.70	20.90	7602	12.14
Seed(5%)	10.34	15.00	18.00	33.76	37.00	22.50	93.26	6.27 (9.35)	6.02 (9.63)	6.08 (9.18)	87.37	92.74	83.29	18.08	2332	73.14
Seed(2.5%)	6.56	14.18	19.00	77.08	78.08	31.26	186.19	5.96 (9.66)	5.74 (9.09)	5.81 (9.33)	93.28	82.63	86.10	19.70	6889	20.65
Control	6.56	12.91	18.76	86.04	75.88	31.02	192.94	5.82 (9.88)	5.72 (9.78)	5.76 (9.45)	96.96	95.65	88.31	20.36	8682	-
CD	2.62	1.87	-	13.20	16.92	6.81	25.73	-	-	-	-	(2.57)	-	(4.58)	NA	-

DAE - Days after adult emergence
I, II, III - (first, second and third gonadotropic cycle)
Figures in parantheses are transformed values: $\sqrt{x}$
NA- Not analysed
*Adults did not survive
Adults did not survive in seed extract 10%

cent seed extract, the other treatments caused significant suppression of fecundity. The number of eggs laid in these treatments ranged from 64 to 110 and 55 to 98 respectively as against 64 to 115 in control, the average being 86.76, 77.08 and 86.04 per female in 10 per cent leaf extract, 2.5 per cent seed extract and control respectively. The fecundity ranged from 17 to 73, 20 to 65 and 18 to 56 per female in 20 and 40 per cent leaf extracts and 5 per cent seed extract treated insects respectively. The average number of eggs laid per female in these treatments were 43.10, 32.84 and 33.76 respectively.

The number of eggs laid during the second gonadotropic cycle was also significantly low. The number of eggs laid in the leaf extract treatments varied from 50 to 112 (10 per cent extract), 18 to 86 (20 per cent) and 40 to 78 (40 per cent), the average fecundity in these treatments being 75.98, 45.74 and 25.60 eggs per female respectively compared to 75.88 eggs in control. While 10 per cent leaf extract was on par with control, the eggs laid per female was significantly reduced in the other two leaf extract treatments. Seed extract treatment (2.5 per cent) with an average fecundity of 78.08 eggs per female was on par with control. Five per cent seed extract was significantly superior to 2.5 per cent leaf extract in reducing the number of eggs laid and the treatment was on par with 20 and 40 per cent leaf extract treatments.

It was observed that only very few treated insects oviposited in the third cycle. None of the insects in 40 per cent leaf extract treatment survived upto the third gonadotropic cycle. While the average number of eggs laid in 20 per cent leaf extract was significantly low (18.86 per female), the number of eggs laid by insects treated with 10 per cent leaf extract was 37.32 per female and it

was on par with control (31.02). Similarly, the average number of eggs in 5 per cent seed extract was significantly low (22.5) and on par with 20 per cent leaf extract. Though only very few females completed the third gonadotropic cycle in 2.5 per cent seed extract treatment, the average number of eggs laid was 31.26 per female and it was on par with control (31.02).

It is evident from the results that the fecundity of treated insects was significantly reduced. While a normal *D. cingulatus* female laid on an average 192.94 eggs during its life time, 20 per cent leaf extract treated females laid 107.70 eggs, 5 per cent seed extract treated females laid 93.26 eggs and 2.5 per cent seed extract treated females laid 186.19 eggs. Ten per cent leaf and 2.5 per cent seed extracts were on par with control. Forty and 20 per cent leaf extracts and 5 per cent seed extract significantly reduced the fecundity of the females.

4.3.8. Effect on incubation period

The incubation period of eggs in the different treatments did not show significant variations in the first, second and third gonadotropic cycles. The incubation period ranged from 5 to 7 days only (Table 4).

4.3.9. Effect on hatching of eggs

No significant variation in the hatching percentage of eggs was seen with respect to the treatments in the first gonadotropic cycle (Table 4). But in the second cycle, the percentage of hatching was significantly low in 40 per cent leaf extract treatment. Only 36.12 per cent of eggs in this treatment hatched. Hatching of eggs in the other treatments was not affected and they were on par with control. No eggs were obtained in the 40 per cent leaf extract

treatment in the third gonadotropic cycle since none of the insects survived upto the cycle. The hatching percentage of eggs of the remaining treatments did not show any significant difference.

4.3.10. Effect on longevity

The life span of adult female bugs in 40 per cent leaf extract was significantly reduced, being 13.10 days only. The longevity of adults in the remaining treatments did not vary significantly and it varied from 18.08 to 20.9 days compared to 20.36 days in control (Table 4).

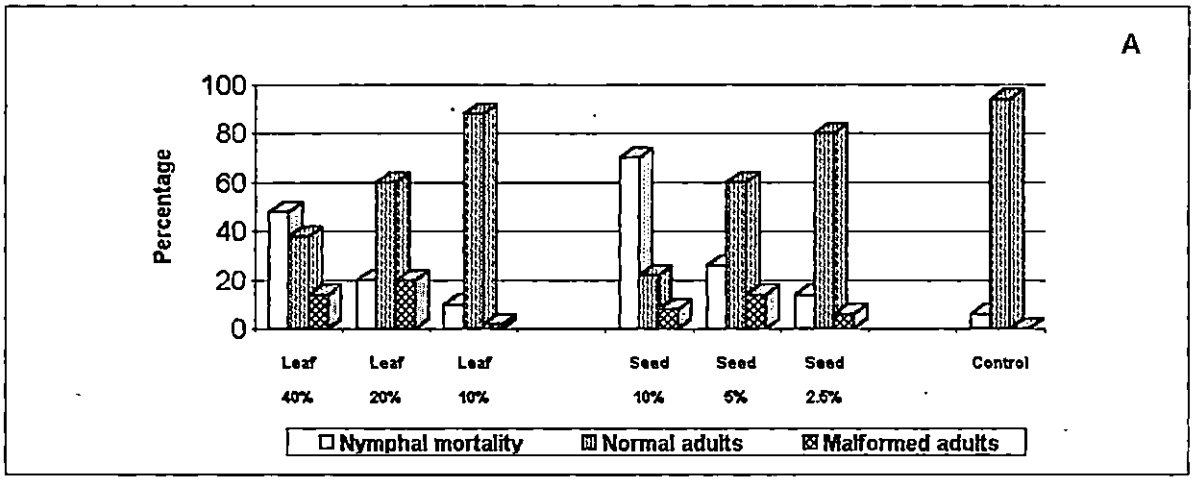
4.3.11. Eggs obtained from fifty insects in each treatment

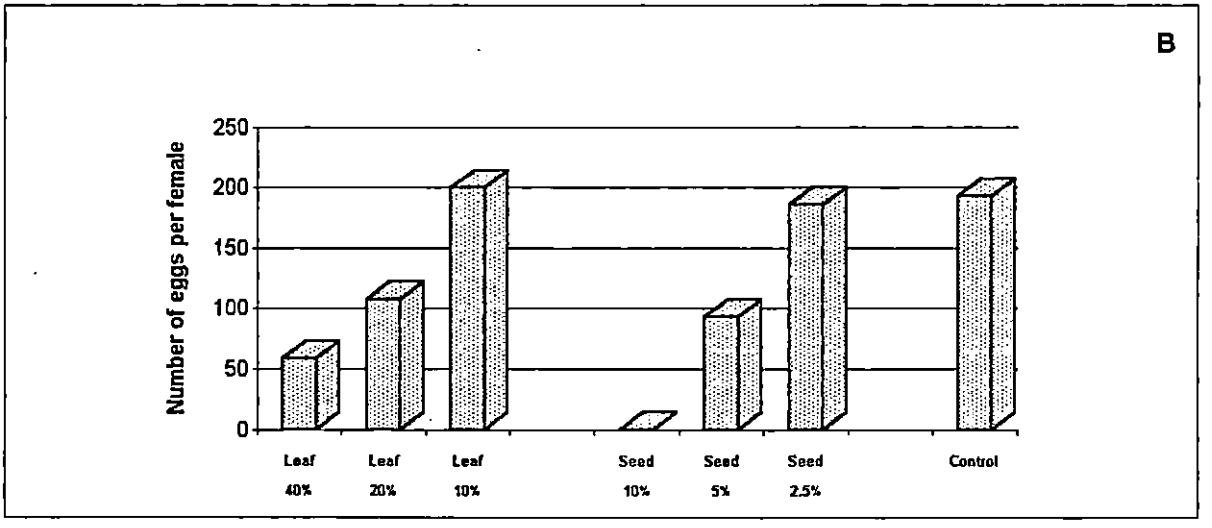
Marked difference was observed in the total number of eggs obtained from a cohort (50 nymphs) of treated insects. Compared to the untreated group where the total number of eggs obtained was 8682, only 584 eggs were obtained in 40 per cent leaf extract treatment. The percentage decrease noticed in the treatment was 93.27. Leaf extract (20 per cent) and seed extract (5 per cent) also resulted in lower number of eggs being 2447 abd 2332 respectively in the treatments. However insects treated with 10 per cent leaf extract showed only limited decrease in egg production, the number of eggs obtained from this group of insects being 7602. The percentage reduction in the eggs laid in the treatment was only 12.14. Seed extract (2.5 per cent) treatment gave 6889 eggs, the percentage reduction over control being 20.65 (Table 4).

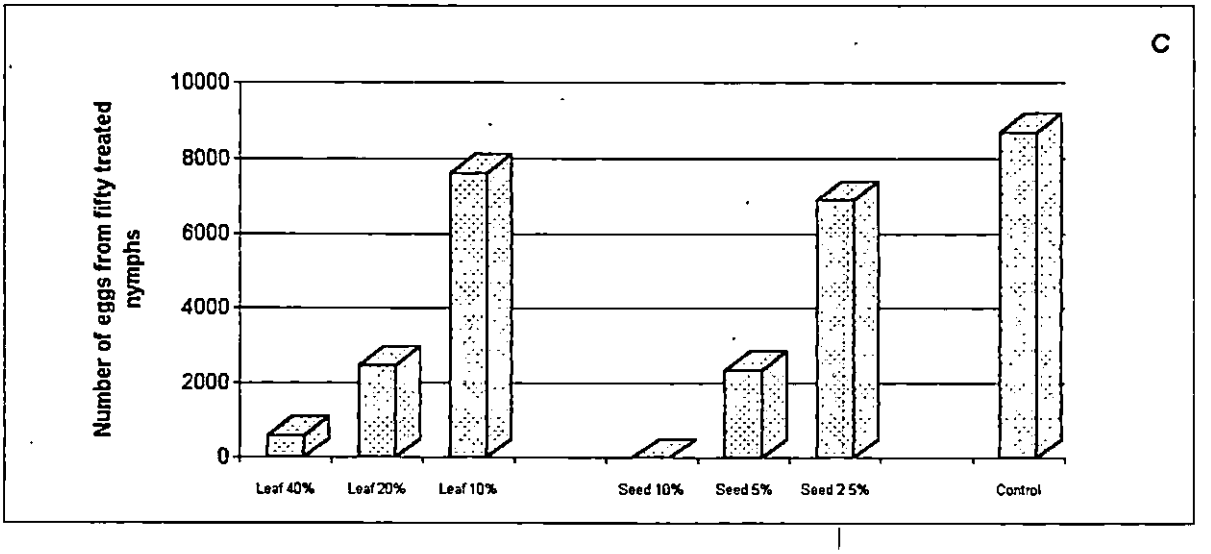
4.3.12. Hormonal/Sterilant effect of different doses of leaf and seed extracts of *T.neriifolia* and their impact on progeny production

The relevant data presented in Fig.2. revealed a significant

Fig . 2. Nymphal mortality, normal and malformed adults and fecundity of *Dysdercus cingulatus* treated with leaf and seed extracts of *Thevetia neriifolia* and total number of eggs obtained from a cohort of fifty treated nymphs







supression of fecundity (Fig. 2B) and the eggs produced in the succeeding generation (Fig. 2C) when a population of 50 nymphs were treated with leaf or seed extract of *T.neriifolia*. The response had a high positive association with the doses. The egg production showed a positive association with the number of normal adults emerging from the nymphs and a negative association with the nymphal mortality. No significant association was observed between the percentage of malformed adults emerging in the population and the numberof eggs produced (Fig.2A). The response of higher doses of leaf extract (20 and 10 per cent) were on par with those of the lower doses of seed extract (5 and 2.5 per cent). At the highest dose (40 per cent leaf extract and 10 per cent seed extract) the response of the leaf extract was higher.

4.3.13. Effect of extracts of *T.neriifolia* on morphology and oocycte development

4.3.13.1. Morphological effects

A number of treated last instar nymphs showed black patches on the abdomen towards the later stage of development. The treated nymphs were rather sluggish during the early days but recovered during later stages. Some treated nymphs failed to moult and ultimately died. Forewings and hindwings of some of the adultoids were seen reduced and it covered only half the length of the body. They died within a few hours after emergence.

4.3.13.2. Effect on ovary

Morphometrics of the ovary of insects subjected to various treatments are presented in Table 5. The length of ovary, number of oocytes per

Table 5 Effect of ethanol extracts of fresh leaf and seed of *Thevetia neriifolia* on ovarian development of *Dysdercus cingulatus* when applied on fifth instar nymphs

	Age of insect (days)									
Treatments	1	2	3	4	5	1	2	3	4	5
A. Length of ovary (mm)						**B. No.of oocytes/ovariole**				
Seed (5%)	1.94	2.15	2.41	2.80	3.72	4.05	4.60	4.75	4.70	5.80
Leaf (20%)	2.01	2.25	2.64	3.29	4.63	4.50	5.05	5.90	5.85	5.60
Control	2.09	2.67	3.13	5.06	6.40	4.75	7.60	7.10	7.30	7.70
CD	-	-	0.153	0.256	0.384	0.501	0.546	0.498	0.792	0.569
C. Length of basal oocyte (mm)						**D. Width of basal oocyte (mm)**				
Seed (5%)	0.19	0.18	0.21	0.31	0.38	0.14	0.14	0.16	0.22	0.29
Leaf (20%)	0.21	0.22	0.28	0.40	0.48	0.15	0.15	0.18	0.27	0.34
Control	0.21	0.24	0.36	0.60	1.07	0.16	0.20	0.28	0.53	0.65
CD	-	0.023	0.032	0.042	0.052	0.013	0.002	0.002	0.003	0.004
E. Length of penultimate oocyte (mm)						**F. Width of penultimate oocyte (mm)**				
Seed (5%)	0.17	0.18	0.20	0.29	0.36	0.14	0.13	0.14	0.20	0.28
Leaf (20%)	0.18	0.21	0.25	0.37	0.42	0.14	0.14	0.16	0.24	0.29
Control	0.22	0.24	0.33	0.59	1.06	0.16	0.19	0.28	0.51	0.65
CD	-	0.024	0.003	0.004	0.005	0.014	0.001	0.002	0.003	0.004
G. Length of antepenultimate oocyte (mm)						**H.Width of antepenultimate oocyte(mm)**				
Seed (5%)	0.16	0.17	0.20	0.27	0.36	0.14	0.12	0.13	0.19	0.27
Leaf (20%)	0.17	0.20	0.23	0.35	0.40	0.14	0.12	0.14	0.22	0.27
Control	0.18	0.23	0.32	0.57	1.07	0.15	0.20	0.28	0.51	0.65
CD	-	0.003	0.003	0.004	0.005	0.001	0.002	0.002	0.003	0.004

ovariole, length and breadth of basal, penultimate and antepenultimate oocytes were observed in detail at different intervals after emergence.

The length of ovary of the newly emerged treated insects did not vary significantly. On the second day after emergence too, there was no significant difference in the length of ovary (2.15 mm, 2.25 mm and 2.67mm in seed and leaf extract treated and control insects respectively). On the third day after emergence, with the onset of vitellogenesis the length of ovary significantly varied. The lengths of the ovaries of the insects treated with seed and leaf extracts were reduced significantly, the lengths being 2.41 and 2.64mm respectively compared to 3.13mm in control. The length of a fully matured ovary on the fifth day after emergence was 6.40mm; while the length of the ovary was significantly reduced in the treatments, these being 3.72mm. and 4.63mm in seed and leaf extract treatments respectively.

The number of oocytes per ovariole also varied significantly in the treatments from the day of emergence till full maturation on the fifth day. While the mean number of oocytes were 4.75, 7.6, 7.1, 7.3 and 7.7 from first to fifth day after emergence in control, the numbers were 4.05, 4.60,4.75,4.70 and 5.80 in seed extract treatment and 4.5, 5.05, 5.90, 5.85 and 5.60 in leaf extract treatment.

No significant difference was also observed in the length of the basal oocytes in treated bugs a day after emergence compared to those of normal insects. But from the second day onwards significant differences could be observed. The lengths of basal oocytes in seed extract treatments were 0.19 mm

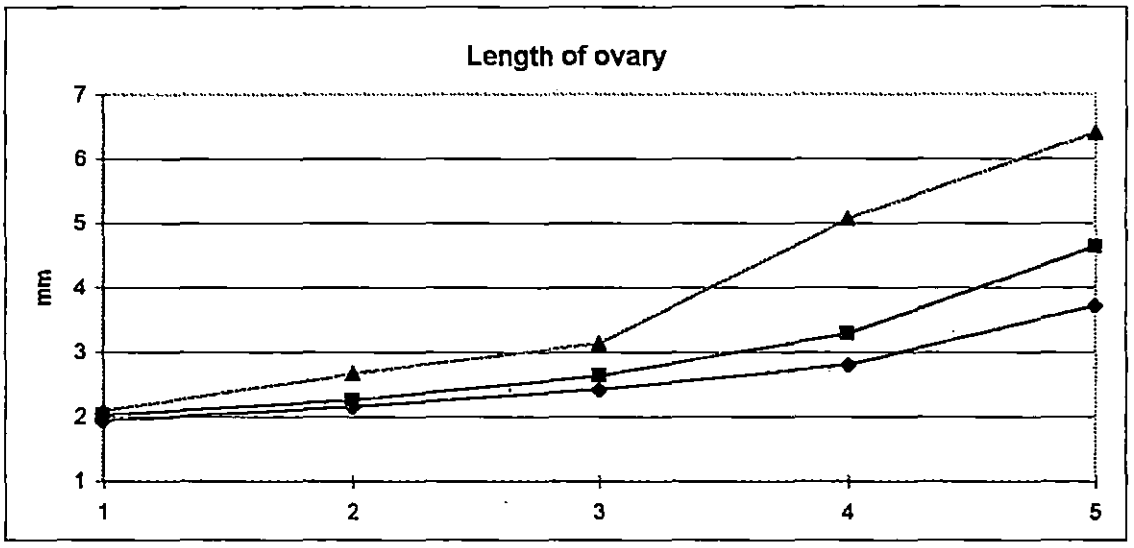
at emergence and it progressively increased to 0.38mm at ovum maturation on the fifth day. In the leaf extract treatment, the initial length of 0.21mm increased to 0.48mm on the fifth day. The growth was significantly lower compared to control in which the ovary length of 0.21 mm at emergence increased to 1.07 mm on the fifth day. The width of the basal oocyte also showed a similar trend. The penultimate and antepenultimate oocytes also showed a similar significant reduction in length and width when treated with seed and leaf extracts of *T. neriifolia*.

The ovaries of insects in the different treatments did not show any significant difference in length on the first day (Fig. 3a). Subsequently (from third to fifth day), the length of ovary increased rapidly in control insects compared to the ovaries of insects in seed and leaf extract treatments. The number of oocytes also increased significantly in the untreated insects. However, in the treated insects the number of oocytes was lesser. Fig.3b and 3c. revealed that the size (length and width) of the basal, penultimate and antepenultimate oocytes attained in the control insects was attained only on the fourth or fifth day in the treated insects. The results thus indicated that the growth of the developing oocytes was only delayed and not suppressed due to treatment with leaf and seed extracts.

4.3.13.3. Effect on the total protein, glycogen and lipid content of fat body, ovary and haemolymph of *D. cingulatus*

The data relating to the study are presented in Table 6.

Fig .3a. Morphometrics of ovary of *Dysdercus cingulatus* treated with leaf and seed extracts of *Thevetia neriifolia*



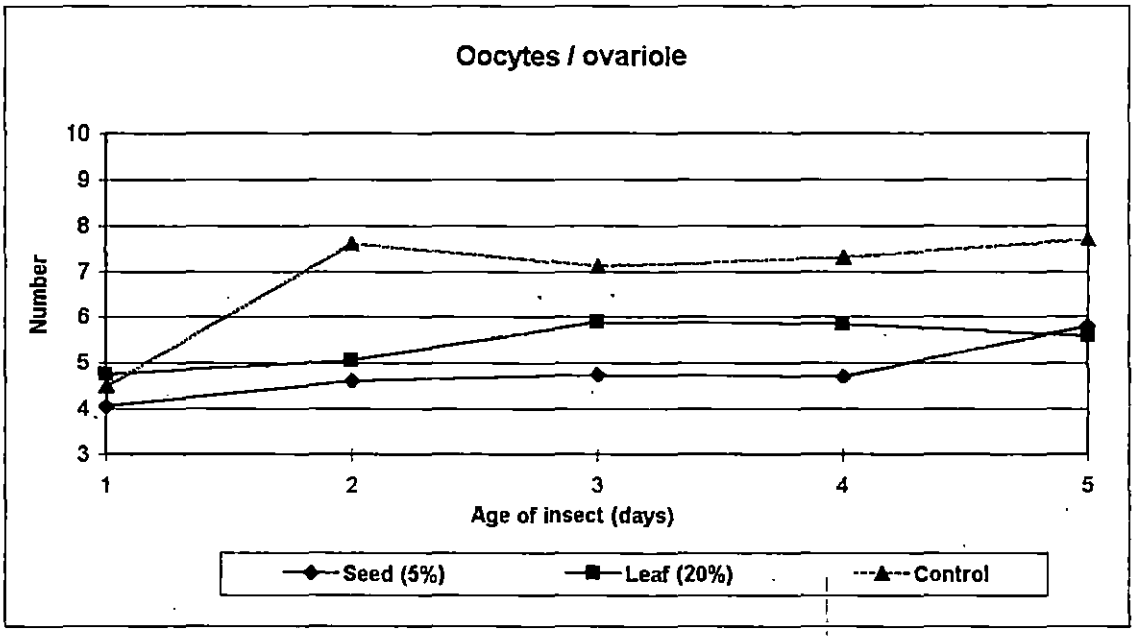
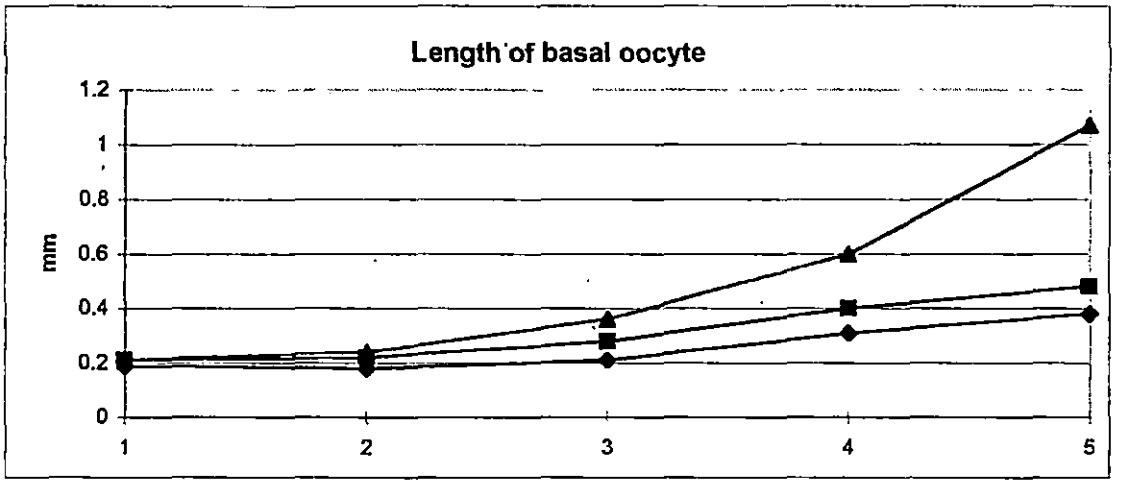
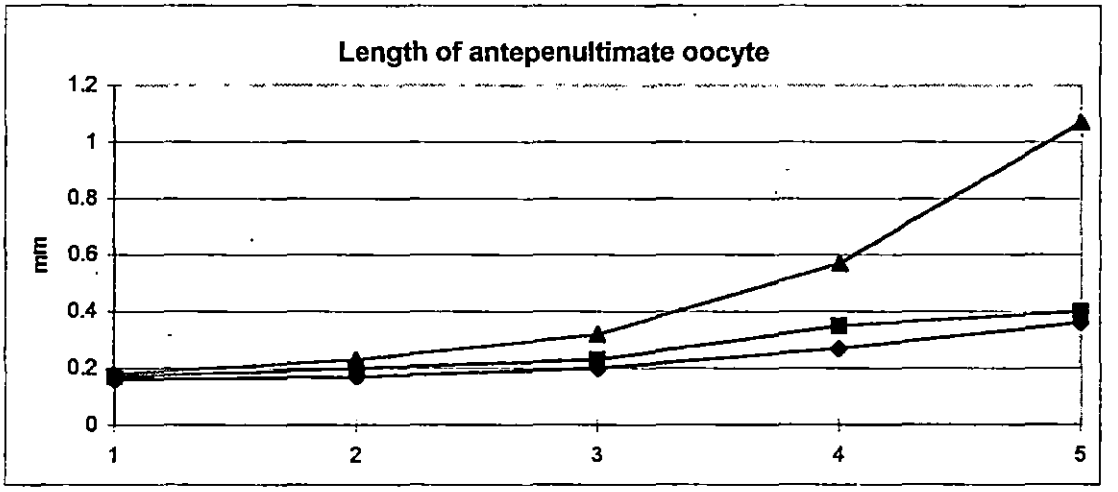


Fig .3b. Morphometrics of ovary of *Dysdercus cingulatus* treated with leaf and seed extracts of *Thevetia neriifolia*





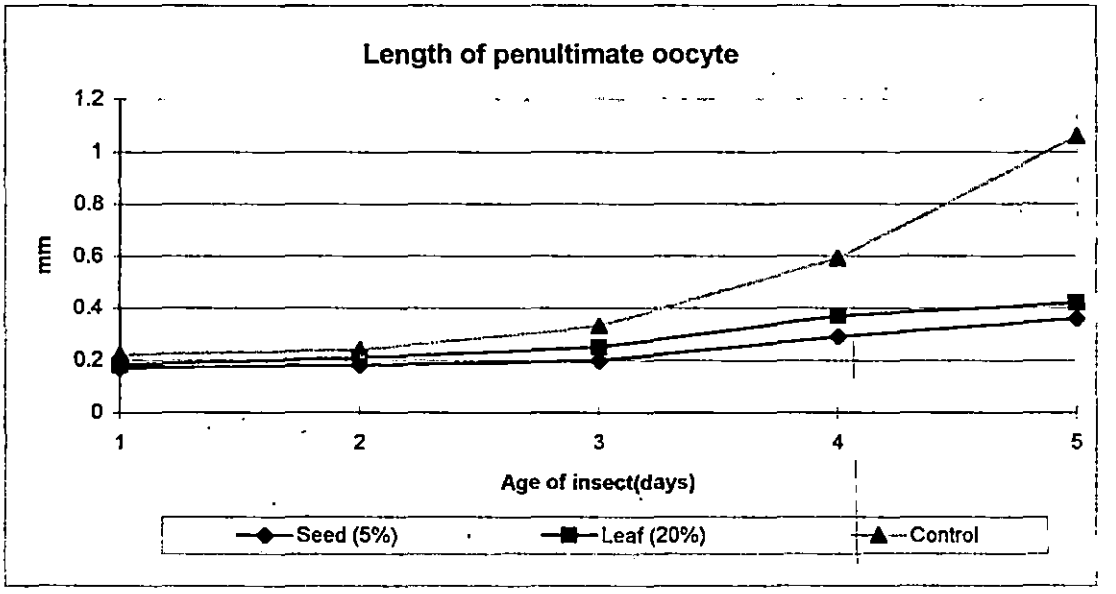
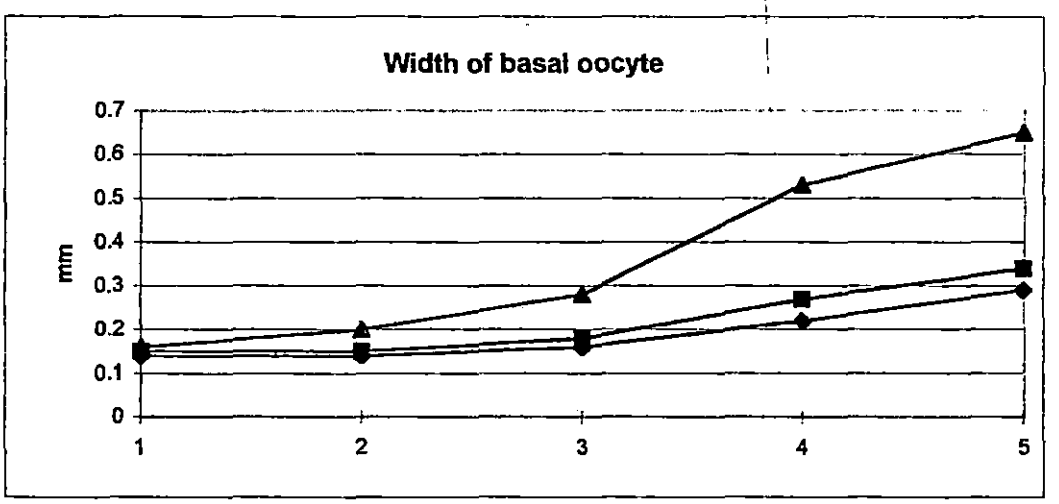
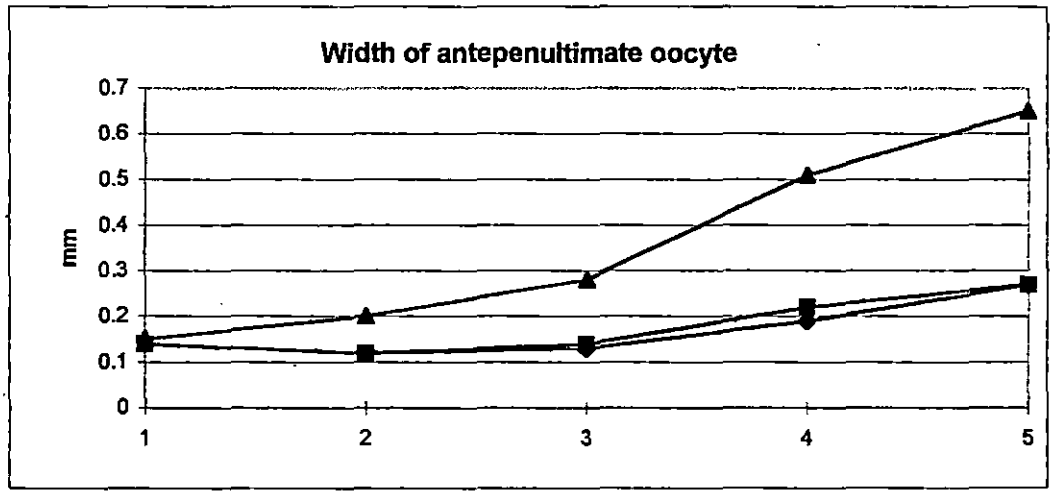
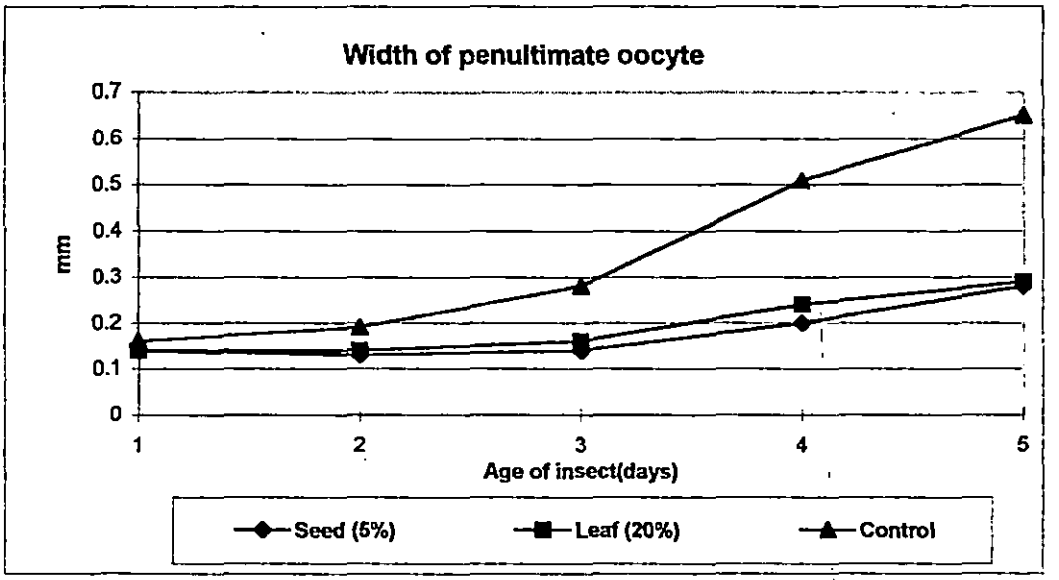


Fig .3c. Morphometrics of ovary of *Dysdercus cingulatus* treated with leaf and seed extracts of *Thevetia neriifolia*







4.3.13.3.1. Total protein

4.3.13.3.1.1. Haemolymph

A gradual increase in the level of protein was observed in the normal insects till third day (15.29 to 77.83 µg/ml) after emergence. On the fourth day, there was a drop in the protein level from 77.83 to 16.44 µg/ml followed by an increase (23.06 µg/ml) on the fifth day. A significant disruption in this normal sequence was observed in the treated insects. In seed extract treatment, the protein level showed a gradual age wise increase from 10.8 µg/ml on the day of emergence to 39.12 µg/ml on the fourth day and then declined to 30.03 µg/ml on the fifth day. In the leaf extract treatment, the protein level increased from 11.84 µg/ml on the day of emergence to 46.49 µg/ml on the third day followed by a decline to 31.95 µg/ml on the fourth day and 27.02 µg/ml on the fifth day after emergence.

At the time of emergence while the protein level was 15.29 µg/ml in the normal insects, significantly low levels of protein (10.8 µg/ml and 11.84 µg/ml) were observed in the seed and leaf extract treated insects respectively. Similarly, the protein levels in the haemolymph were significantly lower (18.73 µg/ml and 23.64µg/ml) in experimental insects on the second day compared to 43.78 µg/ml in the normal insects. On the third day after emergence a peak level of 77.83 µg/ml was observed in the normal insects while lower levels of 32.13 µg/ml and 46.49 µg/ml) were seen in seed and leaf extract treated insects respectively. Contrary to the sharp decline of protein level (16.44 µg/ml) observed in the normal insects on the fourth day, a significantly high level of protein was observed in seed (39.12 µg/ml) and leaf extract (31.95 µg/ml)

Table 6 Effect of ethanol extracts of fresh leaf and seed of *Thevetia neriifolia* on the level of protein, glycogen and lipid in haemolymph, ovary and fat body of *Dysdercus cingulatus* at different intervals after adult emergence when applied on fifth instar nymphs

Treatments	Age of insect (days)					Age of insect (days)					Age of insect (days)				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Protein	**Haemolymph (µg/ml)**					**Ovary (µg/mg)**					**Fat body (µg/mg)**				
Seed (5%)	10.80	18.73	32.13	39.12	30.03	37.37	56.92	91.07	104.43	123.16	36.65	71.42	105.50	122.24	148.38
Leaf (20%)	11.84	23.64	46.49	31.95	27.02	50.14	68.55	115.79	128.45	145.70	41.02	106.37	135.10	141.23	154.75
Control	15.29	43.78	77.83	16.44	23.06	53.63	106.18	143.42	186.58	191.47	51.10	122.30	183.82	87.72	138.61
CD	2.80	6.63	22.94	5.28	-	6.96	11.88	13.11	14.59	11.45	4.40	13.01	18.37	13.54	-
Glycogen	**Haemolymph (µg/ml)**					**Ovary (ug/mg)**					**Fat body (µg/mg)**				
Seed (5%)	0.28	0.51	1.89	2.13	2.38	1.08	1.68	6.14	10.56	11.31	3.33	5.54	9.90	5.73	3.34
Leaf (20%)	0.32	0.68	2.09	2.32	2.09	1.36	2.19	7.41	12.80	13.81	3.74	6.11	13.31	5.42	3.19
Control	0.38	0.89	2.82	1.61	1.06	1.70	2.97	9.17	15.36	15.81	4.15	7.48	16.44	5.86	3.84
CD	0.01	0.21	0.34	0.61	0.92	-	0.70	2.10	3.37	-	-	1.38	3.47	-	-
Lipid	**Haemolymph (µg/ml)**					**Ovary (µg/mg)**					**Fat body (ug/mg)**				
Seed (5%)	3.48	6.38	2.90	2.94	2.08	6.66	10.66	27.94	55.76	75.91	7.98	8.58	20.48	23.32	22.88
Leaf (20%)	3.39	8.14	3.54	4.35	2.62	7.64	11.47	28.29	66.89	87.12	9.42	12.36	25.06	25.32	21.24
Control	3.47	8.44	5.45	5.79	5.47	10.87	18.24	37.10	67.68	106.11	12.30	21.3	41.08	17.24	10.30
CD	-	1.04	0.89	1.04	2.37	2.79	6.92	-	11.57	3.23	3.44	7.71	4.90	5.21	

treatments. There was no significant difference in the protein levels in the treated and normal insects on the fifth day.

4.3.13.3.1.2. Ovary

The level of protein in the ovaries of normal females showed an increase from 53.63 µg/mg tissue from the day of emergence to 191.47 µg/mg tissue on the fifth day after adult emergence. The protein level in the ovaries of seed extract treated insects was only 37.37 µg/mg tissue at the time of emergence and increased upto 123.16 µg/mg tissue on the fifth day while in leaf extract treated insects it was 50.14 µg/mg tissue at emergence,reaching a level of 145.7 µg/mg tissue on the fifth day after emergence. The protein content in the ovaries of plant extract treated insects was significantly low in all the days observed.

4.3.13.3.1.3. Fat body

A significant disruption in the synthesis of protein was observed in the fat body, the site of protein synthesis. While the protein content in the fat body of normal insects increased from 51.1 µg/mg tissue and reached a peak of 183.8 µg/mg on the third day and dropped on the fourth day (87.72 µg/mg tissue), no such sequence was observed in the plant extract treated insects. In these, the level of protein showed a gradual increase from 36.65 µg/mg tissue on the day of emergence to 148.38 µg/mg tissue on the fifth day in the seed extract treatment and 41.02 µg/mg tissue on the day of emergence to 154.75 µg/mg in leaf extract treatment on the fifth day.

4.3.13.3.1.4.Comparison of protein level in haemolymph , ovary and fat body

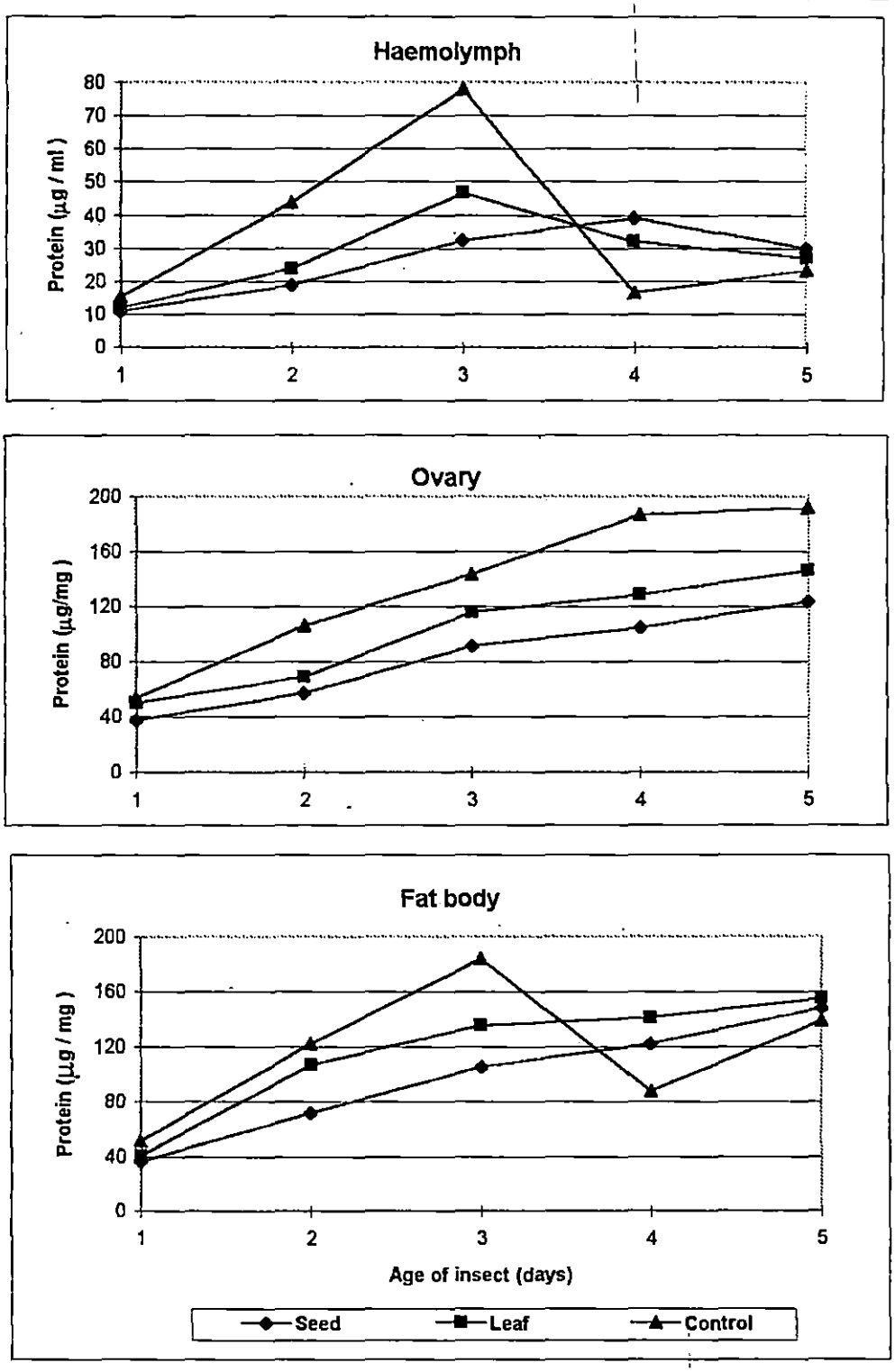
Figure 4 shows that the protein deposition in the ovary increased rapidly from the first to fifth day after emergence while in the treated insects the protein content increased at lesser level upto the fifth day. In the haemolymph and fat body of untreated insects significant rise in protein content was observed upto the third day followed by a sudden decline and then a gradual increase. Leaf extract treated insects showed similar trend in haemolymph but on a lesser level, while in the seed extract treated insects the trend of increase continued upto the fourth day and then there was a decline. The protein content in the fat body showed an increasing trend in treated insects upto the fifth day.

4.3.13.3.2. Total Glycogen

4.3.13.3.2.1. Haemolymph

As observed from the data presented in Table 6, the glycogen content in the normal insect increased from 0.38 to 2.82 µg/ml on third day and then declined to 1.61 and 1.06 µg/ml, on fourth and fifth day after emergence. However in the seed extract treated insects, there was a gradual increase in the glycogen content from 0.28 to 2.38 mg/ml on the fifth day and in leaf extract treatment it increased from 0.32 to 2.32 µg/ml on the fourth day and then declined to 2.09 µg/ml on the fifth day. At corresponding intervals, there were significant differences in the levels of total glycogen in the treated and normal insects.

Fig. 4. Protein level in haemolymph, ovary and fat body of *Dysdercus cingulatus* treated with seed and leaf extracts of *Thevetia neriifolia*



4.3.13.3.2.2. Ovary

The glycogen content of ovaries showed an age related increase in the treated and normal insects. In normal insects, the glycogen level increased from 1.70 to 15.81 µg/mg tissue on the fifth day. In the leaf extract treatment the increase was observed from 1.36 to 13.81 µg/ mg tissue on the fifth day. Seed extract treated insect ovaries showed an increase of glycogen content from 1.08 ug/mg of tissue on the first day of emergence to 11.31 µg/mg tissue on the fifth day. Though significant differences in the levels of glycogen were observed on the second, third and fourth day after emergence between treated and normal insects, seed extract and leaf extract treatments were on par. However no significant difference was observed in glycogen level at the time of emergence and on the fifth day between the treatments and control.

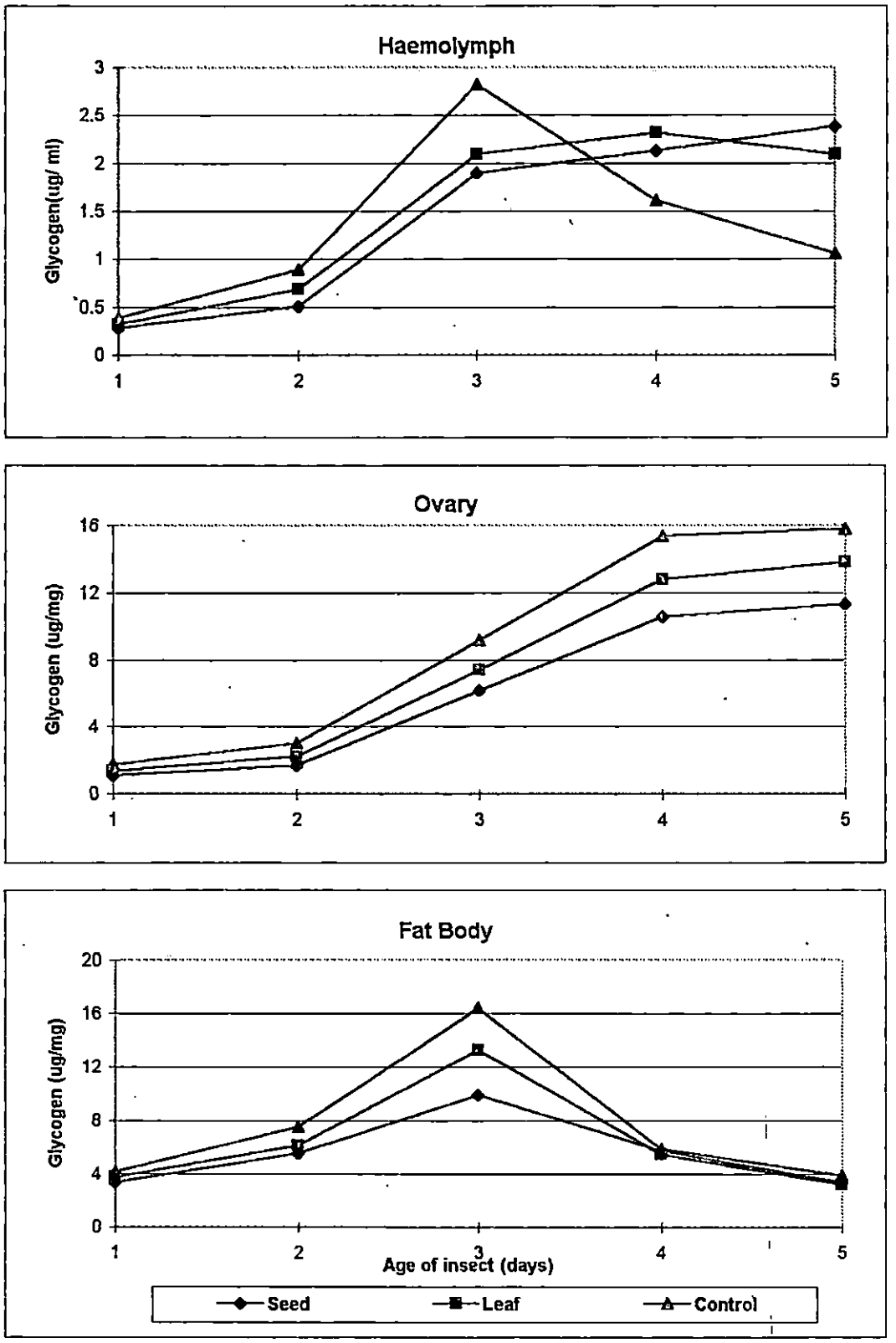
4.3.13.3.2.3. Fat body

Though the glycogen content in seed and leaf extract treated insects and control showed an increasing trend on the first three days, the variations in data were not statistically significant. During the fourth day there was a drastic depletion of glycogen in all the treatments (from 9.9 to 16.44 µg/ mg tissue on the third day to 5.42 to 5.86 µg/mg tissue) and there was a further decrease on the fifth day.

4.3.13.3.2.4. Comparison of glycogen level in haemolymph, ovary and fat body

Glycogen content in the ovary as seen in Fig.5, reached the peak level on the fourth day after emergence in all the treatments including control and then got stabilised though the content in the control was highest at all

Fig.5. Glycogen level in haemolymph , ovary and fat body of *Dysdercus cingulatus* treated with seed and leaf extracts of *Thevetia neriifolia*



intervals. It was followed by leaf and seed extracts. The glycogen in the fat body showed a sudden decline on the fourth day in all the treatments and the same trend was seen in the haemolymph of control insects. But the glycogen content in the haemolymph of leaf and seed extract treated insects showed increase in these treatments on the fourth day.

4.3.13.3.3. Total Lipid

4.3.13.3.3.1. Haemolymph

One day after emergence, the lipid content in the haemolymph did not vary significantly in the treatments and control but differences became significant only between seed extract treatment and control on the second day. While it was 3.47 and 8.44 µg/ml in the normal insects, it was 3.48 and 6.38 µg/ml in seed extract treated insects and 3.39 and 8.14 µg/ml in leaf extract treated insects on the first and second day after emergence. Significant difference was observed in the later stages also (three to five days after adult emergence), the lipid level being significantly lower in treated insects. While it was 5.45, 5.79 and 5.47 µg/ml on the third, fourth and fifth day after adult emergence in normal insects, the content was only 2.9, 2.94 and 2.08 µg/ml in seed extract treatment and 3.54, 4.35 and 2.62µg/ml respectively in leaf extract treatment.

4.3.13.3.3.2.Ovary

The lipid content in the ovary was also significantly low in the treated insects. In the control insects, from an initial level of 10.87 µg/mg in one day old insect, the lipid level gradually increased to 18.24,37.1,67.68 and 106.11 µg/mg on the second, third, fourth and fifth day after emergence. From an initial

low level of 6.66 µg/mg in the newly moulted insect, the lipid level increased only to 75.91 µg/mg in the five day old seed extract treated insect. Similarly, in leaf extract treated insects, the lipid content increased from 7.64 µg/mg to 87.12 µg/mg in five days.

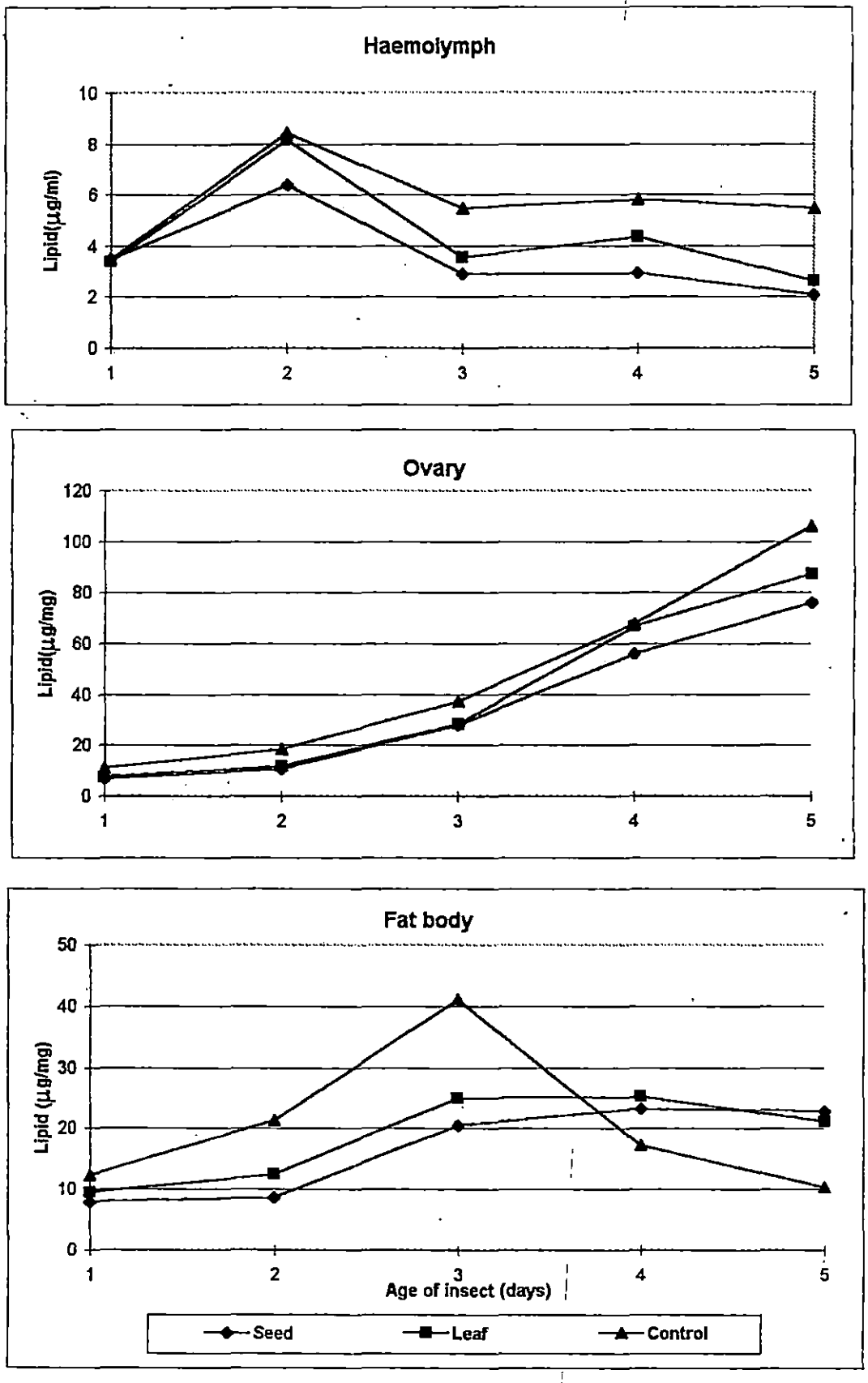
4.3.13.3.3.3. Fat Body

The synthesis of lipids in the fat body was significantly influenced by the treatments. The lipid level in the fat body of normal insects increased gradually from an initial level of 12.3 µg/mg tissue to 41.08 µg/mg tissue on the third day followed by a reduction in the level. In the treated insects, though the lipid level increased considerably upto the third day, the fall, was gradual subsequently. It was 23.32 and 22.88 µg/mg tissue on the fourth and fifth days in seed extract treatment and 25.32 and 21.24 µg/mg in leaf extract treatment respectively compared to 17.24 and 10.30 in control insects.

4.3.13.3.3.4. Comparison of lipid level in the haemolymph, ovary and fat body

The lipid content, as seen in Fig.6, steadily increased from the first to fifth day in the ovary, while in the haemolymph in all the treatments, the lipid content fell sharply on the third day and remained without significant change on the fourth and fifth day. In the fat body of control insects there was a sharp fall on the fourth day which got further reduced on the fifth day, while in leaf and seed extract treated insects the content showed significant rise only on the fourth day. In general the lipid content in control was higher than those of the insects treated with plant extracts.

Fig.6. Lipid level in haemolymph, ovary and fat body of *Dysdercus cingulatus* treated with seed and leaf extracts of *Thevetia neriifolia*



4.4. Insecticidal activity of extracts of *T. neriifolia*

Data relating to the experiment are presented in Table 7. The insecticidal action observed with different plant parts viz. fresh and dried leaves and seed of *T. neriifolia* was very low. Dried leaf extract did not show any insecticidal activity against *S. litura*. The mortality rate was low and ranged from 3.3 to 14.18 per cent only for fresh leaf extract. The toxicity of seed extract against this insect ranged from 4 to 18 per cent. Comparatively fresh leaf and seed extracts were more toxic than dried leaf extract.

Against *H. vigintioctopunctata*, the toxicity of fresh leaf extract ranged from 0 to 10 per cent, acetone extract showing the highest toxicity (10 per cent). Dried leaf extract did not show significant toxicity against grubs of *H. vigintioctopunctata*. Seed extract showed higher insecticidal activity against this pest than *S. litura*. The extent of mortality due to this treatment ranged from 6.7 to 22.5 per cent.

4.5 Antifeedant activity of the chromatographic fractions of ethanol extract of *T. neriifolia*

4.5.1. Antifeedant activity of leaf extract

Antifeedant action of the different fractions isolated from the ethanol extract of fresh leaves at different doses are presented in Table 8.

4.5.1.1. Leaf protection

Results obtained revealed that at 0.5 per cent, fraction VI was significantly superior to all other fractions in protecting the leaves from the feeding of grubs of *H.vigintioctopunctata*. The percentage of leaf protection

Table 7 Contact toxicity of leaf and seed extracts of *Thevetia neriifolia* to the fourth instar larvae of *Spodoptera litura* and the third instar grubs of *Henosepilachna vigintioctopunctata* assessed 48 hours after treatment

Solvents used	Plant parts extracted	Corrected percentage mortality	
		S. litura	*H. vigintioctopunctata*
Acetone	Fresh leaf	3.3	10.0
	Dried leaf	0.0	2.5
	Seed	4.0	12.5
Benzene	Fresh leaf	3.7	7.5
	Dried leaf	0.0	0.0
	Seed	8.0	10.0
Ethanol	Fresh leaf	10.0	0.0
	Dried leaf	0.0	0.0
	Seed	18.0	22.5
Hexane	Fresh leaf	6.7	0.0
	Dried leaf	0.0	0.0
	Seed	8.0	10.0
Methanol	Fresh leaf	3.3	0.0
	Dried leaf	0.0	0.0
	Seed	6.9	20.6
Water	Fresh leaf	14.8	0.0
	Dried leaf	0.0	3.3
	Seed	12.0	6.7

Data not statistically analysed since mortality was very low in all treatments. Fresh leaf, dried leaf and seed were used at 40,20 and 10 per cent concentrations respectively.

given by this fraction was 79.16. This was closely followed by fraction IV which gave 64.84 per cent leaf protection. Fraction V which gave 51.84 per cent leaf protection was on par with EToAc wash (44.26 per cent) in its effect. Fraction I and III, which gave only 31.32 and 27.37 per cent leaf protection were not at all effective. Fraction II did not protect the leaf.

At higher concentration (one per cent), though fraction VI resulted in maximum leaf protection (94.87 per cent) it was on par with fractions IV (84.36 per cent) and V (80.64 per cent). The other fractions viz., I (34.92 per cent) II (12.77 per cent) and III (20.44 per cent) did not give satisfactory leaf protection.

Considering the response at the two doses, fraction I showed no significant difference in the extent of protection when the dose was increased from 0.5 to one per cent. Fractions II, IV, V and VI gave significantly better leaf protection at the higher dose. But fraction III showed slightly better effect at the lower dose (27.37 per cent) than at the higher dose (20.44 per cent). Fraction V gave distinctly higher leaf protection at one per cent (80.64 per cent) than at 0.5 per cent (57.84 per cent).

4.5.1.2. Larval starvation

At 0.5 per cent and one per cent, fraction IV, V and VI resulted in higher activity, the mean larval starvation being 100, 98.78 and 99.49 per cent respectively at one per cent and 68.74, 84.46 and 65.45 per cent respectively at 0.5 per cent. They were on par. The other fractions (I, II and III) showed only low activity. While at 0.5 per cent, fraction II (34.02 per cent) and III (33.67 per

Table 8 **Antifeedant action of chromatographic fractions of ethanol extract of fresh leaves of *Thevetia neriifolia* on third instar grubs of *Henosepilachna vigintioctopunctata***

Fractions isolated	Dose 1%	Dose 0.5%	Mean
Leaf protection (percentage)			
Fraction I	34.92 (5.99)	31.32 (5.68)	33.11 (5.84)
Fraction II	12.77 (3.71)	0 (1.00)	4.57 (2.36)
Fraction III	20.44 (4.63)	27.37 (5.33)	23.80 (4.98)
Fraction IV	84.36 (9.24)	64.84 (8.11)	74.34 (8.68)
Fraction V	80.64 (9.03)	51.84 (7.27)	65.42 (8.15)
Fraction VI	94.87 (9.79)	79.16 (8.95)	86.80 (9.37)
EtoAc Wash	33.58 (5.88)	44.26 (6.73)	38.69 (6.30)
Mean	46.61 (6.90)	36.82 (6.15)	
Larval starvation (percentage)			
Fraction I	30.74 (5.63)	18.50 (4.42)	24.24 (5.02)
Fraction II	34.14 (5.93)	34.02 (5.92	34.08 (5.92)
Fraction III	36.13 (6.09)	33.67 (5.89)	34.89 (5.99)
Fraction IV	100.00 (10.05)	68.74 (8.35)	83.66 (9.20)
Fraction V	98.78 (9.99)	84.46 (9.24)	91.49 (9.62)
Fraction VI	99.49 (10.02)	65.45 (8.15)	81.59 (9.09)
EtoAc Wash	12.72 (3.70)	19.32 (4.51)	15.86 (4.11)
Mean	52.96 (7.35)	43.08 (6.64)	

Figures in parantheses are transformed values : $\sqrt{x+1}$

	Leaf protection	Larval starvation
C.D (0.05) for comparing fractions	0.760	1.167
" " dose	0.423	0.624

cent) where on par, at one per cent fractions I (30.74 per cent), II (34.14 per cent) and III (36.13 per cent) were on par.

With the exception of fractions II and III all other fractions resulted in higher larval starvation at the higher dose (one per cent).

4.5.2. Antifeedant activity of seed extract

Ethanol extract of seeds of *T.neriifolia* showed antifeedant activity in four fractions when tested against third instar grubs of *H.vigintioctopunctata*. The antifeedant activities assessed in terms of leaf protection and larval starvation percentages are presented in Table 9.

4.5.2.1. Leaf protection

Fraction V and fraction VI were found to be highly active when tested at 0.5 per cent giving 100 per cent protection. The other two fractions viz., fraction I and II showed only low activity, the extent of leaf protection being 46.53 and 36.91 per cent respectively and these were much higher than the protections given by the corresponding fractions of leaf extract at 0.5 per cent.

4.5.2.2. Larval starvation

The mean larval starvation was 100 per cent in fractions V and VI and 37.07 per cent in fraction I and 45.40 per cent in fraction II.

4.5.3. Leaf protection and larval starvation as indices of the bioactivity of different fractions of leaf and seed extracts of *T.neriifolia*

Data presented in Fig. 7 showed that the ranking of different fractions of leaf and seed extracts based on leaf protection and larval starvation were broadly the same. Except with reference to larval starvation of fraction V of leaf extract

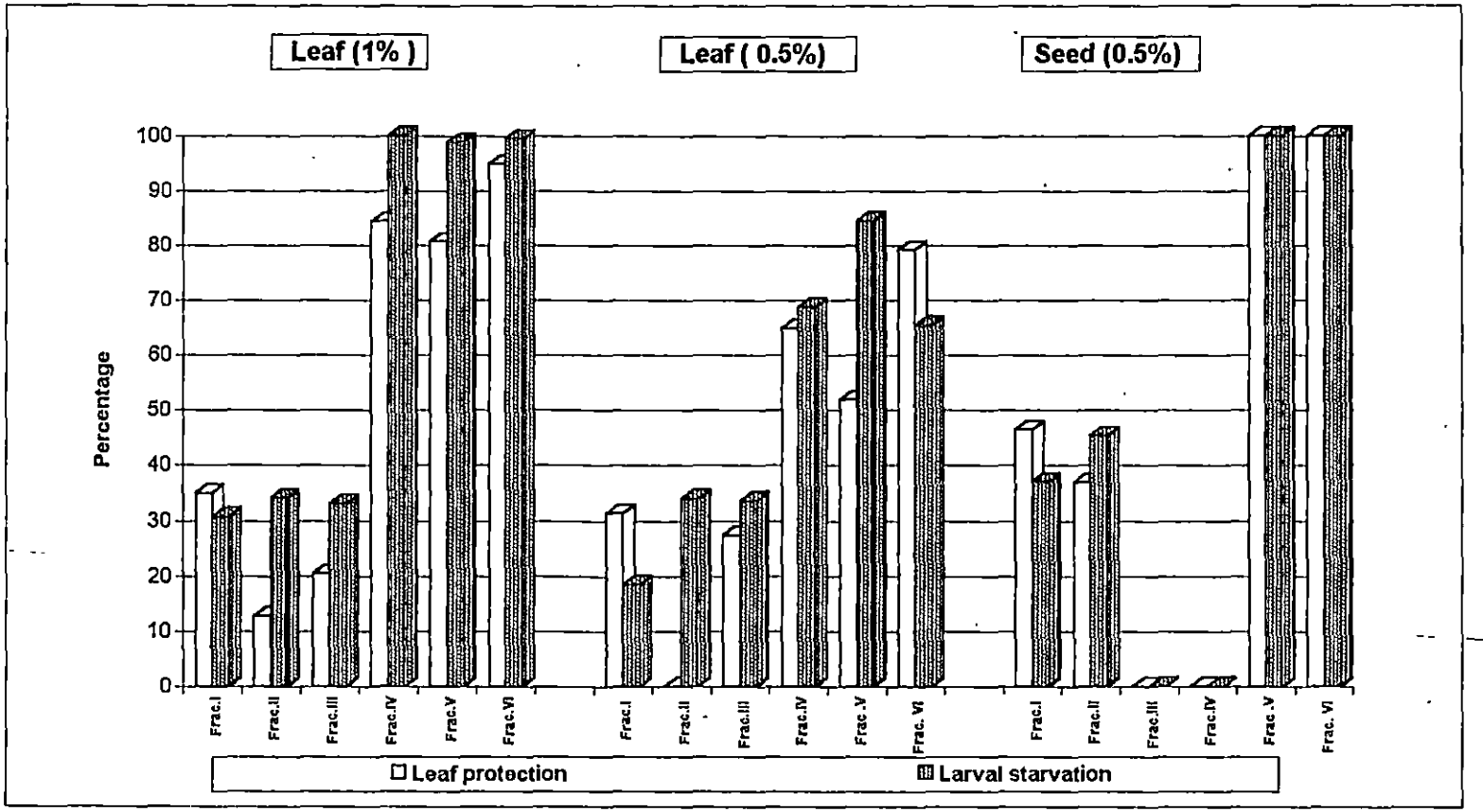
Table 9. Antifeedant action of chromatographic fractions of ethanol extract of seeds of *Thevetia neriifolia* on third instar grubs of *Henosepilachna vigintioctopunctata*

Fractions isolated	Dose 0.5%
Leaf protection (percentage)	
Fraction I	46.53 (6.82)
Fraction II	36.91 (6.08)
Fraction V	100.00 (10.00)
Fraction VI	100.00 (10.00)
CD (0.05)	(0.712)
Larval starvation (percentage)	
Fraction I	37.07 (6.17)
Fraction II	45.40 (6.81)
Fraction V	100.00 (10.05)
Fraction VI	100.00 (10.05)
CD (0.05)	(0.973)

Figures in parantheses are transformed values : $\sqrt{x}/\sqrt{x+1}$

Fractions III and IV were omitted based on TLC findings

Fig : 7. Leaf protection and larval starvation caused by different fractions isolated from leaf and seed extracts of *Thevetia neriifolia*



at 0.5 per cent dose, fractions IV, V and VI of the leaf extracts and fraction V and VI of seed extracts were on par in giving leaf protection and causing larval starvation.

4.5.4. Insecticidal activity

None of the fractions isolated from leaves and seeds showed any contact toxicity to third instar grubs of *H. vigintioctopunctata.*

4.6. Effect of extracts of *T.neriifolia* on the insect parasitoid, *Chrysocharis johnsoni*

Water and ethanol extracts of leaves (fresh and dried) and seed were tested against the parasitoid *C. johnsoni* of *H. vigintioctopunctata.*

4.6.1. Parasitization of *C.johnsoni* on epilachna grubs treated with extracts of *T. neriifolia*

The results obtained on the effect of extracts of *T.neriifolia* on the parasitisation of epilachna grubs and statistical analysis of the data are given in Table 10. Except in seed extract (10 per cent), parasitization of epilachna grubs exposed to other treatments immediately after spraying was not affected as indicated by the number of parasitoids emerging in the different treatments. These treatments were on par with control. Between the two solvents (ethanol and water)there was no significant difference. Seed extract (10 per cent) of both solvents significantly affected the parasitoids. No emergence was observed in grubs exposed to water extract, while the number emerging from ethanol extract was negligible (1.48 parasitoids per grub). The number of parasitoids emerging from water extract of fresh leaf at higher dose was also significantly lower than in other extracts, the number being 7.81 parasitoids per grub. However at two

Table 10 Parasitization of grubs of *Henosepilachna vigintioctopunctata* sprayed with extracts of *Thevetia neriifolia* by *Chrysocharis johnsoni*

Extracts		Mean number of parasites emerged per grub	
		Ethanol	Water
Exposed immediately after drying of spray fluid			
Fresh leaf	(40%)	12.58 (3.69)	7.81 (2.97)
Dried leaf	(20%)	11.05 (3.47)	12.97 (3.65)
Seed	(10%)	1.48 (1.58)	0.00 (1.00)
Fresh leaf	(2%)	15.23 (4.03)	19.37 (4.51)
Dried leaf	(2%)	15.68 (4.08)	12.37 (3.60)
Seed	(2%)	5.87 (2.62)	11.90 (3.59)
Solvent		16.55 (4.19)	17.37 (4.29)
CD (0.05)		(0.79)	(0.79)
Exposed 24 hours after spraying			
Fresh leaf	(40%)	13.25 (3.78)	6.90 (2.81)
Dried leaf	(20%)	17.07 (4.25)	7.65 (2.94)
Seed	(10%)	0.0 (1.00)	2.94 (1.98)
Fresh leaf	(2%)	16.33 (4.16)	11.19 (3.49)
Dried leaf	(2%)	14.71 (3.96)	11.67 (3.56)
Seed	(2%)	12.60 (3.69)	10.49 (3.39)
Solvent		17.73 (4.35)	15.52 (4.06)
CD (0.05)		(0.69)	(0.69)
Exposed 48 hours after spraying			
Fresh leaf	(40%)	13.90 (3.86)	9.12 (3.18)
Dried leaf	(20%)	16.54 (4.19)	9.98 (3.31)
Seed	(10%)	2.96 (1.99)	1.86 (1.69)
Fresh leaf	(2%)	15.72 (4.09)	17.36 (4.29)
Dried leaf	(2%)	17.16 (4.26)	15.07 (4.02)
Seed	(2%)	10.17 (3.54)	11.53 (3.34)
Solvent		17.40 (4.29)	16.66 (4.20)
CD (0.05)		(0.79)	(0.79)

Figures in parantheses are transformed values : $\sqrt{x+1}$.
Two per cent emulsions of fresh and dried leaves and seed were prepared from 40, 20 and 10 per cent stock solutions respectively

per cent concentration this treatment resulted in emergence of parasitoids (15.23 in ethanol and 19.37 in water extracts respectively) and was on par with control (16.55 and 17.37 in water and ethanol respectively).

Exposure of epilachna grubs 24 hours after treatment to ethanol extract of fresh and dried leaves at high and low doses and seed at low dose (2 per cent) did not affect parasitization of the grubs. The number of parasitoids emerging from these treatments (12.60 to 17.07) were on par with those emerging from control (17.73). Seed extract at higher dose (10 per cent) prevented parasitization of the grubs. Water extract of fresh and dried leaves and seed at 2 per cent did not affect parasitization and was on par with control, the number of parasitoids emerging ranging from 10.49 to 11.67 compared to 15.52 in control. Higher doses of the extracts resulted in lesser number of parasitoid emergence (2.94 to 7.65). Between the solvent extracts, ethanol extract was superior to water extract, the extent of parasitoid emergence in this solvent ranging from 12.60 to 17.07. The range of parasitoid emergence in water extract was 6.90 to 11.67 only.

When treated epilachna grubs were exposed to *C. johnsoni* 48 hours after treatment, with the exception of seed extract (10 per cent) all other treatments did not affect parasitization of the grubs. They were on par with control. The number of parasitoids emerging in different ethanol extracts ranged from 10.17 to 17.16 and in water extracts from 9.12 to 17.36.

4.6.2. Contact toxicity

Data relating to the study are presented in Table 11. Excepting 10

Table 11 Contact toxicity of leaf and seed extracts of *Thevetia neriifolia* to the adults of *Chrysocharis johnsoni*

Treatments	Corrected percentage mortality	
	Water	Ethanol
Fresh leaf(40%)	20.00	20.00
Dried leaf(20%)	16.70	20.00
Seed (10%)	40.00	50.00
Fresh leaf (2%)	10.00	0.00
Dried leaf (2%)	6.70	3.30
Seed (2%)	26.70	16.67

Data not statistically analysed
Two per cent emulsions of fresh and dried leaves and seed were prepared from 40, 20 and 10 per cent stock solutions respectively.

per cent extract of seed, the insecticidal action observed on *C. johnsoni* was negligible. Mortality in 2 per cent extract treatments was low. Leaf extracts (fresh and dried) caused low mortality, the mortality percentage being 10 and 6.7 in water extract and 0 and 3.3 in ethanol extract respectively. The mortality percentages in 2 per cent seed extract were 26.7 in water extract and 16.67 per cent in ethanol extract. Higher doses of fresh leaf (40 per cent) and dried leaf (20 per cent) also caused low mortality, the percentages being 20 and 16.7 in water extracts and 20 and 20 in ethanol extracts respectively. Ten per cent seed extract resulted in significantly higher mortality, being 40 and 50 per cent in water and ethanol extracts respectively.

4.7. Effect on higher animals

4.7.1. Acute toxicity

None of the doses of ethanol extract of leaves tested (50, 100, 200, 400, 600 and 1000 mg/kg body weight) showed mortality of animals. The general behaviour of the test animals was normal.

Ethanol extract of seed at 50, 100 and 200 mg/kg body weight did not show any significant mortality. At 400 mg/kg body weight, 50 per cent of the mice died. A few of the remaining animals showed depression preferring to stay cuddled together in the corner of the cage. At 600 and 1000 mg/kg body weight all the treated animals died within four hours.

4.7.2. Chronic toxicity

The relevant data are presented in Table 12.

Table 12 Effect of leaf and seed extracts of *Thevetia neriifolia* administered orally on rats

	Weight of animal			Ratio of weight of organs to body weight		
	Initial (g)	Final (g)	Increase/ decrease over initial wt. (%)	Liver	Kidney	Heart
Seed(400mg/kg)	200	195	-2.5	0.03	0.009	0.005
Leaf (800mg/kg)	190	190	0	0.03	0.008	0.006
Control	205	220	+7.3	0.03	0.009	0.005

	Haematological parameters									Biochemical parameters			
	P.V.C (%)	Total count /mm³	R.B.C million /mm³	Plate count lakhs/mm³	Poly-morph (%)	Lympho-cytes (%)	Eosino-phil (%)	Mono-cyte (%)	Basino-phil (%)	Haemo globin (g%)	Serum cholesterol mg/dl	S.G.O.T. Iu/l	S.G.P.T Iu/l
Seed (400mg/kg)	31.5	6775	3.3	0.8	31.5	63.5	3.5	1.5	0.0	10.4	96	39.5	24.0
Leaf (800mg/kg)	39.5	8300	4.2	1.0	52.5	44.0	2.0	1.5	0.0	13.6	94	59.8	30.5
Control	40.0	8200	4.2	1.0	50.0	49.0	1.0	0.0	0.0	14.4	129	86.0	27.0
Accepted Permissible levels	39 | 53	5000 | 11000	7.3 | 9.6	1.1 | 1.4	9 | 34	65 | 84	0 | 6	0 | 5	0 | 1.5	12.0 | 17.5	120 | 135	86.0 | 92.8	31.4 | 35.0

4.7.2.1. Weights of test animals and internal organs

The seed extract treated animals showed a decrease in weight (2.5 per cent) compared to their initial weights . No change was observed in the weight of leaf extract treated animals while the control animals showed 7.3 per cent increase in weight when observed 14 days after treatment.

Regarding the weights of liver, kidney and heart, no remarkable difference was seen in the treated animals compared to the control animals when expressed as ratio to body weight. The ratio of weight of liver to body weight was 0.03 both in the treated (seed and leaf extracts) and control animals. Similarly, the ratio of wet weight of kidney to body weight was 0.009 and 0.008 in seed and leaf extract treated animals respectively compared to 0.009 in control animals. Again no remarkable difference was observed in the weight of heart in seed and leaf extract treated and control animals, the ratio to body weight being 0.005, 0.006 and 0.005 respectively.

4.7.2.2. Histopathological changes

Histological examination of the major organs viz., aorta, heart, liver and kidney revealed significant changes in animals treated with seed extracts.

Aorta

No visible changes could be observed in the aorta of seed and leaf extract treated animals.

Heart

Major disruption could be seen in the heart tissue of seed extract treated animals. Vacuolation due to fatty changes and cytoplasmic acidophila

were observed in the heart cells of these animals. The tissues in the heart of leaf extract treated animals were similar to those of untreated animals (Plate II).

Liver

In the liver too vacuolation due to fatty changes could be observed in the seed extract treated animals while the tissues of leaf extract treated animals did not show any changes when compared to control (Plate II).

Kidney

Peri-renal fatty tissues showed dense inflammation in seed extract treated animals. Cytological changes were not observed in the tissues.

4.7.2.3. Haematological parameters

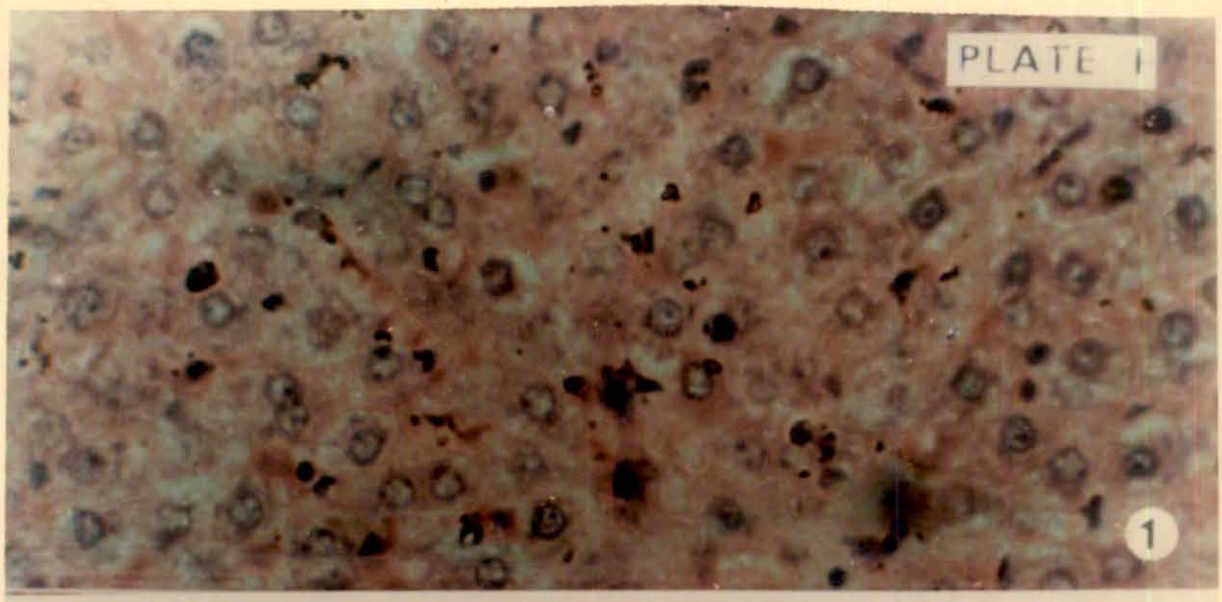
The packed cell volume (PCV) in leaf extract treated rats (39.5 per cent) was close to the range of control animals (40 per cent) and was within the accepted permissible level (39 to 53 per cent). However in seed extract treated animals (31.5 per cent) it was far below the safe range. The total white blood corpuscle count in both seed and leaf extract treated rats were within the normal range (5000 to 11000/mm^3), being 6,775/mm^3 in seed and 8,300/mm^3 in leaf extract treatments respectively. Though within the safe range, the count in seed extract treated rats was low compared to the control animals (8,200/mm^3). The platelet count was reduced in the treated animals to slightly lower level than the normal range (1.1 to 1.4 lakhs/mm^3) in leaf extract treated animals (1.00 lakhs/ mm^3) and still lower in seed extract treated animals (0.8 lakhs/mm^3). The R.B.C count was significantly low in the seed extract treated rats, being 3.3 and leaf extract treatment came on par with control (4.2 million/mm^3).

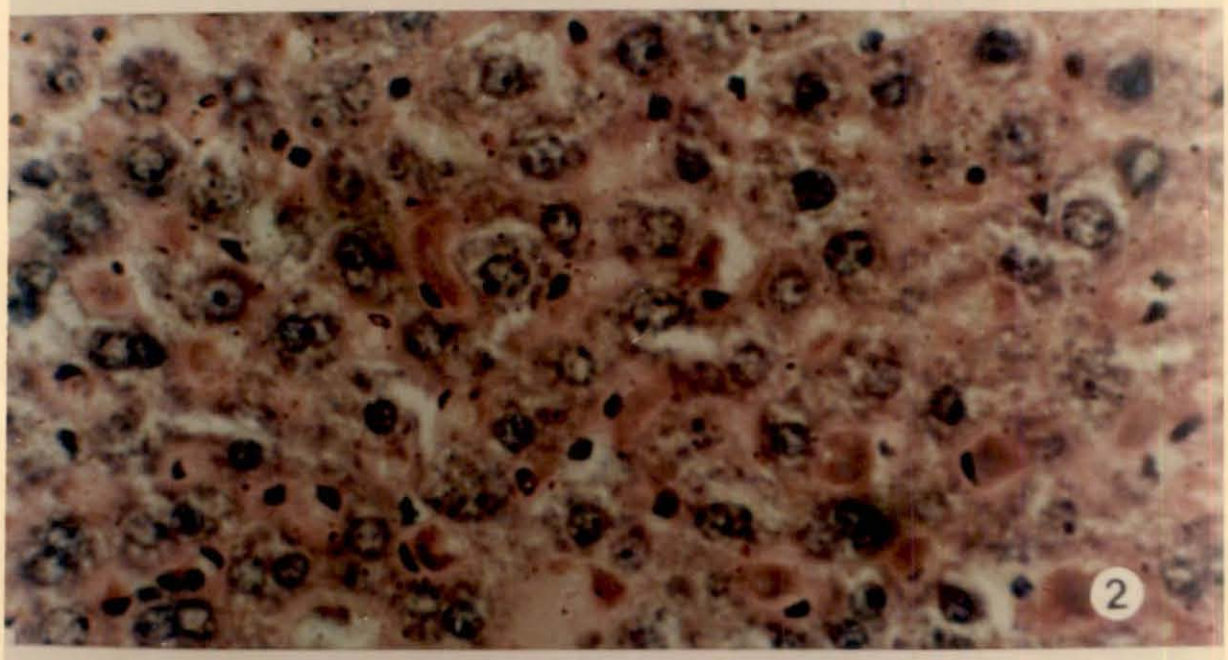
Plate I . Effect of leaf and seed extracts of *Thevetia neriifolia* on the tissues of liver in rat

1. Normal (Untreated)
2. Leaf extract treated
3. Seed extract treated

(Arrows indicate vacuolation in the tissue)

PLATE I





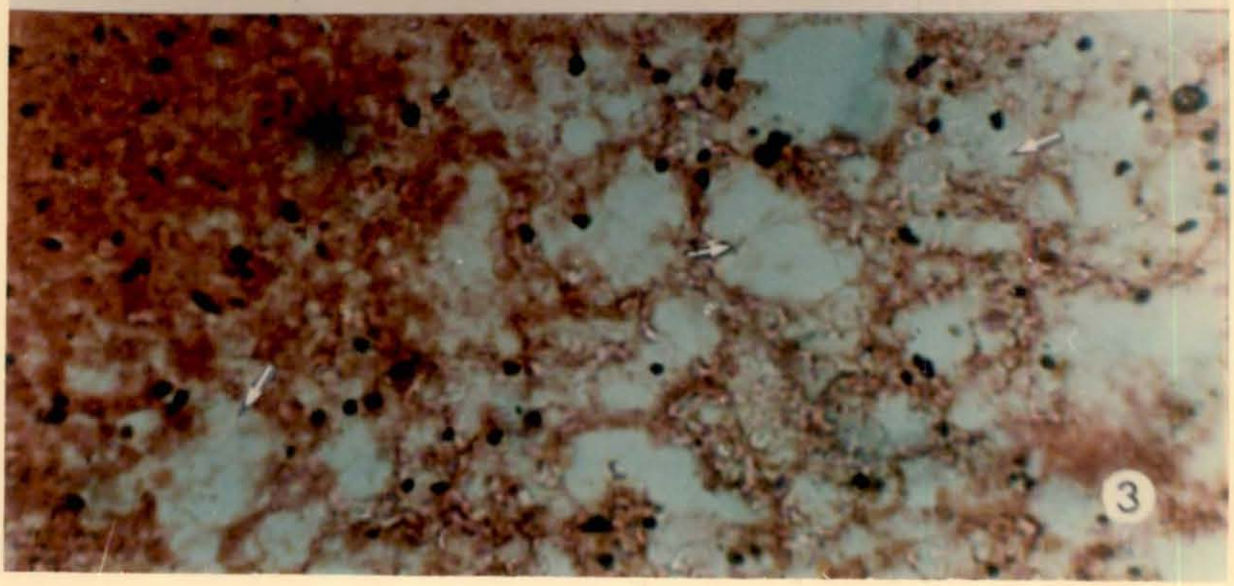
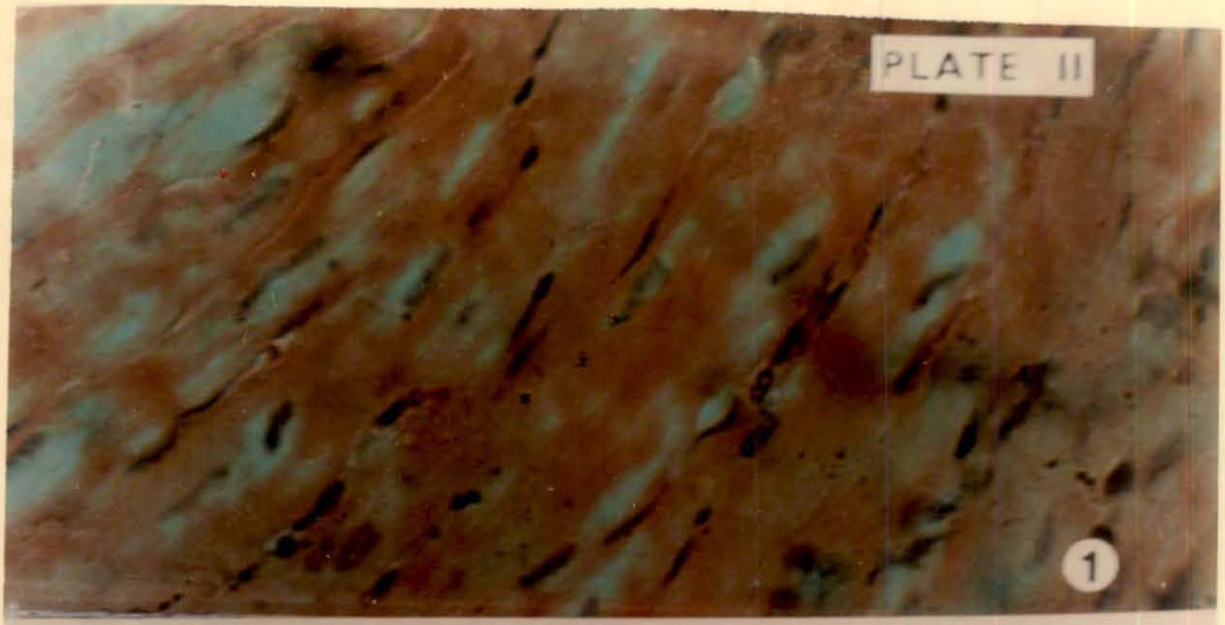


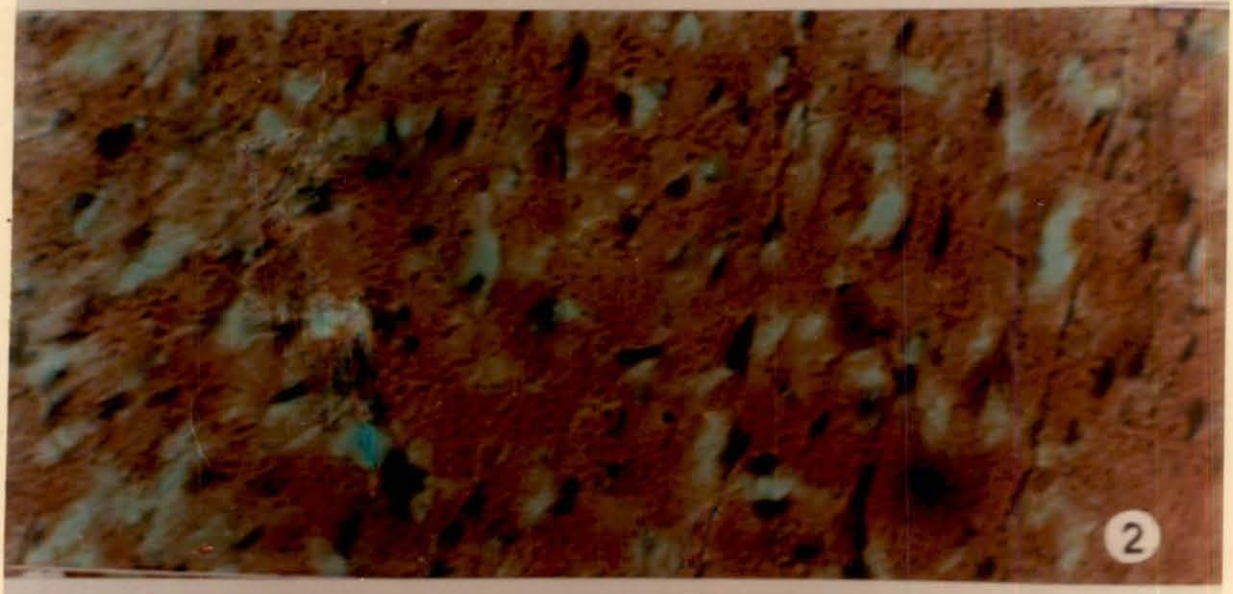
Plate II. Effect of leaf and seed extracts of *Thevetia neriifolia* on the tissues of heart in rat

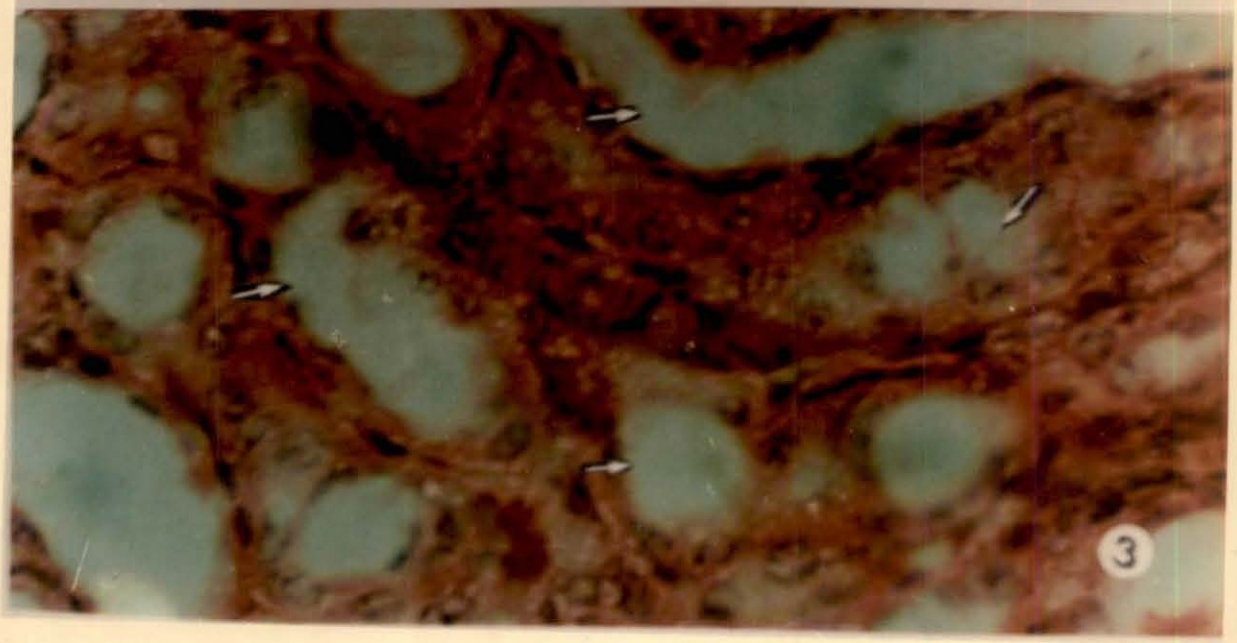
1. Normal (Untreated)
2. Leaf extract treated
3. Seed extract treated

(Arrows indicate vacuolation in the tissue)

PLATE II







Regarding the differential counts, polymorphs in seed extract treated rats was within the normal range(9 to 34 per cent). Lympochyte count was close to the normal range (65 to 84 per cent) in seed extract treated rats (63.5 per cent), but was substantially low in the leaf extract treated rats (44 per cent) and control animals (49 per cent). Values of eosinophil, monocyte and basophil in seed extract treatment (3.5, 1.5 and 0 per cent respectively), leaf extract treatment (2, 1.5 and 0 per cent respectively) and control (1, 0 and 0 per cent respectively) were within the normal range (0 to 6, 0 to 5 and 0 to 1.5 per cent respectively).

4.7.2.4. Biochemical parameters

Haemoglobin was significantly reduced in seed extract treated rats (10.4 g per cent). In leaf extract treated rats, though it was lower (13.6g per cent) than that observed in the control animals (14.4g per cent) it was within the permissible range (12-17.5g per cent). The serum cholesterol level was significantly reduced in the treated animals. While in control animals it was 129 mg/dl comparable to 120 to 135 mg/dl in the safe range, it was only 96 mg/dl in seed extract and 94 mg/dl in leaf extract treated animals. S.G.O.T was also highly reduced in seed extract treated rats (39.5 IU/l) and low in leaf extract treated rats (59.8 IU/l), the value in control animals being (86 IU/l) compared to the normal range of 86 to 92.8 IU/l. Similarly S.G.P.T was also very low in seed extract treated animals (24 IU/l). In leaf extract treated animals, the value (30.5IU/l) was near the normal range (31.4 to 35.0 IU/l).

4.7.3 Effect on heart

Seed extract significantly affected the functioning of frog's heart. Exposure of isolated perfused heart of frog to 10 per cent seed extract resulted in increased contractile activity ultimately leading to cardiac arrest (Plate III). In the case of leaf extract (10 per cent) an initial spurt in the contractile activity was observed and there was a quick recovery and the heart resumed the normal rhythm. Administration of 2 per cent seed extract also led to increased heart beat but soon it returned to normalcy (Plate IV). Two per cent leaf extract did not affect the heart beat. Administration of two per cent seed extract and 10 per cent leaf extract to a failing heart helped in activating the heart and improved the heart beat.

4.8 Efficacy of extracts of leaves and seeds of *T. neriifolia* in controlling *H. vigintioctopunctata* on bittergourd in the field

The efficacy of water and ethanol extracts of seed and fresh and dried leaves of *T. neriifolia* in reducing the population of *H. vigintioctopunctata* on bittergourd was evaluated in field trials done in two successive seasons. The effect was compared with an insecticide, carbaryl. Weekly observations on the incidence of natural enemies and the effect of treatments on yield were also assessed.

4.8.1. Effect of treatments on pest population

4.8.1.1. Eggmass

The data relating to the number of egg masses observed in the different treatments are presented in Table 13.

Plate III. Cardiogram of isolated perfused heart of frog treated with leaf and seed extracts (10 per cent) of *Thevetia neriifolia*

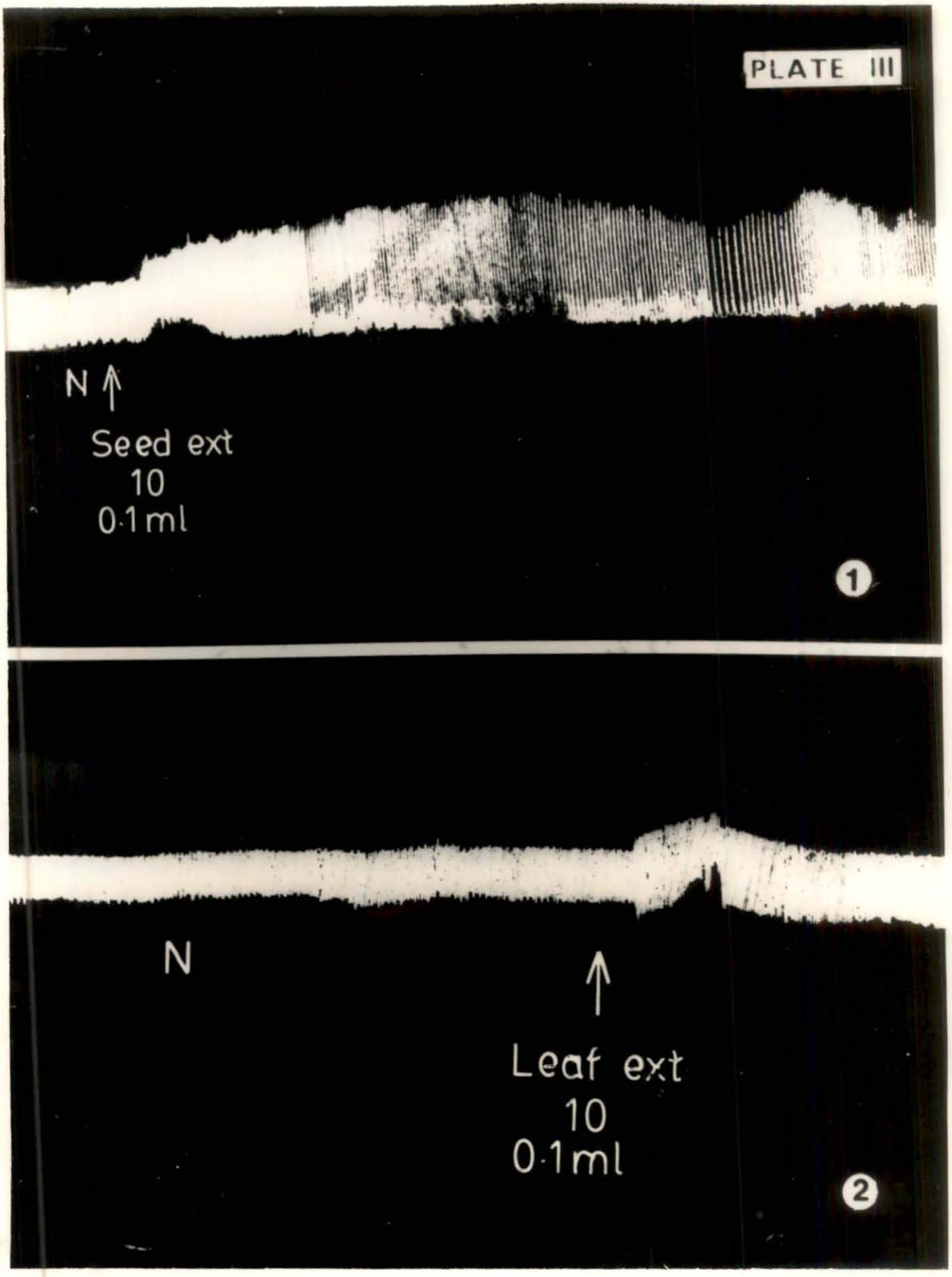


PLATE III
N
Seed ext
10
0·1ml
1
N
Leaf ext
10
0·1ml
2

Plate IV. Cardiogram of isolated perfused heart of frog treated with leaf and seed extracts (2 per cent) of *Thevetia neriifolia*

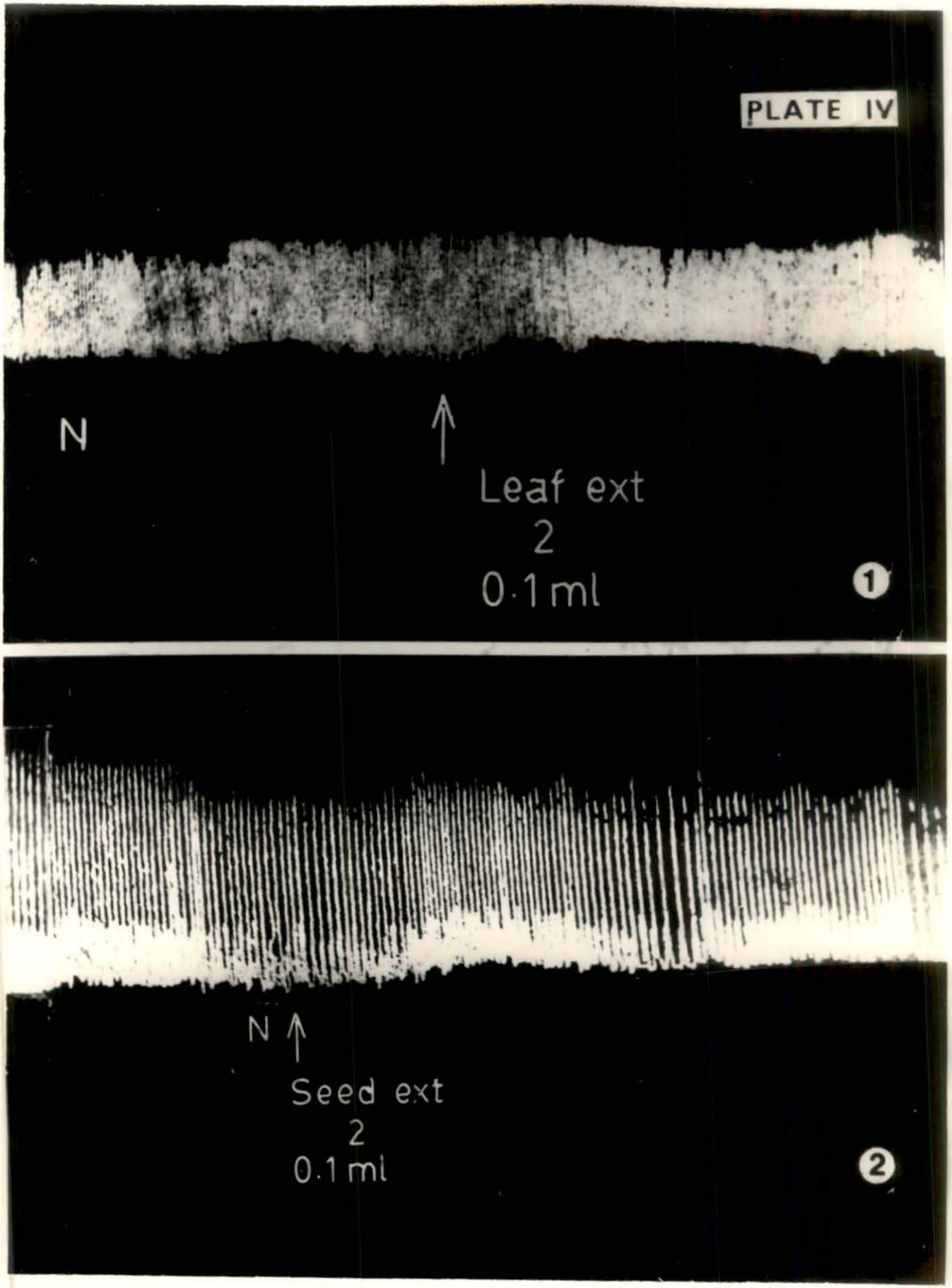


PLATE IV
N
Leaf ext
2
0·1 ml
1
N
Seed ext
2
0.1 ml
2

Pooled data of the fourth week revealed a significant reduction in the number of egg masses in all the treatments compared to the control plots. Spraying of carbaryl (0.15 per cent) was superior to all the treatments, no egg masses being observed in the treatment.

Between the plant parts tested, seed extract was superior to leaf extracts. A significantly low number of egg masses were seen in the seed extract treated plots, the average number of egg masses per plant being 0.10 and 0.14 in water and ethanol extract treated plots respectively when observed four weeks after planting (one week after the first spraying).

Between the fresh and dried leaf extracts, water extract of fresh leaf with 0.66 egg mass per plant was superior to water extract of dried leaf (1.04 egg masses per plant). Ethanol extract of fresh leaf (0.74 egg mass per plant) and dried leaf (0.77 egg mass per plant) were on par.

Considering the efficacy of the solvents, water and ethanol extracts of seed were on par, the number of egg masses observed in these treatments being 0.10 and 0.14 per plant respectively. Similarly water and ethanol extracts of fresh leaf (0.66 and 0.74 egg masses per plant) were on par. Regarding the dried leaf extracts, ethanol extract (0.77 egg mass per plant) was significantly superior to water extract (1.04 egg masses per plant). Ethanol extract of fresh and dried leaves were on par.

Observations recorded five weeks after planting when pooled showed no significant difference between the treatments.

During the sixth week (a week after the third spraying) again a

Table 13 **Effect of leaf and seed extracts of *Thevetia neriifolia* on the eggmasses of *Henosepilachna vigintioctopunctata* on bittergourd in the field**

Treatments	Number of egg masses per plant observed at different intervals after planting (weeks)											
	4	5	6	7	4	5	6	7	4	5	6	7
	Trial I				Trial II				Pooled			
Water extract of dried leaf (2%)	1.10 (1.45)	0.64 (1.28)	0.67 (1.29)	0.27 (1.13)	0.96 (1.40)	0.54 (1.24)	2.20 (1.79)	0.79 (1.34)	1.04 (1.43)	0.59 (1.26)	1.37 (1.54)	0.54 (1.24)
Water extract of fresh leaf (2%)	0.96 (1.40)	0.99 (1.41)	0.68 (1.30)	0.64 (1.28)	0.39 (1.18)	0.62 (1.27)	0.66 (1.29)	0.44 (1.20)	0.66 (1.29)	0.80 (1.34)	0.69 (1.30)	0.54 (1.24)
Water extract of seed (2%)	0.06 (1.03)	0.49 (1.22)	0.23 (1.11)	0.11 (1.05)	0.14 (1.07)	0.02 (1.01)	0.23 (1.11)	0.1 (1.00)	0.10 (1.05)	0.25 (1.12)	0.23 (1.11)	0.06 (1.03)
Ethanol extract of dried leaf (2%)	0.66 (1.29)	0.33 (1.15)	0.44 (1.20)	0.23 (1.11)	0.85 (1.36)	0.79 (1.34)	0.25 (1.12)	0.23 (1.11)	0.77 (1.33)	0.56 (1.25)	0.35 (1.16)	0.23 (1.11)
Ethanol extract of fresh leaf (2%)	0.65 (1.28)	1.15 (1.47)	0.76 (1.32)	0.30 (1.14)	0.85 (1.36)	0.23 (1.11)	0.28 (1.13)	0.14 (1.07)	0.74 (1.32)	0.66 (1.29)	0.51 (1.23)	0.23 (1.11)
Ethanol extract of seed (2%)	0.04 (1.02)	0.26 (1.12)	0.09 (1.04)	0.12 (1.06)	0.23 (1.11)	0.19 (1.09)	0.29 (1.14)	0 (1.00)	0.14 (1.07)	0.23 (1.11)	0.19 (1.09)	0.06 (1.03)
Carbaryl (0.15%)	0 (0.97)	0.03 (1.01)	0 (0.94)	0.07 (1.03)	0.04 (1.02)	0 (0.92)	0 (0.98)	0.02 (1.01)	0 (1.00)	0 (0.97)	0 (0.96)	0.04 (1.02)
Control	2.73 (1.93)	0.64 (1.28)	1.04 (1.43)	0.47 (1.21)	2.10 (1.76)	1.28 (1.51)	0.44 (1.20)	0.90 (1.38)	2.42 (1.85)	0.96 (1.40)	0.74 (1.32)	0.69 (1.30)
CD	(0.22)	(0.27)	(0.22)	-	(0.26)	-	-	(0.12)	(0.08)	-	(0.16)	-

Plant extracts were sprayed at weekly intervals and carbaryl at biweekly intervals commencing from third ~~the~~ week after planting.
Figures in parantheses are adjusted means

significantly low number of egg masses were observed in the treated plots. Fortnightly spraying of insectide(0 egg mass per plant)maintained its superiority. Seed extracts (0.23 and 0.19 egg masses per plant) continued to be superior to the leaf extracts in reducing the egg laying of *H.vigintioctopunctata*

Water extract of fresh leaf with 0.69 egg mass per plant was definitely superior to water extract of dried leaf (1.37 egg masses per plant) which showed even more number of egg masses than the control plots (0.74 egg mass per plant). Ethanol extract of dried leaf with lower number of egg masses (0.35) was on par with fresh leaf extract (0.51).

Ethanol and water extracts of seed were equally effective in reducing the number of egg masses (0.19 and 0.23 respectively). Regarding the fresh and dried leaf extracts, ethanol extracts with 0.51 and 0.35 egg masses per plant respectively were significantly superior to water extracts (0.69 and 1.37 egg masses respectively). Ethanol extract of dried leaf was on par with ethanol and water extracts of seed.

Pooled data of the seventh week did not show any significant difference between the treatments.

Data obtained during each season also showed the effectiveness of the treatments in reducing the egg laying of *H.vigintioctopunctata*. The seed extracts of *T. neriifolia* was on par with the insecticide treatment in reducing the number of egg masses. Considering the effectiveness of leaf extracts, while both fresh and dried leaf extracts showed varying effects during the first season, fresh leaf extracts performed better during the second season.

Ethanol and water extracts of seed did not vary in their effectiveness. Both were equally good and on par with the insecticide treatment in reducing the number of eggs laid during the two seasons. Similarly, both solvent extracts of fresh and dried leaf were on par between themselves during the first season. However during the second season, while ethanol and water extracts of fresh and dried leaves were on par, when observed four weeks after planting, ethanol extract of fresh and dried leaves were superior to the water extracts when observed seven weeks after planting (after the fourth spraying). While water extract of fresh leaf was on par with the insecticide and seed treatment four weeks after planting (after the first spraying), ethanol extracts of fresh and dried leaves were statistically on par with the insecticide and seed extract treatment seven weeks after planting (after the fourth spray).

4.8.1.2. Grubs

The population of grubs of *H.vigintioctopunctata* recorded at weekly intervals during the two seasons and pooled results of the same are presented in Table 14.

All the treatments significantly reduced the population of grubs of epilachna beetle. The insecticide carbaryl, sprayed at fortnightly intervals was significantly superior to all the treatments when observed four, five and seven weeks after planting, the number of grubs seen in the treatment being 4.81, 4.90, and 6.08 respectively.

Among the plant parts evaluated for their relative performance, seed extracts proved significantly better than the leaf extracts when observed

Table 14 **Effect of leaf and seed extracts of *Thevetia neriifolia* on grubs of *Henosepilachna vigintioctopunctata* on bittergourd in the field**

Treatments	Number of grubs per plant observed at different intervals after planting (weeks)														
	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8
	Trial I					Trial II					Pooled				
Water extract of dried leaf (2%)	34.16 (5.93)	47.16 (6.94)	41.38 (6.51)	21.18 (4.71)	5.96 (2.64)	64.28 (8.08)	85.12 (9.28)	49.69 (7.12)	44.96 (6.78)	25.01 (5.10)	48.14 (7.01)	61.25 (7.89)	45.51 (6.82)	32.06 (5.75)	13.98 (3.87)
Water extract of fresh leaf (2%)	31.26 (5.68)	38.32 (6.27)	41.90 (6.55)	28.70 (5.45)	12.32 (3.65)	29.58 (5.53)	22.52 (4.85)	39.70 (6.38)	10.56 (3.40)	7.64 (2.94)	30.47 (5.61)	29.91 (5.56)	40.86 (6.47)	18.62 (4.43)	9.89 (3.30)
Water extract of seed (2%)	20.53 (4.64)	15.16 (4.02)	22.52 (4.85)	10.97 (3.46)	12.39 (3.66)	5.05 (2.46)	18.71 (4.44)	9.11 (3.18)	12.62 (3.69)	5.35 (2.52)	11.60 (3.55)	16.89 (4.23)	15.16 (4.02)	11.82 (3.58)	8.55 (3.09)
Ethanol extract of dried leaf (2%)	39.66 (6.40)	50.98 (7.21)	24.10 (5.01)	27.19 (5.31)	11.88 (3.59)	44.43 (6.74)	65.58 (8.16)	52.73 (7.33)	31.38 (5.69)	10.97 (3.46)	42.16 (6.57)	58.14 (7.69)	37.07 (6.17)	29.25 (5.50)	11.46 (3.53)
Ethanol extract of fresh leaf (2%)	38.19 (6.26)	41.90 (6.55)	32.87 (5.82)	24.30 (5.03)	6.84 (2.80)	31.38 (5.69)	40.08 (6.41)	37.56 (6.21)	24.50 (5.05)	4.95 (2.44)	34.76 (5.98)	40.99 (6.48)	35.24 (6.02)	24.40 (5.04)	5.86 (2.62)
Ethanol extract of seed (2%)	13.28 (3.78)	12.98 (3.74)	26.38 (5.23)	9.11 (3.18)	10.09 (3.33)	17.40 (4.29)	21.09 (4.70)	8.48 (3.08)	10.28 (3.36)	8.95 (2.82)	15.32 (4.04)	16.81 (4.22)	17.31 (4.16)	9.69 (3.27)	9.49 (3.08)
Carbaryl (0.15%)	4.43 (2.33)	4.52 (2.35)	17.23 (4.27)	2.53 (1.88)	14.21 (3.90)	5.20 (2.49)	5.25 (2.50)	2.38 (1.84)	10.83 (3.44)	6.73 (2.78)	4.81 (2.41)	4.90 (2.43)	8.36 (3.06)	6.08 (2.66)	10.16 (3.34)
Control	91.92 (9.64)	117.81 (10.9)	35.12 (6.01)	76.62 (8.81)	7.94 (2.99)	80.00 (9.00)	91.35 (9.61)	129.19 (11.41)	50.98 (7.21)	22.32 (4.83)	85.86 (9.32)	104.27 (10.26)	74.86 (8.71)	63.16 (8.01)	14.29 (3.91)
CD	(2.50)	(3.06)	-	(2.35)	-	(2.44)	-	(3.88)	-	-	(0.84)	(1.03)	-	(0.74)	-

Plant extracts were sprayed at weekly intervals and carbaryl at biweekly intervals commencing from the third week after planting
Figures in parantheses are adjusted means

four weeks after planting (11.6 and 15.32 grubs per plant in water and ethanol extracts respectively). Between the leaf extracts, fresh leaf extracts with 30.47 and 34.46 grubs per plant was highly effective compared to the dried leaf extracts (48.14 and 42.16 grubs per plant). However compared to the control plot (85.86 grubs per plant), dried leaf extracts were definitely effective in reducing the grub population.

Both solvent extracts of seed, fresh and dried leaves were equally good in protecting bittergourd plants from *H. vigintioctopunctata*. Water (11.6 grubs) and ethanol (15.32 grubs) extracts of seed were on par. Fresh leaf extracts with 30.47 grubs per plant (water extract) and 34.76 grubs per plant (ethanol extract) were on par. Similarly water extract of dried leaf (48.14 grubs) and ethanol extract (42.16 grubs) were also on par.

A similar trend was observed after the second spraying (five weeks after planting). Seed extracts continued to give better protection to the plants than the leaf extracts, the number of grubs observed in the treatments being 16.89 and 16.81 respectively. Fresh leaf extracts with 29.91 and 40.99 grubs per plant were superior to dried leaf extracts (61.25 and 58.14 grubs per plant).

The solvents, ethanol and water did not vary in their effect. The least population (16.81 grubs) was observed in ethanol extract of seed treated plots and it was on par with the water extract treatment (16.89 grubs). Water extract of fresh leaf resulted in lower number of grubs (29.91) than ethanol extract (40.99) though they were on par. Ethanol extract of dried leaf with 58.14 grubs per plant was also on par with water extract of dried leaf (61.25). All the treatments were significantly superior to control (104.27 grubs per plant).

Though a similar effect was observed six weeks after planting, the variation among the treatments was statistically not significant.

After the fourth spraying (seven weeks after planting), the least population was observed in carbaryl treated plot (6.08 grubs per plant). Seed extracts were superior to the leaf extracts, the mean number of grubs observed in the plots being 6.08 and 11.82 in ethanol and water extracts respectively. The two solvent extracts were on par. The ethanol extract of seed (6.08 grubs) was as effective as carbaryl and was on par with it.

Fresh leaf extracts continued to protect the plants better than the dried leaves, the mean number of grubs observed in the treatments being 18.62 and 24.40 compared to 32.06 and 29.25 in the dried leaf extract treatment. The dried leaf extracts were statistically superior to the check plots (63.16 grubs per plant).

There was no significant difference between the two solvents in their relative performance. The ethanol extract of seed was on par with the insecticide and ethanol extract of fresh leaf was on par with dried leaf extracts (water and ethanol).

Towards the eighth week after planting there was a natural decline in the grub population consequent to aging of the plants. The variation in the number of grubs ranging from 5.86 to 13.98 per plant seen in the treated plots and 14.29 per plant in the control plot was not statistically significant.

When observed during the two seasons, seed extracts were equally good as carbaryl in reducing the grub infestation during both the seasons.

Considering the effect of the leaf extracts, both fresh and dried leaves were on par in their effect and were significantly superior to control. The solvents also did not show any significant difference in their effectiveness.

During the first season, water extract of dried and fresh leaves and ethanol extract of fresh leaf were found to be on par with water extract of seed, though ethanol extract of seed was superior to the leaf extract treatments. However during the second season fresh leaf extracts were found to be on par with the seed extracts.

4.8.1.3. Pupae

The pupae of *H.vigintioctopunctata* were observed six weeks after planting (Table 15). Generally the total number of grubs reaching the pupal stage was very low in the different treatments.

Pooled data of the sixth week showed that all the treatments were superior in reducing pupal number . The seed extract with ethanol reduced the number of pupae and was on par with carbaryl . Fresh and dried leaf extracts were equally effective in reducing the number of pupae, the number observed in the treatments being 0.82 and 0.80 and 1.34 and 1.07 respectively. The fresh leaf extracts were on par with the seed extracts.

Between the solvents tested, in the case of seed , ethanol (0.35 pupae per plant) was superior to water (0.96 pupae per plant). There was no significant difference between the solvents for the extraction of fresh leaf, the number of pupae observed being 0.82 and 0.80 respectively in water and ethanol extracts. No significant difference was seen in the solvent extracts of dried leaves too (1.34 and 1.07 pupae respectively) using water and ethanol as extractants.

Table 15 Effect of leaf and seed extracts of *Thevetia neriifolia* on the pupae of *Henosepilachna vigintioctopunctata* on bittergourd in the field

Treatments	Number of pupae per plant observed at different intervals after planting (weeks)											
	5	6	7	8	5	6	7	8	5	6	7	8
	Trial I				Trial II				Pooled			
Water extract of dried leaf (2%)	0 (1.00)	0.82 (1.35)	1.15 (1.47)	0.26 (1.12)	0.23 (1.11)	1.89 (1.70)	2.49 (1.87)	0.37 (1.27)	0.12 (1.06)	1.34 (1.53)	1.79 (1.67)	1.32 (1.15)
Water extract of fresh leaf (2%)	0 (1.00)	0.58 (1.26)	1.10 (1.45)	0.12 (1.06)	0.29 (1.14)	1.04 (1.43)	0.37 (1.17)	0.14 (1.07)	0.14 (1.07)	0.82 (1.35)	0.72 (1.31)	0.14 (1.07)
Water extract of seed (2%)	0 (1.00)	0.28 (1.13)	0.28 (1.13)	0.72 (1.31)	0.02 (1.01)	1.76 (1.66)	1.16 (1.47)	0.19 (1.09)	0.02 (1.01)	0.96 (1.40)	0.69 (1.30)	0.44 (1.20)
Ethanol extract of dried leaf (2%)	0.39 (1.18)	0.49 (1.22)	0.85 (1.36)	0.29 (1.14)	0.02 (1.01)	1.76 (1.66)	1.16 (1.47)	1.40 (1.55)	0.21 (1.10)	1.07 (1.44)	1.02 (1.42)	0.85 (1.35)
Ethanol extract of fresh leaf (2%)	0 (1.00)	1.04 (1.43)	0.02 (1.01)	0.82 (1.35)	0.02 (1.01)	0.54 (1.24)	0.39 (1.18)	0.62 (1.27)	0.02 (1.01)	0.80 (1.34)	0.21 (1.10)	0.72 (1.31)
Ethanol extract of seed (2%)	0.08 (1.04)	0 (1.00)	0.29 (1.14)	0.59 (1.26)	0.06 (1.03)	0.72 (1.31)	0.58 (1.26)	0.10 (1.05)	0.08 (1.04)	0.35 (1.16)	0.44 (1.20)	0.35 (1.16)
Carbaryl (0.15%)	0 (1.00)	0.04 (1.02)	0.29 (1.14)	0.59 (1.26)	0.02 (1.01)	0 (0.95)	0.12 (1.06)	0.19 (1.09)	0.02 (1.01)	0 (0.99)	0.21 (1.10)	0.39 (1.18)
Control	0.28 (1.13)	4.80 (2.41)	4.57 (2.36)	0 (0.85)	1.22 (1.49)	9.11 (3.18)	7.29 (2.88)	2.17 (1.78)	0.72 (1.31)	6.84 (2.80)	5.86 (2.62)	0.74 (1.32)
CD	-	(0.36)	(0.48)	-	-	(0.37)	-	-	-	(0.22)	(0.16)	-

Plant extracts were sprayed at weekly intervals and carbaryl at biweekly intervals commencing from third week after planting.
Figures in parantheses are adjusted means.

Pooled data of the pupal population estimated seven weeks after planting showed that the extracts of seed and leaves did not exhibit much variation in their effect, the number of pupae being 0.21 and 0.72 in fresh leaf extract and 0.44 and 0.69 in seed extract and 1.02 and 1.79 in dried leaf extract treatments.

Ethanol and water extract of seeds with 0.44 and 0.69 pupae per plant respectively were on par. Ethanol extracts of fresh (0.21) and dried leaves (1.02) were superior to corresponding water extracts (0.72 and 1.79 in reducing the number of pupae. Ethanol extract of fresh leaf and seed were on par while water extract of fresh leaf and ethanol extract of dried leaf were on par.

The variations in population count taken eight weeks after planting were not significant.

Observations recorded in the first field trial revealed that all the treatments were significantly superior to control in checking the pupal population six and seven weeks after planting. Between the treatments there was no significant difference. The different plant parts and solvents did not show any significant difference in their efficacy in reducing the pupae of *H.vigintioctopunctata.*

In the second trial, the observations recorded seven weeks after planting alone was significant. Again all the treatments were significantly superior to control and among the treatments ethanol extracts of fresh leaf and seed were as good as carbaryl in reducing the number of pupae of *H.vigintioctopunctata.*

4.8.1.4. Effect on damage caused by *H.vigintioctopunctata*

The damage score taken during the two seasons and the pooled data are presented in Table 16.

A week after the first spraying (fourth week after planting) a significant reduction in the extent of damage was noticed in the plants treated with different plant extracts.

Fortnightly spraying of carbaryl (0.15 per cent) was significantly superior to the plant extracts in protecting the plants, the damage score in this treatment being 0.21 . Of the plant extracts tested, seed extract was found to be better than the leaf extracts, the score in the water and ethanol extracts being 0.77 and 0.85 respectively. The two extracts of fresh leaf (1.04 and 0.99) and dried leaf (0.93 and 1.10) were on par.

The extracts with water and ethanol of seeds as well as fresh and dried leaves did not cause any significant variation in the damage. Water (0.77) and ethanol (0.85) extract of seeds were on par with water extract of dried leaf (0.93). Both solvent extracts of fresh leaf were on par with ethanol extract of seed (0.85).

Observations taken five weeks after planting (after second spraying) also showed the superiority of carbaryl in reducing pest damage (0.21). All treatments were significantly superior to control (2.72). Among the plant extracts tested, seed extract with ethanol was superior . Fresh leaf extracts (1.10 and 1.22) were on par and significantly superior to dried leaf (1.79 and 1.53).

Table 16 Effect of leaf and seed extracts of *Thevetia neriifolia* on extent of damage in bittergourd

Treatments	Damage score per plant taken at different intervals after spraying (weeks)														
	4	5	6	7	8	4	5	6	7	8	4	5	6	7	8
	Trial I					Trial II					Pooled				
Water extract of dried leaf (2%)	0.74 (1.32)	1.56 (1.60)	1.49 (1.58)	1.59 (1.61)	1.49 (1.58)	1.13 (1.46)	1.99 (1.73)	2.02 (1.74)	2.27 (1.81)	1.82 (1.68)	0.93 (1.39)	1.79 (1.67)	1.76 (1.66)	1.92 (1.71)	1.66 (1.63)
Water extract of fresh leaf (2%)	1.02 (1.42)	0.96 (1.40)	1.16 (1.47)	1.04 (1.43)	0.87 (1.37)	1.04 (1.43)	0.98 (1.41)	1.46 (1.57)	1.40 (1.55)	1.62 (1.62)	1.04 (1.43)	0.98 (1.41)	1.31 (1.52)	1.22 (1.49)	1.25 (1.50)
Water extract of seed (2%)	0.96 (1.40)	0.96 (1.40)	1.04 (1.43)	1.13 (1.46)	0.96 (1.40)	0.56 (1.25)	1.16 (1.47)	0.93 (1.39)	0.82 (1.35)	0.66 (1.29)	0.77 (1.33)	1.07 (1.44)	0.99 (1.41)	0.99 (1.41)	0.82 (1.35)
Ethanol extract of dried leaf (2%)	1.12 (1.42)	1.19 (1.48)	1.53 (1.59)	1.43 (1.56)	1.31 (1.52)	1.19 (1.48)	2.13 (1.77)	2.09 (1.76)	1.96 (1.72)	1.37 (1.54)	1.10 (1.45)	1.53 (1.59)	1.82 (1.68)	1.69 (1.64)	1.34 (1.53)
Ethanol extract of fresh leaf (2%)	1.02 (1.42)	1.19 (1.48)	1.40 (1.55)	1.19 (1.48)	0.28 (1.13)	0.96 (1.40)	1.22 (1.49)	1.56 (1.60)	1.28 (1.51)	1.92 (1.71)	0.99 (1.41)	1.22 (1.49)	1.50 (1.58)	1.25 (1.50)	1.02 (1.42)
Ethanol extract of seed (2%)	1.02 (1.42)	0.44 (1.20)	1.40 (1.55)	1.13 (1.46)	0.96 (1.40)	0.69 (1.30)	1.07 (1.44)	1.22 (1.49)	0.69 (1.30)	0.66 (1.29)	0.85 (1.36)	0.74 (1.32)	1.31 (1.52)	0.90 (1.38)	0.82 (1.35)
Carbaryl (0.15%)	0.23 (1.11)	0.23 (1.11)	0.56 (1.25)	0.44 (1.20)	0.99 (1.41)	0.18 (1.09)	0.17 (1.08)	0.79 (1.34)	0.66 (1.29)	0.69 (1.30)	0.21 (1.10)	0.21 (1.10)	0.69 (1.30)	0.56 (1.25)	0.85 (1.36)
Control	1.43 (1.56)	2.68 (1.92)	2.68 (1.92)	2.92 (1.98)	2.20 (1.79)	2.06 (1.75)	2.76 (1.94)	3.08 (2.02)	3.41 (2.1)	3.04 (2.01)	1.76 (1.66)	2.72 (1.93)	2.88 (1.97)	3.16 (2.04)	2.61 (1.90)
CD	(0.18)	(0.23)	(0.21)	(0.22)	-	(0.26)	(0.19)	-	(0.32)	-	(0.11)	(0.11)	(0.06)	(0.11)	(0.17)

Plant extracts were sprayed at weekly intervals and carbaryl at biweekly intervals commencing from the third week after planting
Figures in parantheses are adjusted means

Water extract of seed (1.07) was on par with water (0.98) and ethanol (1.22) extracts of fresh leaf and these were significantly superior to water (1.79) and ethanol (1.53) extracts of dried leaf and control (2.72).

Pooled data of sixth week showed that the insecticide treatment (0.69) continued to maintain its superiority in checking pest damage. Water extract of seed (0.99) was superior to ethanol extract of seed (1.31). Between the leaf extracts, fresh leaf extracts (1.31 and 1.50) were significantly more effective than dried leaf extracts (1.76 and 1.82) and was on par with ethanol extract of seed (1.31). The extractants of leaves (water and ethanol) did not show any significant difference in their effect in reducing damage due to pests.

During the seventh week too carbaryl was found to be significantly better in reducing the pest damage (0.56) than the plant extracts. All the treatments were significantly superior to control (3.16). Among the plant extracts seed extract was superior to leaf extracts. Fresh leaf extracts (1.22 and 1.25) were significantly superior to dried leaf extracts (1.92 and 1.69).

No significant difference was seen in the extracts of seed (0.99 and 0.90) fresh leaf (1.22 and 1.25) and dried leaf (1.92 and 1.69) when water and ethanol were used as extractants.

Observations recorded during the eighth week showed that seed (0.82 and 0.82) and fresh leaf extracts (1.02 and 1.25) were as effective as carbaryl (0.85) in reducing pest infestation. Dried leaf extracts (water and ethanol) were on par. Ethanol extract of dried leaf (1.34) was on par with carbaryl (0.85) and fresh leaf extracts (1.02 and 1.25) while water extract was on

par with water extract of fresh leaf (1.25). All the treatments were significantly superior to control.

Results of the trial conducted during the first season showed that all the treatments were definitely superior to control in reducing damage due to pests. Though carbaryl gave better protection than plant extracts when observed four weeks after planting, subsequently (five, six and seven weeks after planting) it was found to be on par with water extracts of seed and fresh leaf.

During the second season, the insecticide and seed extracts (water and ethanol) equally reduced damage four and seven weeks after planting; Five weeks after planting the insecticide was superior to the plant extracts. The seed extracts were on par with fresh leaf extracts. No significant difference could be observed six and eight weeks after planting.

4.8.1.5. Effect on fruit fly infestation

Pooled analysis of observations taken in the field trials conducted in the two seasons (Table 17) revealed a significantly low infestation of fruits by fruit flies in the treated plots. Carbaryl (0.15 per cent) treatment gave the least number of fruits infested by fruit fly (12.91 per cent) and was significantly superior to the plant extract treatments . It was followed by extracts of seeds, the percentage of fruits infested being 22.52 (water) and 25.83 (ethanol) respectively. Leaf extracts too reduced fruit fly infestation significantly. Fresh leaf extracts were superior to dried leaf extracts.

Considering the different extractants used, there was no significant difference between water and ethanol extracts of the different plant

Table 17 Effect of leaf and seed extracts of *Thevetia neriifolia* on infestation of bittergourd fruits by *Dacus cucurbitae*

Treatments	Number of infested fruits (percentage)		
	Trial I	Trial II	Pooled
Water extract of dried leaf (2%)	46.95 (6.92)	30.97 (5.65)	37.56 (6.21)
Water extract of fresh leaf (2%)	36.32 (6.11)	24.22 (5.02)	28.92 (5.47)
Water extract of seed (2%)	21.48 (4.74)	25.69 (5.17)	22.52 (4.85)
Ethanol extract of dried leaf (2%)	43.60 (6.68)	35.59 (5.97)	38.44 (6.28)
Ethanol extract of fresh leaf (2%)	40.18 (6.42)	26.07 (5.20)	31.72 (5.72)
Ethanol extract of seed (2%)	26.00 (5.20)	27.77 (5.36)	25.83 (5.18)
Carbaryl (0.15%)	18.96 (4.47)	9.63 (3.26)	12.91 (3.73)
Control	62.00 (7.94)	35.54 (6.03)	46.89 (6.92)
CD	(0.89)	-	(0.48)

Figures in parantheses are transformed values: $\sqrt{x}$

parts. Water extract of fresh leaf which resulted in 28.92 per cent infested fruits was on par with ethanol extract of seed (25.83 per cent) in its effect in reducing fruit fly infestation. Ethanol extract of fresh leaf resulted in 31.72 per cent infested fruits compared to 46.89 per cent in control plots. The percentage of fruit fly infested fruits in dried leaf extract treated plots were 37.56 and 38.44 when water and ethanol were used as solvents respectively.

A significant reduction in fruit fly infestation was observed in the treated plots in the first trial. Water and ethanol extracts of seed were on par with the insecticide carbaryl in checking fruit fly infestation. This was closely followed by fresh leaf extracts (water and ethanol). But no significant difference was observed in the data during the second season.

Thus among the plant parts tried though seed extracts were superior, fresh leaf extracts too were effective. Both the solvents were equally good as extractants.

4.8.1.6. Effect on yield of bittergourd

The data on the yield of bittergourd obtained during the two seasons and pooled analysis of the same are presented in Table 18. The effect of the treatments on the pest population was reflected in the yield of the plants too.

The number of fruits in treated plants (8.34 to 11.88 per plant) increased significantly compared to the control plants (5.09 fruits per plant). The number of fruits obtained from seed extract treated plants were 11.76 and 10.68 with ethanol and water as at extractants. Fresh leaf extracts gave 11.06 and 10.39 fruits per plant with ethanol and water extracts respectively. Carbaryl

Table 18 Effect of spraying leaf and seed extracts of *Thevetia neriifolia* on the yield of bittergourd and the resultant cost benefit ratio

	Number of fruits/ plant			Weight of fruits/plant(g)			Increase in yield over control		Additional Income/ha	Cost of plant protection	Cost benefit ratio
	Trial I	Trial II	Pooled	Trial I	Trial II	Pooled	per plant(g)	per ha (kg)	Rs.	Rs.	
Water extract of dried leaf (2%)	7.18	9.68	8.43	391.68	486.70	439.19	147.80	369.5	3695*	1500**	1:3
Water extract of fresh leaf (2%)	7.35	13.43	10.39	416.68	766.65	591.67	300.28	750.7	7507	1500	1:5
Water extract of seed (2%)	7.68	13.68	10.68	505.75	792.28	649.02	357.63	894.1	8941	2000	1:5
Ethanol extract of dried leaf (2%)	8.25	8.43	8.34	391.68	498.75	445.22	153.83	384.6	3846	5250	1:1
Ethanol extract of fresh leaf (2%)	7.18	14.93	11.08	402.50	840.00	621.25	329.36	823.4	8234	5250	1:2
Ethanol extract of seed (2%)	7.83	15.68	11.76	487.50	991.25	739.38	447.49	1118.7	11187	5550	1:2
Carbaryl (0.15%)	8.58	15.18	11.88	464.15	898.75	681.45	389.56	973.9	9739	1950	1:5
Control	4.10	4.24	2.30	250.03	333.75	291.89					
CD	-	4.24	2.30	-	258.52	121.28					

* Price of bittergourd - Rs.10/kg (farm rate)

** Labour requirement for preparing and spraying leaf and seed extracts and insecticide were 3,4 and 2 respectively. Wages per man labourer was Rs.100 per day

treatment resulted in 11.88 fruits per plant. Extracts of dried leaf (water and ethanol) yielded only 8.43 and 8.34 fruits respectively.

Pooled analysis of the data of the two seasons showed a significant increase in the yield (weight of fruits) in the insecticide, seed and fresh leaf extract treatments. The maximum yield of 739.38g per plant was recorded from plants treated with ethanol extract of seeds. This was on par with carbaryl (681.45g per plant), water extract of seed (649.02 g per plant) and ethanol extract of fresh leaf (621.25 g per plant). Dried leaf extracts were not effective though these treatments were superior to control. Ethanol and water extracts of dried leaf resulted in only 445.22 g and 439.19 g per plant respectively. The extractants (water and ethanol) did not show any significant difference.

Considering the results of the two successive seasons, the data of the first trial was not significant. The results of the second trial conformed to the findings of the pooled analysis.

Fortnightly spraying of carbaryl (15.18 fruits per plant) and weekly spraying of seed (13.68 and 15.68 per plant) and fresh leaf (13.43 and 14.93 per plant) were superior to dried leaf extracts (9.68 and 8.43 per plant). Between the extractants ethanol and water, there was no significant difference.

On the basis of cost benefit ratio, weekly spraying of water extracts of fresh leaf (1:5) and seed (1:5) were as effective as fortnightly spraying of carbaryl (1:5). Ethanol extracts of fresh leaf (1:2) and seed (1:2) did not prove profitable.

4.8.1.7. Correlation of grub population, damage score and yield

Data presented in Fig. 8 showed that during both the seasons seed extract of *T.neriifolia* was the best for controlling grubs of *H.vigintioctopuntata* on bittergourd in the field followed by fresh and dried leaf extracts. The grub population in all the treatments had a positive association with the damage index. The yield obtained showed a negative association with the pest population and extent of damage caused. Carbaryl gave the best control of pest and maximum yield. All treatments were significantly superior to control.

4.8.2. Effect on parasitisation

4.8.2.1. Egg mass

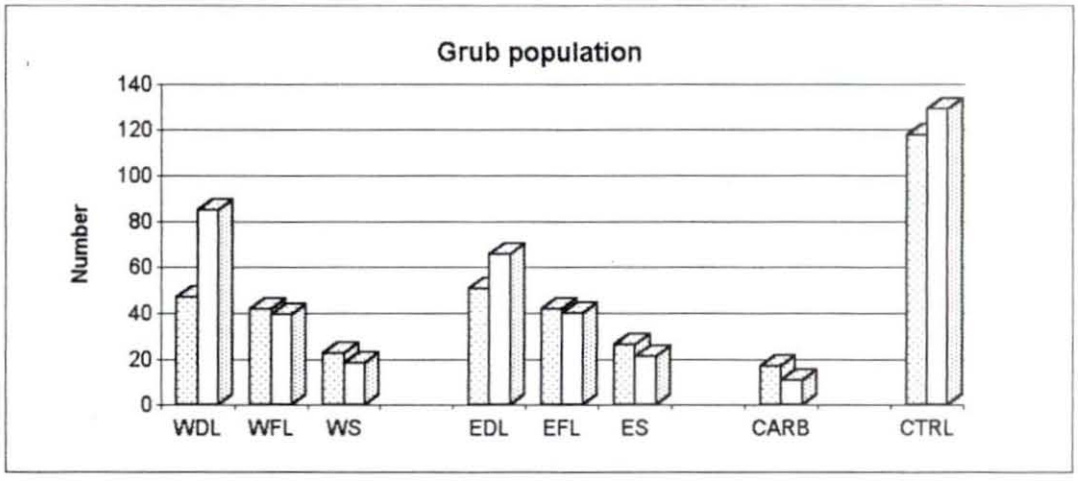
No significant difference was observed in the parasitisation of egg masses of *H.vigintioctopunctata* in the various treatments and control plots under field condition (Table 19). Pooled data showed that parasitisation was totally lacking in the insecticide treated plots. However egg masses in the plant extract treated and control plots were equally parasitized. In the seed extract treated plots, parasitisation of egg masses was low. Contrarily, in leaf extract (fresh and dried) treated plots, egg masses were parasitized to the extent seen in the control plots.

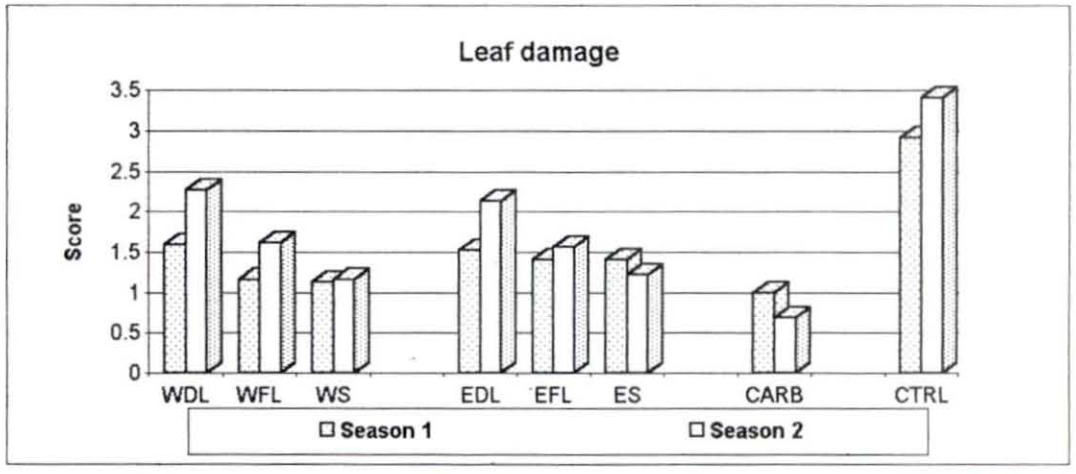
4.8.2.2 Grubs

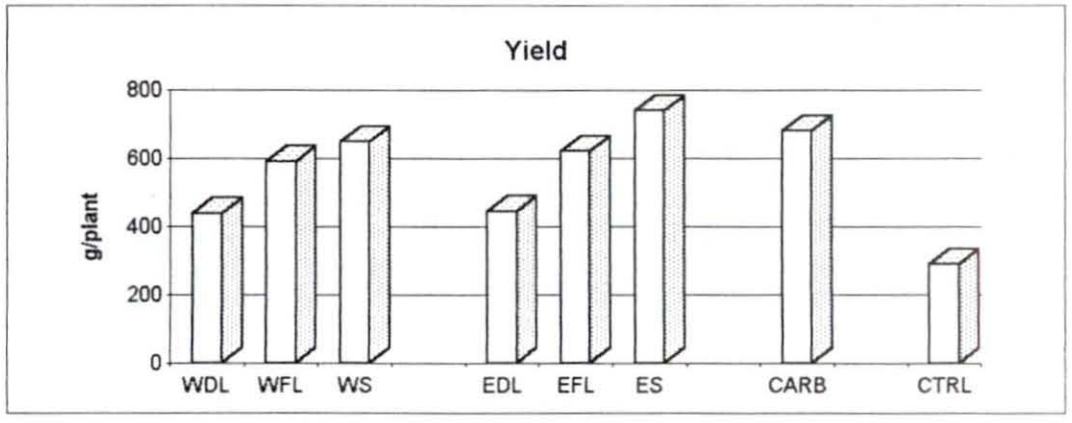
Data on parasitisation of grubs observed during the two seasons and pooled analysis of the same are presented in Table 20.

Pooling of data of the two seasons revealed a significant

Fig.8. Control of *Henosepilachna vigintioctopunctata* on bittergourd with different extracts of *Thevetia neriifolia* and the effect on yield







WDL	:	Water extract of dried leaf (2%)	EDL	:	Ethanol extract of dried leaf (2%)
WFL	:	Water extract of fresh leaf (2%)	EFL	:	Ethanol extract of fresh leaf(2%)
WS	:	Water extract of seed(2%)	ES	:	Ethanol extract of seed(2%)
CARB	:	Carbaryl (0.15 %)	CTRL	:	Water spray

Table 19 **Effect of leaf and seed extracts of *Thevetia neriifolia* on parasitization of egg masses of *Henosepilachna vigintioctopunctata* in the field**

Treatments	Number of parasitized egg masses per plant observed at different intervals after planting (weeks)											
	4	5	6	7	4	5	6	7	4	5	6	7
	Trial I				Trial II				Pooled			
Water extract of dried leaf (2%)	0.23 (1.11)	0.21 (1.10)	0.32 (1.15)	0 (1.00)	0 (1.00)	0.21 (1.10)	0.08 (1.04)	0.23 (1.11)	0.12 (1.06)	0.21 (1.10)	0.21 (1.10)	0.12 (1.06)
Water extract of fresh leaf (2%)	0.25 (1.12)	0.28 (1.13)	0.17 (1.08)	0.32 (1.15)	0.02 (1.01)	0.29 (1.14)	0.44 (1.20)	0.21 (1.10)	1.46 (1.57)	0.30 (1.14)	0.30 (1.14)	0.28 (1.13)
Water extract of seed (2%)	0.08 (1.04)	0.06 (1.03)	0.06 (1.03)	0.15 (1.07)	0.14 (1.07)	0 (1.00)	0 (1.00)	0.14 (1.07)	0.12 (1.06)	0.04 (1.02)	0.04 (1.02)	0.14 (1.07)
Ethanol extract of dried leaf (2%)	0.14 (1.07)	0.35 (1.16)	0.19 (1.09)	0.08 (1.04)	0.12 (1.06)	0 (1.00)	0.29 (1.14)	0.14 (1.07)	0.14 (1.07)	0.17 (1.08)	0.25 (1.12)	0.12 (1.06)
Ethanol extract of fresh leaf (2%)	0 (1.00)	0.12 (1.06)	0.32 (1.15)	0.17 (1.08)	0.06 (1.03)	0.14 (1.07)	0.14 (1.07)	0 (1.00)	0.04 (1.02)	0.14 (1.07)	0.23 (1.11)	0.08 (1.04)
Ethanol extract of seed (2%)	0.02 (1.01)	0.04 (1.02)	0.06 (1.03)	0.06 (1.03)	0.08 (1.04)	0.06 (1.03)	0 (1.00)	0 (1.00)	0.06 (1.03)	0.06 (1.03)	0.04 (1.02)	0.04 (1.02)
Carbaryl (0.15%)	0 (0.99)	0 (0.98)	0 (0.99)	0 (0.99)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (0.99)	0 (1.00)	0 (1.00)
Control	0.17 (1.08)	0.26 (1.12)	0.42 (1.19)	2.50 (1.87)	0.21 (1.10)	0.17 (1.08)	0.14 (1.07)	0.14 (1.07)	0.19 (1.09)	0.21 (1.10)	0.28 (1.13)	1.16 (1.47)
CD	-	-	-	-	-	0.09	-	-	-	-	-	-

Plant extracts were sprayed at weekly intervals and carbaryl at biweekly intervals commencing from the third week after planting
Figures in parantheses are adjusted means

difference in the extent of parasitisation in the treated and control plants when observed five,six and seven weeks after planting. Very few parasitized grubs were observed in the insecticide treated plots (0.04, 0.12 and 0) five, six and seven weeks after planting.

Significantly low number of grubs were found parasitized in the seed extract treated plots (0.28 and 0.12) when observed five weeks after planting. The leaf extracts showed comparatively better parasitisation of grubs than the seed extract.

Water and ethanol extracts of seed were on par, the number of grubs parasitized in these treatments being 0.28 and 0.12 respectively. Water extract of fresh leaf (1.02) was safer than ethanol extract (0.59) resulting in higher parasitization of grubs. It was on par with control (1.02). Water extract of dried leaf (0.66) was also significantly superior to ethanol extract (0.32). The ethanol extract of fresh and dried leaves were on par with water extract of seed.

The extent of parasitization of grubs noted six weeks after planting was negligible in carbaryl treated plots (0.12) and low in seed extract treated plots (0.04 and 0.37) and they were on par.

Water and ethanol extract of seeds did not vary in their effect on parasitization of grubs. However ethanol extract of fresh leaf was superior to water extract, the number of parasitized grubs observed in this treatment being 1.99 and 0.49 respectively. The former was on par with control (1.62). The solvent extracts of dried leaf did not show any significant difference. (0.96 and 0.77 parasitized grubs respectively in water and ethanol extracts).

Table 20 **Effect of leaf and seed extracts of *Thevetia neriifolia* on parasitization of grubs of *Henosepilachna vigintioctopunctata* in the field.**

Treatments	Number of parasitized grubs per plant observed at different intervals after planting (weeks)											
	4	5	6	7	4	5	6	7	4	5	6	7
	Trial I				Trial II				Pooled			
Water extract of dried leaf (2%)	0.15 (1.07)	0.69 (1.30)	0.79 (1.34)	0.52 (1.23)	0.44 (1.20)	0.62 (1.27)	1.10 (1.45)	0.79 (1.34)	0.30 (1.14)	0.66 (1.29)	0.96 (1.40)	0.66 (1.29)
Water extract of fresh leaf (2%)	0.15 (1.07)	1.28 (1.51)	0.54 (1.24)	0.72 (1.31)	0 (1.00)	0.77 (1.33)	0.42 (1.19)	0.21 (1.10)	0.08 (1.04)	1.02 (1.42)	0.49 (1.22)	0.46 (1.21)
Water extract of seed (2%)	0 (1.00)	0.25 (1.12)	0.02 (1.01)	0 (1.00)	0.28 (1.13)	0.28 (1.13)	0.04 (1.02)	0.25 (1.12)	0.14 (1.07)	0.28 (1.13)	0.04 (1.02)	0.12 (1.06)
Ethanol extract of dried leaf (2%)	1.15 (1.07)	1.39 (1.48)	1.10 (1.45)	0.30 (1.14)	0.21 (1.10)	0.23 (1.11)	0.46 (1.21)	1.28 (1.51)	0.19 (1.09)	0.32 (1.15)	0.77 (1.33)	0.77 (1.33)
Ethanol extract of fresh leaf (2%)	0.15 (1.07)	0.49 (1.30)	3.49 (2.12)	0.80 (1.34)	0.15 (1.07)	0.46 (1.21)	0.77 (1.33)	1.02 (1.42)	0.15 (1.07)	0.59 (1.26)	1.99 (1.73)	0.90 (1.38)
Ethanol extract of seed (2%)	0 (1.00)	0.25 (1.12)	0.02 (1.01)	0 (1.00)	0 (1.00)	0 (0.99)	0.74 (1.32)	0 (1.00)	0 (1.00)	0.12 (1.06)	0.37 (1.17)	0 (1.00)
Carbaryl (0.15%)	0 (1.00)	0.08 (1.04)	0.04 (1.02)	0 (0.93)	0 (1.00)	0 (0.99)	0.19 (1.09)	0 (1.00)	0 (1.00)	0.04 (1.02)	0.12 (1.06)	0 (0.97)
Control	0.44 (1.20)	1.31 (1.52)	2.61 (1.90)	2.69 (1.92)	0.14 (1.07)	0.72 (1.31)	0.77 (1.33)	1.13 (1.46)	0.30 (1.14)	1.02 (1.42)	1.62 (1.62)	1.86 (1.69)
CD	-	-	-	-	-	-	-	-	-	(0.13)	(0.22)	(0.15)

Plant extracts were sprayed at weekly intervals and carbaryl at biweekly intervals commencing from the third week after planting
Figures in parantheses are adjusted means

During seventh week after planting seed extracts had negligible number of parasitized grubs (0.12 and 0) and these were on par with the insecticide (0). Significantly higher parasitization was seen in leaf extract (fresh and dried) treated plots.

The solvent extracts of seed and dried leaf did not vary significantly in their effect, However ethanol extract of fresh leaf (0.90 parasitized grubs) was superior to the water extract (0.46 parasitized grubs).

4.9. Efficacy of extracts of seeds and leaves of *T.neriifolia* in controlling leaf webber of amaranthus.

Water and ethanol extracts of seed, fresh and dried leaves of *T.neriifolia* were tested for their efficacy in reducing leaf webber infestation in amaranthus. The results of the trials are presented in Tables 21 to 23.

4.9.1. Population of *H.recurvalis*

The population of *H.recurvalis* observed in the different treatments are presented in Table 21.

Pooled data of the fifth week after planting showed that all the treatments were superior to control (1.99 webbers per plant). Fortnightly spraying of malathion was the best treatment with only 0.66 webbers per plant and was statistically superior to the plant extract treatments. Among the plant parts of *T.neriifolia* tested, seed extracts (1.02 and 1.33 webbers per plant) and fresh leaf extracts (1.16 and 1.31 webbers per plant) were on par and reduced webber population significantly. Fresh and dried leaf extracts also came on par. The solvents (ethanol and water) did not show any significant difference. The

Table 21 **Effect of leaf and seed extracts of *Thevetia neriifolia* on infestation of *Hymenia recurvalis* in amaranthus .**

Treatments	Number of webbers observed per plant at different intervals after spraying (weeks)									
	4	5	6	7	4	5	6	4	5	6
	Trial I				Trial II			Pooled		
Water extract of dried leaf (2%)	0.42 (1.19)	1.37 (1.54)	0.32 (1.15)	2.65 (1.91)	0.59 (1.26)	1.49 (1.58)	0.52 (1.23)	0.52 (1.23)	1.43 (1.56)	0.42 (1.19)
Water extrat of fresh leaf (2%)	0.06 (1.03)	1.13 (1.46)	0.02 (1.01)	1.25 (1.50)	0 (1.00)	1.19 (1.48)	0.52 (1.23)	0.04 (1.02)	1.16 (1.47)	0.25 (1.12)
Water extract of seed (2%)	0.08 (1.04)	0.82 (1.35)	0.08 (1.04)	0.93 (1.39)	0 (1.00)	1.22 (1.49)	0 (1.00)	0.04 (1.02)	1.02 (1.42)	0.04 (1.02)
Ethanol extract dried leaf (2%)	0.51 (1.22)	1.22 (1.49)	0 (1.00)	3.66 (2.16)	0.23 (1.11)	1.49 (1.58)	0.44 (1.20)	0.37 (1.17)	1.37 (1.54)	0.21 (1.10)
Ethanol extract of of fresh leaf (2%)	0.23 (1.11)	0.96 (1.40)	0.02 (1.01)	0.99 (1.41)	0.19 (1.09)	1.69 (1.64)	0.14 (1.07)	0.21 (1.10)	1.31 (1.52)	0.08 (1.04)
Ethanol extract of seed (2%)	0.12 (1.06)	1.22 (1.49)	0 (1.00)	0.82 (1.35)	0.37 (1.17)	1.43 (1.56)	0 (1.00)	0.25 (1.12)	1.33 (1.53)	0 (1.00)
Malathion (0.1%)	0.08 (1.04)	0.56 (1.25)	0.06 (1.03)	0 (0.96)	0.04 (1.02)	0.74 (1.32)	0.02 (1.01)	0.06 (1.03)	0.66 (1.29)	0.04 (1.02)
Control	1.16 (1.47)	2.13 (1.77)	3.62 (2.15)	10.28 (3.36)	1.82 (1.68)	1.82 (1.68)	4.38 (2.32)	1.50 (1.58)	1.99 (1.73)	4.02 (2.24)
CD	(0.13)	-	(0.14)	(0.96)	-	-	(0.11)	-	(0.11)	(0.07)

Plant extracts were sprayed at weekly intervals and malathion at biweekly intervals commencing from the third week after planting.
Figures in parantheses are adjusted means.
Crop was harvested at 6 weeks after planting due to the incidence of leaf spot disease

number of webbers observed in water and ethanol extracts of dried leaf were 1.43 and 1.37 per plant respectively.

Observations recorded six weeks after planting also showed the superiority of seed extracts and ethanol extract of fresh leaf in reducing the pest population. The seed extracts with 0 and 0.04 webbers per plant was on par with the insecticide treatment (0.04 webbers per plant). The leaf extracts were on par and reduced the pest population significantly, compared to control (4.02 webbers per plant).

While ethanol (0 webbers per plant) and water (0.04 webbers per plant) extracts of seed were on par, ethanol and water extracts of fresh and dried leaves significantly differed in their effectiveness. Ethanol extract of fresh leaf (0.08 webbers per plant) was superior to water extract of fresh leaf (0.25 webbers per plant) and was on par with water and ethanol extracts of seed (0.04 and 0 webbers per plant). Similarly ethanol extract of dried leaf (0.21 webbers per plant) was superior to water extract (0.42 webbers per plant).

All the treatments were found to reduce webber infestation significant in the first trial (Table 21). Seed and fresh leaf extracts were as effective as the insecticide in reducing the number of webbers. The dried leaf extracts (0.42 and 0.5 webbers per plant) were on par with ethanol extracts of seed (0.12) and fresh leaf (0.23). The population of the webbers in the treated plots were significantly low (0 to 0.32 webbers per plant) when observed six weeks after planting. While the number of webbers per plant ranged from 0 to 3.66 in the different treatments after the fourth spraying (seven weeks after

transplanting) it was 10.28 per plant in control plot. The second trial did not show any significant effect due to the treatments except at the sixth week after transplanting. Compared to 4.38 webbers per plant in control plots, the population of webbers in the different treatments was significantly low and ranged from 0 to 0.52 per plant only.

4.9.2. Percentage of damaged leaves per plant

Results presented in Table 22 revealed that both malathion and the plant extracts significantly checked the pest damage in amaranthus.

Pooled data showed that the extract of different plant parts viz., water extract of seed and both extracts of leaves were equally effective in reducing leaf damage when observed four weeks after planting and they were also on par with the insecticide treatment. Rest of the treatments were on par and inferior.

Regarding the efficacy of the solvents, water extract of seed with 2.35 per cent damaged leaves was superior to ethanol extract (4.71 per cent). However there was no significant difference between the solvents for leaf extraction (fresh and dried). While the percentage of leaves damaged in fresh leaf extracts were 2.73 (ethanol) and 3.24 (water), it was 5.40 (ethanol) and 5.71 (water) in dried leaf extract compared to 12.03 per cent damage in control.

When observed five weeks after spraying, the extent of leaf damage seen in the different treatments were 4.45 per cent in insecticide treated plants, 4.11 and 4.76 per cents in water and ethanol extracts of seed treated plants, 3.84 and 4.71 per cent in plants treated with water and ethanol extracts of

Table 22 Effect of leaf and seed extracts of *Thevetia neriifolia* on leaf damage in amaranthus

Treatments	Percentage of damaged leaves per plant observed at different intervals after spraying (weeks)								
	4	5	6	7	4	5	6	4	5	6
	Trial I				Trial II			Pooled		
Water extract of dried leaf (2%)	9.14 (3.18)	6.96 (2.82)	10.02 (3.32)	7.26 (2.87)	3.00 (2.00)	10.20 (3.35)	2.61 (1.90)	5.71 (2.59)	8.55 (3.09)	5.81 (2.61)
Water extract of fresh leaf (2%)	6.24 (2.69)	2.11 (1.76)	6.84 (2.80)	3.50 (2.12)	1.06 (1.43)	5.98 (2.64)	2.16 (1.78)	3.24 (2.06)	3.84 (2.20)	4.24 (2.29)
Water extract of seed (2%)	5.59 (2.57)	2.53 (1.88)	6.95 (2.82)	5.10 (2.47)	0.19 (1.09)	5.98 (2.64)	0.64 (1.28)	2.35 (1.83)	4.11 (2.26)	3.20 (2.05)
Ethanol extract of dried leaf (2%)	11.04 (3.47)	10.29 (3.36)	14.06 (3.88)	10.10 (3.33)	1.50 (1.58)	7.01 (2.83)	3.04 (2.01)	5.40 (2.53)	8.61 (3.10)	7.70 (2.95)
Ethanol extract of fresh leaf (2%)	5.66 (2.58)	2.53 (1.88)	6.95 (2.82)	3.04 (2.01)	0.65 (1.28)	7.34 (2.89)	2.88 (1.97)	2.73 (1.93)	4.71 (2.39)	4.76 (2.40)
Ethanol extract of seed (2%)	6.14 (2.67)	2.17 (1.78)	4.22 (2.28)	1.76 (1.66)	3.48 (2.11)	8.06 (3.01)	0.90 (1.38)	4.71 (2.39)	4.76 (2.46)	2.35 (1.83)
Malathion (0.1%)	2.84 (1.96)	0.91 (1.38)	0.80 (1.34)	0.81 (1.34)	1.50 (1.58)	1.40 (1.55)	0 (1.01)	2.13 (1.77)	4.45 (2.11)	3.39 (1.18)
Control	15.97 (4.12)	18.19 (4.38)	24.84 (5.08)	22.18 (4.81)	8.5 (3.09)	12.54 (3.68)	26.98 (5.29)	12.03 (3.61)	15.24 (4.03)	25.94 (5.19)
C D	(0.84)	(0.66)	(0.69)	(0.74)	(0.83)	(0.79)	(0.40)	(0.29)	(0.56)	(0.48)

Plant extracts were sprayed at weekly intervals and malathion at biweekly intervals commencing from third ~~the~~ week after planting .
Figures in parantheses are adjusted means.

fresh leaf and 8.55 and 8.61 per cent in plants treated with water and ethanol extracts of dried leaf respectively compared to 15.24 per cent damage in control plants. Extracts of seed and fresh leaf (3.84 and 4.76) were on par and were also on par with malathion (4.45). Extracts of dried leaf were on par and less effective.

Six weeks after planting,insecticide treatment with 3.39 per cent damage proved superior to the plant extract treatments. Among the plant extracts, seed extracts (2.35 and 3.20 per cent leaf damage) and fresh leaf extracts (4.76 and 4.24 per cent leaf damage in ethanol and water extracts respectively) were on par in their effectiveness in reducing leaf damage. Dried leaf extracts with 7.70 and 5.81 per cent leaf damage in ethanol and water extracts respectively though not as effective as fresh leaf extracts were superior to control (25.94 per cent). The solvent extracts of the plant parts did not show any significant difference.

The individual results obtained in the two field trials agreed with the pooled finding. The insecticide treatment was on par with the seed extracts and fresh leaf extracts while dried leaf extracts were generally inferior. The solvents did not show any significant variation.

4.9.3 Yield

The yield data presented in Table 23 showed the effectiveness of the treatments in reducing pest infestation and consequently increasing yield. Fortnightly spraying of malathion (0.1 per cent) was significantly superior to the plant extract treatment. Among the plant parts tested for their efficacy seed

Table 23 Effect of spraying leaf and seed extracts of *Thevetia neriifolia* on the yield of amaranthus and the resultant cost benefit ratio

Treatments	Mean yield (kg/ plot)			Increase in yield over control		Additional income/ha Rs.	Cost of plant protection Rs.	Cost benefit ratio
	Trial I	Trial II	Pooled	per 4sq.m plot	per ha			
Water extract of dried leaf (2%)	4.19	2.25	3.22	0.47	1175	5,875**	1500*	1:4
Water extract of fresh leaf (2%)	4.94	2.36	3.65	0.9	2250	11,250	1500	1:8
Water extract of seed (2%)	5.00	2.81	3.91	1.16	2900	14,500	2000	1:7
Ethanol extract of dried leaf (2%)	4.38	2.19	3.29	0.54	1350	6,750	5250	1:1
Ethanol extract of fresh leaf (2%)	5.44	2.25	3.85	1.1	2750	13,750	5250	1:3
Ethanol extract of seed (2%)	5.44	2.75	4.10	1.35	3375	16,875	5550	1:3
Malathion (0.1%)	5.94	2.94	4.44	1.69	4225	21,125	1200	1:18
Control	3.94	1.56	2.75					
CD	0.81	0.51	0.24					

** Labour requirement for preparing and spraying leaf and seed extracts and insecticide were 3, 4 and 2 respectively. Wages per man labourer was Rs.100/day
* Price of amaranthus - Rs. 5/kg (farm rate)

extracts of *T. neriifolia* was superior to the leaf extracts,and between the leaf extracts, fresh leaf extracts were significantly better. No significant difference was observed between the solvent extracts.

While the insecticide treatment resulted in an yield of 4.44 kg per plot, seed extract treatments yielded 4.10 (ethanol extract) and 3.91 kg (water extract) per plot. They were followed by the yield in fresh leaf extract treated plots (3.85 and 3.65 kg per plot respectively in ethanol and water extract treatments). The yield obtained from dried leaf extract treated plots was low being 3.29 kg per plot in ethanol extract and 3.22 kg per plot in water extract treated plots. The yield obtained from the check plot was 2.75 kg per plot only.

All the treatments gave significantly better yields than control (3.94 and 1.56 kg per plot) in the trials conducted during the two seasons. Ethanol extracts of seed and fresh leaf were as good as malathion in reducing pest infestation and increasing yield, the yield obtained being 5.94, 5.44 and 5.44 kg per plot respectively in the treatments in the first trial. The seed extracts (5.00 and 5.44 kg per plot) and fresh leaf extracts (4.94 and 5.44 kg per plot) were on par. Dried leaf extracts (4.19 and 4.38 kg per plot) were on par with water extracts of seed (5kg per plot) and fresh leaf (4.94 kg per plot).

In the second trial , seed extracts resulted in significantly better yield (2.81 and 2.75 kg per plot) than other treatments and was on par with malathion (2.94 kg per plot). The leaf extracts were on par. The extractants (ethanol and water) did not show any significant difference.

The cost benefit ratio computed showed the superiority of the

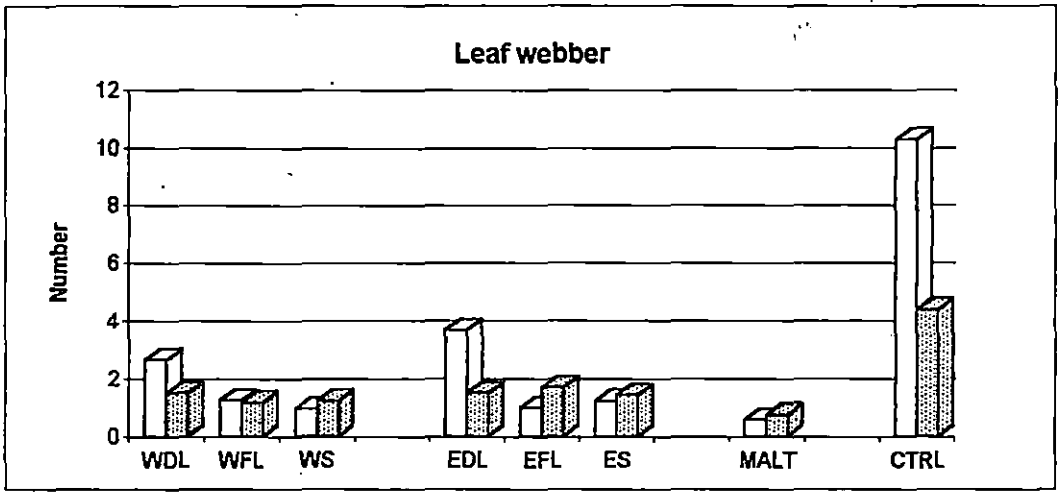
insecticide treatment (1:18). Among the plant parts, fresh leaf and seed were equally effective while between the solvents, water was superior to ethanol.

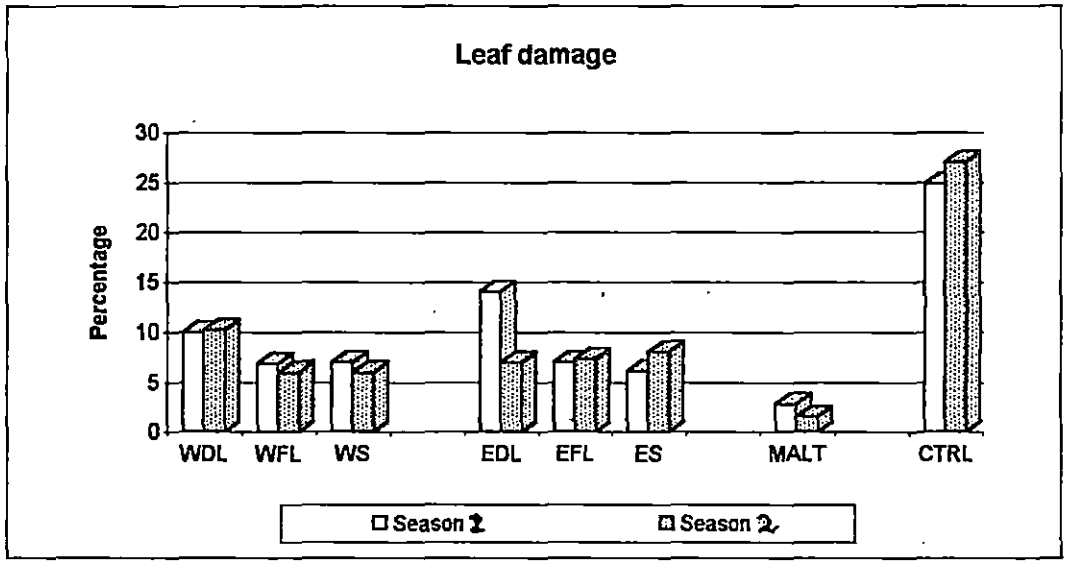
Thus among the plant extract treatments, water extract of fresh leaf with a cost benefit ratio of 1:8 was found to be the best closely followed by water extract of seed (1:7). Dried leaf extracts (1:4 and 1:1) and ethanol extracts of fresh leaf (1:3) and seed (1:3) did not give economic returns.

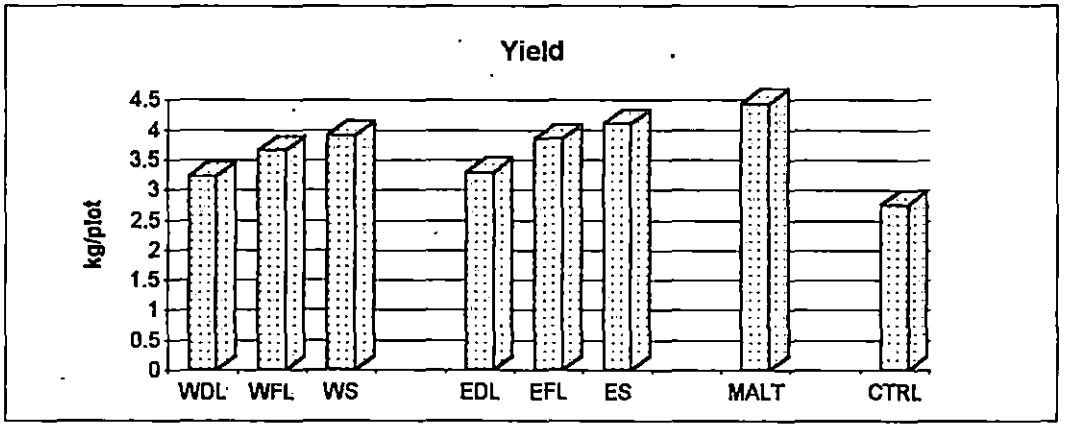
4.9.4. Correlation of leaf webber population, percentage of damaged leaves and yield

Data presented in Fig.9. showed that water and ethanol extracts of leaf and seed did not vary significantly in bioefficacy, during both the seasons. Maximum control of leaf webber was obtained in plots treated with seed extracts and it was followed by fresh and dried leaf extracts. The extent of leaf damage was positively associated with the pest population and the yield showed a negative association with the pest population and extent of leaf damage. Highest yield was obtained in malathion treatment and it was closely followed by seed and fresh leaf extracts, the least effective being the dried leaf extracts. All the treatments were significantly superior to control in reducing pest population and increasing yield.

Fig.9. Control of *Hymenia recurvalis* on amaranthus with different extracts of *Thevetia neriifolia* and the effect on yield







WDL	: Water extract of dried leaf(2%)	EDL	: Ethanol extract of dried leaf(2%)
WFL	: Water extract of fresh leaf(2%)	EFL	: Ethanol extract of fresh leaf(2%)
WS	: Water extract of seed(2%)	ES	: Ethanol extract of seed(2%)
MALT	: Malathion (0.1 %)	CTRL	: Water spray

DISCUSSION

5. DISCUSSION

Synthetic organic insecticides played a key role in solving acute food shortage faced by humanity during the post second world war period. But the intensive and extensive use of these poisonous substances created several side problems to humanity and it became imperative to replace these miraculous agricultural input with effective but more eco-friendly and safer compounds. In this context, the botanical pesticides, which were relegated to the background with the advent of synthetics, got a revival and an universal interest has been evinced in exploiting the secondary metabolites produced by different species of plants for containing pest and disease problems faced by farmers . By the beginning of this decade, more than 1005 species of plants were reported to possess insecticidal properties; 384 antifeedant, 297 repellent, 27 insect attractant and 31 growth inhibitory activities (Jayaraj, 1991). Among these plants, neem has received universal attention of agricultural scientists. A lot of basic information needed for its use in pest control had been generated and many of the products derived from the plant have already been commercialised in India and abroad. The limited availability of this plant species in the world at present renders the search for other fast growing and easily maintainable plant species imperative for meeting the huge pesticide demand and for replacing the undesirable synthetic pesticides at least partly with botanicals. Even in integrated pest management, the most desirable plant protection strategy adopted

at present, insecticides still play an important role . Obviously, from plants already identified as promising ones and new ones to be detected from the vast resource available in nature, best suited ones have to be chosen and the field level use of the effective and safe ones has to be standardised through detailed research.

Among the plants screened out from the rich flora of Kerala (Saradamma, 1989), *Thevetia neriifolia* had been identified as a potential source of phytochemicals suited for insect control. Present investigations aim at detailed information on the bioefficacy, mode of action, field performance and safety of the plant in pest control strategies.

5.1. Antifeedant activity of leaves and seeds of *T.neriifolia* to *H.vigintioctopunctata*.

Antifeedant activity of seed extract of *T. neriifolia* to *Athalia proxima* and of dried leaf extracted with different solvents to *H.vigintioctopunctata* were already reported (Pandey *et al.*, 1977; Saradamma,1989). Maximum activity of dried leaf extracts to *H.vigintioctopunctata* was found with water while with other test insects benzene was found better and hence the latter was concluded as the best solvent for extracting antifeedant factor of *T.neriifolia* against *H.vigintioctopunctata* (Saradamma,1989). In extraction and isolation of active principles of *T.neriifolia* more polar solvents have been used extensively . In the light of the above facts besides benzene and acetone chosen by Saradamma, ethanol, and methanol (two polar solvents) and water were evaluated in the present investigations. Drying of

leaves under shade is recommended as a pre-requisite for extraction of bioactive principles from plants. But it is a time consuming and cumbersome process and hence fresh leaves collected from the plants were also included as treatment in the experiment.

Though leaf is the best portion of the plant to be chosen for use at farm level by virture of its continuous availability round the year, during the months of February to April a large quantity of seeds also becomes available on the plants. With a view to utilising these seeds for plant protection purposes the bioefficacy of the seed extracts also was studied.

The results presented in para 4.1.1.1. showed that among the different parts of the plant evaluated, the seed at one per cent concentration had significantly higher antifeedant effect against *H.vigintioctopunctata* than the dried leaf at two per cent and fresh leaf at four per cent concentrations. The latter two treatments were on par and the doses also could be treated as on par since the loss in weight during drying was around 50 per cent. The results thus showed that leaf of *T.neriifolia* freshly harvested or dried under shade can be used with equal advantage, and seeds when available can also be used at 25 per cent of the quantity of fresh leaves for protecting bittergourd from *H.vigintioctopunctata* infestation. With reference to larval starvation also seed was the best portion of the plant for protecting the treated leaves and it was followed by fresh leaf and dried leaf, the differences among the treatments being statistically significant. The different plant parts of *T.neriifolia* were evaluated for the first time for their antifeedant activity to *H.vigintioctopunctata*. The results shown in para 4.1.1.3

further indicated that the ranking of treatments based on leaf protection and larval starvation were on par and hence one of the criteria could be adopted for reliable screening of plants for their antifeedant potential.

Regarding different solvents, ethanol and methanol were on par and significantly superior closely followed by water with reference to the extent of leaf protection. With reference to larval starvation all the three were on par and effective. Saradamma (1989) observed that benzene and water gave better leaf protection and larval starvation than others and recommended water extraction on cost basis. But methanol and ethanol were not included in her studies. The data from the present studies also showed that on cost cum efficiency basis water extraction can be adopted as an apt technology for extracting different parts of *T.neriifolia* plants for the control of *H. vigintioctopunctata*. On *S. litura* no antifeedant effect was observed in the laboratory even with the highest doses of different extracts used in the experiment. Saradamma (1989) also reported the low response of *S.litura* to the dried leaf extract of *T.neriifolia*. The results highlight the need for the assessment of bioefficacy of plant products to individual pest species in an ecosystem.

5.2. Comparative efficacy of crude and soxhlet methods for extraction of antifeedant principles of *T. neriifolia* against *H.vigintioctopunctata*

The results presented in para 4.2 showed that the simpler crude method of extraction was significantly superior for extracting the leaf and seed of the plant with ethanol compared to soxhlet extraction. In the case of methanol

and benzene both the methods were on par. Obviously for extracting the antifeedant factor from the plants using any of the effective solvents, soaking ground plant tissues well for 48 hours and filtering the same prove to be an apt technology. Pounding the material also may prove effective at farm level. This simple technique and soxhlet extraction were quantitatively compared for the first time though both the methods were being individually adopted by earlier workers.

5.3. Juvenomimetic/Chemosterilant activity of ethanol extract of leaf and seed of *T.neriifolia*.

Many plants showing antifeedant activity are known to posses high juvenomimetic activity too. It is well known that such exogenous juvenile hormones or its analogues applied to sensitive life stages of insects result in the retention of juvenile characters after subsequent moult leading to the development of juvenile adult conditions. The difference in the critical moments of sensitivity to juvenoids in various tissues of developing insects cause variations in the growth and development process. This may result in the prolongation of life stages, the development of extra larvae which may even have ripe eggs in the ovary, emergence of malformed adults, reduction in fecundity and hatchability of eggs laid by emerging adults (Zdarek and Slama, 1972). On these criteria the juvenoid activity of the ethanol extracts of fresh leaf and seed of *T.neriifolia* was assessed in the present investigation.

Data presented in para 4.3.1. showed that the treatments caused post treatment mortality of nymphs to varying extent. The effect was dose

dependant. The lowest doses of 10 per cent leaf and 2.5 per cent seed extracts were ineffective, while 20 per cent leaf and 5 per cent seed extracts were on par. Similarly, 40 per cent leaf and 10 per cent seed extracts were on par. As shown in para 4.3.1.3.1. some of the affected nymphs showed black patches on the abdomen; failed to moult and died. This showed that the death was due to juvenomimetic effect of the treatments. Death due to juvenoid effects of plant extracts had been extensively reported earlier also (Slama and Williams, 1966; Man Singh *et al.*, 1970; Deshpande *et al.*,1974; Prabhu and John, 1975; Jacobson *et al.*, 1975; Schmutterer *et al.*, 1983; Chiu, 1985; EL Sayed, 1985). Schluter (1980) made electron microscopic studies of such black spots appearing on *Epilachna varivestis* grubs treated with some fractions of neem seed extract and found that the epidermis of the larvae below such spots got dissolved and consequently prevented the formation of the pupal cuticle which caused the death of the larvae in due course. The cause of the cuticular disruption was attributed to the fraction of neem playing the role of 'hormone mimics' (juvenile/ ecdysone). Probably the black spots formed on *Dysdercus* due to the application of *T.neriifolia* extracts also might be attributed to a juvenomimetic effect.

Post treatment duration of the surviving nymphs (para 4.3.2) was seen significantly prolonged by the extracts of leaf and seed of *T.neriifolia*. The effect was also dose dependent, the middle and higher doses of leaf extracts being on par with corresponding doses of seed and the effect in the last doses being on par with control. Saradamma (1989) also observed post treatment prolongation of fifth instar nymphs with ether and benzene extract of dried leaves

of *T.neriifolia*. Such prolonged larval durations were reported earlier on a number of other insects with plant extracts (Koul, 1984; Chockalingam *et al.*, 1986; Jamil *et al.*, 1988).

The major effect of juvenoids on insects was observed to be disruption of the normal mechanism of moulting and emergence. The adult deformities observed also could be attributed primarily to this factor rather than to any disturbance of metamorphosis (Outram, 1973). The results presented in para 4.3.3. showed that the treatments significantly limited the number of normal adult emergence and it was also a dose dependent response. Leaf extract(40 and 20 per cent)and seed extract(5 per cent)resulted in significantly higher number of malformed adults. With higher dose of seed extract (10 per cent) malformed adults were low in number but the effect was evident in 100 per cent death of normal and malformed adults before egg laying. Saradamma (1989) found 33 per cent normal adults and 30 per cent malformed adults in *D.cingulatus* treated with 25 per cent dried leaf extract of *T.neriifolia* using benzene as solvent while with acetone extract 15 per cent normal adults and 17 per cent malformed adults were obtained. Obviously the solvents used in the extraction played an important role in the manifestation of the bioactivity on test insects. Extracts of many plants have been reported to have significant effect on morphogenesis of different species of insects (Wellington, 1969; Outram, 1973; Prabhu and John 1975; Kumuda Sukumar and Osmani, 1981; Schmutterer *et al.*, 1983; Chiu, 1985; EL Sayed, 1985; Adeyeye and Blum, 1989; Chockalingam *et al.*, 1992; Jaiswal and Srivastava, 1992; Kumuda Sukumar *et al.*, 1995). This effect of the plant

extracts will have significant adverse effect on the population of the insects in succeeding generations.

As seen from the results in para 4.3.6. the preoviposition period was prolonged by treatment with leaf and seed extracts of *T.neriifolia* the response being higher in 20 per cent leaf and 5 per cent seed extracts. But the interovipositing periods were not seen affected by the treatments. The number of eggs laid by treated insects in all the three gonadotropic cycles were seen significantly reduced in 40 per cent and 20 per cent leaf extracts and 5 per cent seed extract (para 4.3.7). Saradamma (1989) also recorded significant reduction in the fecundity of *D.cingulatus* treated with dried leaf extracts of *T.neriifolia.* Reduced fecundity caused by juvenoids was reported in *C. fumiferanae* (Outram, 1973); *D.cingulatus* (Prabhu *et al.*, 1973; Kumuda Sukumar and Osmani, 1981; Rajendran and Gopalan, 1985)and pulse beetles (EL Ghar and EL Sheikh, 1987). Saxena and Khan, (1986), Hellpap and Mercado (1986), Banerji and Sharma (1993) and Chandraletha (1994) also reported reduced fecundity of various insects due to juvenoid application.

Slama (1974) observed that the juvenoids do not suppress the egg production as is being done by chemosterilants, but females lay eggs of very low or zero hatchability. Data presented in para 4.3.7 to 4.3.10. revealed that the ethanol extracts of leaf and seed of *T.neriifolia* did not significantly affect the longevity of emerging adults or the incubation period and hatching percentage of eggs laid by them. But with acetone and benzene extracts of dried leaves of *T.neriifolia,* significant shortening of longevity of normal adults and adultoids

was observed earlier. The hatching percentage of eggs was lower than that of control with benzene extract while in acetone extract the suppression of egg hatching was marginal (Saradamma, 1989). The variation in the response now observed and the results reported earlier might be attributed to the different solvents used. The bioactive components present in benzene and ethanol extracts may be different since the polarity of substances dissolving in the two solvents should be different.

Post treatment nymphal mortality, reduction in the number of normal adult emergence and lower fecundity resulted in significant reduction of the total number of eggs obtained from a cohort of 50 female nymphs in different treatments. The eggs obtained in 40 per cent leaf extract treatment was just 584 and those of 20 per cent leaf and 5 per cent seed extracts were 2447 and 2332, respectively as against 8682 eggs obtained in control. Though the juvenomimetic activity of the extractants did not cause immediate kill of the target organisms, as done by synthetic organic chemicals, over a few generations the reduction of the pest population caused by the juvenomimetic/ sterilant effect of the leaf / seed extracts of *T. neriifolia* would be high. Obviously *T. neriifolia* is a promising plant as source of juvenoids which can be advantageously exploited in plant protection. Since juvenomimetic effect takes time to manifest and also because the effect largely depends on the occurrence of the receptive life stages in the population, the treatment may not prove as a single effective technique for controlling any pest problem. Such plant derivatives may prove to be ideal components in integrated pest control programmes by virtue of their high

potential in suppressing the population build up, while being eco-friendly unlike the conventional pesticides.

In all the criteria denoting the juvenoid effect, 40,20 and 10 per cent leaf extracts came on par with 10,5 and 2.5 per cent seed extracts of *T.neriifolia*. This indicated that the difference between the two plant parts was quantitative and the seeds were more than four times effective than fresh and dried leaves.

5.4. Effect of ethanol extracts of leaf and seed of *T.neriifolia* on the ovary of *D.cingulatus*

The results presented in para 4.3.13.3 showed that the length of the ovarioles in adults emerging from treated nymphs increased significantly from second to fifth day after emergence of the adult and the increase was comparatively higher in control than in leaf and seed extract treated insects. As a result the size of the ovary in control appeared bigger than those in corresponding treatments on all days of data recording from second to fifth day after emergence. As seen from Fig. 3a to 3c the lengthening of the ovarioles continued upto the fifth day and might have continued up to the eighth day after emergence when egg laying commenced in the plant extract treated insects. In control, the lengthening ceased on the fifth day after emergence while the egg laying took place. The ovariole length reached on third day in control was seen attained on the fourth day in leaf extract treated insects and fifth day in seed extract treated ones. The size of the basal, penultimate and antepenultimate oocytes in the ovary, revealed by their mean length and width, also showed the

same trend. Thus the growth of the ovary and oocytes were just seen delayed rather than suppressed by the plant extract.

Taneja *et al.* (1979) while studying the effect of apholate on the ovarian development of red cotton bug *D.koenigii* observed that in treated insects the egg maturation was delayed by 3 days compared to normal untreated individuals during which period the growth of the ovary was retarded significantly and some of the growing oocytes got degenerated resulting in a reduction in the number of eggs laid. The delay in the ovarian development was accompanied by the suspension of the release of neurosecretory substance from pars intercerebralis for three days. The effect of the delay was manifested in the prolongation of preovipositional period by three days. The effect of the extracts of leaf and seed of *T.neriifolia* on *D.cingulatus* closely resembled the effect of apholate on *D.koenigii* and thus a typical chemosterilant activity could be attributed to the extracts of *T.neriifolia*. While reporting the chemosterilant effect of the alkaloids extracted from *Catharanthus roseus*, Kumuda Sukumar and Osmani (1981) observed that the chemosterilants typically showed the development of black patches on the body and wings of *D.cingulatus* and in the protrusion of genitalia of the males after treatment. Such pigmentation was typically noted in insects treated with ethanol extract of *T.neriifolia* in the present investigations and the extrusion of external genitalia was typically noted with benzene extracts of *T.neriifolia* by Saradamma (1989). These observations indicate the presence of some alkaloids in the extract of *T.neriifolia* exerting a chemosterilant effect on the test insect. Chemosterilant activity of plant extracts

was reported earlier also. *Acorus calamus* extract was conclusively shown to have a strong chemosterilant action on *Trogoderma granarium* in which the mature follicles followed by the remaining parts of the ovary got degenerated (Koul *et al*, 1977). Extracts of *Aristolochia bracteata* exerted haphazard effects on the ovary of *D.koenigii*, *Aedes.aegypti* and *Tribolium castaneum*, causing reduction in fecundity, some of the oocytes being highly vitellogenic and some degenerate (Saxena *et al*., 1979).

As observed by Singh (1990) an important development in neem research was the finding of neem's sterilant effect on insects. Studies on the induction of chemosterilants have been reviewed by several authors (Smith *et al*., 1964; Morgan and La Brecque, 1964; Grover *et al*., 1972; Chaudhry and Tripathi, 1976). They all reported similar cytopathological effects on gonadal embryonic tissues of insects viz., chromatin condensation, pycnotic nuclei, cytoplasmic vacuolization and histolysis of follicular epithelium. In the ovary of *D.cingulatus* treated with *T.neriifolia* extract visible lytic changes were not seen upto 5 days after emergence. Histopathological studies were not included in the present investigations. Based on evidences presented, *T.neriifolia* can be treated as a source of bioactive components having chemosterilant effect on insects. The hazards involved in the field use of chemosterilants, one of the recent class of pesticides, have prompted researchers to look for chemicals from plants which are nontoxic, specific in their action and could be sources of auto sterilants in future. Possibly, *T.neriifolia* will have a place in this category of plants in due course.

5.5. Effect of ethanol extracts of leaf and seed of *T.neriifolia* on the vitellogenesis of *D.cingulatus*.

Data presented in para 4.3.13.3 and Fig. 4 showed that protein, glycogen and lipid contents in the ovary were steadily increasing from first to fifth day after emergence in control as well as in treatments. The increase was much higher in the untreated control and reached the peak on the fifth day preceeding the oviposition date. Though the three nutrients in plant extract treatments showed an increasing trend upto the fifth day the levels were less than that in corresponding controls . As seen in para 4.3.6 the preoviposition period was seen extended due to the plant extract treatment and the eggs laid were normal and viable. Possibly the deposition of vitellin in the growing oocytes might have been delayed and the full deposition might be taking place on the eighth day after emergence ie. the day of oviposition in the treatments.

In the case of fat body where vitellogenin synthesis occurs, protein,glycogen and lipid content generally showed an increasing trend upto the third day after emergence and then it suddenly showed a decreasing trend between the third and fourth day in untreated insects. In seed and leaf extract treatments, the increasing trend continued upto the fourth and fifth days accompanied by the prolongation of the preoviposition period.

Detailed investigations on the ovarian changes and vitellogenesis in *D.cingulatus* caused by extracts of *T.neriifolia* was being studied for the first time. Effect of apholate on carbohydrate, protein and lipid content in fat body, haemolymph and ovary of *D.koenigii* was studied in detail by Taneja *et al.* (1979)

adopting histochemical techniques and they concluded that apholate through an unknown mechanism suspends release of neurosecretory substance from pars intercerebralis of brain for three days during preovipositional period which causes an equivalent delay in the release of vitellogenic components of fat bodies and hence of the nutrients for vitellogenesis in the ovary are made available three days later in treated individuals. During this period growth of ovary gets retarded and some of the oocytes show signs of degeneration. The effect is the prolongation of the preovipositional period and reduction in the egg number. The observation in the present studies were recorded only for five days from the date of emergence of the adults. Changes in the ovary, fat body and haemolymph between fifth and eighth day (till oviposition in the treated insects) are not available and the degeneration of oocytes could not be observed. However the trend in changing content of protein, carbohydrate and lipid in fat body and ovary suggested that extracts of *T.neriifolia* act in a manner similar to that of apholate in *D.koenigii*.

Neem seed extracts and azadirachtin isolated from it are known to have high chemosterilant effect on a variety of insects. Rembold and Sieber (1981) studied the mechanism of action of azadirachtin on *Locusta migratoria* and observed that the substance inhibits vitellogenesis and activation of ovaries, both of which are known to be controlled by juvenile hormone. Schulz (1981) studied the effect of neem kernel extract on the ovarian development of *E.varivestis* and observed that along with the pathological changes of trophocytes, prefollicular cells and follicular epithelium,oogenesis and vitellogenesis were disturbed by

the treatment . Inspite of the intensive work done on the effect of neem products on insects the exact mechanism by which sterilisation is caused in different species of insects is not yet fully known.

In *D.cingulatus* treated with leaf (20 per cent) and seed (5 per cent)extracts using ethanol as solvent the reduction in fecundity was around 50 per cent compared to control. Similar reductions in the egg number was noted for acetone and benzene extracts earlier by Saradamma (1989). The eggs laid by insects treated with benzene extracts had a very low percentage of hatching particularly in higher doses while acetone extracts also affected hatching percentage significantly. Ethanol extract did not influence the hatching of eggs significantly. Obviously leaf and seed extracts of *T.neriifolia* in different solvents affected oogenesis and vitellogenesis of *D. cingulatus* to varying levels. For a proper understanding of this phenomenon the mode of interference in the reproductive physiology of insects have to be elucidated by further indepth studies.

It is well known that the constituents of vitellogenin synthesised in the fat bodies of insects pass into haemolymph and get transported to the ovary through the haemolymph. These pass through the interfollicular spaces (Sahota, 1970; Sahota and Ibaraki, 1973; Highnam, 1979, Gilbert *et al.*, 1980; Riddiford, 1980) and enter the oocyte by micropinocytotic process (Loof, 1971). Among the nutrients appearing in this route, vitellin occurs in female insects only. The production, transportation, passage and absorption by the oocytes are known to be under the control of juvenile hormone secreted by corpus allatum. Juvenile

hormone also activate synthesis of haemolymph proteins which originate in the fat body (Riddiford, 1980). In *D.cingulatus* as in many other insects the median neurosecretory cells (MNSC) of pars intercerebralis also has a decisive role in protein synthesis either directly influencing fat body or through a trophic effect on corpus allatum(Jalaja *et al.*, 1973; Jalaja, 1974; Raji and Muraleedharan,1985). Since the direct effects observed in the size and shape of the ovarioles and on the remaining parts of the reproductive system in *D.cingulatus* females treated with leaf and seed extracts of *T.neriifolia* was slight, the observed reduction in fecundity might be due to the disturbance in oogenesis and vitellogenesis through a direct effect on the ovary and fat body or by a disturbance of the MNSC and/ or corpus allatum activity. The exact reason could not be fixed from the observations.

5.6. Insecticidal action of seed and leaf extracts of *T neriifolia* on *D.cingulatus*

As shown in para 4.4. the insecticidal effects of the leaf and seed extracts of *T.neriifolia*, through a contact action, on *Spodoptera.litura* and *H.vigintioctopunctata* was meagre. When sprayed at a high dose, mortality in *S.litura* ranged from 3.3 to 18 per cent. and in *H. vigintioctopunctata* it ranged from 2.5 to 22.5 per cent only. Obviously, in the field the performance will be much lower. Of the different plant parts tried, seed was most toxic and it was closely followed by fresh leaf. Dried leaf in most treatments was seen totally ineffective. Among the solvents ethanol, methanol and water were almost alike compared to hexane, benzene and acetone.

In bioassay studies Saradamma (1989) also observed that dried leaf extract of *T.neriifolia* using different solvents showed low toxicity to *S.litura* and *D.cingulatus* and the extent of mortality obtained was not sufficient even to assess the LC_{50} values. It was however toxic to *Aphis craccivora* and in this insect the LC_{50} was 34 to 93 per cent in extracts using different solvents. Stomach and systemic toxicity were reported for *T.neriifolia* extracts to European corn borer, (Freedman *et al.*, 1979), A. vittatum (Reed *et al.*, 1982) and *D.cingulatus* (Saradamma, 1989). This aspect has to be investigated further. In general the scope for using *T.neriifolia* as a contact insecticide under field situations was found low.

5.7. Bioefficacy of the different fractions of ethanol extracts of fresh leaf and seed of *T.neriifolia*.

The data on this aspect are presented in para 4.5. . The investigations were taken up with a view to identifying the number and nature of active ingredients in the ethanol extracts of leaf and seed of *T.neriifolia* which showed significantly high antifeedant activity. In TLC separation, six spots were detected and they were isolated as six fractions in a chromatographic column using a mixture of petroleum ether and ethyl acetate in which the concentrations of the latter were 10, 20, 30, 40, 50 and 100 per cent respectively. Observations in TLC indicated the active ingredients to be glycosides. The isolation, identification and characterisation of the glycosides of *T.neriifolia* have already been carried out extensively from the pharmacological angle (Gattefosse, 1949; Voigtlaender *et al.*, 1969; Arora and Rangasami, 1967; Sticher, 1970; Osisiogu,

1975; McLaughlin *et al.*, 1980; Abe *et al* ., 1992; Siddiqui *et al.*, 1992; Perez *et al.*, 1993; Begum *et al.*, 1993 and Johri *et al.*, 1994). Among those identified, thevetin (Gattefosse *et al.*, 1949; Johri *et al.*, 1994) and neriifolin (McLaughlin *et al.*,1980; Freedman *et al.*, 1979) were established to have insecticidal and antifeedant properties. In the light of the vast information available the present studies were restricted to the assessment of antifeedant properties of different fractions using *H.vigintioctopunctata* as test insect.

The results showed that fractions IV, V and VI extracted with the solvents containing 40,50 and 100 per cent of ethyl acetate had high antifeedant effect and they were on par at the higher concentrations of one per cent level and at 0.5 per cent level, fraction VI was more effective than the remaining fractions. The antifeedant effect of fractions I, II and III as well as EToAC wash were very low.

On TLC separation the seed extract revealed only four spots corresponding to the spots of fractions I,II,IV and VI. They were extracted in the solvent mixtures and were bioassayed for antifeedant activity at 0.5 per cent level. Fractions V and VI gave 100 per cent leaf protection indicating the higher content of the active ingredient in seed than in leaf.

On the basis of larval starvation fractions IV,V and VI of leaf extract were found to be on par and highly effective while fraction V and VI of the seed extract also caused 100 per cent larval starvation.

The results indicated the high efficacy of the fractions IV,V and VI in leaf and V and VI in seed.The polar nature of the active ingredients involved

in the antifeedant and larval starvation effects of ethanol extract of leaves of *T.neriifolia* was also evident.

5.8. Effect of leaf and seed extracts of *T. neriifolia* on *Chrysocharis johnsoni*, the predominant parasitoid of *H.vigintioctopunctata* in Kerala

C.johnsoni is the major parasitoid effectively associated with the field population of *H.vigintioctopunctata* in Kerala. At field doses of fresh leaf, dried leaf and seed in ethanol and water, the extracts did not reduce the emergence of the parasitoid significantly when treated grubs were exposed immediately, 24 and 48 hours after spraying. Even with emulsions of higher concentrations the number of parasitoids emerging from fresh leaf and dried leaf extracts ranged from 6 to 17 as against 15 to 17 in control. However at higher doses of seed extracts the parasite emergence was 0 to 3 per grub only. It can thus be concluded that ethanol and water extracts of the plant at lower doses did not interfere with the parasitization of *H.vigintioctopunctata* grubs or subsequent development of the parasitoid inside the host. The result also revealed that ethanol and water did not significantly vary in their influence on the extent of parasitization of *C.johnsoni* on *H.vigintioctopunctata* (vide para 4.6.1).

Results presented in para 4.6.2. also showed that the direct exposure of the parasitoids to dry film of spray fluid of leaf extracts caused only 0 to 10 per cent mortality of *C.johnsoni* and even with 50 times higher dose the mortality was below 20 per cent. With seed extract, the contact toxicity at field dose ranged from 17 to 27 and at higher dose it ranged from 40 to 50 per cent. The level of contact toxicity observed in the laboratory did not indicate possible

adverse effect of the plant extract on the population of the parasitoid under field situations.

Botanicals are generally considered to be safe to natural enemies. The safety of neem and neem products to parasitoids and predators of major crop pests has been reported extensively (Saxena *et al.*, 1981a; Joshi *et al.*, 1982; Wu, 1986., Mansour *et al*., 1987; TNAU, 1992; Patel and Yadav, 1993; Bandara and Kudagamage, 1993). Extracts of other plants like *Eucalyptus sp.* (Tewari and Moorthi, 1985), *Catharanthus sp.* (Shanti and Janardhanan, 1991), *Tagetes erecta* (Mahima Shanthi and Mohana Sundaram, 1992) and *Acorus calamus* (Srinivasa Babu *et al.*, 1993) have been reported to be safe against parasitoids and predators of different crop pests. The results of the study indicated that botanicals are not fully safe to natural enemies. However they are much safer than synthetic organic chemicals. Hence prior to the recommendation of a botanical pesticide for field use, its safety to the natural enemies of the major pests should also be assessed.

5.9 Mammalian toxicity of leaf and seed extracts of *T.neriifolia*

Acute toxicity data presented in para 4.7.1. revealed that leaf extract did not cause any mortality of the mice upto 1000 mg/kg body weight while seed extracts caused 50 per cent mortality of the test population at 400 mg/ kg body weight. Even the surviving animals were sluggish in behaviour.

Acute toxicity of different phytochemicals have been reported earlier. The LD_{50} of usaramine, an alkaloid isolated from *Crotalaria brevifolia*

was reported to be 300 mg/kg body weight in mice (Singh *et al.*, 1969). Bhakuni *et al.* (1969) observed that the LD_{50} of 50 per cent ethanol extract of seeds of *T.peruviana* in mice was 500 mg/kg body weight. LD_{50} of physalin isolated from *Physalis minima* was found to be 2g/kg body weight (Mohana *et al.*, 1979). In general, *T.neriifolia* can be placed on par with plants which are being investigated as potential source of botanical pesticides.

Chronic toxicity data presented in para 4.7.2.1. showed that rats treated with 800 mg/kg body weight of *T.neriifolia* leaf extract did not show increase in body weight and those treated with 400 mg/kg body weight of seed extract lost 2.5 per cent of body weight while in control the body weight increased by 7.5 per cent in 14 days after treatment. With reference to the ratio of weight of liver, kidney and heart to body weight the treated animals did not show any significant difference from the untreated ones.

Extracts of *Adhathoda vasica* (Pahwa *et al.*, 1987) and *Balanite roxburghii* (Shah *et al.*, 1994) did not cause any loss in body weight even when fed with extracts for 90 and 30 days respectively. But 200 mg/kg body weight of *Ocimum sanctum* extract, a plant known for its safety and medicinal properties was found to cause weight loss in rats (Khanna *et al.*, 1986).

As seen in para 4.7.2.2. and Plates I and II, clear vacuolation and cytoplasmic acidophilia of the tissues of liver and heart of seed extract treated rats were evident at the end of 14 days. In the kidney, perirenal fatty tissues showed dense inflammation.

Pahwa and Chatterjee, (1990) reported inflammatory and degenerative changes in the liver and kidney of rats fed with crushed ground seeds of *Thevetia* spp. when fed at 20 and 30 per cent concentrations for 10 days. Chronic administration of neem oil to adult albino rats for eight days showed microscopic lesions in liver and kidney (Badri Srimannarayana *et al.*, 1993). But histopathological examination of major organs of rats and monkeys treated with vasicine isolated from *A.vasica* for 6 months did not reveal any abnormality (Pahwa *et al.*, 1987).

As seen from para 4.7.2.3. among haematological parameters, seed extract alone caused a significant reduction in packed cell volume. A fall in the count of RBC, platelets and lymphocytes was observed in leaf and seed extract treatments. But such reductions were observed in control also and hence cannot be attributed to the effect of treatments.

Significant reductions in red blood cell count, total leukocyte count and neutrophils and increased lymphocytes were observed in *Rattus rattus* fed with 20 and 30 per cent concentrations of crushed seeds for 10 days (Pahwa and Chatterjee, 1990). Chronic administration of neem oil (a plant product acceptable as perfectly safe for human consumption) for eight days showed marked reduction in haemoglobin (Badri Srimmannarayana *et al.*, 1993). GuptaMalaya *et al.* (1994) reported that low and moderate dose of *Clerodendron colebrookianum* (20 to 40 mg/kg) leaf extract did not affect haematological parameters.

Among the biochemical parameters (para 4.7.2.4), haemoglobin level was lower than the permissible limits in seed extract treatment alone. Serum cholesterol and SGOT levels were below minimum permissible levels in both the treatments. The level of SGPT was lower in both the treatments. But it was on par with control. The adverse effects observed in haematological and biochemical parameters were marginal in both the treatments.

Pahwa and Chatterjee, (1990) observed significant reductions in blood glucose and serum proteins and increased BUN, AST and LDH in rats fed on crushed seeds of *Thevetia* spp. Blood glucose, serum proteins, transaminases and serum cholesterol were significantly reduced in albino rats administered with neem oil for 8 days (Badri Srimannarayana *et al.*, 1993). Sharma and Mahanta (1995) reported that extracts of *Plumbago rosea* and *Shorea robusta* significantly altered the total glycogen protein and RNA content in uterus tissue of rats.

It is seen from the present investigation and earlier reports that plant extracts of *T.neriifolia*, as well as those plants known for their safety to human beings are capable of causing adverse effects on higher animals. Obviously the relevance of the data in evaluating botanical pesticides for plant protection purposes will depend on the assessment of the persistence of the extracts on treated plant parts and in the ecosystem. Since the active ingredients involved in each plant extract are many in number, their characterisation and the standardisation of techniques for residue estimation will be too laborious and expensive. It is seen in field experiments that the bioactivity of the sprayed

extracts under field situation drops drastically within a week. Obviously the residue hazards from *T.neriifolia* extracts under field situation will be low. It is also observed that leaf extract of *T.neriifolia* is comparatively safer than seed extract and the latter also can probably be used at lower doses without adverse effect on non target organisms and the ecosystem.

5.10. Field evaluation of extracts of *T.neriifolia* for the control of pests of bittergourd.

The objectives of the field experiment were to assess the efficacy of fresh leaf, dried leaf and seed extracts of *T.neriifolia* for the control of pests of bittergourd and to assess the effect of treatments on the extent of crop damage and yield. The comparative efficacy of water and ethanol used for the extraction of the plant parts was also envisaged.

One week after spraying, egg mass number in all treatments were on par and significantly lower than in control. In all the subsequent observations, carbaryl ranked top among the treatments with no eggmass in the plots and it was closely followed by ethanol and water extracts of seed. These were followed by water and ethanol extracts of fresh leaf and ethanol extract of dried leaf. Water extract of dried leaf was least effective. In general, water was found to be as good a solvent as ethanol for reducing egg laying of *H.vigintioctopunctata* on bittergourd. The lower number of eggs in treated plots might be due to the larval starvation caused by antifeedant, juvenomimetic and/or chemosterilant action of the plant extracts (vide para 4.8.1.1).

With reference to the grub population presented in para 4.8.1.2. all the treatments were found significantly superior to control. Seed extract was the best treatment for controlling the grubs and it was closely followed by fresh leaf and dried leaf was least effective. As extractants, ethanol and water were found equally effective. While there was a fluctuating population in the control plots, the grub population in all the treatments were continuously declining during the eight week duration of the experiment. During the period, pest population in carbaryl treated plots remained almost at zero level. A gradual check of the pest population through larval starvation, juvenilising and sterilising effects on the pest was thus evident in the result.

Overall effect of the treatment on the number of pupae of *H.vigintioctopunctata* observed at different intervals revealed the growth inhibitory effect of *T.neriifolia* extract on the pest populaton. The number of pupae was much lower in treatments than in control, the variations among the different treatments being statistically insignificant.

The extent of leaf damage observed in different treatments (vide para 4.8.1.4.) also revealed the efficacy of *T.neriifolia* extracts in protecting bittergourd against *H.vigintioctopunctata*. In all the observations, reduced leaf damage was seen in the treatments compared to control. The least damage was noted in carbaryl treated plots and it was significantly less than the damage in other treatments. In general, seed ranked top among the plant parts and it was followed by fresh leaf and dried leaf, the differences among the three treatments being statistically significant. Though the pest population in carbaryl treated plots

was remaining near 'zero' level in all observations, leaf damage was seen on these plants too. Leaf protection obtained in the treated plots, was the cumulative effect of the lower pest population and the leaf protection given by the antifeedant effect of the treatments.

Effect of different treatments on the infestation of fruits by fruit fly was evident in the reduced number of damaged fruits in treated plots. Best control was obtained with carbaryl and it was closely followed by seed extract and the latter by fresh leaf extract. Dried leaf extract was less effective. The two solvents were performing equally well with all the plant parts (vide para 4.8.1.5).

The extent of reductions in the population of *H.vigintioctopunctata* and on the leaf damage were reflected in the yield of the crop. The maximum number of fruits was obtained from carbaryl treated plots and this was on par with seed and fresh leaf extract treatments while the dried leaf extract was on par with control. The two solvents were on par. On weight basis, the dried leaf extracts (ethanol and water) were inferior and on par with control. Highest yield was obtained in ethanol extract of seed treated plots. The remaining treatments were on par. On cost benefit basis, water extract of fresh leaf and seed were found to be on par with carbaryl and could be ranked high.

Data presented in para 4.8.2 showed that the level of parasitisation of eggs and grubs of *H. vigintioctopunctata* in treated plots was on par with that of control while in seed extract treated plots it was low and totally absent in carbaryl treatment. While the low parasitoid activity in carbaryl treated plots was due to its direct toxic effect, in seed extract treated plots, the low activity may be due to low host density consequent to the antifeedant effect of the treatment.

Benzene and water extracts of *T.neriifolia* were evaluated against *H.vigintioctopunctata* infesting brinjal and bittergourd by Saradamma (1989). She found that two per cent emulsion of 25 per cent stock extract of dried leaf kept the population effectively under control for one week. This was a microplot study. Assessment of the performance of the extracts of *T.neriifolia* through a replicated field experiment was being done for the first time. The evaluation of different plant parts, as potential source of insecticide, also was included in the treatments and this aspect also had not been studied earlier. Data obtained from the two seasons showed the high efficacy of fresh leaves and seeds of *T.neriifolia* as a source of botanical pesticide for the control of *H.vigintioctopunctata* on bittergourd and to a limited extent the fruit fly,*Dacus cucurbitae*. On cost benefit basis the treatments were found highly attractive to the farmers.

5.11. Control of pests of amaranthus with leaf and seed extracts of *T.neriifolia* in the field.

Results presented in para 4.9.1 showed that the population of leaf webber catepillars observed in the check treatment (malathion 0.1 per cent) and in plots treated with different plant extracts were significantly lower than that of control. Maximum reduction was in insecticide treated plots and it was closely followed by seed and fresh leaf extracts. Dried leaf extract was least effective among the treatments in all the observations. In the case of leaf extracts, ethanol was found significantly superior to water as an extractant and in the case of seeds both the solvents were on par. Though variations in the data were statistically significant, the levels of infestations in the two treatments were very low for a conformation of this finding.

The leaf damage caused by the pest para (4.9.2) also was seen significantly reduced in all the treatments, compared to control. All treatments were on par at the fourth week after planting. But in the subsequent observations the seed and fresh leaf extracts came on par and significantly superior to dried leaf extract treatment. Ethanol and water did not show significant variations in the extent of leaf damage.

On the basis of yield, ethanol extracts of seed and fresh leaf proved as good as insecticide check while water extracts of seed and fresh leaf were on par with water and ethanol extracts of dried leaf. On cost benefit basis water extracts of fresh leaf and seed ranked higher than the rest of the treatments.

The field performance of dried leaf extract of *T.neriifolia* was evaluated earlier in a field experiment against the pests of amaranthus (Srinath,1990). It was observed that two and four per cent emulsion of eight per cent stock extract of dried leaf protected the crop from the attack of *Attracotomorpha crenulata* and *Psara basalis* and the treatments gave significant increase in yield. In the present investigation too the dried leaf extract was found to give significant control of the leaf webbers of amaranthus. But the fresh leaf and seeds of *T.neriifolia* were found significantly superior to the dried leaf.

From the two field experiments conducted for two seasons it may be concluded that *T.neriifolia* is a potential source of botanical pesticide. The control of the pest population observed with the seed and fresh leaf extracts, though inferior to the standard insecticide check, was adequate to reduce the

population effectively and to boost the yield. This attribute render the plant source as an ideal substitute of chemical pesticides in an integrated pest management concept where the reduction of pest population is the aim rather than the total eradication of the species from the ecosystem. Since botanicals are known to be more selective than synthetic insecticides in action the efficacy of the plant extracts against the different species of pests in the ecosystem have to be studied in detail for exploiting the plant for plant protection purposes. The mammalian toxicity observed, particularly with seed extract of *T.neriifolia* indicates the necessity for further toxicological studies, for confirming the safety of the products to human health and to the ecosystem. Low persistence of the plant extracts under field situations limits the possible hazards from these plant products, in comparison with any of the available synthetic insecticides and hence a provisional utilisation of the leaf and seed extracts of *T.neriifolia* for pest control programme can be considered favourably.

SUMMARY

SUMMARY

The quest for fast growing and easily maintainable indigenous plant species with pesticidal properties for inclusion in integrated pest management practices led to the identification of several potential plants. *Thevetia neriifolia* is one such plant reported from Kerala. Fresh and dried leaves and seed of the plant were extracted with different solvents (acetone, benzene,ethanol, hexane, methanol and water) and assayed for antifeedant, juvenomimetic/sterilant and insecticidal activities on different test organisms. The bioactive principles in ethanol extract of seed and fresh leaf were fractionated chromatographically and their antifeedant and larval starvation effects were bioassayed using *Henosepilachna vigintioctopunctata* as test organism. Toxicology of the seed and leaf extracts was also studied. The efficacy of the crude extracts of the plant for controlling major pests of bittergourd and amaranthus was studied in two seasons under field conditions.

The major findings of the investigations are summarised below:

1. Based on the percentage of leaf protection in bittergourd against *H.vigintioctopunctata*, seed extracts were found significantly superior to leaf extracts, fresh and dried leaves being on par. With reference to larval starvation, seed extracts were found superior to leaf extracts and fresh leaf extracts were better than dried leaf extracts.

2. Response to the extracts of different plant parts based on leaf protection and larval starvation were broadly the same and hence any one of the two criteria may be a reliable index of antifeedant potential of plants.

3. Among the solvents tested, ethanol and methanol were found significantly superior to water, benzene and acetone based on percentage of leaf protection while methanol, ethanol and water came on par with reference to larval starvation. On cost and efficacy basis water is to be chosen as the best solvent for extracting antifeedant principles from different parts of *T.neriifolia* plants.

4. Soaking powdered plant materials in solvents for 48 hours and filtering the extract was as good as soxhlet extraction in the recovery of bioactive principles of *T.neriifolia*.

5. Different extracts of *T.neriifolia* plants showed varying juvenomimetic/sterilant effects on *D.cingulatus*. The post treatment mortality of fifth instar nymphs of *D.cingulatus* was high in seed extract followed by fresh leaf. The response was dose dependent.

6. Post treatment duration of nymphs was prolonged significantly. The effect was dose dependent, the middle and higher doses of leaf being on par with corresponding doses of seed.

7. Number of abnormal adults, though generally low in all the treatments, showed a dose dependent response. Leaf extract 40 and 20 per cent and seed extract 5 per cent resulted in significantly higher number of malformed adults.

8. The preoviposition period was prolonged significantly in leaf and seed extract treated insects, the response being higher in 20 per cent leaf and 5 per cent seed extract treatments. But the interovipositing periods were not affected by the treatments.

9. Fecundity of insects exposed to 40 and 20 per cent leaf extracts and 5 per cent seed extract was reduced significantly. The longevity of emerging adults, incubation period and hatching of eggs were not affected.

10. Post treatment nymphal mortality, reduction in the number of normal adult emergence and lower fecundity resulted in a drastic reduction in the total number of eggs obtained in the succeeding generation.

11. Lengthening of ovarioles and development of oocytes during the pre-oviposition period was seen delayed.

12. Fluctuations in protein, glycogen and lipid content of the ovary, fat body and haemolymph during preoviposition period in treated and control insects revealed that the plant extracts delayed vitellogenesis and consequent egg maturation. Pre-oviposition period was also correspondingly extended. This is a typical chemosterilant effect. But juvenomimetic effect on the neurosecretory cum corpus allatal control of vitellogenesis is also indicated.

13. Contact insecticidal action of the extracts was very low on *S.litura* and *H.vigintioctopunctata,* even a very high dose of 40 per cent giving less than 50 per cent mortality.

14. Chromatographic isolation of ethanol extract of fresh leaf and seed yielded six active fractions from the leaf and four from the seed. Fractions III and IV were absent in seed. Fractions IV, V and VI showed high antifeedant activity and larval starvation. The three fractionswere broadly on par. The study indicated the involvement of a number of active ingredients in causing antifeedant effect on *H.vigintioctopunctata.* Higher activity of the seeds might be due

to higher quantity of the available active ingredients and not any principle specifically available in seeds.

15. Water and ethanol extracts of leaf and seed did not adversely affect the level of parasitization or development of *Chrysocharis johnsoni*, an important parasite of *H.vigintioctopunctata*. Even fifty times higher concentrations of the field dose did not prevent parasitization though there was some reductions. For field application leaf and seed extracts were found fully safe.

16. Acute toxicity studies on albino mice indicated that seed extract at doses above 400 mg/kg body weight was toxic to the animals. Leaf extract did not show any toxicity even at the dose of 1000 mg/kg body weight.

17. Continuous feeding of rats with 400 and 800 mg/kg body weight of seed and leaf extracts respectively did not result in any gain in body weight in leaf extract treated rats while 2.5 per cent loss was recorded in seed extract treated ones. Control animals recorded 7.5 per cent increase in weight during the period.

18. Histopathological studies showed vacuolation due to fatty changes and cytoplasmic acidophilia in the heart and liver tissues and

severe inflammatory changes in the kidney of seed extract treated animals.

19. Haematological parameters like packed cell volume and biochemical parameters like haemoglobin, SGOT and serum cholesterol were significantly low in seed extract treated animals while in leaf extract treated animals, SGOT and serum cholesterol were reduced. From the above results and literature on toxicological studies on plant extracts reviewed, it is evident that botanicals are not fully safe for plant protection purpose. But the data have to be examined in relation with the field persistence of plant extracts and the residue levels on treated plants. Since their persistence is very low, the hazard is likely to be meagre. Still it has to be investigated in detail.

20. Replicated field experiments conducted for two seasons showed that the number of egg masses, grub and pupal population of *H.vigintioctopunctata* and extent of leaf damage caused by the pest were significantly reduced by ethanol and water extracts of seed, fresh leaf and dried leaf. Ethanol and water were on par in relative efficacy. Two season replicated experiments on amaranthus proved that seed, fresh leaf and dried leaf extracts were effective in controlling leaf webber caterpillars, the dried leaf being less

effective in relative efficacy. Seed extract was almost on par with insecticide. It may be possible to bring fresh leaf also on par by increasing the dose since it was observed that the effects of the treatments were dose dependent. Water was found to be as good as ethanol for extraction of different plant parts.

T.neriifolia is a potential source of promising botanical pesticide. The control of the pest population observed with fresh leaf and seed extracts, though inferior to the standard insecticide check, was adequate to reduce the pest population effectively and to boost the yield. They can fit well in integrated pest management strategies.

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* Originals not seen

ABSTRACT

Fresh and dried leaves and seeds of *Thevetia neriifolia* were evaluated for their antifeedant activity against *Henosepilachna vigintioctopunctata*, using acetone, benzene, ethanol, hexane, methanol and water as extractants. Based on the percentage of leaf protection, seed extracts were superior to the leaf extracts. Fresh leaf and dried leaf extracts were on par. Among the solvents, ethanol and methanol gave maximum leaf protection closely followed by water. Based on larval starvation, seed extracts were superior to leaf extracts and fresh leaf extracts were significantly better than dried leaf extracts. Water, methanol and ethanol were on par for extracting plant tissues.

Soaking of powdered plant material well in solvents for 48 hours and filteration was found as effective as soxhlet method for extracting antifeedant components from leaves and seeds of *T.neriifolia*.

Leaves and seeds of the plant showed conspicuous hormonal/ sterilant activity on *Dysdercus cingulatus* and the response was dose dependent. This was evident in post treatment larval mortality, prolonged preovipositional period, emergence of malformed adults and reduced fecundity. Adult longevity, incubation period and hatching percentage of egge were not affected. Forty per cent and ten per cent of leaf and seed extracts as well as 20 and 5 per cent extracts of the same came on par in juvenomimetic effect. The results showed that seeds were four times more effective than the leaves in their juvenomimetic effect on *D.cingulatus*.

The length of ovary, number of oocytes per ovariole and size of basal, penultimate and antepenultimate oocytes were significantly lower in seed and leaf extract treated females of *D.cingulatus*. Closer examination of the data revealed that growth of ovary was delayed rather than suppressed by the plant extracts.

Levels of protein, glycogen and lipid contents observed in ovary, fat body and haemolymph of treated and untreated insects from first to fifth day after emergence indicated a delay in vitellogenesis and oocyte development in treated insects. Consequently preoviposition period was prolonged. The effect observed is similar to the effect of chemosterilants reported on some insects earlier. Results indicated that reduction in fecundity of *D.cingulatus* may be due to the chemosterilant action of *T.neriifolia* extracts.

Bioassay studies in the laboratory revealed the low contact toxicity of leaf and seed extracts of *T.neriifolia* to *S.litura* and *H.vigintioctopunctata*. Chromatographic isolation of different fractions of ethanol extracts of fresh leaf and seed yielded six bioactive fractions from the leaf and four from the seed. Fractions III and IV were absent in seeds. Fractions IV, V and VI obtained from leaves and V and VI from seeds had far higher activity than the remaining fractions and they were on par in their antifeedant effect and larval starvation on *H.vigintioctopunctata*. Available active fractions were more concentrated in seeds than in leaves. Laboratorty studies showed the safety of leaf and seed extracts of *T.neriifolia* at field doses to *Chrysocharis johnsoni*, the most important parasite of *H.vigintioctopunctata* in Kerala.

Toxicological studies in albino mice and rats revealed that 400 mg/kg body weight was the LD_{50} dose of seed extract of *T.neriifolia* while doses upto 1000mg/kg body weight of leaf extract was not toxic to the animals.

Haematological picture of animals chronically exposed to the extracts showed marginal deviations in the haemoglobin content, RBC count, serum cholesterol, SGOT and SGPT levels in seed extract treated animals from the permissible safe range. Leaf extract treatment caused deviations in SGOT and serum cholesterol.

Vacuolation, cytoplasmic acidophilia and degenerative changes in the liver and heart tissues and inflammation of kidney were observed in animals treated with seed extract. Adverse effects of leaf extract were negligible.

Replicated field experiments were conducted for two seasons on bittergourd and amaranthus to evaluate the efficacy of crude extracts of *T. neriifolia* in controlling their major pests. All the extracts viz., ethanol and water extracts of dried and fresh leaves and seed reduced the pest population and the extent of leaf damage. Seed extract was on par with the insecticide check, carbaryl (0.15 per cent) in bittergourd and malathion (0.1 per cent) in amaranthus. Fresh leaf extract was on par with the seed extract and was superior to extracts of dried leaf in reducing pest population and increasing yield of both the crops. Water extracts of leaves and seed were found equally good for checking the pest population and increasing yield. On cost benefit basis water extracts were found more advantageous to the farmer. Parasitization of egg masses, grubs and pupae in treated plots were on par with control while they were totally missing in the

insecticide treated plots. Extracts of *T.neriifolia* were thus found to be safe to the non-target organisms too.

An overall assessment of the results obtained revealed that water extracts of fresh leaf and seed in appropriate doses can be effectively and safely used for the control of important pests of bittergourd and amaranthus without any health hazards and adverse effect on the non target organisms in the agroecosystem. *T.neriifolia* can be considered as a plant suited for developing effective plant protection chemicals for replacing undesirable synthetic insecticides, especially in integrated pest control calender.