STUDIES ON THE EFFECTS OF PHYTOCHEMICALS IN THE EXTRACTS OF *VITEX NEGUNDO* LINNAEUS (VERBENACEAE) ON THE STORED PRODUCT PEST, *TRIBOLIUM CASTANEUM* HERBST (COLEOPTERA : TENEBRIONIDAE)

Thesis
submitted to the University of Calicut
in partial fulfilment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY
in Zoology

By

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Dedicated

To *my whole family,*

Who dreams a lot for each step of my achievements . . . .

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CERTIFICATE

This is to certify that this thesis entitled **"Studies on the Effects of Phytochemicals in the Extracts of *Vitex negundo* Linnaeus (Verbenaceae) on the Stored Product Pest, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)"** is a bonafide record of work done by Mr. P. Haridasan, in the Laboratory of Insect Physiology and Biochemistry of this Department, under my supervision and guidance. Further certified that no part of this thesis has been presented elsewhere for the award of any other degree/diploma.

(Dr. M. Gokuldas)

DECLARATION

I do hereby declare that this work has been originally carried out by me under the guidance and supervision of Dr. M. Gokuldas, Professor, Department of Zoology, University of Calicut, and that this has not been submitted elsewhere for any other degree or diploma or other similar titles.

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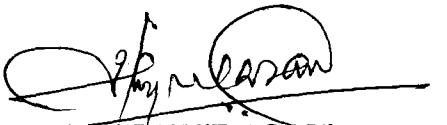

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ABBREVIATIONS

AEI	:	Adult Emergence Index
CC	:	Column Chromatography
conc.	:	concentration(s)
^{o}C	:	degree Celsius
ECI	:	Efficiency of Conversion of Ingested food
FDI	:	Feeding Deterrence Index
FID	:	Flame Ionization Detector
FRM	:	Film Residue Method
g	:	gram(s)
h	:	hour(s)
HPLC	:	High Performance Liquid Chromatography
IDCC	:	Inverted Dry Column Chromatography
IR	:	Infrared
LC	:	Lethal Concentration
mg	:	milligram
min	:	minutes
µl	:	microlitre
µm	:	micrometre
ml	:	millilitre
MS	:	Mass Spectrometry
NMR	:	Nuclear Magnetic Resonance
RCR	:	Relative Consumption Rate
RGR	:	Relative Growth Rate
r.h.	:	relative humidity
sp.	:	species
TAM	:	Topical Application Method
TLC	:	Thin Layer Chromatography / Chromatogram
UV	:	Ultraviolet
VME	:	*Vitex negundo* Methanol Extract
VPE	:	*Vitex negundo* Petroleum ether Extract

STATISTICAL NOTATIONS

ANCOVA	:	Analysis of Covariance
ANOVA	:	Analysis of Variance
df	:	degrees of freedom
F	:	F-ratio
MS	:	Mean Square
P	:	Probability
R	:	Regression coefficient
SD	:	Standard Deviation
SEM	:	Standard Error of Mean
χ^2	:	Chi-square
x	:	intercept of the regression line
y	:	slope of the regression line

Chapter 1

GENERAL INTRODUCTION

1.1. Pest control: Historical perspectives

Pest problems and actions to alleviate them are as old as human attempts to grow crops for meeting his food requirements. In order to combat the situation man has come up with various methods from magic and quackery to alchemy. Besides, from the early ages, rational pest control using crude chemical preparations has also been devised and put to use. The history of insecticides goes as far back as 2500 B.C. Sumerians knew sulphur to have acaricidal and insecticidal properties. In China, in about 1200 B.C., chalk and wood ash were used to control insects in enclosed spaces, plant extracts for treatment of stored grains and arsenic sulphide to control human lice.

Ancient Greeks and Romans utilized sulphur, fumigants, oil sprays, oil and bitumen, sticky bands, oil and ash and other preparations for insect control. Progress in the use of insecticides came with the introduction of botanicals, as pyrethrum, derris, quassia and tobacco leaf infusion around the 16^{th} century, but this may have been the extension of a usage, which may have originated thousands of years earlier. In Japan, whale oil was used to control insects in paddy. With the growth of knowledge, better ways were desirable and the principal interest began to focus on the use of various chemical concoctions for the control of insect pests.

1.2. Losses due to pests

Assessment of crop losses due to pests has been a difficult and often controversial subject. The average crop losses resulting from animals (mostly insects), diseases and weeds have been calculated to be as high as 42 per cent of the potential production of all crops and is quite high both in developed as well as in developing countries. In North America, Europe and Japan, losses are estimated to be in the range of 10-30 per cent, but in developing parts of the world, they are substantially higher (Edwards, 1986). Crop losses due to pests and plant diseases of the order of 40 per cent are common in these areas, and losses of as much as 75 per cent have been reported. Even greater and often more significant losses occur after the crop is harvested, which are caused by pests that attack the stored products, particularly in tropics (FAO, 1985).

Micro-organisms, fungi, insects, rodents, birds, temperature, humidity and storage structures are the factors which are responsible for reducing the qualitative and quantitative losses during grain storage. Annual post-harvest losses caused by insect damage, microbial deterioration and other factors are estimated to be in the order of 10-25% world wide (Mathews, 1993)

Hence, one of the challenges facing us today is to produce adequate food for the expanding population. In this pursuit to achieve the objective, we have to exploit all the available natural resources on this planet without disturbing the ecosystem.

1.3. Limitations of pesticide usage

Synthetic insecticides are primarily used for the management of the pests of field crops and stored products for years. While a triumph in science and technology, the development of modern pesticides has not been without its problems. Over time, excessive use of synthetic insecticides has resulted in serious problems including human pesticide poisoning, development of insect resistance to insecticides, insecticide-induced resurgence of insect pests, hazards of pesticide residues and the adverse effects on non-target organisms, namely, parasitoids, predators, honey bees, pollinators, fishes, birds, cattle and human beings. In addition, phytotoxicity, environmental pollution and an alarming increase in the cost of pesticides have dictated the need for effective and biodegradable pest control materials with greater selectivity.

1.4. Ecologically rational methods for pest control

For the past two decades, research on pest control approaches that are ecologically rational, yet effective, has received high priority worldwide. Innovative developments include use of microbial pesticides, breeding of resistant plant varieties, sterile male techniques, behavioural interruptions and the use of natural products to kill or regulate growth and behaviour of insects. Now-a-days, it is highly relevant to go for ecologically sound methods on the use of natural compounds in an integrated approach to pest management.

1.5. Occurrence of pesticidal plants

"Nature's chemical factories", the plants, offer an excellent source of biologically active natural products, which can limit insect populations. There are more than 3,08,000 plant species. How many of these have pesticidal property? Scanning of literature reveals that pesticidal plants reported so far are distributed in about 190 plant families. Grainge and Ahmed (1988) reported 2400 plant species as pesticidal (insecticide, acaricide, nematicide, fungicide etc.) distributed in these families. Singh *et al.* (1998) listed 10 important plant families viz., Asteraceae, Apocyanaceae, Euphorbiaceae, Fabaceae, Leguminosae, Myrtaceae, Rananculaceae, Rutaceae, Rosaceae and Meliaceae, where maximum number of pesticidal plants occur. Since then several new plants have been reported to possess high insecticidal activity and these are mainly concentrated in the plants of family Meliaceae which has more than 500 species. Isman *et al.* (1995) screened about 100 species either for antifeedant or growth regulatory activity or both and most of them showed high to very high bioactivity against insect pests.

The number of plants (2400) listed by Grainge and Ahmed (1988) seem to be far less than the actual number of naturally occurring pesticidal plants as this is just 0.77 per cent of the total of 3,08,000 plants or 0.87 per cent of 2,75, 000 species of flowering plants.

1.6. Role of phytochemicals

Plant chemicals can be classified as primary or secondary constituents, depending on whether or not they have an essential role in plant metabolism and

are universally present in all plants. Primary constituents include the common sugars, the proteins, the purines and pyrimidines of nucleic acids, the chlorophylls and so on. Secondary constituents make up all the remaining plant chemicals from alkaloids to terpenoids and acetogenins to phenolics. These substances do not appear to have an essential role in metabolism and vary in their distribution from plant to plant.

Most of the plant secondary metabolites have no recognised role in the maintenance of fundamental life processes in plants. However, they have a key role in protecting the plant from environmental pressures. They also act as plant growth substances, floral pigments and odours, anti-herbivore and anti-fungal agents. Similarly, some plants are also reported to contain chemical compounds having animal hormonal activity (e.g., silkworm moulting hormone, 20-hydroxyecdysone in the leaves of *Taxus buccata* and the fronds of the fern *Polypodium vulgare*; the mammalian hormones oestriol and oestrone in the seeds of the apple and the pomegranate) and establishing symbiotic associations (e.g., Lichen; legume-rhizobium interaction).

Some of the secondary metabolites derived from plants affect insect behaviour, growth, health or physiology and many possess abilities of repellence, feeding deterrence, toxicity or other adverse effects. Knowledge of the action of these chemical constituents in natural plant communities paves the way for development of new strategies for highly selective insect pest control models.

1.7. Advantages of plant derived pesticides

In search for safer and more congenial alternatives, attention has been focussed on botanicals. Plant derived insecticides have distinct advantages over organic synthetic insecticides. They do not leave poisonous residues in food chains and are more readily biodegradable. Therefore, they are less likely to contaminate the environment and may be less toxic to mammals and other useful organisms. They fit in well in a sustainable agriculture because of their renewability. Moreover, village co-operatives can take up the formulation of locally available plants and thus poor and marginalised farmers can save money spent on costly synthetic agrochemicals.

1.8. Plant products for storage pest management

Several pesticidal plant materials have been used for the control of storage pests as in the control of agricultural pests. It has been an age old practice in rural areas to mix dried neem leaves with grain meant for storage for long periods. Different parts of neem (*Azadirachta indica*) like leaves, seeds and bark from which oil cake and extracts are prepared have been reported to possess fungicidal, nematicidal, insecticidal, insect repellent and antifeedent properties (Ketkar *et al.*, 1976). Much work has been done on neem and its products and these are effective protectant against most of the stored product pests. Like storage pests, agricultural pests also have been controlled by neem and its products.

Besides neem, several other plants such as sweetflag, *Acorus calamus,* custard apples, *Annona squamosa*, *A. reticulata*, garlic, *Allium sativum,* turmeric,

Curcuma longa, fenugreek, *Trigonella foenum-graecum,* chillies, *Capsicum* sp., black pepper, *Piper nigrum*, ajuba, *Adhatoda vasica* etc., have been extensively used against a variety of insect pests.

1.9. Relevance and objectives of the present investigation

Majority of grain storing farmers are not fully aware of the utility of plant products for the purpose of grain protection. There is a necessity for finding effective and locally available plant products for grain protection, which is stored in small quantity for small duration. The active principles of insecticidal or repellent compounds having the grain protecting property are to be investigated in detail so that active chemical components can be characterised before they are synthesized and marketed. In addition, farmers need to be motivated to use plant products for grain protection because of several advantages including the safety of the consumers.

These considerations led us to investigate the possibility of using the local, abundantly available plant, *Vitex negundo* against the stored product pest, *Tribolium castaneum*. This study, therefore, explores the management of stored product pests with indigenous plant materials in an innocuous way.

Present investigations cover the following aspects:

i) Assessment of repellent effects of petroleum ether and methanol extracts of *Vitex negundo* (VPE and VME) against *T. castaneum* adults.

ii) Determination of nutritional and feeding deterrence indices for *T. castaneum* adults to these extracts.

iii) Assessment of the effects of the extracts on emergence, development and progeny reduction in *T. castaneum*.

iv) Assessment of the effects of the extracts on adult emergence.

v) Evaluation of toxicity of the extracts against *T. castaneum* adults.

vi) Thin layer chromatographic separation of the extracts of *V. negundo* and evaluation of toxicity of various fractions against *T. castaneum*

vii) Identification and analysis of the toxic chemical constituents of extracts of *V. negundo*..

Chapter II

REVIEW OF LITERATURE

Now-a-days indigenous plant materials, plant products and chemical constituents are found to attract much attention of many who are involved in the insect pest management. These materials act as repellents, antifeedants, toxic/insecticidal, ovicidal and grain protectants against various agricultural and stored product insects. Moreover, they have some adverse effects on reproduction, development, growth, oviposition, egg hatching and other biological activities. Various aspects of activities of such plant derivatives on various insect pests are briefly reviewed as follows.

2.1. Repellent activity

Chemicals that prevent insect damage to plants or animals by rendering them unattractive, unpalatable or offensive are called repellents. These substances act as stimuli that elicit "avoiding reaction" in the insects. Leaves, flowers, seeds, barks, stems, rhizomes, roots, oils etc. from various plants have been reported to possess repellent properties against insects.

2.1.1. Plant materials/extracts

Jilani and Su (1983) reported that plant materials such as rhizomes of *Curcuma longa,* and leaves of *Azadirachta indica* and *Trigonella foenum-graecum* were showing repellent activity against adults of *T. castaneum*, *Sitophilus granarius*, and *Rhizopertha dominica.* They found that turmeric powder was the most effective among the three against *S. granarius* and *R. dominica* and also the

most effective of the solvent extract against *T. castaneum.* Similarly, Malik and Naqvi (1984) have screened seven indigenous plants for their repellent activity against *T. castaneum* and their studies suggested that *Saussurea lappa* possessed the best repellent property. Likewise, repellent properties of extracts of 19 local plants against *T. castaneum* (Qureshi *et al.*, 1988), neem extracts against the larvae and adults of *Spodoptera litura* (Ayyangar and Rao, 1989) and *Melia toosendan* seed kernel extract against *T. castaneum* (Xiong and Deng, 1992) had been extensively studied.

It was seen that powder and vapour of turmeric were effective repellents against adults and larvae of *T. castaneum* (Parveen and Mondal, 1992). Similarly, the repellency of ground leaves, bark, seeds and four different seed extracts of pithraj, *Aphanamixis polystachya* against *C. chinensis* (Talukder and Howse, 1994) and *T. castaneum* (Talukder and Howse, 1995) were reported. Leaf dust of *Solanum xanthocarpum* (Hussain, 1995) and extracts of *Polygonum hydropiper* and *Annona squamosa* also acted as repellents against adults of *T. castaneum* (Hussain *et al.* 1995). Similarly repellent effect of azadirachtin and neem extracts (Xie *et al.*, 1995 a) and bark extracts of *Melia toosendan* (Xie *et al.*, 1995 b) against *Cryptolestes ferrugineus, S. oryzae* and *T. castaneum* had been reported.

It was observed that extracts of *Lantana camara, Adenoclyma sliviana, Crinum bulbispermum, Rauwolfia serpentina* and *Aloe vera* had the repellent activity to the larvae of *Statherotis luecaspis*. Here, *A. sliviana* was found to be the most effective repellent (Singh *et al.*, 1996). Similarly, various plant extracts had been extensively studied for their repellent activity against many stored product

insects (Novo *et al.*, 1997; Ho *et al.*, 1997 a, c; Suss *et al.*, 1997; Pascual, 1998; Pradeep and Radhakrishnan, 1999; Rahman *et al.*, 1999). It was demonstrated that leaf extracts of *Azadirachta indica, Eucalyptus rostrata, Lantana camara, Pongamia pinnata* and *Allium sativum* possess repellent property against the shoot and fruit borer, *Earias vitella* (Shukla *et al.*, 1997). Likewise, Egwunyenga *et al.* (1998) evaluated the repellent activity of *Dennettia tripetala* powder and extracts in acetone, ethanol and water against the larvae of the leather beetle, *Dermestes maculatus* and compared the activity with that of a pyrethrum standard. It was seen that seed powder of this plant showed higher repellency than pyrethrum.

Repellent properties of the leaf extracts of *Eucalyptus, Dalbergia, Aegle, Lawsonia* and *Bignonia* against the 4th instar larvae of the diamond back moth *Plutella xylostella* were evaluated by Dwivedi and Mathur (1999). They observed that extracts of *Eucalyptus* was found to be the strongest repellent while the least repellency was exhibited by *Bignonia* acetone extract. Similarly, Chander *et al.* (1999, 2000) studied the repellent effects brought about by the extracts of *Acorus calamus, Saussurea lappa, Curcuma longa, Murraya* sp. and *A. indica*, crude mustard oil, two commercial neem formulations (nimbicidin, repellin) and one synthetic pyrethroid (cypermethrin) on *T. castaneum* and they used these as prophylactic sprays to protect bagged grain. Abubaker *et al.* (2000) studied the repellent effect of the rhizome extract of *Cyperus articulatus* against *T. castaneum.*

Similarly, repellent activities of methanol extract of *Ferronia elephantum* leaves (Venkatachalam and Jebanesan, 2001) and different fractions isolated from *L. camara* flowers against *Aedes* mosquitoes (Dua *et al.*, 2003) have been

extensively studied recently. Nazrul *et al.* (2002) reported that extracts of three indigenous plants, *Aphanamixis polystachya, Colocasia esculenta* and *Eichhornia crassipes* were showing repellent effect on *C. chinensis. Colocasia esculenta* was found to be more effective than other plant extracts. Another studies were conducted to evaluate the effect of a protein-enriched pea (*Pism sativum* var. *bonneville*) flour extract against *S. oryzae* in its repellency. Among the tested concentration, protein-enriched pea flour treated milled rice at 1% would be highly effective in preventing the infestation of *S. oryzae* (Pretheepkumar *et al.*, 2004).

2.1.2. Oils / phytochemical constituents

Oils, chemical constituents and some natural products derived from plants cause repellent activity in insects. Singhamony *et al.* (1984) reported that natural products such as oils of clove, cedar wood, karanja and acetone extract of black pepper seeds acted as repellents against adult *T. castaneum*. Here, cedar wood, karanja and pepper products were found to be more potent than the standard repellent, dimethyl phthalate. Saim and Meloan (1986) reported 15 volatile compounds in bay leaves that acted as repellents against adults of *T. castaneum* when added to wheat flour. They found that three compounds, viz., benzaldehyde, piperidine and geraniol had maximum activity.

Oils from various plants have been extensively investigated for their repellent activity on insects. It was observed that 17 locally available plant oils were showing repellent properties against *T. castaneum* (Muhiuddin *et al.*, 1987). Similarly, repellent activities of oils of turmeric, sweetflag, neem and the commercial pesticide, Margosan-O against *T. castaneum* (Jilani *et al.*, 1988) and *R.*

dominica (Jilani and Saxena, 1990) had been reported. Repellent properties of oils of *Chenopodium ambrosioides* against adults of *C. chinensis, T. confusum, S. oryzae* and *Lasioderma serricorne* (Su, 1991), and fruit oil of *Piper retrofractum* against *T. castaneum, Spilosoma obliqua* and *S. litura* had also been studied (Tripathi *et al.*, 1997). Gu *et al.* (1997) reported that oils of *Zanthoxylum bungeanum, Ginkgo biloba, Ricinus communis, Melia azedarach, Citrus reticulata* and *Allium sativum* extracted in petroleum ether caused repellency to adult *T. castaneum*. They observed that oils of *Z. bungeanum, C. reticulata* and *A. sativum* were the most effective repellents. Crepuscular mosquitoes (*Armigeres subalbatus*) were controlled by using oils of citronella, lemon grass, tulsi, eucalyptus, neem and neem oil mixed with coconut oil (Devi and Pandian, 1999). They found that all these plant oils exhibited a reasonable protection time similar to synthetic repellents available in the market and therefore these oils were recommended as mosquito repellents. Singh and Singh (1991) screened the repellent properties of 31 essential oils extracted from different plants against *Musca domestica*. The essential oil extracts of six Malaysian plants, viz., *Curcuma longa, Zingiber officinale, Pandanus odorus, Cinnamomum zeylanicum, Syzygium aromaticum* and *Cymbopogon citratus*, had been evaluated for repellent activity against *Periplaneta americana* (Ahmad *et al.*, 1995). It was also seen that dose-dependent repellency ranging from 57.1 to 100% was exhibited by all the six extracts at the lowest concentration tested (12 ppm).

Similarly, repellent activity of essential oils of *Ocimum suava* against *S. zeamais, R. dominica* and *Sitotroga cerealella* (Bekele *et al.*, 1996) and 1,8-cineole,

eugenol and camphor from the essential oils of *Ocimum kenyense, O. suave* and *O. killimandscharicum* against *S. granarius, T. castaneum, Prostephanus truncates, Lasioderma serricorne* and *Stegobium paniceum* (Obeng-Ofori *et al.*, 1997 a) have been studied. Essential oils from some indigenous plant species were found to have repellent activity against a variety of insects (Singh and Mehta, 1998). Liu and Ho (1999) reported that essential oils from *Evodia rutaecarpa* were found to have repellent activity against the larvae and adults of *S. zeamais* and *T. castaneum*. Similarly, the repellent activity of the essential oils of *Lippia alba* var. *kavach* rich in linalool against *C. maculatus, R. dominica, S. oryzae* and *T. castaneum* (Verma *et al.*, 2000) and from several herb species on *S. oryzae* and *T. castaneum* (Padin *et al.*, 2000) have also been demonstrated.

Six essential oils from the leaves of *Citrus tangerina, C. aurantium, C. bergamia, Pinus sylvestris, Cupressus funebris* and *Eucalyptus citriodora* were found to act as repellents against *Liposcelides bostrychophila* (Wang *et al.*, 2001) and it was found that *C. funebris* oil was the most effective repellent. Likewise, Papachristos and Stamopoulos (2002) screened 13 essential oils from plants for their repellency in their vapour form against *Acanthoscelides obtectus*.

The insect repellent, quwenling, a product of China derived from the extracts of *Eucalyptus maculata citriodon* was showing repellent activity against various species of mosquitoes (Schreck and Leonhardt, 1991). 'Rotundial', a new potent natural mosquito repellent isolated from the fresh leaves of *Vitex rotundifolia* was also extensively studied (Watanabe *et al.*, 1995). Repellent activities were shown by the smoke of the leaves of *Adhatoda vasica, A. indica* and

Ocimum sanctum against *Armigeres subalbatus* and *C. quenquefasciatus* (Selvaraj *et al.*, 1995). Wood smoke and topical applications of various plant products were found to be repellents against the biting of anophiline and culicine mosquitoes (Paru *et al.*, 1995). Likewise, mosquito repellent properties of leaf callus-derived pyrethrins from *Chrysanthemum cinerariifolium* were studied by Rajasekharan *et al.* (1996). Effects of various plant-derived formulations have been extensively studied (Braverman *et al.*, 2000).

Diterpenoid and norditerpenoid alkaloids obtained from *Delphinium consolida* and *Aconitum* species have been reported to possess repellent activity against *T. castaneum*, among which the highest activity was found in diterpene alkaloid, hetisine and the lowest activity in venulol (Ulubelen *et al.*, 2001). Owusu (2001) reported the effect of some Ghanaian plant components for control of *T. castaneum* and *S. oryzae* and found that *Chromolaena odorata* and *Ocimum viride* showed strong repellent activity. The repellent effects of various components of pea seeds on stored product insects have been studied, in which wheat kernels dusted with fractions rich in protein and fibre showed maximum protection (Fields *et al.*, 2001). In similar studies, Mohan and Fields (2002) tested some natural products, i.e., diatomaceous earth (DE), ground peas, protein rich pea flour, pea starch and pea fibre for repellency against *S. oryzae, T. castaneum* and *Cryptolestes ferrugineus* and found that except pea starch, all of them showed repellent activity.

2.2. Antifeedant activity

Antifeedants or feeding deterrents are those chemicals that inhibit the insect from feeding on grain or other products leading to starvation and consequent death.

Various chemicals are involved in the inhibition of feeding and some of them are among the normal constituents of plants. Inhibitors may function by blocking the input from receptors, normally responding to phagostimulants or by stimulating specific 'deterrent' cells. The former may have a general effect on all insects, but chemicals in the latter category will only be effective if the insect has neurons capable of responding to them. A wide variety of plants offer good sources of antifeedants against insects.

2.2.1. Plant materials/extracts

McMillian *et al.* (1969) reported that leaf extracts of chinaberry tree and *Melia azedarach* acted as feeding deterrents to the larvae of *Heliothis zea* and *Spodoptera frugiperda.* Antifeedant properties of extracts of some indigenous plant materials had been extensively studied against mustard sawfly, *Athalia proxima* (Pandey *et al.*, 1977; Banerji *et al.*, 1982) and *R. dominica* (Malik and Naqvi, 1984). The chemistry and biological activity of insect feeding deterrents from certain weed and crop plants against scale insects, mites and white flies (Jacobson *et al.*, 1978) and phagodeterrency induced by leaves and leaf extracts of *Catharanthus roseus* on the larvae of *S. littoralis* (Meisner *et al.*, 1981) had also been investigated. Tripathi and Singh (1994) screened several indigenous plants for antifeedant activities against *Spilosoma obliqua.* Similarly, crude fractions of aerial parts of *Andrographis paniculata* were studied for antifeedant activity against *Spilarctia obliqua* (Tripathi *et al.*, 1999). Talukder and Howse (1993, 2000) isolated secondary plant compounds from the sub-fractions of pithraj, *Aphanamixis*

polystachya seed extract and found that one of the sub-fractions had high feeding deterrency against *T. castaneum*.

Roychoudhury (1993) reported the antifeedant properties of the extracts of *Didymocarpus podocarpus, Coriaria nepalensis* and *Clerodendron fragrans* against *S. oryzae*. Antifeedant properties of North-Western Himalayan plants such as *Artemisia brevifolia, Eupatorium adenophorum, Lantana camara, Melia azedarach* and *Rumex nepalensis* against cabbage caterpillar, *Pieris brassicae* (Mehta *et al.*, 2002) have been reported.

Methanolic extracts of leaves of *Aloe vera* and *Lantana camara* var. *aculeate* have been screened for antifeedant properties against the teak skeletonizer, *Eutectona machaeralis* (Kulkarni *et al.*, 1997). It was found that extracts of both the plants were equally effective in reducing the food consumption rate. Similarly, antifeedant properties of *Nerium indicum, Thevitia peruviam, A. indica* and *Ricinus communis* (Dhanapakiam and Shanazbegun, 1995), extracts of *A. indica, Citrus sinensis, V. negundo* and *Zingiber officinale* (Sahayaraj, 1998) and extracts of *Trichilia americana* against *Spodoptera litura* (Wheeler and Isman, 2001) have been reported.

Joseph (2000) reported that neem seed kernel extract have antifeedant effects against the last instar larvae of *Ailanthus* defoliator, *Eligma narcissus indica*. Likewise, antifeedant activities of aqueous extracts of *Gnidia glauca* and *Toddalia asiatica* against *Helicoverpa armigera* (Sundararajan and Kumuthakalavalli, 2001), rhizome extract of sweetflag, *Acorus calamus* against *T.*

castaneum (Chandel *et al.*, 2001) and *Chromolaena odorata* and *Ocimum viride* against *T. castaneum* and *S. oryzae* (Owusu, 2001) have also been investigated.

Rao *et al.* (2002) reported that *A. indica* in combination with *Acorus calamus* and *Pongamia glabra* have strong antifeedant effect on *Earias vittella.* Similarly, antifeedant activity of extracts of *Adhatoda vasica* against *S. littoralis* (Sadek, 2003), *A. squamosa* against *Crypsiptya coclesalis* (Kulkarni *et al.*, 2003) has been extensively studied.

2.2.2. Oils/phytochemical constituents

The activity of plant extracts as repellents and other deterrent activities are due to their various chemical constituents present in them. Several components in the plants are reported to possess antifeedant properties against many insects. Gilbert *et al.* (1967) reported that juglone (5-hydroxy-1,4-naphthoquinone) from the bark of *Carya ovata* showed feeding deterrency to *Scolytus multistriatus.* Chapman (1974) in his review described a variety of chemicals, which inhibited the feeding of phytophagous insects. Similarly, antifeedant properties of azadirachtin in the seed extracts of *A. indica* against *Schistocerca gregaria* (Butterworth and Morgan, 1971) and the European cornborer, *Ostrinia nubilalis* have been reported (Arnason *et al.*, 1985). Azadirachtin reduced the rate of feeding, growth and utilization of food in *S. gregaria* (Rao and Subrahmanian, 1986), which also affected food consumption, and utilization in *S. mauritia* (Jagannadh and Nair, 1996).

Nerifolin, a known cardiotonic glycoside isolated from the seeds of *Thevetia thevetioides* acts as an active feeding deterrent against the stripped cucumber beetle, *Acalymma vittatum*, the codling moth, *Laspeyresia pomonella* and the Japanese beetle, *Popillia japonica* (Reed *et al.,* 1982). Antifeedant activity of 13 quassinoids against the Mexican bean beetle, *Epilachna varivestis* and the southern army worm, *Spodoptera eridania* were reported (Leskinen *et al.,* 1984).

Sharma *et al.* (1990) screened seven different natural essential oils for their antifeedant activity against *Spodoptera litura.* Antifeedant activities of essential oils of *A. calamus* against the variegated cut worm, *Peridroma saucia* (Koul and Isman, 1990) and oils of turmeric, sweetflag, neem and a neem based insecticide, Margosan-O against *R. dominica* (Jilani and Saxena, 1990) have been evaluated. *T. castaneum* and *S. zeamais* were tested for their feeding response after the application of the essential oils of nutmeg seeds (Huang *et al.*, 1997), cinnamaldehyde from *Cinnamomum aromaticum* (Huang and Ho, 1998) and the essential oils of *Elletaria cardamomum* (Huang *et al.*, 2000). Liu and Ho (1999) reported that essential oils from *Evodia rutaecarpa* elicited antifeedant activity in *S. zeamais* and *T. castaneum.* Similarly, the feeding deterrent effects of neem seed oil on the egg parasitoid, *Trichogramma chiloris* (Raguraman and Singh, 1999) and neem oil on the 3rd instar grubs of *Oryctes rhinoceros* (Padmasheela and Delvi, 2002) have been reported. Besides the investigations with crude plant extracts, Tripathi and co-workers have tested various essential oil components from plants for their antifeedant activity in various insects. The antifeedant properties of 1,8-cineole from *Artemisia annua* against *T. castaneum* (Tripathi *et al.,* 2001 b),

essential oils of *C. longa* against *R. dominica* and *S. oryzae* (Tripathi *et al.*, 2002) and essential oils of *Lippia alba* against 3 crop pests, *Spilarctia obliqua, Spodoptera litura* and *Heliothis armigera* (Tripathi, 2002) have been extensively studied.

Limonoids are reported to act as antifeedants against many insects. Potential antifeedants like citrus limonoids (limonin, deoxylimonin and citrolin) were tested against spruce budworm, *Choristoneura fumiferana* (Alford and Bentley, 1986) and ten structurally modified limonins against larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Bentley *et al.*, 1988). Bentley *et al.* (1987) also reported that isopongaflavone, tephrosin, rotenone and tetrahydroisopongaflavone were active antifeedants against *Spodoptera exempta, Maruca testulalis* and *Eldana saccharina.* These insects also exhibited antifeedant response to other limonoids like limonin, deoxylimonin, citrolin, obacunone, harrisonin and acetoxyharrisonin (Hassanali *et al.*, 1986). Abdelgaleil and Nakatani (2003) isolated fifteen B, D-secolimonoids from extracts of *Khaya senegalensis* among which khayalactol, 1-0-acetylkhayanolide A, 2-hydroxyseneganolide, khayanolide A, khayanolide D and methyl angolensate displayed strong antifeedant activity against *S. littoralis.* Hassanali *et al.* (1987) also reported pedonin, a spirotetranortriterpenoid insect antifeedant from *Harrisonia abyssinica.* Similarly, neem (*A. indica*) limonoids, viz., azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin were found to have potent antifeedant activity against the cotton bollworm, *Helicoverpa armigera* (Murugan *et al.*, 1998).

Various alkaloids from plants exhibit antifeedant activity against insects. Saxena *et al.* (1986) reported that major alkaloids such as vasicine, vasicinol, vasicinone, deoxyvasicine and deoxyvasicinone from *Adhatoda vasica* had antifeedant activity against *Aulacophora foveicollis* and *Epilachna vigintioctopunctata.* Similarly, antifeedant activities of alkaloids such as tylophorine, tylophorinine and pergularinine isolated from the leaves of *Nicotiana tabacum* on the larvae of *T. castaneum* (Archana *et al.*, 1995) and alkaloids from *Dioscorea hispida* on larvae of the diamond back moth, *Plutella xylostella* have been studied (Banaag, 1996). Liu *et al.* (1990) evaluated the antifeedancy of four new sesquiterpene alkaloids isolated from *Celastrus angulatus* against *Pieris rapae, Ostrinia furnacalis* and *T. castaneum.* Six amide alkaloids of *Piper guineense* were tested for antifeedant activity against the 5th instar larvae of *Chilo partellus* (Torto *et al.*, 1992). Isoquinoline alkaloids identified in *Coptis japonica* roots were found to possess antifeedant activity against *Hyphantria cunea* and *Ageiastica coerulea* (Kwon *et al.*, 2000).

Srivastava *et al.* (1990) found that major sesquiterpene lactone and encelin from *Encelia actoni* and *E. asperifolia* have shown antifeedant activity against *S. littoralis.* Likewise, antifeedant activities of grayanoid diterpenes, viz., rhodojaponin III, grayanotoxin III and kalmanol isolated from *Rhododendron molle* on the larvae of *Leptinotarsa decemlineata* and *S. frugiperda* (Klocke *et al.,* 1991), dihydro β-agarofuran sesquiterpenes from Celastraceae on *S. littoralis* (Gonzalez *et al.,* 1992) and one *furanocoumarin* and five biogenetically related furanochromones isolated from the extract of *Pimpinella monoica* on *S. litura*

(Luthria *et al.*, 1992) have been reported. Cucurbitacins, the bitter triterpenes common to all cucurbitaceae have been shown to act as potential feeding deterrents against all insects, which are not adapted for exploiting cucurbits (Tallamy *et al.*, 1997). Neo-clerodane diterpenoid from *Ajuga reptans* had been found to act as an antifeedant against *S. littoralis* (Bremner *et al.*, 1998). Chiam *et al.* (1999) reported that allyl disulphide, a volatile compound from *Allium sativum* has antifeedant activity against adults and larvae of *T. castaneum* and adults of *S. zeamais*.

Jannet *et al.* (2000) reported that neo-clerodane diterperoids isolated from *Ajuga pseudoiva* leaves were found to show antifeedant activity against the larvae of *S. littoralis*. Similarly, antifeedant activity of quassinoids from *Samadera indica* against *S. litura* (Govindachari *et al.*, 2001) and three new tetranortriterpenoids from *Trichilia pallida* against 4 species of Lepidoptera (Simmonds *et al.*, 2001) have been reported. Likewise, antifeedant activities of quinones from *Ventilago madaraspatana* against *Henosepilachna vigintioctopunctata* and *S. litura* (Krishnakumari *et al.*, 2001) and components isolated from *Zingiber officinale* rhizomes against *Spilosoma obliqua* (Agarwal *et al.*, 2001) were studied. Morimoto *et al.* (2002) reported the antifeedant activities of anthroquinone aldehyde, nor-damnacanthol (1,3-dihydroxy-anthiraquinone-2-al), identified in *Galium aparine* against *S. litura*. Similarly, antifeedant properties of some plant derivatives viz., oils, a fatty acid and a terpineol and their combinations in different proportions against larvae of *S. litura* have been reported (Bhonde *et al.*, 2002).

2.3. Effect on reproduction, hatching of eggs and development

Saxena and Mathur (1976) reported that vapours of *A. calamus* oils had profound influence on *D. koenigii* where higher concentration of vapours impeded copulation and lower doses hampered the maturation of ova resulting in partial loss of fecundity. Different plant oils were showing adverse effects on egg laying and survival of adult beetles of *C. chinensis* and seeds treated with these oils did not permit adult beetles to lay eggs (Ali *et al.*, 1983). Similarly, Mishra and Kumar (1983) evaluated the susceptibility of the different developmental stages of *T. castaneum* to *Mentha piperita* oil as a fumigant. Sharma and Srivastava (1984) studied the efficacy of groundnut oil on embryonic development of *C. chinensis* and they found that density of eggs laid by the insects was reduced on treatment.

Maheswaran and Ganesalingam (1988) investigated the efficacy of volatile substances from *A. indica* seeds on the reproductive biology of *T. castaneum*. These substances reduced the number of eggs laid by females, prolonged the development period from egg to larva and reduced the number of larvae emerged from the eggs. Similarly, reduction of progeny of *C. chinensis*, *S. oryzae*, *S. granarius* and *T. confusum* in response to vapours of *A. calamus* oils (Schmidt *et al.*, 1991) and adverse effects on fecundity and progeny emergence of *C. maculatus* by volatile oils of *Lippia adoensis*, *Cymbopogon citratus*, *Eugenia uniflora*, *L. camara* and *Chromolaena odorata* (Gbolade and Adebayo, 1993) have been reported.

Don-Pedro (1989 a) reported that application of oils of ground nut, traditional coconut, industrial coconut, palm, and shark liver to exposed surface of

dried trout significantly reduced the development of the progeny of *Dermestes maculatus*. It was seen that in *C. chinensis* there was a suppression of egg hatching (Kachare *et al.*, 1994). Similarly, treatment of seeds with some vegetable oils caused oviposition deterrent effects, delayed development and reduction of adult emergence (Singh *et al.*, 1994). Pongam oil from *Pongamia pinnata* was found to decrease egg laying of *C. chinensis* (Negi *et al.*, 1994). Nadira *et al.* (1994) reported that caffeine and castor oils reduced the fecundity and fertility of *T. castaneum* and *Annona squamosa* seed oil was reported to lengthen the development of *T. castaneum* (Malek and Wilkins, 1995). Four vegetable oils and ten botanical powders significantly reduced the number of egg laid and longevity of the adults of *C. maculatus, C. chinensis* and *C. rhodesianus* (Rajapakse and Emden, 1997).

Obeng-Ofori and Reichmuth (1997) reported that eugenol, a major component of essential oils of *Ocimum suave* completely inhibited the development of eggs and immature stages of *S. granarius, S. zeamais, T. castaneum* and *Prostephanus truncatus* inside the grain kernels. Similarly, aromatised kaolin powders with essential oils of *Tagetes minuta, H. suaveolens, Ocimum* spp. significantly affected egg laying and adult emergence of *C. maculatus* and reduced egg hatching and adult emergence (Keita *et al.*, 2000).

Volatile oil constituents of *Mentha* sp. were found to inhibit development of *T. castaneum* and *C. maculatus* (Tripathi, *et al.*, 2000 b) and 1,8-cineole isolated from *Artemisia annua* reduced the hatching of eggs and the subsequent progeny production of *T. castaneum* (Tripathi *et al.*, 2001 b). Similarly, essential oils of *C.*

longa caused oviposition deterrent and ovicidal effects and adversely affected egg hatching and progeny production in *T. castaneum* (Tripathi *et al.*, 2002). Huang *et al.* (2000) reported that essential oil from *E. cardamomum* was found to reduce egg hatching and adult emergence of *S. zeamais* and *T. castaneum*. Lale and Yusuf (2001) in their study revealed that significantly fewer *T. castaneum* beetles developed in grains/products treated with *P. guineense* seed oil. Papachristos and Stamopoulos (2002) tested 13 essential oils in their vapour form against *Acanthoscelides obtectus* and found that most of the oils reduced fecundity and egg hatchability and increased neonate larval mortality and adversely influenced offspring emergence of this insect.

Ladd *et al.* (1984) studied the effects of azadirachtin from *A. indica* on the development of the larvae of *Popillia japonica*. Pandey *et al.* (1985) reported that mixing of neem (*A. indica*) oil, powder of kernels, cake, leaves and flowers and babul (*Acacia arabica*) germ with wheat seed proved to be protectants against *C. cephalonia* and caused adverse effects on development such as increased developmental period, higher mortality, lower percentage of adult emergence with less number of females. Similarly, azadirachtin adversely affected development and endocrine events in the larvae of the tobacco horn worm, *M. sexta* (Schluter *et al.*, 1985), caused moult inhibition and reduction of body weight in *S. gregaria* (Rao and Subrahmanyam, 1986) and interrupted longevity, hatchability of eggs, reproduction and adult development of *Oncopeltus fasciatus* (Dorn, 1986). Singh (2003) found that aqueous extract of neem seed kernel extract (NSKE) and azadirachtin inhibited fecundity, fertility and post embryonic development of the

melonfly *Bactrocera cucurbitae.* Likewise *E. vittella* larvae fed on fruits treated progressively with higher concentrations of the extracts of *A. indica* and *M. azedarach* had correspondingly lower weight, prolonged duration and decreased pupation, adult emergence and fecundity than those fed on untreated food (Gajmer *et al.*, 2003). Similarly, neem compound reduced the fecundity and longevity of *C. capitata* (Ilio *et al.*, 1999) and neem limonoids significantly inhibited reproduction, development, longevity and fecundity of *H. armigera* (Jeyabalan and Murugan, 1997).

Several other components in the plants are also reported to have some effects in insects. Vijay and Singh (1991) found that protein fractions (albumin, gobulin, prolamin and glutalin) from cashew nut kernels (*Anacardium occidentale*) reduced survival and slowed the development of *T. castaneum* and *O. surinamensis*. Some terpenoid lactones were found to reduce the fecundity and hatching of eggs in *T. castaneum* (Gursharan and Singh, 1999). Extracts of *Polygonum hydropiper* leaf and *Aphanamixis polystachya* seed coat significantly reduced the fecundity and fertility of *T. confusum* (Khanam and Talukder, 1993). Rani and Jamil (1989) reported that crude extracts of *E. crassipes* mixed with the diet of insects, retarded development and mortality of the 4th instar larvae of *T. castaneum* and *C. cephalonica.* Kaur *et al.* (1989) reported that *Chrysanthemum indicum* acted as an effective development inhibitor of *Dysdercus similis.* Margosan-O, a commercial neem based insecticide, prolonged the development of the larvae of *S. littoralis* (Haubruge *et al.*, 1994).

Chiranjeevi and Sudhakar (1996) evaluated some indigenous plant materials for their effects on fecundity, development and adult emergence in *C. chinensis*. The reproductive suppression properties of root bark powder of *Zanthoxylum zanthoxyloides* against *C. maculatus* was evaluated and compared with neem seed powder and primiphos-methyl (Ogunwolu and Odunlami, 1996). Sesquiterpene lactone isolated from the extract of *Sphaeranthus indicus* affected hatching of eggs and metamorphosis of larvae of *Culex quinquefasciatus* (Sharma, 1996). Similarly, Pemonge *et al.* (1997) reported that seeds and leaves of *Trigonella foenum-graecum* decreased the fecundity of *Acanthoscelides obtectus* and *T. castaneum*. Aqueous extracts of eight plants were found to affect the longevity and fecundity of the tea mosquito bug, *Helopeltis theivora* (Deka *et al.*, 1998 a) and solvent extracts of *L. camara* and *P. orientale* significantly affected both the fecundity and viability of eggs of tea mosquito bug (Deka *et al.*, 1998 b). Chanda and Chakravorty (1998) observed that food containing neem oil was found to have adverse effects on the life and development of the rice moth, *C. cephalonica*. Babu *et al.* (1999) reported that aqueous extracts of some indigenous plants reduced the egg laying in *C. maculatus*. Extracts of *Ageratum conyzoides* adversely affected developmental period, pupation and adult emergence and reproduction of *S. litura* (Singh and Rao, 2000) and powders of *Eichhornia crassipes, Citrus sinensis* and *Chromolaena odorata* also inhibited fecundity hatchability of eggs, developmental period and adult longevity of *C. cephalonica* (Allotey and Azalekor, 2000). Mukherjee and Joseph (2000) reported that commercially available extracts of *A. calamus, Rauwolfia serpentina, Sapindus trifoliatus* and *Commiphora mukul* affected the post embryonic development and adult emergence of *T. castaneum*.

Egg hatching and larval development of the seed weevil, *Caryedon serratus* were found to be affected by some botanicals (El-Atta and Ahmed, 2002). *Melia azedarach* root extract prolonged larval and pupal period, reduced pupation and adult emergence of *Earias vittella* (Mahla *et al.*, 2002). Similarly, *azadirachtin* was found to influence the development and reproduction of *Nezara viridula* (Riba *et al.*, 2003).

2.4. Effect on adult emergence

Several plant constituents are reported to have adverse effects on the emergence of adult insects. Chander and Ahmed (1986) reported that powdered rhizomes of *A. calamus*, leaves of *Clerodendron inerme, Tylophora asthmatica, Justicia betonica* and *Cestrum necturnum* significantly reduced adult emergence of *C. cephalonica*. It was shown that turmeric powder and mustard oil in different combinations acted as protectants of milled rice against *T. castaneum* infestation. Here, it suppressed the development of progeny of insects (Chander *et al.*, 1992). Similarly, reduction of adult emergence of *R. dominica* in response to indigenous plant products had been evaluated (Sosamma and Sheila, 1993). Maheshwari and Dwivedi (1996) reported that leaf powders of *Cassia occidentalis, Tephrosia appolinea, Calotropis procera, Datura metel* and *Croton bonplandianum* significantly reduced the adult emergence of *T. castaneum*. Likewise, reduction of adult emergence of *C. maculatus* by *P. nigrum, A. reticulata, Dillenia retusa* and *O. sanctum* (Rajapakse, 1996) and *C. chinensis* by indigenous plant materials (Chiranjeevi and Sudhakar, 1996) have been studied in detail. Wongo (1998) reported the biological activity of tannin extracts of *Sorghum bicolor* on *S. oryzae,*

S. cerealella and *T. castaneum* and he found that this extract prolonged pupal stage and reduced the number of adult progency.

Sharma (1999) reported that neem products such as neem seed kernel powder (NSKP), neem leaf powder (NLP) and neem oil acted as protectants for maize by reducing the emergence of F_1 and F_2 progeny of *S. oryzae, S. cerealella, R. dominica, T. granarium* and *T. castaneum*. Babu *et al.* (1999) reported that aqueous extracts of some indigenous plants reduced adult emergence of *C. maculatus*. Similarly, reduction of adult emergence in *S. litura* by *Ageratum conyzoides* extracts (Singh and Rao, 2000) and that of *T. castaneum* by commercially available extracts of *A. calamus, R. serpentina, Sapindus trifoliatus* and *Commiphora mukul* (Mukherjee and Joseph, 2000) have been evaluated. Reduction of adult emergence in *Earias vitella* by root extract of *M. azedarach* (Mahla *et al.*, 2002), the seed weevil, *Caryedon serratus* by some botanicals (El-Atta and Ahmed, 2002) and of *C. chinensis* by extracts of *Peganum harmala* (Srivastava and Mann, 2002) have also been reported.

Oils are also reported to reduce adult emergence in insects. Ten vegetable oils mixed with pigeon pea (Khaire *et al.*, 1992), pongam oil treated onto green grams (Negi *et al.*, 1994) and crucifer oil treated onto pigeon pea (Sharma *et al.*, 1999) were reported to reduce the adult emergence of *C. chinensis*. Similarly, edible oils of cotton seed, sunflower, ground nut, soy bean and mustard mixed with cowpea (Ramzan, 1994) and volatile oils of *Ageratum conyzoides* mixed with beans (Gbolade *et al.*, 1999) were found to inhibit the adult emergence of *C. maculatus*. Keita *et al.* (2000) reported that kaolin powders aromatized with essential oils of

Tagetus minuta, Hyptis suaveolens, Ocimum canum and *O. basilicum* significantly reduced the adult emergence of *C. maculatus*. Keita *et al.* (2001 b) also evaluated the reduction in adult emergence of *C. maculatus* in response to the essential oils of *O. basilicum* and *O. gratissimum*.

2.5. Effect on growth

Various chemical constituents present in the plants act as growth inhibitors in insects, which disrupts their life cycles and hence are found to be effective in insect pest management. Deshmukh and Renapurkar (1987) reported the insect growth regulating properties of petroleum ether extracts of 10 indigenous plants against *Culex pipiens fatigans* and *M. domestica nebula*. Similarly, growth inhibitory properties of *Chrysanthemum indicum* against *Dysdercus similis* (Kaur *et al.*, 1989) and seed extracts from *Melia volkensii* and *M. azedarach* against *C. pipiens molestus* (Al-Sharook, *et al.*, 1991) have been investigated. Joseph *et al.* (1994) evaluated the growth inhibition and impairment of reproductive potential in *T. castaneum* by commercially available plant extracts of *A. calamus, R. serpentina, S. trifoliatus* and *C. mukul*. Similarly, growth inhibitory effect of a neem-based insecticide, Margosan-O on *S. littoralis* (Haubruge *et al.*, 1994) and growth regulatory effects of neem extracts on *C. quinquefasciatus* (Singh, 1996) have been reported.

Extracts of Solanaceae and Compositae plants were found to act as growth inhibitors on the larvae of *T. castaneum* (Pascual, 1998). Similarly, growth inhibitory activity of *Andrographis paniculata* against *Spilarctia obliqua* (Tripathi *et al.*, 1999) and essential oils of *Lippia alba* against *Spilarctia obliqua, S. litura*

and *H. armigera* (Tripathi *et al.*, 2002) have been reported. Parvathi and Jamil (1999) reported that *Gliricidia sepium* leaf showed growth inhibitory activity against *Dysdercus koenigii*, *Achaea janata* and *S. litura*. Neem seed kernel extract and neem in combination with sweetflag and pongam extracts caused growth inhibition in *Eligma narcissus indica* and *E. vitella* (Joseph, 2000; Rao *et al.*, 2002) respectively.

Growth inhibitory activity of diterpene acids from *Helianthus annus* against the lepidopteran species, *Heliothis virescens, H. zea and Pectinophora gossypiella* (Elliger *et al.*, 1976) and the isolated triterpenoid ($C_{46}H_{80}O_2$) from the bark of *Santalum album* against some forest insects (viz., *Atteva fabriciella, Eligma narcissus, Eupterote germinata* etc.) (Shankaranarayana *et al.*, 1980) had been investigated. Duffey and Isman (1981) investigated the inhibition of larval growth of *H. zea* by phenolics in glandular trichomes of tomato leaves. Isman (1993) also reported the comparative efficacy of azadirachtin as a larval growth inhibitor of six species of noctuids of economic importance. Isman and Rodriguez (1983) reported that natural products isolated from various species of *Parthenium* inhibited larval growth in *H. zea* and *Spodoptera exigua*. Similarly, azadirachtin adversely affected growth in the larvae of tobacco hornworm, *M. sexta* (Schluter *et al.*, 1985). Alford and Bentley (1986) reported that citrolin was found to act as growth disruptor in *Choristoneura fumiferana* where it extended larval development. It was also reported that (.)-3-epicaryoptin isolated from the leaves of *Clerodendron inerme* was showing growth inhibitory effect on *M. domestica* and *C. quinquefasciatus* (Pereira and Gurudutt, 1990). Jilani *et al.* (1988) reported that turmeric oil,

sweetflag oil, neem oil and Margosan-O acted as growth inhibitors which produced fewer and underweight larvae, pupae and adults in *T. castaneum*. Ishaaya *et al.* (1991) observed the growth inhibitory effect of mimosine, a non-protein amino acid on the larvae of *T. castaneum*. Growth inhibitory activity of grayanoid diterpenes from *Rhododendron molle* on *L. decemlineata* and *S. frugiperda* (Klocke *et al.*, 1991), rocaglamide, a benzofuran isolated from the twigs of Chinese rice flower bush *Aglaia odorata* on *Peridroma saucia* and *S. litura* (Janprasert *et al.*, 1993) were reported. Similarly, insect growth inhibitory effect of cardinolide glycosides from *Anodendron affine* has been tested on *B. mori* (Fukuyama *et al.*, 1995).

Chenchaiah and Bhattacharya (2000) reported that phenolic extract of seed coat of red gram adversely affected the growth of *Creatonotus gangis*. Similarly, Agarwal *et al.* (2001) evaluated the growth inhibitory activity of components isolated/derived from rhizome of *Zingiber officinale* against *Spilosoma obliqua*. Growth regulating activity of quassinoids from *Samadera indica* against *S. litura* (Govindachari *et al.*, 2001) and of arjunoic acid isolated from the *Cornus capitata* against the 4th instar larvae of *Spilarctia obliqua* (Prajapati and Kumar, 2002) have also been reported.

2.6. Toxic/insecticidal activity

Now-a-days indigenous plant materials, products, chemical constituents etc. attract much attention as toxic/insecticidal materials with activities against agricultural and stored product pests in addition to their other biological effects. Several studies on the effectiveness of such plant derivatives as insecticidal/toxic materials against insect pest species have been conducted.

2.6.1. Raw plant materials /crude extracts

Much of the earlier works on toxicity of plant materials against insects used crude preparations. *Acorus calamus* had been used as an indigenous insecticide for the control of household insects (Subrahmanyam, 1942). Similarly, 45 plants in Mysore state were screened for their insecticidal activity (Puttarudriah, 1956). Atwal and Pajni (1964) in their preliminary studies revealed the insecticidal properties of drupes of *Melia azedarach* against caterpillars of *Pieris brassicae.* Su (1977) reported the insecticidal properties of *Piper nigrum* against *S. oryzae* and *C. maculatus*. Similarly, *Piper guineense* and *Capsicum* species have been reported to possess insecticidal activity against *C. maculatus* (Ivbijaro and Agbaje, 1986). Insecticidal properties of some indigenous plants such as *A. calamus, Euphorbia royleana, Crinum bulbispermum, L. camara* and *Aloe vera* against mustard saw fly, *Athalia proxima* (Pandey *et al.*, 1977) had been reported. Teotia and Pandey (1978) reported that rhizome extract of *A. calamus* was showing insecticidal activity against *S. oryzae*.

Toxic effect of flower extract of pink *L. camara* on *Dysdercus cingulatus, M. domestica* and *S. zeamais* and the volatile oil isolated from flowers against *D. cingulatus, M. domestica, S. zeamais, Spodoptera exempta* and *R. dominica* have been reported (Morallo and Tantengco, 1986). Rani and Jamil (1989) evaluated the petroleum ether extract of *Eichhornia crassipes* for contact toxicity against *T. castaneum*, *S. oryzae, C. maculatus* and *C. cephalonica.*

Insecticidal activities of *Calotropis procera* leaf powder against *T. confusum* (Jahan *et al.*, 1991), *Datura metel* leaf extracts (Khalequizzaman and Islam, 1992)

and *Thevetia nerifolia*, *Ricinus communis*, *Arachis hypogea* and *Carissa carandas* seed oil extracts (Malek *et al.*, 1996) against *T. castaneum* have been tested. Aerial parts of *L. camara* (Saxena *et al.*, 1992) and seed extracts of *Piper guineense* (Mbata *et al.*, 1995) were also found to have insecticidal activity against *C. maculatus*. Similarly, *Aphanamixis polystachya* seed extracts were evaluated for contact toxicity against *C. chinensis* (Talukder and Howse, 1994) and *T. castaneum* (Talukder and Howse, 1995). Insecticidal properties of the extracts of *Clerodendron siphonanthus* and *C. nepalensis* have been evaluated against *S. oryzae* by Roychoudhury (1993).

Mosquito larvicidal activities of some mangrove plant extracts against *A. aegypti* have been reported by Thangam and Kathiresan (1992). Pandian *et al.* (1994) in their study found that the smoke of the leaves of *V. negundo* and *Leucas aspera* were more toxic to *C. quinquefasciatus* than synthetic mosquito mats which contain d-allethrin. Smoke of leaves of *Adhatoda vasica, A. indica* and *Ocimum sanctum* were also showing toxicity against *Armigeres subalbatus* and *C. quinquefasciatus* (Selvaraj *et al.*, 1995). Efficacy of some plant extracts for the control of mosquitoe larvae had also been reported (Pushpalatha and Muthukrishnan, 1999).

Contact and fumigant toxicity of powdered and intact dried leaves of *Ocimum canum* had been evaluated against adult *Zabrotes subfasciatus* (Weaver *et al.*, 1994 a). Similarly, insecticidal activities of floral, foliar and root extracts of *Tagetes minuta* against *Zabrotes subfasciatus* (Weaver *et al.*, 1994 b), and fractionated floral extract of this plant against *S. zeamais* (Weaver *et al.*, 1997) had

been reported. Toxic effects of powder and extracts of *M. azedarach* against *T. castaneum* adults (El-Lakwah *et al.*, 1994) and that of *Capsicum* sp., *Clerodendrum inerme, Eucalyptus globulus* and *Duranta plumicri* against adults of *C. maculatus* (El-Lakwah *et al.*, 1996) have been reported. El-Lakwah *et al.* (1997) also evaluated the toxicity of the extracts of *Withania somnifera* leaves and fruits on *S. oryzae, R. dominica* and *T. castaneum.*

Toxic effects of bark extracts of *Melia toosendan* and azadirachtin and 3 neem extracts against *Cryptolestes ferrugineus, S. oryzae* and *T. castaneum* have also been reported (Xie *et al.*, 1995 a; 1995 b). Sahayaraj and Sekhar (1996) reported that leaves of *A. indica, V. negundo, Citrus sinensis* and rhizome of *Zingiber officinale* were showing toxicity against the larvae of *S. litura.* Insecticidal activities of extracts of seeds and leaves of *Trigonella foenum-graecum* against *A. obtectus* have been shown by Pemonge *et al.* (1997). Perveen *et al.* (1998) reported that methanol extracts of *Calotropis gigantea* and *Ipomoea nil* showed toxicity to *S. oryzae, T. castaneum* and *Cryptolestes ferrugineus* and *Gliricidia sepium* against *D. koenigii, A. janata* and *S. litura:* (Parvathi and Jamil, (1999). Efficacies of aqueous extracts of some plant species belonging to 7 families have been evaluated for their insecticidal properties against *C. maculatus* (Babu *et al.*, 1999). Similarly, Adedire and Lajide (1999) reported that crude methanolic extracts of *Aframomum melegueta, Erythrophleum quineense, Allium cepa, Hyptis suavolens, Piper umbellatum, Eugenia aromatica* and *Cyperus rotundus* have insecticidal activity against *C. maculatus.*

The mosquitocidal effects of extracts made from 11 S. American medicinal plants on *A. aegypti* have been tested (Ciccia *et al.*, 2000). Similarly, seed extracts of *Annona squamosa* (Mehra and Hiradhar, 2000) and extracts of *A. calamus, Allium sativum* and *Gardenia gummifora* were shown to have larvicidal activity against *C. quinquefasciatus* (Suryadevara and Khanam, 2002).

Umoetok (2000) investigated the toxicity of the powder of *A. calamus* against *S. oryzae, T. castaneum* and *R. dominica.* The results indicated that only *S. oryzae* and *R. dominica* were susceptible to the test products and there was no mortality in *T. castaneum.* Similarly, the effects of crude extracts of *Andrographis paniculata* on mortality of *C. chinensis* (Bright *et al.*, 2001) and larvicidal activity of some plant extracts against *C. cephalonica* (Dwivedi and Garg, 2001) have also been evaluated.

Jaswanth *et al.* (2002) reported the insecticidal activity of *A. squamosa* against *S. oryzae.* Similarly, Srivastava and Mann (2002) screened extracts of various parts of *Peganum harmala* on adult mortality of *C. chinensis* and found that, leaves were the most effective parts, followed by fruit, root and stem. El-Atta and Ahmed (2002) studied the relative effects of some botanicals extracted from the leaves and seed kernel of *A. indica* and *Eucalyptus camaldulensis* for the control of seed weevil *Caryedon serratus.* Three indigenous plants, *Aphanamixis polystachya, Colocasia esculenta* and *E. crassipes* were reported to have toxic effects on *C. chinensis.* Extract of *C. esculenta* was found to be more effective than other plant extracts (Nazrul *et al.*, 2002). Aqueous extracts of *Rhazya stricta, Calotropis procera* and *Franeocuria crispa* have been tested against the larvae and

eggs of *Culex pipiens* (Al-Doghairi and Elhag, 2002), and aqueous latex extracts of *Jatropa gossypifolia* and *Euphorbia tirucalli* against *C. quinquefasciatus* larvae (Yadav *et al.*, 2003). Dwivedi and Sharma (2003) evaluated the toxicity of *A. squamosa* extracts to various stages of *T. granarium*. Similarly, efficacy of certain botanicals as toxic substances against *Aphis gossypii* have been reported by Devi *et al.* (2003). Reena and Singh (2003) demonstrated the insecticidal properties of various products of garlic, *Allium sativum* against various pests of public health importance, stored grain and field and plantation crops. Extracts of *Adhatoda vasica* (Sadek, 2003), and eight species of medicinal plants exhibited insecticidal activity against *S. littoralis* (Pavela, 2004). Badshah *et al.* (2004) reported that extracts of *Calotropis procera* have toxic effects on termites, *Heterotermes indicola* and *Coptotermes heimi.* Protein enriched pea (*Pisum sativum* var. *bonneville*) flour extract mixed with milled rice at various concentrations have shown high toxicity against *S. oryzae* (Pretheepkumar *et al.*, 2004). Okonkwo (2005) reported that plant materials from the families of Annonaceae, Piperacceae and Rutaceae have been used for the protection of stored products against insect pests in Nigeria.

2.6.2. Oils/phytochemical constitutents

Oils from plants are also reported to have insecticidal activities against some insect pests. Amonkar and Reeves (1970) reported that mosquitoes were controlled by oil fraction of *Allium sativum.* Similarly, insecticidal activity of garlic oil to *M. domestica nebulo* and *T. granarium* had been studied (Thomas and Pal, 1974). Essential oils of *Cedrus deodara* were found to be toxic against *C.*

chinensis (Singh and Rao, 1985). Singh and Mehta (1998) screened different plant extracts and essential oils for their insecticidal properties against *C. chinensis* and *M. domestica*. They found that essential oils of *Cedrus deodara* and *Matriculata chamomilla* were the most effective ones. Su (1991) evaluated the toxic effects of essential oils from *Chenopodium ambrosioides* against *C. maculatus, T. confusum, S. oryzae* and *Lasioderma serricorne*. An insecticidal activitiesof some vegetable oils against *D. maculatus* on dried trout was investigated by Don-Pedro (1989 a). Toxicity of powdered sun-dried orange and grape fruit peels to *C. maculatus* and *D. maculatus* (Don-Pedro, 1989 b) and fumigant toxicity of six citrus peel oils against adult and immature stages of storage insect pests like *C. maculatus, S. zeamais* and *D. maculatus* (Don-Pedro, 1996) have also been reported. Haubruge *et al.* (1989) studied the toxicity of five essential oils extracted from *Citrus* sp. to *S. zeamais, Prostephanus truncatus* and *T. castaneum*. Similarly, insecticidal activity of essential oils from *Clausena dunniana* against *S. zeamais, T. molitor, R. dominica* and *T. castaneum* (HanHong *et al.*, 1994), *Illicium verum* (HanHong *et al.*, 1996 a) and essential oils from *Cinnamomum micranthum* (HanHong *et al.*, 1996 b) against *T. castaneum* have also been evaluated.

Ansari and Mishra (1990) studied the toxicity of essential oils of *Callistemon lanceolatus* and *Eupatorium capillifolium* to adults of *C. maculatus* in the laboratory. Plant extracts/essential oils of *Mentha piperita, A. calamus, Anethum sowa, P. nigrum, Pongamia glabra* and *A. indica* exhibited pesticidal activity against *A. aegypti, Dysdercus koenigii, S. oryzae, C. chinensis, Stegobium panicem* and *M. domestica* (Singh and Upadhyay, 1993). Tripathi *et al.* (1997)

reported the insecticidal properties of *Piper retrofractum* fruit oil against *T. castaneum*, *Spilosoma obliqua* and *S. litura*. Similarly, toxic effect of oil from *Artemisia annua* on *T. castaneum* and *C. chinensis* (Tripathi *et al.*, 2000 a) and from *Mentha* sp. on *T. castaneum* and *C. maculatus* (Tripathi *et al.*, 2000 b) have been reported.

Essential oils of *Anethum sowa* and *C. longa* and 1,8-cineole isolated from *Artemisia annua* were found to have toxic effects on *C. maculatus* and *R. dominica*, *T. castaneum* and *S. oryzae* (Tripathi *et al.*, 2001 a, 2001 b, 2002).

Huang *et al.* (1997) reported the contact and fumigant toxicity of essential oils extracted from nutmeg seeds against *T. castaneum* and *S. zeamais*. Similarly, toxicity of cinnamaldehyde from *C. aromaticum* (Huang and Ho, 1998) and essential oils of *Elletaria cardamomum* against *S. zeamais* and *T. castaneum* (Huang *et al.*, 2000) have also been evaluated. Shaaya *et al.*, (1997) reported that essential oils extracted from various spices, herb plants and a number of edible oils exhibited toxicity against many major stored product insects viz., *T. castaneum*, *S. oryzae*, *R. dominica* and *O. surinamensis*. Namrata *et al.* (1997) evaluated contact and fumigant toxicity of volatile essential oil of *Murraya koenigii* against *C. chinensis*. Similarly, 1,8-cineole, eugenol and camphor from essential oils of various *Ocimum* sp. were found to have toxicity against *S. granarius*, *S. zeamais*, *T. castaneum*, *Prostephanus truncatus*, *Lasioderma serricorne* and *Stegobium paniceum*. (Obeng-Ofori and Reichmuth 1997; Obeng-Ofori *et al.*, 1997 a, 1997 b, 1998).

Passino *et al.* (1999) examined the toxic effects of ingestion of formulations containing essential oils from aromatic plants, viz., *Rosmarinus officinalis, Salvia officinalis, Cinnamomum zeylanicum* and *Thymus* sp. on adult *C. capitata*. Contact toxicity of neem seed oil against egg parasitoid, *Trichogramma chilonis* have been reported by Raghuraman and Singh (1999). Chiam *et al.* (1999) reported the contact and fumigant toxic effect of allyl disulphide, a volatile compound from *Allium sativum* against adults and larvae of *T. castaneum* and adults of *S. zeamais*. Similarly, fumigant toxicity of volatile oil of *Ageratum conyzoides* has been evaluated against adults of *C. maculatus* (Gbolade *et al.*, 1999).

Thomas *et al.* (2000) from their studies revealed that the essential oils of *Cannabis sativa* had larvicidal effects on *Culex tritaeniorhynchus, Anopheles stephensi, Aedes aegypti* and *C. quinquefasciatus*. Essential oils of *Lippia alba* var. *kavach* rich in linalool have been shown to have contact and fumigant toxicity on *C. maculatus, R. dominica, S. oryzae* and *T. castaneum* (Verma *et al.*, 2000). Keita *et al.* (2000) reported that fumigation with pure essential oils and aromatized kaolin powders of four West African plant species viz., *Tagetes minuta, Hyptis suaveolens, Ocimum canum* and *O. basilicum* resulted in adult mortality in *C. maculatus*. Similarly, insecticidal activities of essential oils of *Thuja occidentalis* (*Keita et al.*, 2001 a), *O. basilicum* and *O. gratissimum* have been studied on *C. maculatus* (Keita *et al.*, 2001 b). Lee *et al.* (2000) studied the larvicidal activity of totarol, a diterpenoid phenol isolated from *Podocarpus totaria* root bark against *Culex pipiens*. Fumigant toxicity of volatile constituents of essential oils from Korean spices and medicinal plants against *S. oryzae* and of lavender and ylang-

ylang essential oils against a chlorpyrifos-methyl resistant strain and an insecticide-susceptible resistant strain of *Oryzaephilus surinamensis* have been studied (Lee *et al.*, 2001; Lee, 2002).

Wang *et al.* (2001) reported that six essential oils from the leaves of *Citrus tangerina*, *C. aurantium*, *C. bergamia*, *Pinus sylvestris*, *Cupressus funebris* and *Eucalyptus citriodora* have high level of toxicity on *Liposcelis bostrychophila* adults. Similarly, contact and fumigant toxic activities of essential oil constituents derived from the fruit of fennel, *Foeniculum vulgare,* against adults of *S.* oryzae, *C. chinensis* and *L. serricorne* have been reported (Kim and Ahn, 2001). Bauda *et al.* (2001) reported that essential oils from *A. conyzoides, L. camara* and *C. odorata* were showing mortality on *S. zeamais*. Fumigant toxicity of 13 essential oils extracted from plants against *Acanthoscelides obtectus* (Papachristos and Stamopoulos, 2002) and neem oil against the 3rd instar grubs of *Oryctes rhinoceros* (Padmasheela and Delvi, 2002) have been reported. Kim *et al.*, (2003 a) reported that some aromatic plant extracts and essential oils were found to have contact and fumigant activities against *Lasioderma serricorne*. Similarly, insecticidal activities of extracts and essential oils from some aromatic plant species against *S. oryzae* and *C. chinensis* had also been reported. Extracts from *Cinnamomum cassia, C. sieboldii* and *Foeniculum vulgare* as well as oils from cinnamon, horse radish and mustard were highly effective against both species (Kim *et al.*, 2003 b).

Several other phytochemical constituents having insecticidal properties are also reported from plants. Isman and Duffey (1982) reported the toxic effects of chlorogenic acid and rutin, the major phenolic constituents of tomato foliage and

phenolic rich aqueous extract of tomato foliage on *Heliothis zea.* Prakash and Mathur (1985) reviewed the use of some active principles from plant products for insect pest management in stored grain and these active principles of plant products were found to have promising grain protection against insect infestation. Insecticidal property of azadirachtin against European corn borer, *Ostrinia nubilalis* was reported by Arnason *et al.* (1985). It was also reported that plumbagin, a naturally occuring chitin synthesis inhibitor from the African medicinal plant, *Plumbago capensis* exhibited high contact toxicity against *Dysdercus koenigii* (Gujar and Mehrotra, 1988).

Srivastava *et al.* (1990) studied the toxicity of 3 major sesquiterpenes from *Encelia actoni* and *E. asperifolia.* The sequiterpene encelin that contained a α-methylene-γ-lactone moiety was the most active compound. Similarly, insecticidal activities of the constituents, grayanoid diterpenes from *Rhododendron molle* on the larvae of *Leptinotarsa decemlineata* and *Spodoptera frugiperda* (Klocke *et al.*, 1991) and rocaglamide, isolated from the twigs of the Chinese flower bush, *Aglaia odorata* on *Peridroma saucia* and *S. litura* have been reported (Janprasert *et al.*, 1993). Rice and Coats (1994) have shown the insecticidal properties of several monoterpenoids against *M. domestica, T. castaneum* and *Diabrotica undecimpunctata.* Toxicity of 6 monoterpenoids, 2 alkaloids and 1 hydrocarbon derived from plant or ant semiochemicals were evaluated against formosan subterranean termite, *Coptotermes formosanus* (Cornelius *et al.*, 1997). Monoterpenoid alcohols, particularly eugenol, were the most effective termiticide. Similarly, Prates *et al.* (1998) reported the insecticidal activity of monocyclic

monoterpenes, 1,8-cineole (eucalyptol) from *Eucalyptus* sp. and R-(+)-limonene from *Citrus* sp. against *R. dominica* and *T. castaneum*.

Alkaloid containing fractions isolated from the leaves of *Nicotiana tabacum* exhibited insecticidal effect against *T. castaneum* (Archana *et al.*, 1995). Similarly, some alkaloids isolated from *Glycosmis pentaphylla, Murraya koenigli* and *Piper nigrum* and their derived products were evaluated for toxicity against *C. quinquefasciatus* (Das *et al.*, 1996). Here, the alkaloid, piperine obtained from *P. nigrum* was found to have the highest toxicity on mosquito larvae. Tsao *et al.* (1996) investigated the insecticidal activity of glucosinolate-containing extracts from the seeds of *Crambe abyssinica* and found it as a potential control agent for certain agricultural and public health insect pests. Ho *et al.* (1997 b) in their study revealed that trans-anethole, a potential insecticide obtained from *Illicium verum* exhibited toxicity against *T. castaneum* and *S. zeamais*. It was also found that *T. castaneum* adults were more susceptible to both the fumigant and contact action of anethole than *S. zeamais*. Recent research on the insecticidal effects of plant derived substances on stored product pests indicate that the components 1,8-cineol and R-(+)-limonene of essential oils of *Eucalyptus camaldulensis, E. cameroni* and of the peel of *Citrus aurantium* have significant insecticidal action, being lethal to *R. dominica, T. castaneum, S. oryzae* and *S. zeamais* (Santos *et al.*, 1997).

Gikonyo *et al.* (1998) studied the toxic activity of acetone extracts of *Polygonum senegalense* leaves, its flavonoid component 2'6'-dihydroxy-4' – methoxy dihydrochalcone and the internal tissue flavonol quercetin against *A. aegypti* larvae. Wenjun *et al.* (2001) isolated 3 insecticidal sesquiterpene polyol

esters from petroleum ether extract of the root bark of *Celastrus angulatus*. Recently, goniothalamin, a potent mosquito larvicide was isolated from *Bryonopsis laciniosa*, which was highly effective against *C. quinquefasciatus* (Kabir *et al.*, 2003).

2.7. Antimicrobial/acaricidal/nematicidal activity

Several plant-derived substances are also reported to possess antibacterial, antifungal, antiviral, acaricidal and nematicidal effects.

Antibacterial properties of essential oils from *Vitex* sp., *Zingiber officinale, Cymbopogon citratus, C. longa, P. nigrum* and *Colcus aromaticus* against 20 strains of bacteria have been reported by Nycin *et al.* (1996). Antifungal activity of the flavonoids such as cabruvin and quercetin isolated from the roots of *Clerodendron infortunatum* inhibited spore germination of *Alternaria carthami* and *Heminthosporium oryzae* (Roy *et al.*, 1996). Similarly, Verma *et al.* (1997 a) in their studies on *in vitro* antimiocrobial activity of various triterpenoids isolated from *Lantana* sp. against number of microorganisms (bacteria and fungi) revealed that it inhibited growth. A rare antibacterial flavone glucoside isolated from the leaves of *L. camara* was evaluated against a wide range of gram +ve and gram –ve bacteria by Verma *et al.*, (1997 b) in their investigation.

The antibacterial triterpenoids such as lantic acid, camaric acid, camarinic acid and lantanilic acid, isolated from *L. camara,* were evaluated against gram +ve and gram -ve bacteria. This study revealed that lantic acid has strong antibacterial activity (Mahmoud *et al.*, 1999). Agarwal *et al.* (2001) reported antifungal activity

of certain components isolated from *Zingiber officinale* rhizomes against *Rhizoctomia solani.*

Omer *et al.* (2000) reported the antiviral activity of extracts of *Aconitum nasutum, Daphne glomerata, Hypericum androsaneum, Laurus nobilis, Nerium oleander, Olea europea, Prunus laurocerasus, Punica granatum, Rhododendron caucasicum* and *Urtica dioica* against *Autographa californica* nuclear polyhedrosis virus grown in *Spodoptera frugiperda* cell culture. Similarly, inhibition of HIV-1 integrase by galloyl glucoses from *Terminalia chebula* and flavonol glycoside gallates from *Euphorbia pekinensis* was reported by (Ahn *et al.*, 2002).

Mansingh and Williams (1998) reported that ethanol extracts of the leaves of 43 Jumaican plants produced varying degrees of multiple acaricidal effects on engorged *Boophilus microplus* including mortality, inhibition of oviposition and embryogenesis. Acaricidal activity of butylidenephthalide identified in *Cnidium officinale* rhizome has been tested against *Dermatophagoides farinae* and *D. petronyssinus* (Kwon and Ahn, 2002).

The constituents lantanoside, lantanone, linaroside and camarinic acid, isolated from the aerial parts of *Lantana camara* were found to have nematicidal activity against *Meloidogyne incognita* (Sabira *et al.*, 2000).

2.8. Oviposition deterrent/ovicidal activity

Besides the above mentioned activities, plants materials also have oviposition deterrent and ovicidal effects on insects. In laboratory and field tests, aqueous extracts from a few indigenous plants were showing oviposition deterrent

effects against *Heliothis virescens* (Tingle and Mitchell, 1984). Saxena *et al.* (1992) reported the oviposition deterrent effect of aerial parts of *L. camara* on *C. chinensis*. Plants such as *P. nigrum, A. reticulata, Dillenia retusa, O. sanctum* (Rajapakse, 1996), *P. nigrum, A. reticulata, A. indica, Capsicum annum* and *Citrus limon* (Rajapakse *et al.*, 1998) were found to supress oviposition of *C. maculatus.* Sharma *et al.* (1997) in their studies with extracts of 10 plant species found that these extracts were showing oviposition deterrent and ovicidal activity against *Phthorimaea operculella.* Oviposition deterrent effects of extracts of five plants *Rhazya stricta, A. indica, Syzygium aromaticum,* and *Heliotropium bacciferum* against *C. maculatus* on chick peas have been evaluated (Elhag *et al.*, 1999). Tripathi *et al.* (1999) reported the oviposition deterrent activity of crude extract of aerial parts of *Andrographis paniculata* in *Spilarctia obliqua.* Ovicidal and oviposition deterrent effect of the essential oils of *Anethum sowa* and its three major fractions against *C. maculatus* (Tripathi *et al.*, 2001 a) and essential oils from the leaves of *C. longa* on *T. castaneum* (Tripathi *et al.*, 2002) have also been evaluated. Gajmer *et al.* (2002) evaluated oviposition deterrent activities of methanolic extracts of *A. indica* and *M. azedarach* seeds on in *Earias vitella.* Similarly, Srivastava and Mann (2002) reported that extracts of *Penganum harmala* reduced the egg laying in *C. chinensis.*

Oils from plants are also reported to possess oviposition deterrent effects on insects. Javer *et al.* (1987) reported pine oil as an oviposition deterrent for onion maggots, *Delia antiqua.* Essential oils from geranium, cypress, eucalyptus and bitter almond were tested in their vapour form against *Acanthoscelides obtectus* for

oviposition deterrent effect. It was found that eucalyptus oil strongly reduced fecundity of this insect (Stamopoulos, 1991). Kachare *et al.* (1994) reportd that different vegetable oils applied on seeds showed significant repellent action on egg laying by *C. chinensis* adults. Similarly, oviposition deterrent effect of neem seed oil on the egg parasitoid, *Trichogramma chilonis* (Raguraman and Singh, 1999) and crucifer oil against *C. chinensis* (Sharma *et al.*, 1999) have been reported. Verma *et al.* (2000) reported that essential oil of *Lippia alba* var. *kavach* rich in linalool was showing oviposition deterrent effect on *C. maculatus, R. dominica, S. oryzae* and *T. castaneum.*

In addition to oils, alkaloids from plants are also reproted to possess oviposition deterrent properties. Zhao *et al.* (1998) reported quinolizidine-containing alkaloid extracts and isolated quinolizidine alkaloids from 2 Chinese plants, *Sophora alopecuroides* and *Thermopsis lanceolata* that deterred oviposition of spruce budworm.

Ovicidal activities of some plant materials have been reported. Agarwal (1990) reported that 5 phytochemical components extracted from *A. indica, Swietenia macrophylla* and *Calophyllum inophyllum* exhibited ovicidal activity against eggs of *Myllocerus undecimpustulatus.* Similarly, ovicidal activity of some plant extracts on eggs of *S. litura* and *Dysdercus koenigii* (Suryakala *et al.*, 1995) and sesquiterpene lactone isolated from extracts of *Sphaeranthus indicus* on *C. quinquefasciatus* (Sharma, 1996) have been evaluated. Su and Mulla (1998) investigated the ovicidal activity of neem products on *Culex tarsalis* and *C. quinquefasciatus.* Ovicidal activity of essential oil vapours from *Pimpinella*

anisum, Cuminum cyminum, Eucalyptus camaldulensis, Origanum syriacum var. *bevanii* and *Rosmarinus officinalis* against *T. confusum* and *Ephestia kuehniella* (Tunc *et al.*, 2000) have been reported. Extracts of *Cassia fistula, Acacia nilotica, L. camara* and *Tagetes nilotica* tested on the eggs of *T. granarium* revealed that these botanicals have ovicidal activity (Dwivedi and Bajaj, 2001). Mahla *et al.* (2002) in their investigation revealed that *M. azedarach* root extract possessed ovicidal activity against *Earias vitella.*

2.9. Efficacy as grain protectant

Several plant materials/products have been used as grain protectants against the infestation of stored product insects.

Chander and Ahmed (1983) reported the relative efficacy of some indigenous plant products to control the infestation of *S. oryzae, T. granarium* and *C. chinensis*. Powder of *A. calamus* rhizome was the most promising protectant against these pests. Similarly, effectiveness of turmeric powder and mustard oil in different combinations as protectants for milled rice against infestation by *T. castaneum* have been also evaluated by Chander *et al.* (1992). Here, these plant materials were found to suppress the pogeny of this insect. It was proved that extracts of *A. indica, L. camara, Thevetia nerifolia* and *Ipomoea carnea* mixed with green gram seeds are safe protectants against the infestation of *C. chinensis* (Pandey *et al.*, 1986). Adebayo and Gbolade (1994) reported the efficacy of some plant products (leaf powders and volatile oils) in protecting cow pea seeds from the attack of *C. maculatus* during storage (Talukder and Howse, 1995).

Rouf *et al.* (1996) evaluated individual and combined effects of leaf powders of *A. indica, V. negundo* and *Polygonum hydropiper* for protection of lentil seeds from *C. chinensis* infestation. It was seen that *V. negundo* leaf powder was most effective in reducing oviposition and adult emergence of the insect. Singh *et al.* (2001) reported the efficacy of the leaf powder of lantana, sadabahar, neem, madar and kali tulsi and oils of castor, neem and mahua as grain protectants against *C. chinensis*. Leaf powder of dharek and sadabahar, oils of coconut and mustard and neem products (Umrao and Verma, 2002) had been shown to have protective effect against *C. chinensis* infesting grains. Oils of clove, cedarwood and karanja and pepper extract were reported to provide protection to wheat against *S. oryzae* and *R. dominica* (Sighamony *et al.*, 1986).

Efficacy of pigeon pea protection by 10 vegetable oils (Khaire *et al.*, 1992) and some other essential oils (basil, geranium, rue, lemon grass, citronella, eucalyptus and lemon) (Richa *et al.*, 1993) from *C. chinensis* have been studied. Coconut oil was found to protect different pulse grains from *C. chinensis* (Sosamma, 1994) and oil of *Allium sativum* was found to be a potential grain protectant against *T. castaneum* and *S. zeamais* (Ho *et al.*, 1997 a). Similarly, several indigenous oils such as soybean, linseed, sunflower, castor, sesamum, *Toria* (T_9) mustard, *Karad, Taramira* and safflower oils were evaluated against *S. oryzae* on barley (Uttam *et al.*, 2002).

2.10. Other biological effects

Certain plant constituents are reported to possess various biological effects on insects in addition to the above mentioned effects. Azadirachtin, for example,

exhibited inhibition of moulting in the last larval instar of *Locusta migratoria* (Sieber and Rembold, 1983) and the larvae of the face fly, *Musca autumnalis* (Gaabous *et al.,* 1984) due to the interference in the endocrine control. Marco *et al.* (1990) noticed that there was a depletion of ecdysteroid by azadirachtin in *Tenebrio molitor* pupae. Saxena *et al.* (1986) reported that various alkaloids (vasicine, vasicinol, and vasicinone) from the extracts of *Adhatoda vasica* showed antifertility properties against *Dysdercus koenigii* and *T. castaneum.* However, plumbagin from *Plumbago capensis* showed a wide range of morphogenetic effects and also interference with the neuro endrocrine system and its integration with moulting process in *Dysdercus koenigii* (Gujar and Mehrotra, 1988).

Mimosine, a non-protein amino acid, inhibited enzyme system in *T. castaneum* (Ishaaya *et al.,* 1991). Inhibition of cuticule formation and elytral aberration of *T. castaneum* and *C. cephalonica* resulted with extracts of *Eichhornia crassipes* (Rani and Jamil, 1995). Ramanathan *et al.* (1997) studied leaf extract of *Pongamia glabra* on histological changes of fat body of *P. americana* adult male. Moult inhibiting activity of limonoids such as limonin, nomilin and obacunone from *Citrus reticulata* in the mosquito, *C. quinquefasciatus* larvae have been investigated by Jayaprakasha *et al.* (1997). Similarly, antifertility effects of *Clerodendron siphonanthus* leaf extract on female *C. chinensis* (Pandey and Khan, 1998) have also been evaluated. Raguraman and Singh (1999) investigated the insect growth regulatory effects of neem seed oil against the parasitoid, *Trichogramma chilonis.* Padmaja and Rao (2000) studied the efficacy of oils of *Artemisia annua, Ageratum conyzoides*, and *A. indica* on the hemolymph proteins

of final instar larvae of *Helicoverpa armigera* and found that these oils affected the number and prominence of the major protein bands in the electrophoretic protein profiles of haemolymph.

2.11. *Vitex negundo*

Among various plant species, *Vitex negundo*, the experimental plant used in the present investigation also contain several phytochemicals. The chemical constituents of this plant have medicinal, behavioural, biological and other properties. So, it is hoped that this plant can be effectively used in the strategy of insect pest management.

2.11.1. Chemical constituents:

Chemical constituents of *V. negundo* have been investigated by several workers. Chandra and Babber (1987) isolated 5,4'-dihydroxy-7,8,3'5'-tetramethoxyflavone from *V. negundo.* They also isolated two isomeric pentaoxygenated flavononcs from this plant. Phytochemical studies on the leaves of *Vitex negundo* carried out by Dayrit *et al.* (1987) revealed that it contains the casticin, chrysosplenol D, luteolin, p-hydroxybenzoic acid, D-fructose, isoorientin and flavonoids. Dayrit *et al.* (1994) also identified four iridoids, 2'-p-hydroxybenzoyl mussaenosidic acid, 6'-p-hydroxybenzoyl mussaenosidic acid, angnuside and lagundinin in the pharmacologically active fraction of this plant. Banerji *et al.* (1988) isolated 4, 4'-dimethoxy-trans-stilbene and 5 flavonoids from the leaves and twigs. Physico-chemical analysis of seed oil of the plant revealed that it contained fatty acid components (Ahmad *et al.,* 1989). Chawla *et al.* (1991)

investigated the unsaponifiable matter from the oil and yielded a new antiinflammatory diterpene, 5-β-hydro-8,11,13-abietatrien-6α-ol, a triterpene, lanostan-8,25-dien-3-β-ol and a flavonoid, artemetin. The chloroform extract of the seeds of this plant yielded four triterpenoids that exhibited antiinflammatory activity (Chawla *et al.*, 1992 a). Similarly, seeds also afforded a lignan, characterised as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde by spectroscopic methods (Chawla *et al.*, 1992 b). Reddy and Radhakrishnaiah (1992) reported that *Vitex altissima, V. negundo, V. peduncularis, V. pinnata* and *V. trifolia* contained the chemical constituents, p-coumaric, benzoic, ellagic and vanillic acids, vanillin and other unidentified phenolic compounds. Das and Das (1994) in their review mentioned 51 chemical compounds isolated from *Vitex negundo* and their medicinal properties. Mallavarapu *et al.* (1994) analysed the composition of essential oils of *V. negundo* leaves by GC and GC-MS. This study revealed that the oil contained 65 known compounds including sabinene, p-cymene, beta-phellandrene, gamma-terpinene, terpinen-4-ol, beta-caryophellene, alpha-quaiene, spathulenol, beta-caryophyllene oxide, globulol, viridiflorol, bis- [1,1-dimethyl] methylphenol and abieta-7,13-diene. Several minor, unidentified compounds were also detected. Similarly, essential oils of the leaves of *V. negundo* var. *negundo* and *V. negundo purpurescens* were investigated by GC-FID, GC-MS and olfactometry. This revealed that monoterpenes such as terpinen-4-ol, p-cymene, a-terpineol and sabinene as well as sesquiterpenes such as b-caryophyllene, globulol, spathulenol, b-farnesene and bis [1,1-dimethyl] methyl phenol were the main essential oils of the 2 varieties of the plant (Jirovetz *et al.*, 1998). Ragasa *et al.* (1999) reported that

vitexilactone and casticin, isolated from the chloroform extract of air dried leaves of *V. negundo* were found to inhibit growth of bacteria and fungi. The volatile constituents of the leaves of *V. negundo* were analysed by GC-MS and was found to contain 66 compounds. Thirty five compounds, constituting 74.96% of the oil, were identified. The main compounds were viridiflorol, b-caryophyllene, sabinene, 4-terpineol, g-terpiene, caryophyllene oxide, 1-oceten-3-ol and globulol (Singh *et al.*, 1999). Singh *et al.* (2003) also investigated some other chemical constituents of leaves of this plant viz., viridiflorol, squalene, β-sitosterol, 5-hydroxy-3,6,7,3'4'-pentamethoxy flavone, 5-hydroxy-3, 7, 3', 4'-tetra methoxy flavone, 5, 3'-dihydroxy-7, 8, 4'-trimethoxy flavanone, p-hydroxybenzoic acid, 3,4-dihydroxy benzoic acid, luteolin, 7-glucoside, isoorientin, agnuside and 2'-p-hydroxy benzoyl mussaenosidic acid. They were isolated and characterised by spectral data (UV, IR, NMR & MS) from the different extractives of the leaves. GC-MS analysis of the oil by Singh *et al.* (2000) indicated the presence of 94 compounds, of which 28 compounds, constituting 57.53% of the oil were identified. The main compounds are viridiflorol, β-caryophyllene, 4-terpineol, linalool, globulol, elemol, β-farnesene and aromadendrene.

2.11.2. Medicinal properties and other effects

Medicinal properties and some effects of this plant have been revealed by some workers. Bhargava (1986, 1989) isolated the flavonoids, 5, 7, 3'-trihydroxy, 6,8,4'-trimethoxy-flavone from seeds of *V. negundo* having antifertility effects in dogs. Here, it disrupted the different stages of spermatogenesis. Balboa and Lim (1993) reported the antigenotoxic effects of drug preparation from this plant. These

drug preparations inhibited the genotoxicity of dimethylnitrosamine, methylmethane sulphonate and tetracyclin. *V. negundo* and some medicinal plants were also found to reduce the incidence of skin tumours in experimental mice (Balboa and Lim, 1995).

Nair and Saraf (1995) reported the inhibition of antigen and compound 48/80 induced contraction of guinea pig trachea by the ethanolic extract of the leaves of *V. negundo*. This extract was found to significantly inhibit both the initial and later phases of contraction of the trachea. The studies also revealed that this extract inhibited the release of histamine and products of arachidonic acid metabolism. Ethanolic extract of root of this plant exhibited hepatoprotective activity in carbontetrachloride treated rats (Srinivas *et al.*, 1999). Analgesic and anti-inflammatory activities of hydroalcoholic extract of leaves of this plant have been studied by Telang *et al.* (1999). This study indicated the significant decrease of rat paw oedema volume and also significant decrease in the formation of granuloma pouch in rats. This extract was also found to have inhibitory action on oxytocin-induced contractions in isolated horns of uterus primed with oestradiol. The analgesic and antiinflammatory action of this extract was attributed to its flavonoid contents, which are known to act through inhibition of prostaglandin biosynthesis. Gupta *et al.* (1999) investigated the CNS depressant of methanol leaf extract of this plant. This extract potentiated significantly the sleeping time induced by pentobarbitone sodium, diazepam and chlorpromazine in mice.

2.11.3. Repellent effect

Vitex negundo is found to be repellent against some insects. Hebbalkar *et al.* (1992) in their investigation observed that oil obtained from *V. negundo* leaves and fractioned by column chromatography, showed repellent activity against *A. aegypti.* This fraction contained mixture of monoterpenes and sesquiterpenes, monoterpenes and squiterpene alcohols which affored mosquito repellency. Fifty one plant species including *V. negundo* were found to have repellent activity against *L. serricorne* (Ambadkar and Khan, 1994). Pradeep and Radhkrishnan (1998) screened a few plant species including *V. negundo* leaf extracts in aqueous, acidic and alkaline media. These were showing repellent properties against *T. castaneum.* It was also observed that the larval responses to the extracts were low when compared to adults. The chloroform fraction of the aqueous extract of fresh leaves of *V. negundo* yielded rotundial, which showed potent mosquito repellent activity (Amancharla *et al.,* 1999).

2.11.4. Antifeedant effect

Antifeedant activity of this plant has been investigated by some authors. Premeela and Muraleedharan (1995) reported the inhibition of food digestion by certain phytochemicals in the extracts of *V. negundo* and *Eupatorium odoratum* in *D. cingulatus.* Similarly, extracts of *V. negundo* and some other plants were evaluated for their antifeedant activity on *S. litura. V. negundo* was showing feeding deterrent activity in this insect. (Sahayaraj, 1998). Sharma *et al.* (2000) found that essential oils of *V. negundo* and some medicinal plants were showing antifeedant effects in *S. litura.*

2.11.5. Effect on reproduction and development

This plant is also found to have some effects on reproduction and development. Sukumaran *et al.* (1987) reported that *V. negundo* was a potential plant for the control of rice pests. It was found that petroleum ether extract and dried powder of the leaves produced malformation of pupae and failure of adult emergence in the rice leaf folder *Cnaphalocrosis medinalis* and angoumois grain moth, *Sitotroga cerealella.* Kalavathi *et al.* (1991) reported that emergence of adults of *Epilachna septima* was inhibited when pupae were treated with the extract of this plant. Effectiveness of some indigenous plant materials was also tested on *C. chinensis* and found that *V. negundo* leaf powder was very effective in reducing oviposition and adult emergence (Miah *et al.*, 1992, 1993). Singh *et al.* (1996 a) investigated the effectiveness of the extracts of *V. negundo,* neem, garlic, oranges, *Eucalyptus hybrida, L. camara, Ferula assafoetida* and "pudinhara" on the fecundity and adult emergence of *R. dominica* in wheat grains. There was lower fecundity, adult emergence and prolonged duration of generations. Essential oils of *V. negundo* and some medicinal plants were found to show growth inhibitory effects on *S. litura* (Sharma *et al.*, 2000). Raja *et al.* (2000 b) studied the efficacy of solvent residues of *V. negundo* and *Cassia fistula* leaves on egg laying and adult emergence of *C. maculatus* and its larval parasitoid *Dinarmus vagabundus.* Here, it was seen that *V. negundo* significantly reduced the number of eggs, emergence of F_1 adults. Both these plants did not affect the parasitoid.

2.11.6. Toxic/insecticidal effect

Some workers reported the insecticidal activity of *V. negundo*. Kalyanasundaram and Babu (1982) studied the larvicidal action of petroleum ether extracts of 7 indigenous plants on the 4th instar larvae of *C. quinquefasciatus*. Of these extracts, *Cleome viscosa, O. basilicum* and *V. negundo* were found to be effective at 60, 100 and 120 ppm dosage levels. Similarly, insecticidal properties of extracts of *V. negundo* and other indigenous plants against *S. litura* (More *et al.* 1989) and to the larvae of *T. confusum* (Khanam *et al.*, 1990) were reported. Kalavathi *et al.* (1991) investigated the toxicity of the extracts of *V. negundo* against insect pests such as *Earias vitella, Diaphania indica and Epilachna septima.* Here, acetone extract of the leaf was showing the most toxic effect on the insects. Eight plant species including *V. negundo* leaves were used for the control of potato tuber moth, *Phthorimaea operculella* in stores (Kashyap *et al.*, 1992). Durairaj and Venugopal (1993) tested the extracts of neem and *V. negundo* for their insecticidal activity and the effects against *Leptocorisa acuta* on rice in the field. Pandian *et al.* (1994) reported that smoke of the leaves of *V. negundo* and *Leucas aspera* were found to be toxic on *C. quinquefasciatus* than the synthetic mosquito mat. Anti-pest compounds from the volatile oils from the leaves of *V. negundo* caused ovicidal effect in *Plutella xylostella* on topical application (Dayrit *et al.*, 1995). Pushpalatha and Muthukrishnan (1995) reported the larvicidal activity of *V. negundo* leaf extract and a few plant extracts on different instars of *C. quinquefasciatus* and *Anopheles stephensi*. Efficacy and persistence of different plant extracts including *V. negundo* for their toxic effect on *R. dominica* (Singh *et*

al., 1996 b) were reported. Similarly, toxicity of leaves of *V. negundo* and some plant extracts against *S. litura* (Sahayaraj and Sekhar, 1996), against *H. armigera* (Sahayaraj and Paulraj, 2001) were also studied. Senguttuvan and Dhanakodi (1999) reported the efficacy of indigenous plant extracts including *V. negundo* in controlling the ground nut leaf miner, *Aproaerema modicella*. Similarly, larvicidal activity of some botanicals including *V. negundo* against *C. quinquefasciatus* (Arivoli *et al.* 2000) and insecticidal activity of essential oils of some medicinal plants and *V. negundo* against *S. litura* (Sharma *et al.*, 2000) have been evaluated.

2.11.7. Efficacy as grain protectant

Vitex negundo is found to show grain protectant activity against some stored product insects. Prakash and Rao (1989) reported that leaves of *V. negundo* were found to act as a pulse grain protectant against *C. chinensis*. Here, it was observed that oviposition and adult emergence of the insect decreased significantly. Similarly, leaf powder and whole dried leaves of *V. negundo* were found to have seed protectant activity against *S. zeamais* (Buiyah and Quiniones, 1990). Senguttuvan *et al.*(1995) tested a range of plant products and edible oils for their efficacy in protecting ground nuts against *C. cephalonica* among which *V. negundo* leaf powder, neem leaf powder and neem oil were most effective followed by neem seed kernel powder. Miah *et al.* (1996) found application of leaf powders and oils of *V. negundo* as protectants of lentil seeds against *C. chinensis*. The result showed that *V. negundo* leaf powder significantly reduced oviposition and adult emergence of this insect. The use of jute bags treated with some plant extracts to protect seeds of cowpea from the infestation and damage by *C. maculatus* were investigated by

Raja *et al.* (2000 b). Here, treatment of jute bags with leaf extract of *V. negundo* reduced egg laying, adult emergence and seed damage.

The foregoing account dealt in detail the various useful aspects of several locally available plants that could be adopted in IPM strategies for insect pests. The present project, aimed at investigations on the possibilities of using *Vitex negundo* in controlling the stored product pest, *Tribolium castaneum* explores the various activities of the plant extracts prepared in two solvents on different stages of the insect, mainly on adults.

Chapter III

MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Experimental Plant : *Vitex negundo* Linnaeus (Verbenaceae)

(MAL. – Vellanochi; HINDI – Sambhalu, Shivari; BENG. – Nishinda, Nirgundi; ORIYA – Beyguna; GUJ. – Nagoda).

The experimental plant, *Vitex negundo* (Plate III.1 a) was collected from the Calicut University Campus for the purpose of preparing extracts of leaves in nonpolar and polar solvents and for testing various biological effects on the test insect. The plant was identified from the herbarium of Botany Department, University of Calicut.

Vitex negundo is an aromatic shrub that occurs throughout the greater part of India, up to an altitude of 1500 m including the outer Himalayas. It is widely planted as a hedge plant along the roads and between the fields and is usually not browsed by cattle. *Vitex* can be propagated by vegetative cutting. It is also found in Afghanistan, tropical Africa, Myanmar, China, Malaysia, Pakistan, Philippines and Sri Lanka.

It is a large shrub or sometimes a smaller slender tree; bark thin, grey; branchlets quadrangular, whitish with a fine tomentum, up to 4.5 m in height. Leaves 3-5 foliolate; leaflets lanceolate, entire or rarely crenate, terminal leaflets 5-10 cm x 1.6-3.2 cm, lateral leaflets smaller, all nearly glabrous above, white tomentose beneath. Flowers bluish purple, small, in peduncled cymes, forming

PLATE III. 1

a

Vitex negundo Linnaeus

b

Tribolium castaneum Herbst

large, terminal, often compound, pyramidal panicles. Drupes globose, black when ripe, 5-6 mm in diameter, invested at the base with enlarged calyx.

The shrub is one of the common plants used in Indian medicine. Almost all its parts are employed, but the leaves and the roots are more important and are sold as drugs. Leaf extracts of this shrub are claimed to be an effective drug against rheumatic swelling of the joints, catarrhal fever and also as a tonic and vermifuge. Smoke of its leaves is used to get relief from headache. An extract of leaves shows anti-cancer activity against *Ehrlich ascites* tumour cells. The roots possess tonic, febrifugal, expectorant and diuretic properties. They are used in dyspepsia and rheumatism, and also for boils. The powdered root is prescribed as an antihelminthic, and as a demulcent in dysentery; it is also given for piles. The flowers are astringent and are used in fever, diarrhoea and liver complaints. The fruits are prescribed in headache, catarrh and watery eyes; when dried they are considered vermifuge.

The plant is reported to possess insecticidal activity against stored product pests, mosquitoe larvae, houseflies and tobacco leaf eating larvae. Oils from the plant are shown to have repellent action against mosquitoes, stored product pests and some other pests. The extract of the leaves and twigs shows antibacterial activity against *Micrococcus pyogenes* var. *aureus* and *Escherichia coli*.

The essential oils, especially mono- and sesquiterpenes, are responsible for the characteristic odour as well as for some other properties reported in folk medicines. The freshly collected leaves yield a pale greenish yellow oil (0.04 – 0.07%), the maximum yield of which is during October – November, just before

flowering. The constituents of the oil are: aldehydes and ketones, 22.5%; phenolic derivatives, 15% and cineol, 10%. The other chief phytochemical constituents of the plant are complex terpenoids, vitexilactone, casticin, chrysoplenol D, Isoorientin, flavonoids, iridoids, 2 alkaloids nishindine ($C_{15}H_{21}ON$) and hydrocotylene ($C_{22}H_{33}O_8N$), glucononitol, *p*-hydroxybenzoic acid, 5-hydroxyisophthalic acid, 3,4-dihydroxybenzoic acid, an amorphous glucoside ($C_{20}H_{26}O_{11}$), tannic acid, aucubin, agnuside ($C_{22}H_{26}O_{11}$), orientin, α-D-glucoside of a tetrahydroxy- monomethyl flavone ($C_{22}H_{24}O_{12}$), 5-hydroxy-3,6,7,3',4'-pentamethoxyflavone ($C_{20}H_{20}O_8$), vitamin C and carotene (all in leaves), lignan (seeds) and volatile oils (flowering twigs).

3.1.2. Test Insect: *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

(Rust-red four beetle, bran bug)

The stored product insect, *T. castaneum* adults (Plate III.1 b) were used for studying the various biological effects of *Vitex negundo* leaf extracts.

Identifying features: Three apical segments of antennae forming a distinct club; eyes are large and no ridge is present above the eyes; on under side of head, the width of each eye is equal to the distance separating them; margin of the head is not notched; length 2.3 to 4.4 mm; wings functional (Wilbur, 1962; Grist and Lever, 1969; Prakash *et al.*, 1987).

Distribution: It is distributed world wide, being abundant in tropics.

Host products: It is a voracious feeder and a serious pest of stored products, viz., milled products, wheat flour, milled rice, rice bran (hence bran bug), suji, flour,

cereals, pulses and processed food (Plate III. 2 a & b). It also feeds up on dry fruits, prepared cereal foods and is also observed feeding on gur and cotton seed in both field and storage.

Nature of damage: Both larvae and adults cause damage. The greatest damage is during the hot and humid monsoon season. It does considerable damage to flour and flour products and also to grains damaged by other pests. The larvae are negatively phototactic and are always found hidden in the food. The adults, however, are active creatures, capable of short flights but are mostly found concealed in flour. In severe infestation, the flour turns greyish and mouldy and has a pungent, disagreeable odour making it unfit for human consumption.

Adult: The adult beetle is small, elongate, reddish brown in colour measuring to about 2.3 to 4.4 mm in length and 1.2 mm in width. Adult is very active, moving rapidly when disturbed. The adult can survive up to 2 or more years (Metcalf *et al.*, 1962) but has a life of only 30 days at 34°C and 75% r.h. (Atansov, 1978). Mating occurs after 2 days of emergence and oviposition begins 3 to 5 days after mating (Pajni and Virk, 1982 a).

Egg: The females lay white or dirty white, transparent, cylindrical eggs in the flour, or in the frassy material among the grains and other foodstuff. The surface of freshly laid eggs is sticky and therefore, flour or dust particles easily adhere to them. Eggs measure about 0.1 to 0.3 mm and are laid singly or in groups of 5-10 eggs. A single female may lay 400-500 eggs during its life span. Egg laying continues up to 5-6 weeks. Egg hatches in 5-10 days.

PLATE III.2

a

Infested suji

b

Infested wheat flour

c

Tribolium castaneum : Life cycle

d

Rearing of *T. castaneum* colony

Larva: Larva is very active, generally creamy or white coloured and measures 5-10 mm in length. It is cylindrical, worm-like and with paired appendages on the terminal end. Larval stages, which comprises of 6-8 instars (normally 7) survive for 18-19 days at 30°C and 70% r.h. Larva moults by making a Y-shaped split on the head, which usually extends to the thorax and first 3 abdominal segments (Pajni and Virk, 1982 b).

Pupa: Pupa is whitish to yellowish brown in colour, measuring 5-8 mm in length. Normally, pupal stage lasts for 5-9 days. Sometimes it lives for 1-2 weeks. Pupae reared at 30°C become normal adults at 20°C but they do not emerge as adults at 17.5°C unless they spend 2 days or more at 30°C. Pupae can be sexed by presence of prominent genital lobes in females (Pajni and Virk, 1982 b).

Life cycle: The developmental period from egg to adult is 25-30 days in summer (Plate III.2 c). However, this may be extended up to 4-12 weeks under unfavourable conditions. The adult beetles are known to live up to 2 or more years. The optimum conditions for its development are a temperature of 35°C and r.h of 70%. Its life cycle is longer under unfavourable conditions of temperature, humidity, atmosphere, food-quality and population density.

3.1.3. Equipments and other materials

Laboratory equipments and other materials used for the experiments were the following.

1. Electronic balance (ANAMED, SARTORIUS).
2. Centrifuge (REMI).

3. Automatic Shaker (TOSHNIWAL).
4. Hot air oven (KEMI).
5. Oven (ADCO).
6. Vacuum pump.
7. Büchner funnel.
8. Glass plates (20 cm x 20 cm).
9. Spreader (TOSHNIWAL).
10. TLC Spotting guide (TOSHNIWAL).
11. TLC aligning tray (110 cm).
12. TLC developing jar with lid (25 cm x 10 cm x 25 cm).
13. Micropipette of different volumes.
14. Glass spray bulbs.

3.1.4. Chemicals

Laboratory chemicals and organic solvents (Analytical/Guaranteed Grade) were obtained from local suppliers. Glass-distilled water was used for the preparation of all aqueous solutions and reagents.

1. Petroleum ether (B.P. 60° – 80°C) [GLAXO]
2. Methanol [MERCK]
3. Acetone [BDH]
4. Anisaldehyde [MERCK]
5. Conc. Sulphuric acid [BDH]
6. Acetic anhydride [BDH]
7. Ethyl alcohol [BDH]

8. Perchloric acid [SRL]
9. Vanillin [SRL]
10. Potassium ferricyanide [SRL]
11. Ferric chloride [BDH]
12. Ceric ammonium nitrate [SRL]
13. Conc. Nitric acid [BDH]
14. Aluminium chloride [SRL]
15. Silica Gel G [MERCK]

3.2. METHODS

3.2.1. Insect culture

Adults of the rust-red flour beetle, *Tribolium castaneum* were collected from local granaries and reared in the insectary of the Department of Zoology, University of Calicut (Plate III.2 d). Insects were reared in plastic jars (size 13.5 cm height x 10 cm diameter) containing the diet (150 g wheat flour). Insect culture was maintained at room temperature (26 $\pm$ 2°C) and 70-80% r.h. Every month, newly emerged larvae, pupae and adults were separated by sifting the wheat flour with fine mesh and were transferred into clean jars containing fresh food. Larvae, pupae and adults of known age were used for the experiments.

3.2.2. Preparation of plant extracts

Leaves of *Vitex negundo* were collected from the Calicut University Campus. Fresh leaves were thoroughly washed in distilled water and shade dried at room temperature for a week and it was further dried for one day in a hot air oven

at 35-38°C. The dried leaves were then powdered using a domestic grinder and sifted through fine mesh of a sieve. The leaf powder was kept in air- tight glass bottles and stored at about 4°C. This dried leaf powder was used for the preparation of petroleum ether (nonpolar solvent) and methanol (polar solvent) extracts of *V. negundo*.

For preparing petroleum ether extract of *Vitex negundo* (VPE), 200 g of powdered material was extracted with 1400 ml of petroleum ether. Powdered plant material 50 g each were taken in 4 conical flasks of 500 ml capacity and 200 ml of petroleum ether were added to each conical flask. Mouth of the conical flasks were covered with aluminium foil and polythene cover and fastened by rubber bands. The mixtures were agitated on an automatic shaker for 24 h at room temperature and the extract was filtered through Whatman No.1 filter paper by negative pressure using a Büchner funnel. The filtrate was kept in a fridge. The residues were reextracted using another 150 ml of petroleum ether and filtered after 24 hour. The filtrates were combined and stored in glass containers.

The combined filtrate was allowed to dry in a hot air oven maintained at 40°C. The weight of the dried residue was determined. After ascertaining the final weight of the residue, 10% stock solution was prepared in petroleum ether and stored in an air- tight standard flask and kept refrigerated until used.

For preparing the working extracts, the stock extract was diluted to different concentrations (0.625%, 1.25%, 2.5% and 5%) with petroleum ether and stored in air-tight glass containers in a refrigerator.

Preparation of methanol extract of *Vitex negundo* (VME) was done in the same method as mentioned above for VPE, using methanol as the extracting solvent instead of petroleum ether.

3.2.3. Repellency bioassay (Two choice test)

Repellent effects of VPE and VME on *T. castaneum* adults were studied as detailed below.

Repellent activity of VPE of different concentrations (0.625, 1.25, 2.5 and 5%) on *T. castaneum* was evaluated by a modified method of Mc Donald *et al.* (1970). Testing of repellency was conducted in a 'choice chamber'. This consisted of glass Petri dishes at the bottom of which was placed untreated and treated filter paper semi circles. One half of the paper was soaked with 0.5 ml of the extract and the other half in petroleum ether (control). Four separate sets were kept with the 4 different concentrations of the extract. Solvents in the filter paper were allowed to evaporate off completely by keeping them open for at least 10 min. The semicircular papers were joined in the middle using clear adhesive tape. The circular edge was pasted onto the Petri dishes to prevent insects moving underneath the filter paper.

Forty healthy adult insects were randomly selected from the colony and 10 adults each were released onto the centre of the test arena of the four 'choice chambers' which provided equal area of untreated and treated paper. Each 'choice chamber' was covered with the lid of the Petri dish inside of which was pasted a correctly fitting black paper. The complete set of Petri dishes was kept in a plastic

tray at room temperature. The number of insects moved onto the untreated and treated areas were recorded at 1.5, 3, 6, 24, 48 and 72 h intervals. The experiment was replicated 6 times.

The percentage repellency was calculated for each replicate at the above-mentioned intervals and the means for different treatment periods were taken. The percentage of repellency was calculated as follows.

$$\% \text{ Repellency} = \frac{C-T}{C} x100$$

where C = number of insects on control paper;

T = number of insects on treated paper

Repellency to *T. castaneum* adults by VME was evaluated by the same method mentioned in the case of VPE. Here, different concentrations of methanol extract were used instead of petroleum ether extract.

3.2.4. Nutritional and feeding deterrence indices bioassay

Nutritional and feeding deterrence indices for *T. castaneum* adults to the VPE and VME were studied by the method of 'no choice' test using extract incorporated diet in the chamber.

Nutritional and feeding deterrence indices for *T. castaneum* adults with VPE were determined by a modified method of flour disc bioassay of Xie *et al.* (1996). Testing of this nutritional effect was conducted in the 'no choice' chambers. This consisted of separate plastic vials with perforated lids (3.2 cm diameter x 4.5 cm

height) containing diets incorporated with 3 concentrations of VPE (treated) and petroleum ether alone (control). The vials were labeled properly.

The extract incorporated diet was prepared by mixing 150 µl each of 1.25, 2.5 and 5% concentrations of VPE added drop-wise into 150 mg of each wheat flour taken in watch glasses. Similarly, same volume of petroleum ether alone was added into same quantity of wheat flour in a fourth watch glass (control). Thoroughly mixed the contents of all the watch glasses with separate glass rods and allowed the solvent to evaporate completely at room temperature for about 3 h. The 4 'no choice' chambers were weighed separately. Then 100 mg each of diet treated with different concentrations of VPE (1.25%, 2.5% and 5%) were added into the four vials (3 treated and 1 control).

Forty healthy adults, pre-starved for 24 h before the test, were weighed as 4 groups of 10 and released into the 4 pre-weighed vial ('no choice' chambers) containing the diet. They were kept in the insectary at room temperature for one day for consumption of the diet.

After 24 h, all insects were removed from the 'choice chambers' and weighed the live insects separately as 4 groups. Mortality of insects, if any, was recorded. Also weighed each vial with lid plus flour diet to determine the decrease in weight of flour diet in control and treated. Six replicates were used for each treatment and control.

The experiment was repeated with the other extract, VME, in the same pattern as with VPE. The diet in control was treated with methanol alone.

Nutritional indices were calculated as described by Manuwoto and Scriber 1982; Farrar *et al.*, 1989 with some modifications.

$$\mathbf{RGR = (A - B)/B \times day}$$

RGR = Relative Growth Rate

Where A = Weight of live insects on one day (mg)/ number of live insects on 1 day.

B = Initial weight of insects (mg)/ initial number of insects.

$$\mathbf{RCR = D/B \times day}$$

RCR = Relative Consumption Rate

Where D = biomass ingested (mg)/ number of live insects on 1 day.

$$\mathbf{ECI\ (\%) = (RGR/RCR) \times 100}$$

ECI % = Percentage of Efficiency of Conversion of Ingested food.

Antifeedant activity (feeding deterrence activity) was calculated as Feeding Deterrence Index (Isman *et al.*, 1990).

$$\mathbf{FDI\ (\%) = [(C–T)/\ C] \times 100}$$

FDI % = Percentage of Feeding Deterrence Index

Where C = Consumption of control diet

T = Consumption of treated diet.

3.2.5. Effects of the extracts on emergence, development and progeny reduction

In this bioassay, the effect of wheat flour diet treated with different concentrations of VPE and VME on emergence, development and progeny reduction (mortality of larvae, pupae and adults) in *T. castaneum* had been evaluated.

Twenty grams each of wheat flour diet was taken in 4 beakers and 10 ml each of 2.5%, 5%, 10% of the extract (treated) and petroleum ether alone (control) added to the wheat flour in the beakers and thoroughly mixed with separate glass rods. They were kept at room temperature overnight to evaporate off petroleum ether. Wheat flour samples were transferred to labeled plastic vials (13.5 cm diameter x 5 cm high). Healthy adults of *T. castaneum* of 1-2 weeks old (20 nos) were released into each treated and control wheat flour taken in the plastic vials. They were kept in the insectary at 26 ± 2°C and 70-80% r.h. for an oviposition period of seven days. After 7 days, adults were removed from the treated and control wheat flour by sifting and the wheat flour with eggs were kept in the insectary at the same conditions for another 60 days. Observations were made for the emergence of larvae, pupae and adults starting from 15th day. Subsequently, number of larvae, pupae and adults present in the diet were noted after 25, 30, 40, 50 and 60 days. Mortalities of the progeny at various stages were also recorded. Progeny of the larvae, pupae and adults were separated at these intervals by sifting the wheat flour through fine mesh of a sieve. After enumeration, larvae and pupae

were again transferred to respective vials but adults were removed. Four replicates of each treated and control were used for this bioassay.

The experiment was repeated to study the effect of VME on emergence, development and progeny reduction in *T. castaneum* using the same procedure used for VPE. Here, wheat flour diet treated with VME at 2.5, 5 and 10% concentration were used instead of VPE and the control diet was treated with methanol alone.

3.2.6. Bioassay for effect on adult emergence

Effects of VPE and VME on emergence of *T. castaneum* adults during 25, 30, 40 , 50 days and the total number of adults emerged during 50 days were evaluated. Adult emergence index and the reduction or inhibition of progeny for this period was also studied. In this bioassay, 4 replicates each of the three treated and control of the experiment mentioned in the section 3.2.5 were used. Effects of different concentrations of (2.5%, 5% and 10%) VPE (treated) and petroleum ether alone (control) on adult emergence of *T. castaneum* at intervals of 25, 30, 40 and 50 days were determined. From the total number of adults emerged during 50 days, adult emergence index and the percentage of inhibition of the progeny during 50 days were calculated. Adults (alone) were removed from treated and control wheat flour medium at intervals by sieving.

The experiment was carried out with VME also in a similar way using various concentrations of VME (experimentals) and the solvent methanol (control).

The mean number of adults emerged from the replicates of the treated and those from the untreated control during 50 days and per cent inhibition or reduction of adult emergence was calculated by the formula used by Mian and Mulla (1982).

Per cent inhibition of progeny = 100 (1 – t/c)

where, t = number of adults in the treated diet;

c = number of adults in the untreated diet (control).

3.2.7. Toxicity bioassay

Evaluation of toxic effects of VPE and VME on *T. castaneum* adults was done by film residue and topical application methods.

3.2.7.1. Evaluation of toxicity of VPE against *T. castaneum* by the film residue method (FRM)

Films of petroleum ether extract at concentrations of 0.625, 1.25, 2.5, 5 and 10% were prepared in separate Petri dishes (8.5 cm diameter) by 1 ml of the extract of the respective concentrations which was poured and smeared inside the dish and the lid of each Petri dish set. They were swirled to get a uniform coating of the extract on the inside surface and then air dried for about 30 min for the complete evaporation of the solvent. A control was also prepared in another set of Petri dish using 1 ml of petroleum ether alone.

Sixty healthy adult insects (1-2 week old) were selected from the colony and 10 adults each was released into each Petri dish and then covered with the lid. A black paper was placed over the tray. This was allowed to remain for 7 days in the

laboratory conditions at 26 ± 2° C temperature and 70-80% r.h. in a plastic tray. Observations of mortality of insects in each Petri dish were made everyday for a period of one week. Insects that did not move or respond to gentle touches were considered as dead. 10 replicates were maintained in each of the treated and the control.

Mortality values were converted to corrected percentage mortality by Abbott's (1925) formula.

$$\textbf{Corrected \% mortality} = [\,(T - C) / (100 - C)\,] \times 100$$

where T = percentage mortality in treatment;

C = percentage mortality in control.

3.2.7.2. Evaluation of toxicity of VPE by the topical application method (TAM)

For evaluating the toxicity of VPE on *T. castaneum* adults by the topical application, different concentrations of VPE, viz., 0.625, 1.25, 2.5, 5 and 10% were taken for treatment. Petroleum ether alone was used in the controls.

Sixty healthy adult insects (1-2 weeks old) were selected from the colony and 10 adults each were released into separate Petri dishes. Samples of 5 µl of different concentrations of the extract (0.625, 1.25, 2.5, 5 and 10%) were applied topically on the dorsum of the thorax of each insect of all the groups except the control, with the help of a micropipette. Petroleum ether alone was topically applied in the case of control. Insects were kept under an electric ceiling fan for about 15 min for evaporation of the solvent. The dishes were covered with lids and

kept for 96 h. These six Petri dishes were covered with a black paper and kept in the laboratory under conditions of 26 ± 2°C and 70-80% r.h. Mortality of adult insects in each treated and control Petri dishes at intervals of 1.5 h, 3 h, 6 h, 24 h, 48 h, 72 h and 96 h were recorded. The insects, which did not move or respond to gentle touch, were considered as dead. Ten replicates were maintained in each treated and control of this experiment.

Mortality values were converted to corrected percentage mortality by Abbott's (1925) formula as mentioned in the section 3.2.7.1.

3.2.7.3. Evaluation of toxicity of VME against *T. castaneum* by FRM

The method adopted to study the toxicity of VME on adult *T. castaneum* by the film residue method was similar to that described in section 3.2.7.1 Here, various concentrations of VME were used instead of VPE.

3.2.7.4. Evaluation of toxicity of VME against *T. castaneum* by TAM

The method adopted to study the toxicity of VME on *T. castaneum* adults by the topical application method were similar to that adopted in experiments described in 3.2.7.2. Here, also methanol extract at different concentrations were used instead of VPE.

3.2.8. Thin layer chromatographic separation of *V. negundo* extracts

Thin layer chromatography (TLC) was employed for the separation of various constituents of VPE and VME and their further phytochemical analysis.

a. Preparation of plates: Glass plates (20 cm x 20 cm) were washed thoroughly with detergent and water and cleaned by cotton soaked in alcohol. Placed clean dry glass plates on a TLC aligning tray over a plane surface. A slurry of silica gel G (adsorbent) in 10% methanol was prepared. For 400 μm thickness of the adsorbent, 75 g silica gel G in 140 ml of 10% methanol was used. Air-dried plates were stored in desiccated cabinet. Plates were activated at 110°C in an oven for 60 min just before use.

b. Sample application: Extracts of *Vitex negundo* prepared in petroleum ether or methanol were used as sample for application on the plates for their thin layer chromatographic separation. After activation, the plate was taken from the oven and it was kept at room temperature for 15 minutes for cooling. Sample of 5% was applied as bands on the silica gel plate 2 cm above the lower end of the plate using a capillary tube. About 1.0 ml of the extract was loaded on to the plate and properly dried.

c. Developing chromatogram: The developing solvent was ethyl acetate – petroleum ether (60-80 °C) in 1:4 ratios. The developing solvent (about 250 ml) was poured into a glass tank (25 cm x 10 cm x 25 cm). Filter paper lining kept in the tank to a depth of about 15 cm maintained tank saturation. After about 30 min of saturation, loaded TLC plates were introduced into the tank. Care was taken to keep the level of solvent in the tank below the sample band. Kept the tank covered with its lid and allowed the solvent to move up to 1 cm below the top of the TLC plate. The separation of the components occurred as the solvent moved upward.

This took about 30-35 minutes. The plate was then removed from the tank and air-dried.

Both VPE and VME were separated on TLC using the same procedures as described above.

3.2.9. Evaluation of toxicity of TLC fractions

a. Preparation of TLC fractions of the extracts for toxicity bioassay

Silica gel (from 10 plates) corresponding to various fractions (F_1-F_{13} and F_0, 14 fractions in the case of VPE; F_1-F_{17}, and F_0, 18 fractions in the case of VME) were scrapped off and pooled and eluted in 50 ml each of acetone on an automatic shaker. The mixtures were centrifuged to remove the gel. All the supernatants were reduced to a final volume of 2.0 ml (stock solution) and kept in air-tight containers.

b. Testing the toxicity of TLC fractions by TAM

The method adopted to evaluate the toxicity of various fractions of the extracts (separated on TLC) by topical application was same as that described in the section 3.2.7.2 and 3.2.7.4. Various fractions as mentioned in 3.2.9 a (Plate IX.1 a,b; Table IX.1,2) were used for testing their toxicity.

3.2.10. Methods for the phytochemical analysis and identification of the chemical constituents on thin layer chromatogram

The classes of chemical constituents present in VPE and VME were identified by the colour developed on the chromatogram after spraying with various

chemical reagents specific for different classes of the constituents. Important chemical constituents present in *V. negundo* are known from literature, which was used for the identification of the toxic phytochemical constitutents. R_f values of the fractions of VPE and VME on the chromatogram were noted before and after spraying the visualizing reagents. Preparation and application of the spray reagents were done according to the methods described by Krebs *et al.* (1969) and Touchstone and Dobbins (1978).

a. Separation and identification of the chemical constituents of the extracts

The method for the preparation of plates for TLC was same as in the section 3.2.8 a. The plates were prepared with a thickness of 300 μm. Here, 55 g silica gel G was used for the preparation of slurry in 100 ml 10% methanol. Samples of the extracts (0.25 ml of 5%) were applied on the chromatogram and the method of application and development of chromatogram were similar to those described in 3.2.8 b and c).

Spray reagents and analysis of phytochemical constituents

i) ***Anisaldehyde – sulphuric acid*** **(for identification of terpenes, steroids, etc.):** Concentrated sulphuric acid (1.0 ml) was added to a solution of 0.5 ml anisaldehyde in 50 ml acetic acid and mixed thoroughly. After spraying this mixture on the chromatogram, the plate was heated at 100-105°C until the spots attained maximum colour intensity. Freshly prepared reagent was used every time.

ii. ***Acetic anhydride – sulphuric acid (Liebermann – Burchard reagent)*** **(to detect the presence of triterpene glycosides and steroids):** This reagent was

prepared by mixing 5.0 ml of acetic anhydride with 5.0 ml conc. sulphuric acid under cooling. This mixture was added cautiously to 50 ml absolute ethanol with cooling. After spraying, the TLC plate was heated for 10 min at 100°C. The reagent was freshly prepared before use.

iii. ***Perchloric acid*** **(for the detection of steroids):** Sprayed the chromatogram with 20% aqueous perchloric acid and heated for about 10 min at 150°C until maximum colour of the spots was reached.

iv. ***Vanillin-sulphuric acid*** **(for identification of essential oils, higher alcohols, phenols and steroids):** Vanillin (1.0 g) dissolved in 100 ml conc. sulphuric acid sprayed on the chromatogram. Heated the TLC plate at 120°C for 10-20 min. until the spots attained maximum colour intensity.

v. ***Potassium ferricyanide – ferric chloride*** **(for identification of phenols and amines):** Equal volumes of 1% aqueous potassium ferricyanide and 2% aqueous ferric chloride solutions were mixed and this reagent sprayed on the TLC plates. The colours were intensified by subsequent spraying with 2 N hydrochloric acid.

vi. ***Aluminium chloride*** **(for the detection of flavonoids):** Sprayed 1% aluminium chloride solution in ethanol on the chromatogram and viewed the plates under long-wave UV lamp.

vii. ***Ceric ammonium nitrate*** **(for the detection of alcohols (polyalcohols):** Ceric ammonium nitrate (6%) in 2 N HNO3 was prepared and sprayed on the

chromatogram. Dried the plate for 5 min at 105°C until the spots attained maximum colour intensity

b. Further separation and identification of the chemical constituents of F_0 fraction of VME

The method for the preparation of plates was same as that described in section 3.2.8. a. The plates were prepared with a thickness of 300 μm. Here, 0.25 ml of F_0 of VME (origin) was the sample applied on the plate. Ethyl acetate alone was the developing solvent used for the separation and the method of development of the thin layer chromatogram was similar to that described in section 3.2.8 c.

The chromatograms were sprayed with various spray reagents for identification of various chemical constituents in the F_0 fraction in a way similar to that described in section 3.2.10 a above.

3.2.11. Statistical analysis of data

In the present investigation, the data obtained for various biological effects of VPE and VME on *T. castaneum* were subjected to appropriate statistical analysis. Different variables such as extracts, concentrations and durations of treatment had to be compared for their effect on insects and determined their significance level.

In Chapter IV, Analysis of Variance (ANOVA) was employed for testing the significance of difference between the activities of the two extracts, difference between the effects of different concentrations of the extracts and various time periods (hours) on repellent activity in *T. castaneum* adults.

In Chapter V, the data of the effects of VPE and VME, their comparison and effects of different concentrations on relative growth rate and relative consumption rate for *T. castaneum* were subjected to Analysis of Covariance (ANCOVA) to find out their significance level. The analysis of covariance is a technique that combines the features of analysis of variance and regression. However, significance in the comparison of two extracts, effects of different concentrations on efficiency of conversion of ingested food and feeding deterrence indices for the insects were analysed by ANOVA. For finding the significance of ECI and FDI for the insects, tests for homogeneity of variances such as Hartley's, Cochran's and Bartlett's were used.

In Chapter VI and VII, the data were subjected to ANOVA for finding significance in the effects of VPE and VME on emergence, development and mortality of the progeny and adult emergence of *T. castaneum*.

In Chapter VIII, the data of contact toxicity of VPE and VME against *T. castaneum* were subjected to Probit Analysis (Finney, 1971) for calculating the regression equation and LC_{50}, LC_{80} and LC_{95} values of the extracts.

In Chapter IX, Pearson Chi-square (χ^2) test was adopted to find the significance of the difference among toxic effects of TLC fractions of VPE and of VME against *T. castaneum*.

Chapter IV

BIOASSAY FOR REPELLENCY OF *VITEX NEGUNDO* ON *TRIBOLIUM CASTANEUM*

4.1. Introduction

Insects often cause extensive damage to stored grain products, which reduce the quantity and quality of grain during storage. Although food grains are commonly protected by insecticides and fumigants, such practice pose health risks unless the chemicals used are safe to mammals. Due to such disadvantages of pesticides, other ways of controlling insects are becoming more important.

Insect repellents offer a group of new and potential agents for protecting stored grains from insect pests, that help us to establish new control practices, which are quite specific, and having low mammalian toxicity and low persistence in the environment. In fact, management of stored product pests using substances of natural origin is nowadays the subject of much research. Dethier *et al.* (1960) defined insect repellents as chemical substances, which cause insects to make oriented movements away from the source of the substance. Repellents have the potential for exclusion of stored product pests from grain and have been used to prevent insect feeding and oviposition. To improve the protection of stored products from insect attacks, there is a continuing need for repellents that are more effective, more persistent, more economical and more practical to use, than the existing synthetics available. More than 1400 such compounds have been evaluated for their repellency (Anon., 1959). Similarly, some active naturally occurring materials have been extracted from plants and chemically identified.

Several plant extracts have been reported to possess repellent properties against many stored product insects. Jilani and Su (1983) reported that solvent extracts of rhizomes of *Curcuma longa* and leaves of *A. indica* and *Trigonella foenum-graecum* were effective to protect cereal grains against *T. castaneum*, *S. granarius* and *R. dominica*. Similarly, the repellent effects of extracts of several plant species (Malik and Naqvi, 1984; Qureshi *et al.* 1988) and *Melia toosendan* seed kernel extract (Xiong and Deng, 1992) have been evaluated against *T. castaneum*. Ground leaves, barks, seeds and seed extracts of *Aphanamixis polystachya* were reported to possess repellent effect against *C. chinensis* (Talukder and Howse, 1994) and *T. castaneum* (Talukder and Howse, 1995).

The repellent activities of azadirachtin and neem extracts (Xie *et al.*, 1995 a) and bark extracts of *Melia toosendan* and the extract containing toosendanin (Xie, *et al.*, 1995 b) against *Cryptolestes ferrugineus, S. oryzae* and *T. castaneum* have been investigated. Similarly, extracts of several other plants have been successfully evaluated against many stored product insects (Gu *et al.*, 1997; Ho *et al.*, 1997 c; Novo *et al.*, 1997; Suss *et al.*, 1997; Egwunyenga *et al.*, 1998; Pascual, 1998; Pradeep and Radhakrishnan, 1999; Rahman *et al.*, 1999). Repellency of the extracts of sweetflag rhizome, kut root, curry leaf, kinnow peel and crude mustard oil, two commercial neem formulations (nimbicidin and repellin) and one synthetic pyrethroid (cypermethrin) have been evaluated by Chander *et al.* (2000) and found to be effective as prophylactic spray to protect bagged grain against *T. castaneum*. Abubakar *et al.* (2000) have investigated the repellent properties of petroleum ether and methanol extracts of the *Cyperus articulatus* rhizome against *T. castaneum*.

Natural oils such as olive oil, cedar wood oil and karanja oil, and black pepper extract (Sighamony *et al.*, 1984), volatile oil compounds such as benzaldehyde, piperidine and geraniol from bay leaves (Saim and Maloan, 1986) and diterpenoid alkaloids from *Delphinium consolida* and *Aconitum* species (Ulubelen *et al.*, 2001) have been found to act as repellents against *T. castaneum.*

Repellent effects of turmeric oil, sweetflag oil, neem oil and Margosan-O have been evaluated against *T. castaneum* (Jilani *et al.*, 1988) and *R. dominica* (Jilani and Saxena, 1990). Su (1991) reported the repellent activity of *Chenopodium* oil against *C. chinensis, T. confusum, S. oryzae* and *L. serricorne.* Essential oil constituents such as 1,8-cineole, eugenol and camphor from *Ocimum kenyense, O. suave* and *O. killimandscharicum* have been evaluated by Obeng-Ofori *et al.* (1997 a) for their repellent activities against *S. granarius, T. castaneum, P. truncatus, L. serricorne* and *S. paniceum.* Similarly, many essential oils have been investigated for their repellent properties against several stored product pests (Obeng-Ofori and Reichmuth, 1997; Obeng-Ofori *et al.*, 1997 b, 1998; Liu and Ho, 1999; Padin *et al.*, 2000; Verma *et al.*, 2000). Papachristos and Stamopoulos (2002) reported 13 essential oils from plants in their vapour form, which showed repellency against *Acanthoscelides obtectus.*

The experiments described in this chapter are to evaluate the repellent effects of petroleum ether and methanol extracts of *V. negundo* on the rust-red flour beetle, *T. castaneum.*

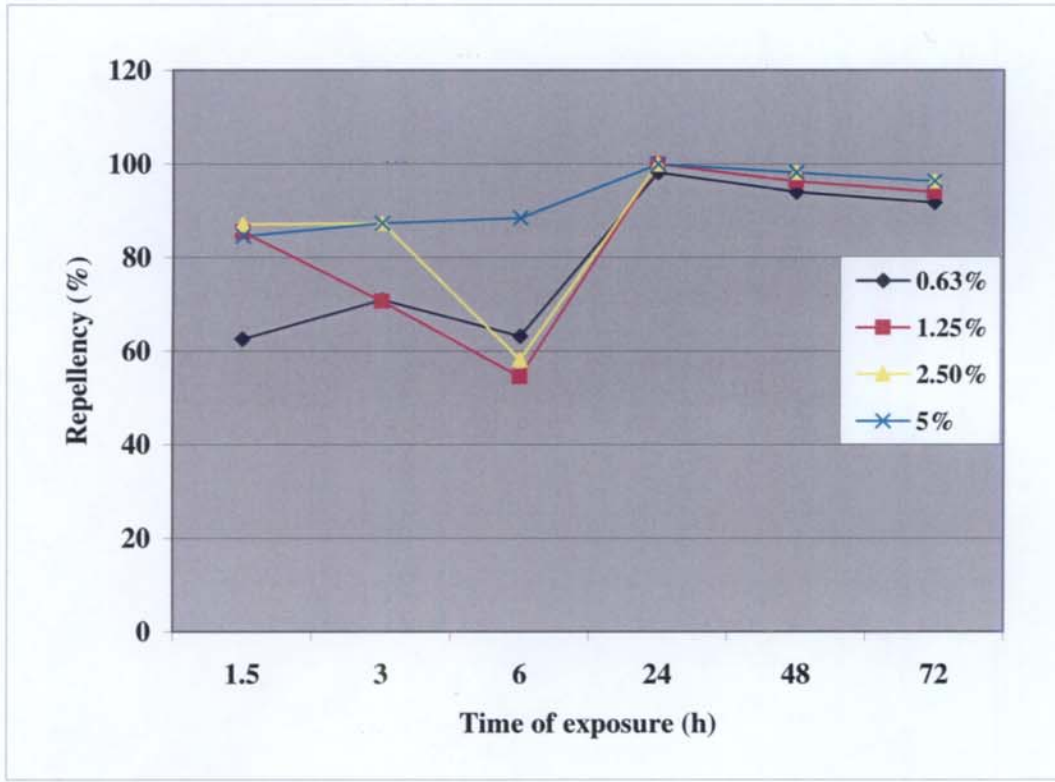
TABLE IV.1

Repellency of various concentrations of VPE against *T. castaneum* adults

Conc. of VPE (%)	Corrected percentage repellency rates at different durations of exposure (hours)					
	1.5	3	6	24	48	72
0.625	62.50 ± 5.54	70.90 ± 4.27	63.09 ± 1.54	98.15 ± 0.76	93.98 ± 1.72	91.67 ± 2.15
1.25	85.45 ± 2.43	70.63 ± 5.55	54.50 ± 3.42	100	96.30 ± 0.96	93.98 ± 1.72
2.5	87.04 ± 4.45	87.30 ± 2.62	58.13 ± 3.40	100	98.15 ± 0.76	96.30 ± 0.96
5	84.52 ± 3.03	87.30 ± 2.62	88.43 ± 1.32	100	98.15 ± 0.76	96.30 ± 0.96

Values are expressed as Means ± SEMs (n = 6).
Sample: 10 insects x 4 conc. x 6 replications = 240 insects.
See section 3.2.3 of Chapter III for repellency formula and other details.

Fig. 4.1. Time-course repellent activities of various concentrations of VPE against *T. castaneum* adults



4.2. Methods

The method described in 3.2.3 was adopted to evaluate the repellency of VPE and VME on *T. castaneum* adults.

4.3. Results

4.3.1. Repellent effect of VPE on *T. castaneum*

The repellent effects of different concentrations of VPE on *T. castaneum* adults during different time periods were evaluated using two choice bioassay. In this experiment, when the insects were released at the centre of the filter paper of the 'choice chamber', it was observed that they moved about on the treated and control areas for some time. Then, after different time intervals (1.5, 3, 6, 24, 48 and 72 h), it was observed that majority of insects (> 80%) were repelled from the treated area. They moved towards the control area and remained there as a group. Insects remaining on the treated area were seen moving over the filter paper randomly.

From the results of this experiment, it was found that all the concentrations of the extract (0.625, 1.25, 2.5 and 5% of VPE) strongly repelled *T. castaneum* adults and the percentage repellency rates were between 55-100% during different time intervals.

Table IV.1 provides the corrected percentage repellency rates of various concentrations of VPE against *T. castaneum* adults during different time intervals. The pattern of activity is represented in Figure 4.1. From a study on repellent activity, the following results were obtained. The low concentration (0.625%) of

VPE during an exposure of 1.5 h showed a repellency of 62.50 ± 5.54 agaisnt *T. castaneum* adults. During 3 h exposure time, repellent activity increased to 70.90 ± 4.27, which however, showed a decline of activity (63.09± 1.54) when the exposure period was extended to 6 h. The repellency rate at this period was almost equal to the value that was seen at 1.5 h exposure. After 6 h, the activity was found to show a sharp increase (98.15 ± 0.76) during an exposure period of 24 h. However, afterwards there was slight decline of repellency during 48 h (93.98 ± 1.72) to 72 h (91.67 ± 2.15).

With 1.25% VPE, high repellency rate (85.45 ± 2.43) was observed at 1.5 h exposure period compared to that shown by 0.625%. However, repellency rates decreased considerably to 70.63 ± 5.55 and 54.50 ± 3.42 when the exposure time was increased to 3 h and 6 h respectively. At the same time, when the exposure period was increased to 24 h, 1.25% showed a reverse trend and the percentage repellency reached a maximum (100). With 48 h and 72 h exposure, activity was found to slightly decrease to 96.30 ± 0.96 and 93.98 ± 1.72 respectively as in the case of low concentration of the extract.

The repellent activity of 2.5% VPE, during the exposure times of 1.5 h and 3 h, were some what similar (87.04 ± 4.45 and 87.30 ± 2.62 respectively) and the activity was found to be better than that of 1.25% VPE. However, repellency was found to decrease sharply during 6 h exposure period (58.13 ± 3.40) and was having similar pattern as that of other lower concentrations. During 24 h, repellent activity reached maximum (100) and was similar to that of 0.625 and 1.25% VPE. There was a sudden and steep increase in activity. However, during 48 h and 72 h

of exposure, activities were found to be maintaining higher plateau similar to that found with other concentrations (98.15 ± 0.76 and 96.30 ± 0.96).

The highest concentration (5% VPE) also showed strong repellency (84.52 ± 3.03) during 1.5 h exposure. The rates of repellency gradually increased to 87.30 ± 2.62 and to 88.43 ± 1.32 respectively for the exposure periods of 3 and 6 h. When compared to that of 2.5% VPE, activity of 5% VPE was found to be similar and high at 3 h, and very high at 6 h exposure. Unlike other concentrations of the extract, 5% VPE was not showing decrease of activity during 6 h. During 24 h of exposure, activity reached maximum (100). Beyond 24 h, repellency was found to be slightly declining during 48 h (98.15 ± 0.76) and 72 h (96.30 ± 0.96) following the same pattern of 2.5% VPE.

Figure 4.1 gives the time-course of repellent activities of various concentrations of VPE against *T. castaneum*. This graph clearly shows that the extent of initial repellency observed during early periods of exposure (1.5 to 3 h) dropped considerably in about 6 h of exposure. After 6 h, the activity showed a reverse trend and at 24 h maximum repellency was reached which was more or less maintained through out the 72 h of exposure. This was more or less applicable to the different concentrations of the extract.

4.3.2. Repellent effect of VME on *T. castaneum*

Two choice bioassay was also employed for evaluating the repellency of VME against *T. castaneum* adults. In this test also, general behaviour of insects observed in the 'choice chamber' of the treatment was similar to that in the test of

VPE and the majority of insects were repelled away from the treated area towards the control area where they remained as a group. From this experiment also, it was shown that all the concentrations of VME (0.625, 1.25, 2.5 and 5%) had higher repellency rates, which ranged between 62-100% during different time periods of treatment.

Table IV.2 shows the corrected percentage repellency rates of *T. castaneum* adults brought about by various concentrations of VME during different time intervals. The pattern of activity of the extract on insects is provided in the Figure 4.2. From the investigations on repellency, the following results were obtained. With 0.625% of VME, the repellency was found to be 62.3 $\pm$ 4.38 during 1.5 h exposure. When the exposure was increased to 3 h, there was an increase in the repellency (79.17$\pm$ 3.97). However, the activity decreased to 66.93 $\pm$ 4.95 after 6 h. During 24 h of exposure, a reverse trend in activity was observed. The repellency increased to 82.87 $\pm$ 4.37, which further increased to 86.84 $\pm$ 2.93 after 48 h exposure. After this period, there was a sudden decline of activity at 72 h of observation (72.22 $\pm$ 7.38).

With 1.25% of VME, the repellent activity at 1.5 h (77.78 $\pm$ 6.42) increased to 82.87 $\pm$ 4.37 after 3 h exposure period. After this period, activity of 1.25% VME was found to cause a sudden decline at 6 h (68.06 $\pm$ 4.57). It was seen that there was an increase in activity to 85.71 $\pm$ 3.69 during 24 h exposure period. The pattern of activity of this concentration up to 24 h was similar to that observed with 0.625% VME. However, 1.25% VME showed a slight decline at 48 h (81.75 $\pm$ 4.88) and an increase of activity at 72 h (87.04 $\pm$ 4.45) of exposure time.

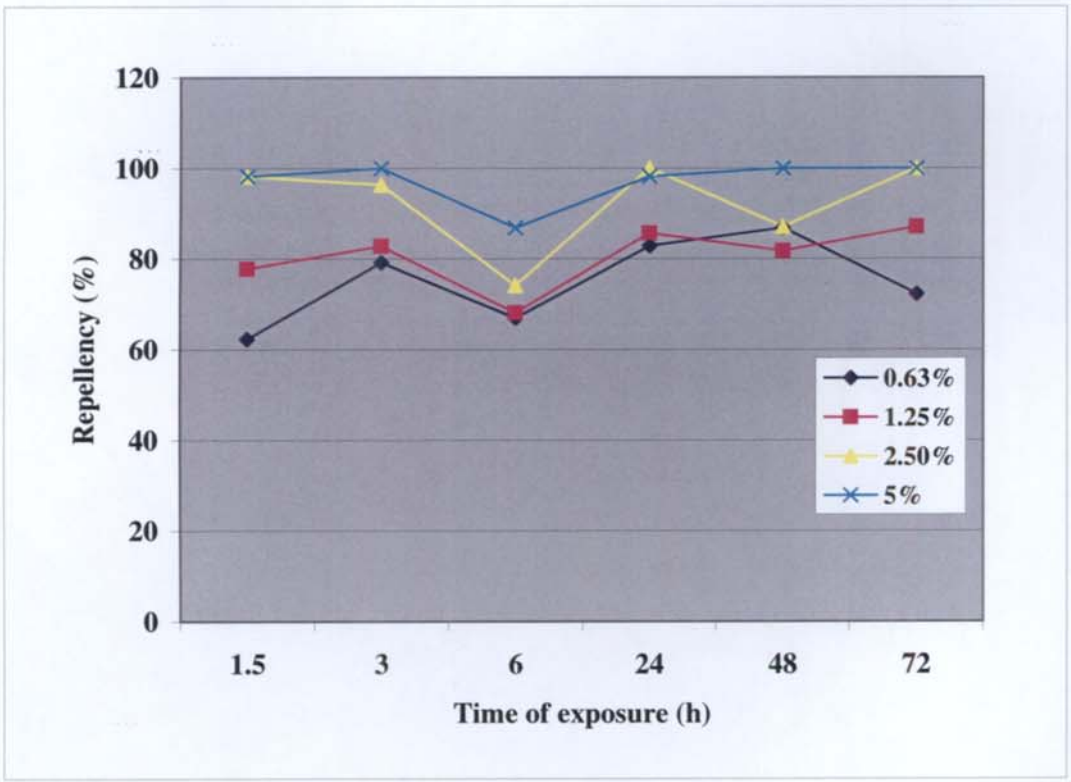
TABLE IV.2

Repellency of various concentrations of VME against *T. castaneum* adults

Conc. of VME (%)	Corrected percentage repellency rates at different durations of exposure (hours)					
	1.5	3	6	24	48	72
0.625	62.3 ± 4.38	79.17 ± 3.97	66.93 ± 4.95	82.87 ± 4.37	86.84 ± 2.93	72.22 ± 7.38
1.25	77.78 ± 6.42	82.87 ± 4.37	68.06 ± 4.57	85.71 ± 3.69	81.75 ± 4.88	87.04 ± 4.45
2.5	98.15 ± 0.76	96.30 ± 0.96	74.07 ± 5.32	100	87.04 ± 4.45	100
5	98.15 ± 0.76	100	86.84 ± 2.93	98.15 ± 0.76	100	100

Values are expressed as Means ± SEMs (n=6).
Sample: 10 insects x 4 conc. x 6 replications = total 240 insects.
See the section 3.2.3 for repellency formula and other details.

Fig. 4.2. Time-course repellent activities of various concentrations of VME against *T. castaneum* adults



Methanol extract at 2.5% was showing strong repellency of 98.15 ± 0.76 initially during the period of 1.5 h exposure than other low concentrations of VME. After this period, activity was found to show a slight drop at 3 h (96.30 ± 0.96) and sharp decline at 6 h (74.07 ± 5.32), which were however showing high rates of repellency compared to other lower concentrations of the extract during these periods. Like other concentrations, here also there was a reversal of activity with this concentration at 24 h exposure period (100). However, a decline of activity at 48 h (87.04 ± 4.45) was seen, which was immediately followed by a sharp increase to reach maximum repellency at 72 h exposure period (100).

With 5% VME, repellent activity was found to be high and similar (98.15 ± 0.76) to that of 2.5% during 1.5 h but maximum (100) at 3 h exposure time. However, activity of VME was found to decline (86.84 ± 2.93) during 6 h treatment, which was similar to that of other concentrations, but it had the highest rate of activity. During 24 h, the percentage repellency was found to be increasing gradually (98.15 ± 0.76) and steadily through 48 h up to 72 h to reach 100% repellency.

Figure 4.2 provides the time-course of repellent activities of various concentrations of VME against *T. castaneum*. Like VPE, here also the extent of initial repellency of VME on *T. castaneum* during early periods of treatment (1.5 to 3 h) was reduced considerably in about 6 h treatment where the repellency went down considerably. After 6 h, there was a reversal of activity and the extent of repellency at 24, 48 and 72 h showed various levels. However, repellency with 5%

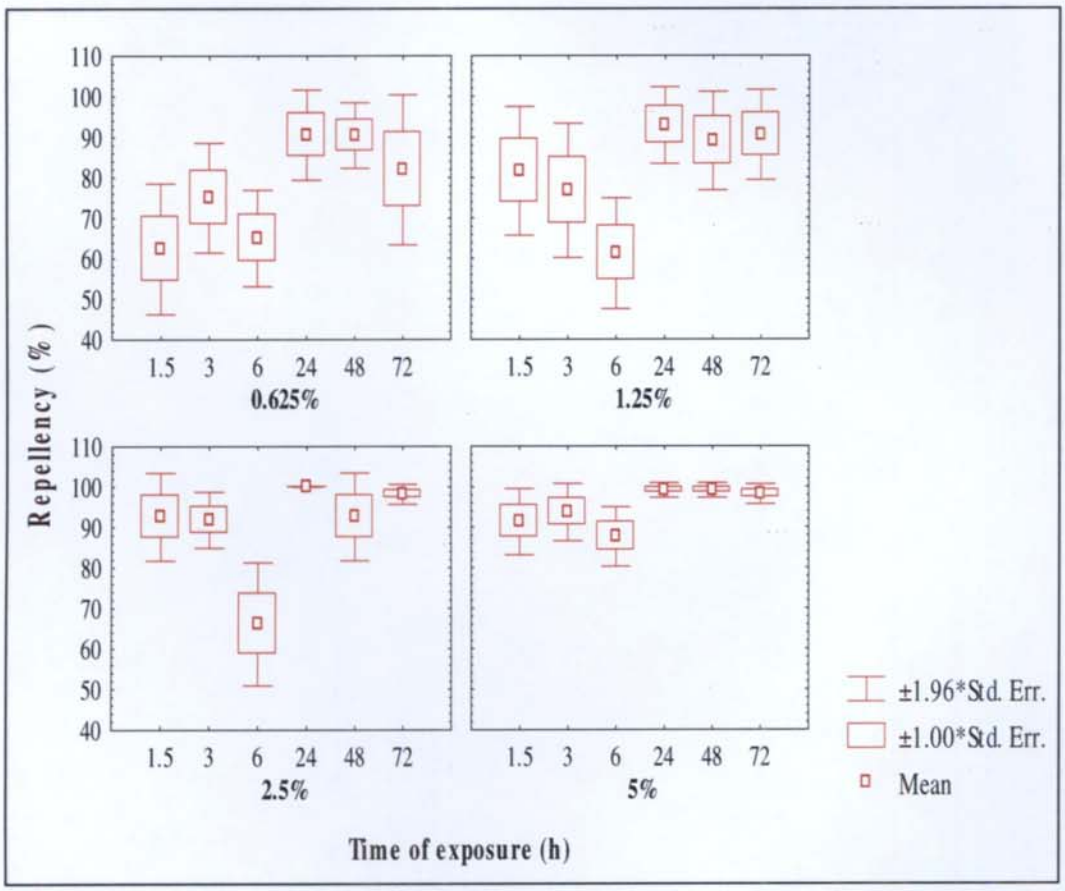
TABLE IV.3

ANOVA of data showing the repellent effects of VPE and VME on *T. castaneum* adults

Source of variables	df	MS	df Error	MS Error	F	P level
Extracts	1	11.18	240.00	382.46	0.03	0.86
Conc.	3	4385.21	240.00	382.46	11.47	<0.001**
Time	5	4331.21	240.00	382.46	11.32	<0.001**

**Shows very high significance.

Fig. 4.3. Categorised plot for variable of percentage repellency of VPE and VME on *T. castaneum* adults



Data represent the pooled values for the repellent effects of VPE and VME for different concentrations used.

The difference between the activities of the two extracts was not significant. (ANOVA, P = 0.86)

concentration remained at a higher plateau and that with 0.625% went to a lower plateau.

From the data of repellency (Table IV.2; Fig. 4.2) of VME on *T. castaneum* it seems apparent that the extract of various concentrations in the case of VME, maintained more or less a steady activity (between 60-100%) with slight variation as the time of exposure is increased. However, these effects were found to be statistically significant (Table IV.3).

4.3.3. Statistical analysis of data

The data obtained from studies on the repellent effects of VPE and VME on *T. castaneum* adults were subjected to ANOVA for testing the significance of repellent effects of these extracts on this insect. ANOVA of data indicates that both VPE and VME are showing high repellency rates and the difference between the repellent effects of these two extracts on insects was not significant ($P=0.86$) [$df=1$; $F=0.03$]. However, difference among concentrations of the extracts ($P<0.001$) [$df=3$; $F=11.47$] and different times of exposure ($P<0.001$) [$df=5$; $F=11.32$] on repellency were highly significant (Table IV.3).

Figure 4.3 presents the categorized plot for the variable of percentage repellency of various concentrations of VPE and VME on *T. castaneum* adults during different time periods.

4.4. Discussion

Experiments carried out to study the repellent effects of VPE and VME on *T. castaneum* adults have shown promising results. It is evident from the result that

both the extracts of *V. negundo* are showing strong repellent activities on *T. castaneum* adults. It is also apparently seen from the results of repellency bioassay that almost all concentrations of VPE and VME strongly repelled *T. castaneum* within a range of 55 to 100% during 1.5 to 72 h of exposure (Table IV. 1 & 2). Here, the difference between two extracts with respect to their repellent effect on insects was not significant (P = 0.86) (Fig. 4. 3.). The strong repellency of the two extracts in this study are reflected by a gradual decrease in the number of insects approaching the treated area in the 'choice chamber' which in turn is the result of the chemosensory effects of the active components in these two extracts. Fields *et al.* (2001) explained the repellent effects which they observed in the case of several stored product insects on wheat to fractions of pea seed, as due to the chemosensory effect of the fractions, either on the olfactory or gustatory receptors.

The repellency observed in our experiment was found to be significantly dose-dependent (Tables IV.1 & 2; Figs. 4.1 & 2) ($P< 0.001$). It was found that the rate of repellency of the insects increased with the increase in concentration of the extracts during different periods of treatment. In the case of repellent effect of VPE, maximum repellency was reached in about 24 h of treatment and it was also found that 2.5 and 5% concentrations had almost the same effect especially at 24, 48 and 72 h. Hence, it is inferred that further increase of concentration from 2.5% did not have much influence on repellency (Table IV.1). In the case of VME also, 2.5 and 5% were also showing higher rate of repellency (98-100%) at 24 h and had almost maintained the effect (87-100%) afterwards during 48 and 72 h (Table IV.2).

The dose-dependent repellency of these extracts is in agreement with the findings of various authors. Jilani *et al.* (1988) demonstrated the repellency of sweetflag oil, neem oil and Margosan-O against *T. castaneum,* which increased with an increase in the concentration of the test materials. Jilani *et al.* (1990) also reported similar effects of these materials against *R. dominica*. Similar observations were made by Tripathi *et al.* (1997) on the repellent effects of fruit oil of *Piper retrofractum* on *T. castaneum* and by Wang *et al.* (2001) in their studies on repellency of six plant essential oils against *Liposcelis bostrychophila*.

The repellency was found to be time-dependent for both the extracts. The time course curve (Fig. 4.1) of VPE showed that the extent of initial repellency (between 85 and 90% except the lowest concentration of 0.625%) observed during early periods of exposure (1.5 and 3 h) dropped considerably in about 6 h of exposure where the repellency went down to about 55-60% in all the different concentrations tested, except the lowest concentration (0.625%) where until 6 h treatment, the repellency was almost steady (between 63 and 71% repellency). However, the highest concentration (5%) was having exceptionally high activity, which did not show the drop in activity after 6 h. There was a steady and sudden increase of activity within 24 h of treatment from about 85 to nearly 100%. Beyond 24 h, repellency with all the concentrations reached and almost maintained a plateau with a slight and gradual decline up to 72 h.

The time-course curve of VME also showed a similar pattern as that of VPE. The extent of initial repellency observed at early periods of exposure (1.5 to 3 h) was reduced considerably in about 6 h of exposure. Repellency was regained

during further exposure for 24 h. It was seen that there was a sudden increase of activity during 24 h for all the concentrations of VME. Beyond 24 h, 5% VPE was showing steady pattern of repellency (100%) during 48 and 72 h. Whereas 2.5 and 5% concentrations were showing slightly different pattern of repellency with a decline of activity at 48 h (to 87%) and it attain maximum repellency at 72 h (100%). Repellency in the case of 0.625 and 1.25% concentrations were always showing lower rates than the other two higher concentrations (Fig. 4.2).

From the results, it is thus inferred that the extract contains several volatile compounds having various degrees of volatility. The initial high repellency during smaller duration of exposure may be due to presence of highly volatile low molecular weight compounds, which escapes soon from the experimental chamber and also from the insects' receptors. This leads to the insects return onto the treated parts of the filter paper. Between 6 and 24 h, the insects get further doses of low volatile high molecular weight compounds that are characteristically persistent. This repellency thus, lasts long and has been found in the 24-72 h period of treatment. The slow decrease in repellency in the high activity plateau may probably be due to the slow acclimatisation of the insects to the volatile compounds. Decrease in repellent activity after prolonged treatment (more than 5 h) with the essential oil from *Evodia rutaecarpa* against *T. castaneum* and *S. zeamais* have been reported by Liu and Ho (1999), which is comparable to the result we obtained in this experiment. Similar decrease in repellency on *R. dominica* by turmeric oil and sweetflag oil over an 8-week period was also reported by Jilani and Saxena (1990).

The present study thus provides evidence for the repellent activity of compounds present in *V. negundo* against *T. castaneum* and the results agree with the repellency studies of *V. negundo* on other insects. Hebbalkar *et al.* (1992) reported that the column chromatographic fractions of the oil obtained from the steam distillate of *V. negundo* leaves showed repellent activity against *Aedes aegypti*. They also found that these active fractions contained a mixture of monoterpenes, sesquiterpenes, monoterpene and sesquiterpene alcohols responsible for repellency. The protection period against bites by polar fractions ranged between 1-3 h. Amancharla *et al.* (1999) reported a mosquito repellent, rotundial, isolated from the chloroform fractions of an aqueous extract of fresh leaves of *V. negundo*. Repellent properties of fresh leaf discs as well as dried leaf discs of 51 plant species including *V. negundo* against *Lasioderma serricorne* were reported by Ambadkar and Khan (1994). Similarly, Pradeep and Radhakrishnan (1998) screened leaf extracts in aqueous, acidic and alkaline media of 28 locally available plant species including *V. negundo* for repellent properties against *T. castaneum*, most of which were found to be effective repellents.

It is seen that the repellent effects of VPE and VME are presumably due to the active components such as oils, monoterpenes, sesquiterpenes, mixture of monoterpene and sesquiterpene alcohols, rotundial, volatile oils, essential oils etc. This observation is in agreement with results obtained by various authors, from their studies using *V. negundo*. The components of the essential oils of different varities of *V. negundo* (*V. negundo* var. *negundo, V. negundo* var. *purpurescens)* have been studied and found to be consisting of monoterpenes [terpinen-4-ol, p-

cymene, a-terpineol and sabinene] and sesquiterpenes [b-caryophyllene, globulol, spathulenol, b-farnesene and bis [1, 1-dimethyl] methyl phenol] (Mallavarapu *et al.,* 1994; Jirovetz *et al.*, 1998). Similarly, Singh *et al.* (1999) reported the presence of 66 compounds in the volatile constituents of *V. negundo* leaves. The major compounds are viridiflorol, b-caryophyllene, sabinene, 4-terpineol, g-terpinene, caryophyllene oxide, 1-oceten-3-ol and globulol. These active components may be responsible for the repellency to *T. castaneum.*

Several other reports on the repellent activities of volatile oils / essential oils against stored product insects have appeared during the last 2 or 3 decades (Saim and Meloan, 1986; Jilani *et al.*, 1988; Jilani and Saxena, 1990; Su, 1991; Tripathi *et al.*, 1997; Obeng-Ofori *et al.*, 1997 a; Liu and Ho, 1999; Padin *et al.*, 2000; Papachristos and Stamopoulos, 2002). Likewise, several compounds present in plants such as quwenling, azadirachtin, rotundial, trichomes, diterpenoid, nor-diterpenoid alkaloids etc. are also reported to possess repellent activities against various insects. [Schreck and Leonhardt, 1991; Watanabe *et al.*, 1995; Ulubelen *et al.*, 2001).

The findings in this study thus revealed that both petroleum ether and methanol extracts of *V. negundo* leaves show strong repellency to *T. castaneum* adults. This also suggested that there may be different constituents in both the extracts responsible for repellency which is active either individually or synergistically in a dose-dependent manner. So, further analytical studies should be carried out to determine the exact chemical structure of the bioactive components in the extracts of *V. negundo* to understand how each of the

constituents influences the physiology and behaviour of the insects. Moreover, it might be worthwhile if the insect- repellent components of *V. negundo* are produced commercially so that their potential in controlling stored product pests can be fully exploited.

Chapter V

STUDIES ON NUTRITIONAL AND FEEDING DETERRENCE INDICES

5.1. Introduction

Antifeedants or feeding deterrents are those materials, which inhibit or prevent animals from feeding. In the case of insects, antifeedants prevent them from feeding on vegetation, grain or other products leading them to starvation and subsequent death. Various compounds are found to be effective in the inhibition of feeding in insects. Besides many chemicals, a wide variety of higher plants may provide new sources of natural pesticides and antifeedants (Grainge and Ahmed, 1988; Arnason *et al.*, 1989; Ananthakrishnan, 1992). Antifeedant activity is one of the principal bioactivities brought about by plant constituents on insects, which lend impetus to incorporation of feeding deterrence strategy in integrated pest management (IPM) programmes. Most of the plants contain different types of secondary compounds, which have deterrent effects on many insect species. However, since most of these antifeedant principles, derived from plants are non-toxic in nature, their environmental compatibility is an important advantage. Antifeedants of plant origin have the additional advantage of quick biodegradability and are friendly to non-target organisms. Hence, insect antifeedants have become the subject of considerable interest.

Many plant materials have been effectively used against stored product insects. Roychoudhury (1993) reported the antifeedant properties of the extracts of *Didymocarpus podocarpus, Coriaria nepalensis* and *Clerodendron fragrans*

against *S. oryzae.* Talukder and Howse (1993) investigated the antifeedant effect of pithraj, *Aphanamixis polystachya* on *T. castaneum*. Talukder and Howse (2000) also isolated secondary plant compounds from the sub-fractions of *A. polystachya* seed extract and found that a subfraction A-142 contained strong feeding deterrents that acted against *T. castaneum.* Similarly, antifeedant properties of the rhizome extract of *Acorus calamus* have been investigated against *T. castaneum* (Chandel *et al.,* 2001) and extracts of *Chromolaena odorata* and *Ocimum viride* against *T. castaneum* and *S. oryzae* (Owusu, 2001). Jilani and Saxena (1990) evaluated oils of turmeric, sweetflag and neem and the neem based insecticide, Margosan-O for their antifeedant effect on *R. dominica.* Similarly, antifeedant activities of the essential oils from nutmeg seeds (Huang *et* al., 1997), cinnamaldehyde from *C. aromaticum* (Huang and Ho, 1998), *Evodia rutaecarpa* (Liu and Ho, 1999) and *Elletaria cardamomum* (Huang *et al.*, 2000) against *T. castaneum* and *S. zeamais* have been reported. Studies on the antifeedant activities of 1,8-cineole from *Artemisia annua* against *T. castaneum* (Tripathi *et al.,* 2001 b) and essential oils of *C. longa* against *R. dominica* and *S. oryzae* (Tripathi *et al.,* 2002) have also been conducted.

Liu *et al.* (1990) reported the antifeedant activities of four sesquiterpene alkaloids isolated from *Celastrus angulatus* against *T. castaneum.* Antifeedancy of alkaloid containing fraction isolated from the leaves of *Nicotiana tabacum* against the larvae of *T. castaneum* has been reported (Archana *et al.,* 1995). Chiam *et al.* (1999) evaluated allyl disulphide, a volatile compound from *A. sativum*, for the antifeedant activity against adults and larvae of *T. castaneum* and *S. zeamais*.

The experiments described in this section are carried out to evaluate the nutritional and feeding deterrence indices for *T. castaneum* adults brought about by the petroleum ether and methanol extracts of *V. negundo.*

5.2. Methods

Methods adopted for the experiments are described in section 3.2.4.

5.3. Results

5.3.1. Nutritional and feeding deterrence indices for *T. castaneum* adults treated with VPE

Results of the experiments to determine the nutritional and feeding deterrence indices for *T. castaneum* adults using VPE by 'no-choice' feeding bioassay are recorded in the Table V.1. A perusal of the table reveals that all the concentrations of the extract (1.25, 2.5 and 5%) exhibit feeding deterrent activity or antifeedant activity against *T. castaneum.* This extract thus correspondingly affect/influence the nutritional indices such as relative growth rate (RGR), relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI %).

Nutritional indices for VPE

The relative growth rate (RGR) of insects for 24 hours were showing slight reduction in treated samples compared to control and the RGR decreased with increase in concentration of the extract. Thus, 1.25, 2.5 and 5% concentrations afforded the RGR of 0.040, 0.033 and 0.028 mg/mg/d respectively where as in control the value was 0.052 mg/mg/d (Table V.1).

TABLE V.1

Nutritional and feeding deterrence indices for *T. castaneum* adults treated with VPE

Conc. of VPE(%)	RGR (mg/mg/d)	RCR (mg/mg/d)	ECI (%)	FDI (%)
Control	0.052 ± 0.004	0.23 ± 0.022	23.19± 2.07	-
1.25	0.040 ± 0.004	0.161 ± 0.004	25.30 ± 2.20	34.48
2.5	0.033 ± 0.003	0.079 ± 0.007	43.17 ± 6.36	68.52
5	0.028 ± 0.008	0.067 ± 0.006	41.21 ± 9.91	73.88

Values are expressed as Means ± SEMs (n = 6).
Sample : 10 insects x 4 treatments x 6 replications = Total 240 insects.
RGR: Relative Growth Rate.
RCR: Relative Consumption Rate.
ECI : Efficiency of Conversion of Ingested Food.
FDI: Feeding Deterrence Index.
Calculated as described in section 3.2.4 of Chapter III.

Similarly, the relative consumption rate (RCR) of insects were significantly reduced in a dose-dependent manner. The RCR of the insects were 0.161, 0.079 and 0.067 mg/mg/d for 1.25, 2.5 and 5% concentrations of VPE. In the case of control, the RCR was 0.23 mg/mg/d (Table V.1).

On the other hand, the percentage of efficiency of conversion of ingested food (ECI %) of the insects increased with increase in the concentration of VPE. Higher ECI% was shown by 2.5% (ECI = 43%) and 5% (ECI = 41%) concentrations of the extract. Values of ECI calculated for a concentration of 1.25% of VPE was almost equal to the value (25%) obtained for the control (23%) (Table V.1).

Feeding deterrence index for VPE

Significant feeding deterrent activity or antifeedant activity against *T. castaneum* adults was exhibited by VPE at different concentrations in a dose-dependent manner. The feeding deterrence indices (FDI %) increased gradually from about 34% to 69% and 74% respectively with VPE of 1.25, 2.5 and 5% concentrations (Table V.1).

5.3.2. Nutritional and feeding deterrence indices for *T. castaneum* adults treated with VME

The 'no-choice' feeding bioassay was employed for evaluating the nutritional and feeding deterrence indices for *T. castaneum* in the presence of different concentrations of VME. Table V.2 provides the nutritional and feeding deterrence indices for *T. castaneum* adults in response to VME. It is evident from the Table

TABLE V.2

Nutritional and feeding deterrence indices for *T. castaneum* adults treated with VME

Conc. of VME(%)	RGR (mg/mg/d)	RCR (mg/mg/d)	ECI (%)	FDI (%)
Control	0.046 ± 0.004	0.169 ± 0.023	27.77± 1.02	-
1.25	0.044 ± 0.007	0.113 ± 0.007	38.89 ± 5.20	36.75
2.5	0.024 ± 0.006	0.073 ± 0.007	31.13 ± 5.27	58.43
5	0.027 ± 0.005	0.063 ± 0.008	48.34 ± 12.20	65.96

Values represent means ± SEMs (n=6).
Sample: 10 insects x 4 treatment x 6 replications = Total 240 insects.
RGR: Relative Growth Rate.
RCR: Relative Consumption Rate.
ECI: Efficiency of Conversion of Ingested food.
FDI: Feeding Deterrence Index.
Calculated as described in section 3.2.4 of Chapter III.

that like VPE, all the different concentrations of this extract (1.25, 2.5 and 5%) also shows significant antifeedant activity against *T. castaneum* adults compared to the control. It is thus obvious that methanol extract influenced the relative growth rate (RGR), relative consumption rate (RCR) and efficiency of conversion of ingested food (ECI %) of the insects.

Nutritional indices for VME

The relative growth rate of the insects were inhibited by different concentrations of VME in a dose-dependent manner. A concentration of 1.25% of VME brought about only a small decrease in RGR from the control value (from 0.046 to 0.044 mg/mg/d). Higher concentration (2.5%) was found to be very effective in reducing the RGR to 0.024 mg/mg/d. The highest concentration of 5% of the extract was also showing a similar effect. There was only a very slight increase in the RGR (0.027 mg/mg/d) (Table V.2).

Similarly, relative consumption rate (RCR) also significantly decreased with increasing concentrations of the extract. With a concentration of 1.25% of the extract the control value of 0.169 mg/mg/d was reduced to 0.113 mg/mg/d, which was then reduced to 0.073 mg/mg/d with 2.5% of the VME. However, the RCR showed only a slight decrease when the concentration was further increased to 5%. The value was 0.063 mg/mg/d (Table V.2).

In the case of the percentage of efficiency of conversion of ingested food (ECI %) of the insects, there was an increase in the case of 1.25% of VME (39%) from the control value of 28%. This value was seen to drop to 31% when the

concentration of the extract was increased to 2.5%. However, this drop was overcome when the concentration was increased to 5%. The ECI value was 48% in this case (Table V.2).

Feeding deterrence index for VME

Like VPE, VME also exhibited significant feeding deterrent activity against *T. castaneum*. It was seen that here also, the feeding deterrence index (FDI %) increased in a dose-dependent manner. The FDI % were 37, 58 and 66% respectively for 1.25, 2.5 and 5% of the extract (Table V.2).

5.3.3. Statistical analysis of data

Data obtained from the nutritional studies of *T. castaneum* by using petroleum ether and methanol extracts of *V. negundo* were subjected to ANCOVA and ANOVA.

ANCOVA of the data on the effects of VPE and VME on RGR of *T. castaneum* showed that difference between the effects of these two extracts were not significant ($P=0.49$; $F=0.4858$). Similarly, difference among the various concentrations of each extract on RGR of the insect was not significant ($P=0.37$; $F=1.0882$). ANCOVA of the data on effects of these two extracts on RCR of *T. castaneum* showed that difference between their activities was not significant ($P=0.80$, $F=0.0643$). However, difference between the activities of the different concentrations of the two extracts was significant ($P<0.05$; $F=2.8002$). Here, the covariate was consumption of the food (mass) by the insect (Table V.3; Fig. 5.1).

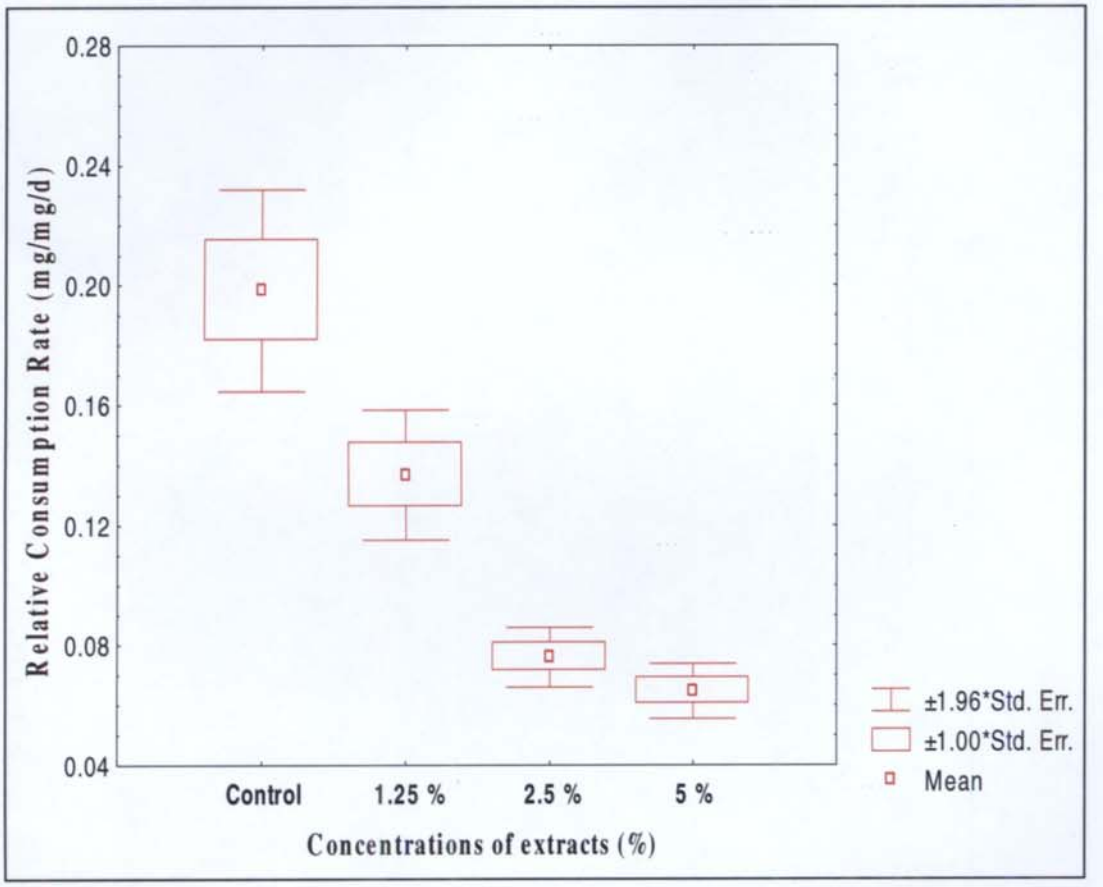
TABLE V.3

ANCOVA of data showing the effects of VPE and VME on RGR and RCR of *T. castaneum* adults

Parameters	Source of variables	df	MS	df Error	MS Error	F	P level
RGR	Extracts	1	7.82E-05	39	0.0002	0.4858	0.49 (NS)
	Conc.	3	0.0002	39	0.0002	1.0882	0.37 (NS)
RCR	Extracts	1	4.02E-06	39	6.25E-05	0.0643	0.80 (NS)
	Conc.	3	0.0002	39	6.25E-05	2.8002	< 0.05

RGR : Relative Growth Rate.
RCR : Relative Consumption Rate.
Covariate : Consumption.
NS : Not Significant.

Fig. 5.1. Categorised plot for variable of RCR for *T. castaneum* for different concentrations of VPE and VME



Data represent the pooled values for the effects of VPE and VME for different concentrations used.

The difference between the activities of the two extracts was not significant. (ANCOVA, P = 0.80)

TABLE V.4

ANOVA of data showing the effects of VPE and VME on ECI (%) and FDI (%) for *T. castaneum* adults

Parameters	Source of variables	df	MS	df Error	MS Error	F	P level
ECI (%)	Extracts	1	27.5899	40	267.2598	0.1032	0.75 (NS)
	Conc.	3	1134.008	40	267.2598	4.2431	< 0.05
FDI (%)	Extracts	1	377.1487	30	107.8557	3.4968	0.07 (NS)
	Conc.	2	3677.776	30	107.8557	34.0991	< 0.001**

ECI (%) : Percentage of Efficiency of Conversion of Ingested food.
FDI (%) : Percentage of Feeding Deterrence Index.
** Shows very high significance.
NS : Not Significant.
Calculated as described in section 3.2.4 of Chapter III.

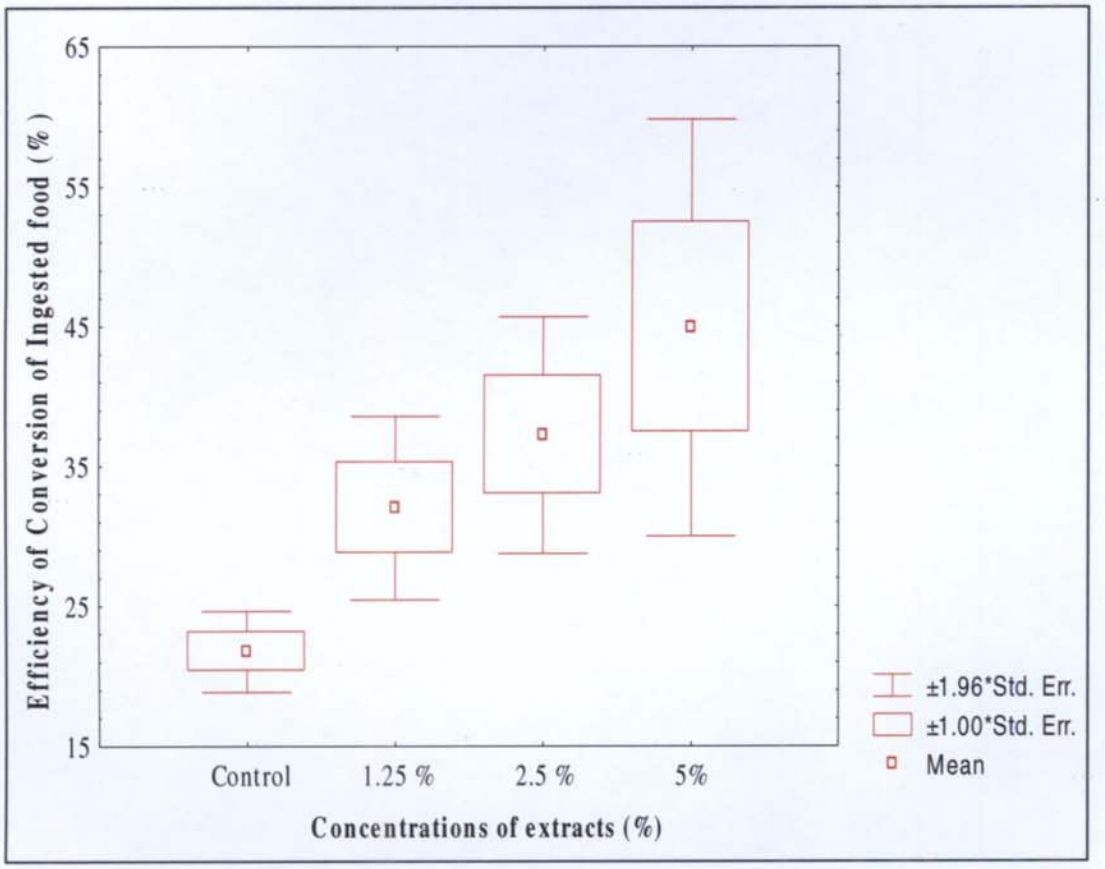
TABLE V.5

Homogeneity of variances of data on ECI (%) and FDI (%) for *T. castaneum* adults

Parameters	Hartley F-max	Cochran C	Bartlett Chi-sqr	df	P level
ECI (%)	34.8717	0.4221	28.1415	7	< 0.001**
FDI (%)	24.9351	0.4541	12.0937	5	< 0.05

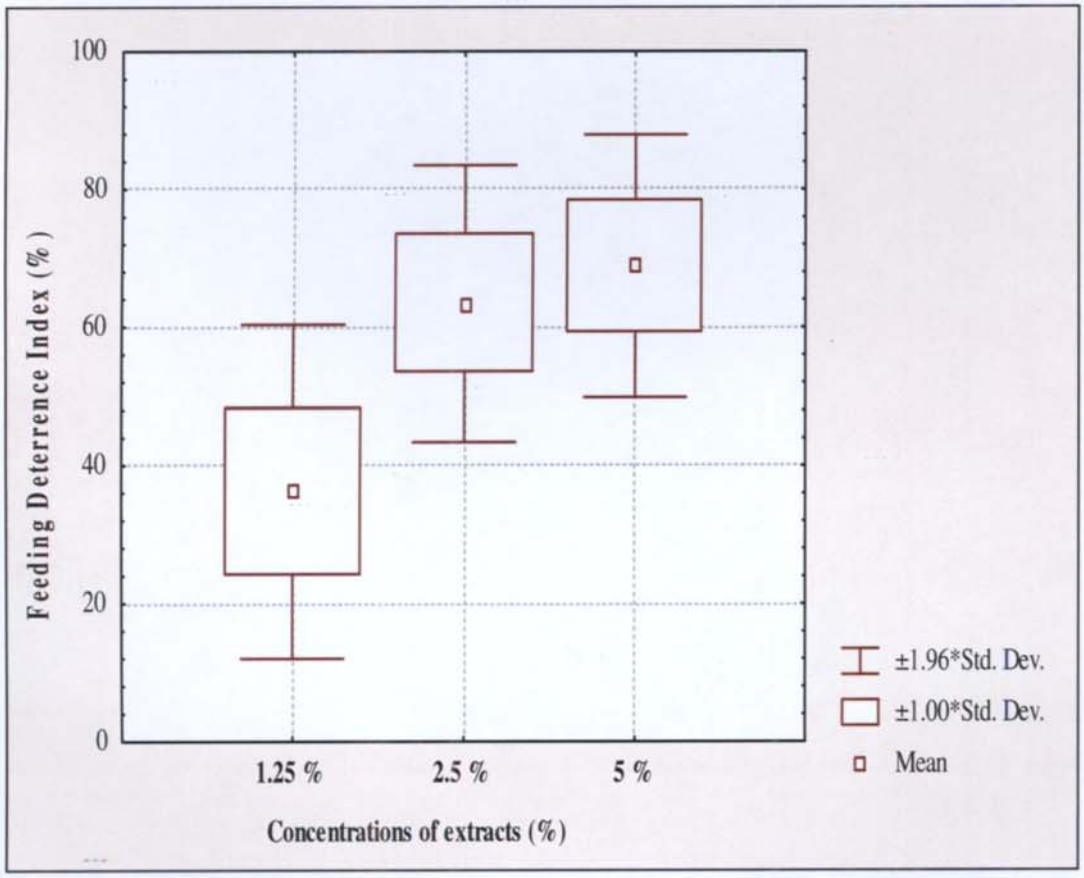
** Shows very high significance.

Fig. 5.2. Categorised plot for variable of ECI % for *T. castaneum* for different concentrations of VPE and VME



Data represent the pooled values for the effects of VPE and VME for different concentrations used.
The difference between the activities of VPE and VME was not significant. (ANOVA , P = 0.75).

Fig. 5.3. Categorised plot for variable of FDI % for *T. castaneum* for different concentrations of VPE and VME



Data represent the pooled values for the effects of VPE and VME for different concentrations used.
The difference between the activities of VPE and VME was not significant. (ANOVA , P = 0.07)

ANOVA of the data on ECI (%) of *T. castaneum* for the effects of VPE and VME reveal that difference between the activities of the two extracts was not significant (P=0.75; 0.1032). However, difference among the various concentrations of the extracts on ECI (%) of the insect was significant (P<0.05; F=4.2431) (Table V.4; Fig. 5.2). When the homogeneity of variances of the data were tested, it was found that ECI (%) of the insect was highly significant (P<0.001) (Table V.5).

ANOVA of the data for FDI (%) showed that difference between the activities of the two extracts was not significant (P=0.07; F=3.4968). On the other hand, the difference of FDI (%) among different concentrations of both the extracts was statistically highly significant (P<0.001; F=34.0991) (Fig. 5.3; Table V.4). The tests for homogeneity of variances of the data (Table V.5) showed that FDI (%) for the insects was significant (P<0.05).

5.4. Discussion

The results of the nutritional studies on *T. castaneum* carried out for 24 h clearly indicated that VPE and VME significantly affected the growth, food consumption and utilisation and brought about antifeedancy in *T. castaneum* (Tables V.1 & 2).

From the present study, it appears that the two extracts brought about slight reduction of growth rate in the insect, compared to that in the control. The reduction of RGR was more prominent for 2.5 and 5% of both the extracts. Difference between the effects of VPE and VME on RGR of the insects was not

significant (P=0.49). There was a progressive decline in the food consumption (RCR) (Fig. 5.1; Tables V.1 & 2) with increasing concentrations of the extract compared to control. The difference between the activities of the extracts was not significant. However, different concentrations of the extracts showed significantly different effects (P<0.05).

The reduction of RGR and RCR of *T. castaneum* by both the extracts observed in this study are in agreement with similar studies of various other workers. Reduction of growth and food consumption of *T. castaneum* and *S. zeamais* was brought about by essential oils of nutmeg seeds (Huang *et al.*, 1997) and by cinnamaldehyde from *C. aromaticum* (Huang and Ho, 1998). Allyl disulphide, a volatile compound from garlic, *Allium sativum* (Chiam *et al.*, 1999) and 1,8-cineole from *A. annua* (Tripathi *et al.*, 2001 b) were found to cause significant reduction of RGR and RCR of *T. castaneum*. Similar results have been reported in certain phytophagous insect pests. Effects of *A. calamus* oil on *Peridroma saucia* (Koul and Isman, 1990), neem limonoids on *H. armigera* (Murugan *et al.*, 1998) and neem in combination with sweetflag and pongam extracts on *Earias vitella* (Rao, 2002) are also in tune with the reduction of growth and consumption observed in *T. castaneum* brought about by *V. negundo*.

The reduction of growth and food consumption of *T. castaneum* observed in this study may presumably be due to the antifeedant action of the extract incorporated into the diets. This study also reveals that the quantity of food material converted to body matter is reduced and that the consumption of lesser quantity of the treated food is likely to be the main cause of growth inhibition.

In the nutritional studies, it was also seen that with both the extracts, increase in concentration resulted in increase in the efficiency of conversion of ingested food (ECI %) significantly ($P<0.05$). It was seen that the difference between the activities of these two extracts on ECI was not significant (Fig. 5.2; Tables V.1 & 2).

Significantly higher ECI values over the control were obtained with all concentrations of the extracts in the present study. Moreover, there appeared to be a dose-dependency for this effect (Fig. 5.2; Tables V.1 & 2). Similar observations were reported in *Crocidolomia binotalis* (Fagoonee, 1984) and *Spodoptera litura* (Sahayaraj, 1998). Similarly, Joseph (2000) reported higher ECI % from his studies of antifeedancy and growth inhibitory effects of neem seed kernel extract on *Ailanthus* defoliator, *Eligma narcissus indica.* Present studies using *V. negundo* against *T. castaneum* show higher ECI values which presumably reflect the compensation for antifeedant effect as suggested by Fagoonee (1984) who reported the neem related high ECI and ECD values in *Crocidolomia binotalis*. Huang *et al.* (1997), Huang and Ho (1998), Tripathi *et al.* (2001 b), Koul and Isman (1990), Murugan *et al.* (1998) and Rao *et al.* (2002) in their reports revealed that ECI% decreased when the concentration of the components/extracts of the plants were increased.

It has been also found that the extracts exerted significant feeding deterrence (antifeedancy) on this insect (Fig. 5.3; Tables V.1 & 2). The effect was found to dose-dependent and the increases by all the concentrations of the two extracts were

found to be significant ($P<0.001$). The difference between the activities of the extracts was not significant.

Antifeedant effects of *V. negundo* have been reported earlier. Premeela and Muraleedharan (1995) reported that certain phytochemicals in the extracts of *V. negundo* caused significant reduction in the levels of all the three digestive enzymes (midgut protease, invertase and amylase) and thus inhibited the food digestion in the red cotton bug. *Dysdercus cingulatus*. Extracts of *V. negundo,* (Sahayaraj, 1998) and essential oils derived from this plant (Sharma *et al.*, 2000) have been reported to exhibit antifeedant activities against *Spodoptera litura.*

Antifeedant activities of a variety of plant materials against stored product insects have been reported by earlier workers. Essential oils from nutmeg seeds (Huang *et al.*, 1997) and cinnamaldehyde from *C. aromaticum* (Huang and Ho, 1998) have antifeedant properties against *T. castaneum* and *S. zeamais*. Similarly, allyl disulphide from *A. sativum* (Chiam *et al.*, 1999), secondary plant compounds from *A. polystachya* (Talukder and Howse, 2000), 1,8-cineole from *A. annua* (Tripathi *et al.*, 2001 b) and rhizome extract of *Acorus calamus* (Chandel *et al.*, 2001) have feeding deterrent effect against *T. castaneum.*

Several essential oil constituents contained in the petroleum ether and methanol extracts of *V. negundo* leaves may be responsible for the antifeedant activity against *T. castaneum*. GC and GC-MS analysis of the essential oils of *V. negundo* leaves (Mallavarappu *et al.*, 1994) revealed that this oil contain 65 known compounds, including sabinene p-cymene, beta-phellandrene, gamma-terpinene, terpinene-4-ol, beta-caryophyllene, alpha-guaiene, spathulenol, beta-caryophyllene

oxide, globulol, viridiflorol, bis [1,1-dimethyl]-methylphenol, abieta-7, 13-diene and several minor unidentified compounds. Jirovetz *et al.* (1998) investigated the essential oil components of the leaves of *V. negundo* var. *negundo* and *V. negundo* var. *purpurescens* by GC-FID, GC-MS and olfactometry. They isolated monoterpenes (terpinen-4-ol, p-cymene, a-terpineol, sabinene) and sequiterpenes (b-caryophyllene, globulol, spathulenol, b-farnesene and bis [1,1-dimethyl] methyl phenol) from the leaves of the two varieties of *V. negundo*. Hebbalkar *et al.* (1992) reported that oils from *V. negundo* leaves when analysed by column chromatography and IDCC technique revealed that it contained several components such as α- terpenine, γ-terpenine, p-cymene, mixture of sesquiterpene hydrocarbons, 4-terpineol, monoterpenes, sequiterpines and a mixture of monoterpene and sesquiterpene alcohols. The majority of components present in the fractions exhibited repellent activity against *A. aegypti*. These essential oil constituents present in the leaf extracts presumably are responsible for antifeedant activity against *T. castaneum*. Several volatile constituents of *V. negundo* leaves were analysed by GC-MS by Singh *et al.* (1999). The main components detected were viridiflorol, b-caryophyllene, sabinene, 4-terpineol, g-terpinene, caryophyllene oxide, 1-oceton-3-ol and globulol. These components in the leaves may also act as antifeedants against *T. castaneum*.

Antifeedant activities of the essential oils derived from *A. calamus* against *Peridroma saucia* (Koul and Isman, 1990), *E. rutaecarpa* against *S. zeamais* and *T. castaneum* (Liu and Ho, 1999) and *C. longa* against *R. dominica, T. castaneum* and *S. oryzae* (Tripathi *et al.*, 2002) have been reported.

Many earlier reports have shown various chemical constituents of plants, responsible for antifeedancy against stored grain and other agricultural insect pest species. Azadirachtin (Butterworth and Morgan, 1971), nerifolin (a cardiotonic glycoside) (Reed *et al.,* 1982), quassinoids (Leskinen *et al.,* 1984), isopongaflavone, tephrosin, rotenone, tetrahydroisopongaflavone (Bentley *et al.,* 1987), limonoids (limonin, deoxylimonin, citrolin, obacunone, harrisonin and acetoxyharrisonin) (Hassanali *et al.,* 1986) and pedonin (a spirotetranortriterpenoid) (Hassanali *et al.,* 1987) from various plants have been reported for their antifeedant properties against various insect pest species. Various alkaloids such as vasicine, vasicinol, vasicinone, deoxyvasicine and deoxyvasicinone (Saxena *et al.,* 1986), tylophorine, tylophorinine and pergularinine (Verma *et al.,* 1986), sesquiterpene alkaloids (Liu *et al.,* 1990), amide alkaloids (Torto *et al.,* 1992) and isoquinoline alkaloids (Kwon *et al.,* 2000) also act as antifeedants against insects. Other components such as sesquiterpene lactone encelin (Srivastava *et al.,* 1990), grayanoid diterpenes (Klocke *et al.,* 1991), furanocoumarin and furanochromones (Luthria *et al.,* 1992), cucurbitacins (the bitter triterpenes) (Tallamy *et al.,* 1997), neo-clerodane diterpenoids (Jannet *et al.,* 2000) and tetranortriterpenoids (Simmonds *et al.,* 2001) also possess antifeedant properties.

The mechanism of perception of inhibitory chemicals in insects have been reviewed by Chapman (1974) and Schoonhoven (1982). On the basis of behavioural and electrophysiological studies, parts of the body on which the relevant receptors are located and the mechanism by which insects differentiate inhibitory and stimilatory materials have been established (Chapman, 1974).

Antennae in insects are generally considered to be the main organ for perception of smell in insects (Dethier, 1963) and they would be expected to be involved in responses to olfactory repellents. The experiments of Haskell and Mordue (1969) suggested that sensilla on the tip of the labrum have a primary role in the detection of inhibitory chemicals in *S. gregaria.* These mechanisms of perception of inhibitory chemicals in feeding may also occur in *T. castaneum* adults in response to *V. negundo* leaf extract-incorporated wheat flour diets. Various chemical components extracted from *V. negundo* in petroleum ether and methanol presumably inhibit the feeding of the insect and perception of these chemicals are effected by the receptors like sensilla on the antennae, labrum, maxillary and labial palps of *T. castaneum.*

Electrophysiological studies have indicated that inhibitory phytochemicals may be involved in the antifeedancy in insects. These chemicals may either directly inhibit the input from the phagostimulant receptor or the signals may be interpreted as inhibitory at the central nervous system (Ma, 1969, 1972; Schoonhoven, 1973; Ishikawa *et al.*, 1969). Similar electrophysiological mechanism of perception of inhibitory chemicals may also be active in *T. castaneum* with regard to the response to the extract incorporated diets. Active phytochemicals in the extracts presumably suppress the activity of the receptor cells of these insects or input signal from the receptor neuron can be perceived by the central nervous system in which it is interpreted as inhibitory and may cause antifeedant effect.

The present study reveals that both petroleum ether and methanol extracts of *V. negundo* are showing antifeedancy to *T. castaneum* and thus it can effectively be used for the protection of grains from insects. Since antifeedant action offers a valuable weapon against this pest, further study on the effect of these extracts on other stored product insects and phytophagous insects should be carried out. Further research into the bioactivity of their chemical constituents against stored product insects is needed before commercial production and their application are considered. Antifeedants make it possible to develop methods of grain protection as well as crop protection without, or with much less of organic insecticides. The main advantage of antifeedants lies in their relatively high specificity. It is also realised that antifeedants would have fewer undesirable effects in the environment and less adverse effects on non-target organisms.

Chapter VI

STUDIES ON EMERGENCE, DEVELOPMENT AND PROGENY REDUCTION IN *T. CASTANEUM*

6.1. Introduction

The intricate problem with regard to the management of pests in stored grains and grain products is that this cannot be solved by using synthetic insecticides due to obvious health hazards. It has therefore, been always a subject of interest to find cheap and non-hazardous methods of combating the menace. Exploitation of plant products for this purpose has received growing attention now-a-days.

Many plant chemical constituents are toxic, repellent, and/or antifeedant materials and they have significant effects on reproduction, fecundity, hatching of eggs, development and emergence of the progeny of insect pest species. The effectiveness of many plant derivatives used against stored product insects have been studied in detail. Pandey *et al.* (1985) reported that mixing of neem (*A. indica*), oil powder of kernels, cake, leaves and flowers and babul (*Acacia arabica*) gum with wheat seed proved to be protectants against *C. cephalonica* resulting in adverse effects on the development, viz., increased developmental period, higher mortality and less percentage of adult emergence with less number of females of the pest. Rani and Jamil (1989) observed that crude extracts of *Eichhornia crassipes* mixed with the diet of the 4th instar larvae of *T. castaneum* and *C.*

cephalonica retarded the development and mortality. Similarly, *Polygonum hydropiper* and *A. polystachya* seed coat extracts significantly reduced the fecundity and fertility of *T. confusum* (Khanam and Talukder, 1993). Bark extract of *Melia toosendan* was found to reduce fecundity of *Cryptolestes ferrugineus*, *S. oryzae* and *T. castaneum* (Xie *et al.*, 1995 b). Seeds and leaves of *Trigonella foenum-graecum* was found to reduce the fecundity of *A. obtectus* and *T. castaneum* (Pemonge *et al.*, 1997). Rajapakse *et al.* (1998) evaluated the efficacy of powders of *P. nigrum*, *A. reticulata*, *A. indica*, *Capsicum annum* and *Citrus limon* on adult emergence of *C. maculatus* infesting cowpea. Similarly, extracts of *Coleus aromaticus*, *Morinda tinctoria* and *Cassia siamea* on *C. maculatus* (Babu *et al.*, 1999) and *Peganum harmala* on *C. chinensis* (Srivastava and Mann, 2002) were found to reduce the adult emergence of these insects. Allotey and Azalekor (2000) reported that powder made of *E. crassipes*, *Citrus sinensis* and *C. odorata* affected fecundity, hatchability of eggs, developmental period and adult longevity of *C. cephalonica*.

Oils in the plants also adversely affected the bio-activities of insects. Sharma and Srivastava (1984) found that ground nut oil reduced the fecundity and embryonic development in *C. chinensis*. Maheswaran and Ganesalingam (1988) investigated the efficacy of volatile substances from *A. indica* seeds on the reproductive biology of *T. castaneum*. It was found that these substances reduced the number of eggs laid by females, prolonged the developmental period and reduced the number of larvae emerging from eggs. Similarly, application of oils of ground nut, traditional coconut, industrial coconut, palm and shark to exposed

surface of dried trout significantly reduced the development of the progeny of *D. maculatus* (Don-Pedro, 1989 a).

Chander *et al.* (1992) reported that turmeric powder and mustard oil reduced the progeny of *T. castaneum* in stored milled rice. It has been also reported that volatile oils of *Lippia adoensis, Cymbopogon citratus, Eugenia uniflora, L. camara* and *Chromolaena* were found to reduce the fecundity and adult emergence of *C. maculatus* (Gbolade and Adebayo, 1993). Similarly, ten vegetable oils (Khaire *et al.*, 1992), pongam oil (Negi *et al.*, 1994) and crucifer oils (Sharma *et al.*, 1999) reduced the adult emergence of *C. chinensis*. Rajapakse and Emden (1997) reported four vegetable oils and powders of ten plants that significantly reduced oviposition and longevity of adults of *C. maculatus, C. chinensis* and *C. rhodesianus*. Effect of essential oils of *Tagetes minuta, Hyptis suaveolens, Ocimum canum, O. basilicum* and *O. gratissimum* on egg hatching and adult emergence of *C. maculatus* have been reported (Keita *et al.*, 2000). Tripathi *et al.* (2002) reported the effects of essential oils of *C. longa* on the oviposition, hatching and the progeny of *R. dominica, S. oryzae* and *T. castaneum*.

Various constituents of plants also have significant impact on bio-activities of stored product insects. Vijay and Singh (1991) found that protein fractions from cashew nut kernels (*Anacardium occidentalis*) reduced survival and also slowed the development of *T. castaneum* and *O. surinamensis*. Some terpenoid lactones reduced the fecundity and hatching of eggs in *T. castaneum* (Gursharan and Singh, 1999).

The aim of the experiments described in this chapter is to study the effect of leaf extracts of *V. negundo* on emergence, development and reduction of *T. castaneum* progeny.

6.2. Methods

Methods adopted for this study are described in section 3.2.5.

6.3. Results

6.3.1. Effect of VPE on emergence, development and progeny reduction in *T. castaneum*

Results of the studies on emergence and development of larvae, pupae and adults of *T. castaneum* in diets mixed with different concentrations of VPE at different intervals (days) are provided in the Table VI.1 and Figures 6.1-3. From the Table and Figure, it is evident that the time for transformation of larvae into pupae and adults were delayed and their number was reduced in treated diets compared to control. In this experiment, it was also observed that the size of the larvae were generally smaller in the treated diets than in the control.

On the 15th day, 4% of initial number of larvae (415) were transformed into pupae in the control whereas no pupae were formed in the diet treated with 10% of VPE. With 2.5 and 5% of VPE, only 2% were transformed into pupae (column 3, Table VI.1). During this period no adults were formed. However, on the 25th day, 8% adults emerged in control whereas very few or no adults were formed in the treated diets. Although, higher percentage of larvae was formed, their transformation to pupae and adults were delayed or prevented. It was observed that

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TABLE VI.1

Effects of various concentrations of VPE on emergence and development of larvae, pupae and adults of *T. castaneum* during different periods of exposure

1	2	Mean % progeny transformation during different periods																	
		3			4			5			6			7			8		
Conc. of VPE (%)	Initial no. of larvae	15 days			25 days			30 days			40 days			50 days			60 days		
		L	P	A	L	P	A	L	P	A	L	P	A	L	P	A	L	P	A
Control	415	96	4	0	30	30	8	11	16	27	3	1	**19**	1	1	1	**1**	0	0
2.5	440	98	2	0	34	15	1	11	18	10	3	2	**17**	1	0	1	**1**	0	0
5	407	98	2	0	47	10	0	18	18	6	4	2	**22**	1	0	3	**1**	0	0
10	431	100	0	0	80	3	0	54	4	0	16	4	**1**	9	0	4	**3**	0	0

All values are means rounded off to the nearest whole number (n = 4).
L = Larva; P = Pupa; A = Adult.

Fig. 6.1. Effects of various concentrations of VPE on emergence and development of *T. castaneum* larvae exposed for different durations

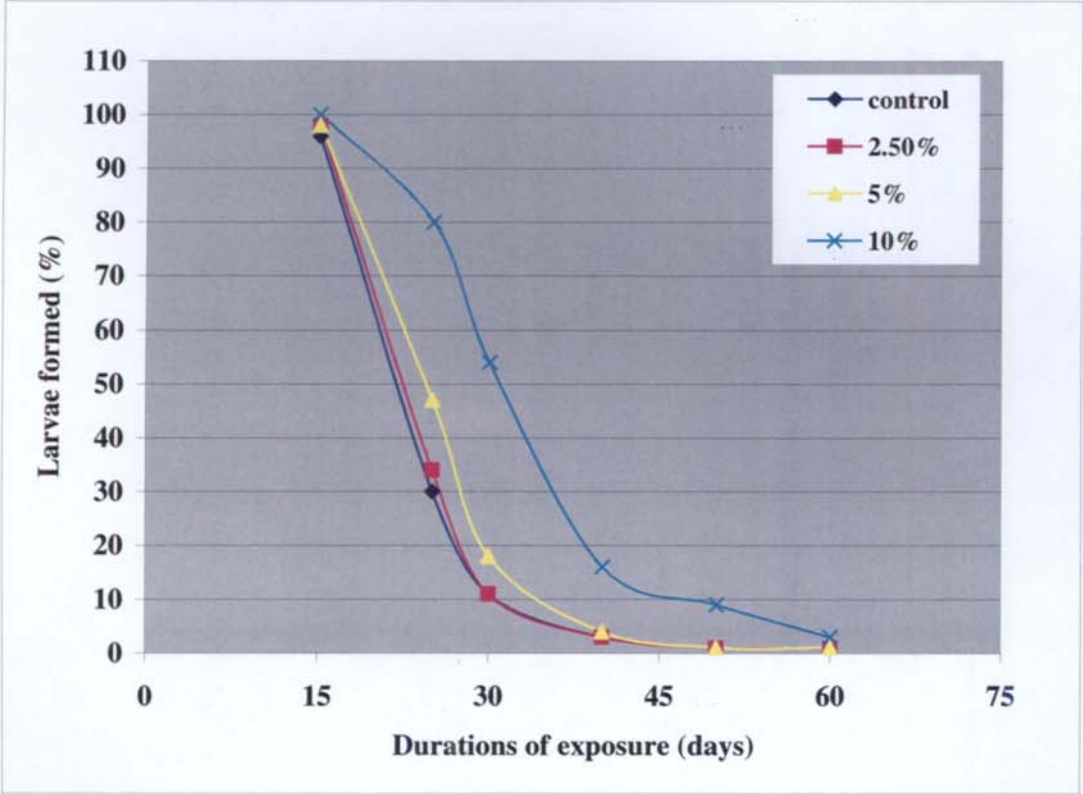


Fig. 6.2. Effects of various concentrations of VPE on pupation in *T. castaneum* exposed for different durations

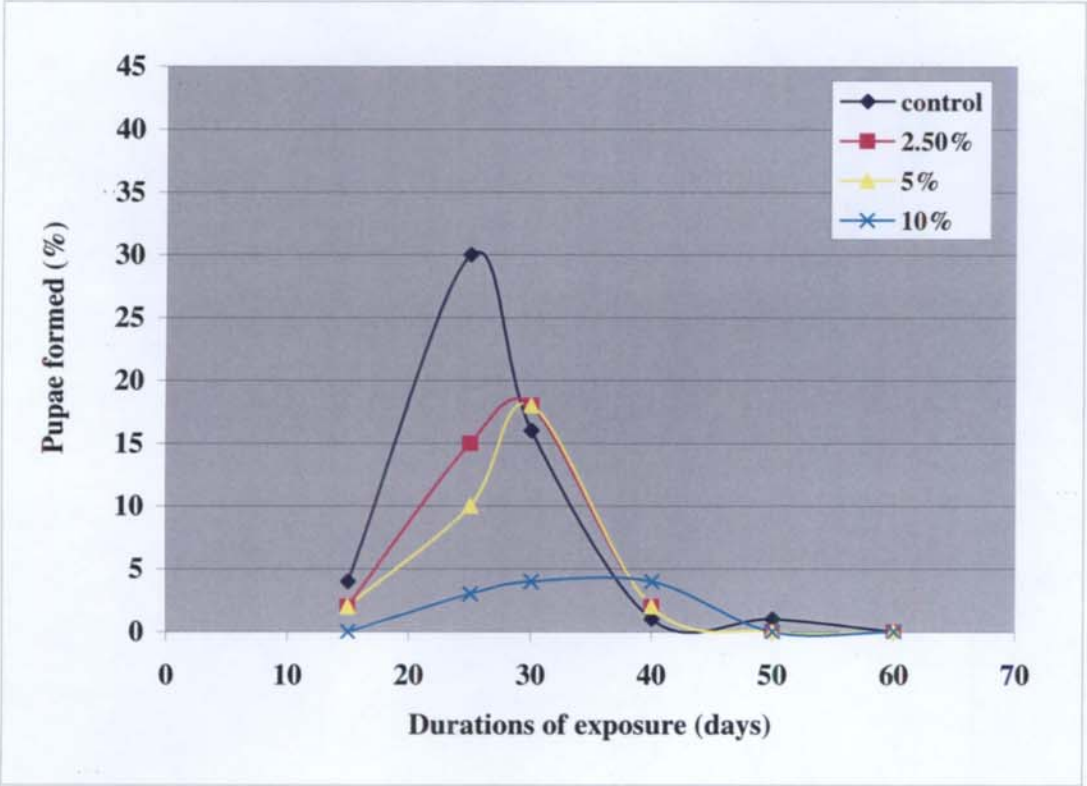
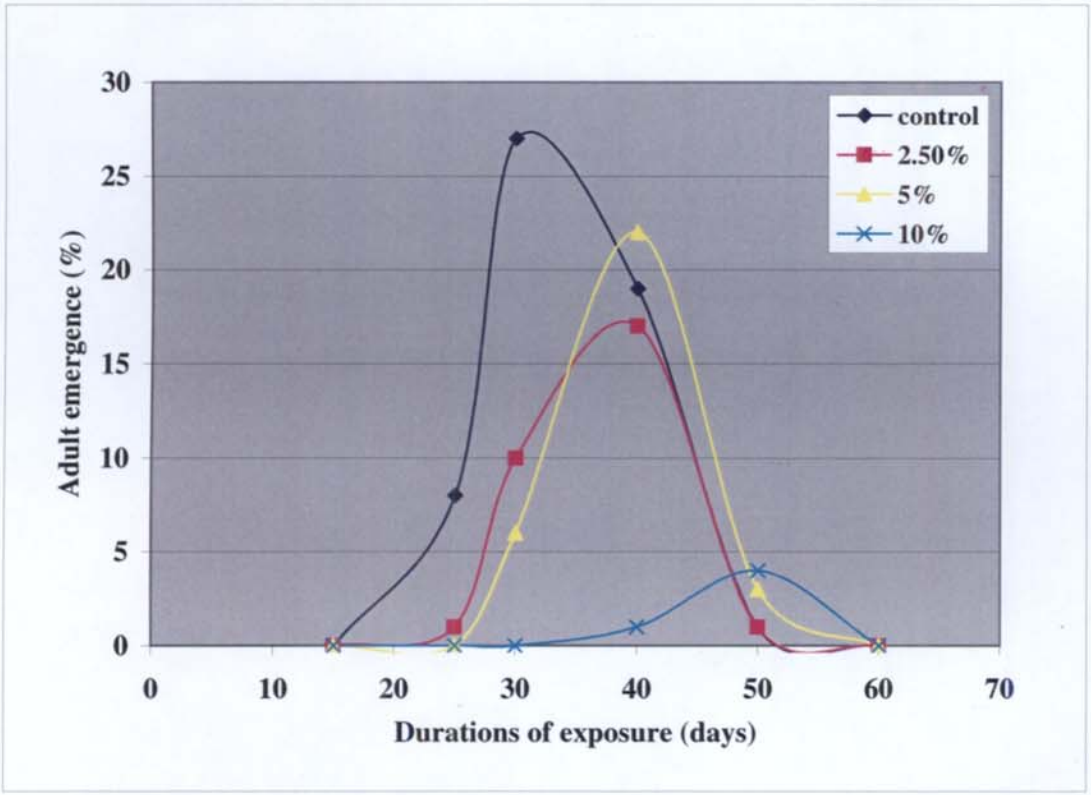


Fig. 6.3. Effects of various concentrations of VPE on adult emergence of *T. castaneum* exposed for different durations



the percentage of larvae retained increased with increasing concentrations of the VPE mixed with diets. Maximum percentage of larvae (80%) was found in the diet treated with 10% VPE. The percentage of pupae, on other land, showed a reverse effect, i.e., with an increasing concentration of the extract there was a decreasing percentage of pupae. In the control, 30% pupae were formed whereas it was only 3% in diet treated with 10% VPE. During the period, a few adults also had been seen (column 4, Table VI.1).

Observation made on the 30th day revealed that more adults were formed (27%) in the control diet and there were no adults in the diet treated with 10% of VPE. Percentage of adults emerged in the diets treated with 2.5% and 5% of VPE showed a corresponding decrease with an inverse relation (column 5, Table VI.1). There were concomitant changes in the number of larvae present in the respective diets. It was also observed that in the diet treated with 10% VPE, the percentage of pupae formed were very low (4%).

On the 40th day, more adults were formed except in the diets treated with 10% VPE, where only 1% adults were formed. In the case of control, there were 19% adults, which was however slightly lesser than what was formed on the 30th day (27%). In the diets treated with 2.5 and 5% of VPE, maximum percentage of adults was formed during this period (17% and 22% respectively). It was also observed here that there was a concomitant reduction in the percentage of larvae and pupae including the control. However, in the case of diet treated with 10% VPE, there were more larvae (16%) pupae (4%) were prevented from transformation (column 6, Table VI.1).

Observations made on the 50th and 60th days revealed that larval-pupal adult transformations were almost complete in the case of control and diet treated with the lowest concentration (2.5% of VPE). Only about 1% adults were found on the 50th day and there were no adults on the 60th day. On the other hand, in the diets treated with 5 and 10% of VPE, more adults were found to be emerging even at this time (3 and 4% respectively). In the case of 10% VPE, it was also noted that there was a higher percent of larvae (9%) present in it (column 7, Table VI.1). A few larvae were present in this diet on the 60th day of observation (3%) (column 8, Table VI.1).

Table VI.2 presents an account of the mortality of larvae, pupae and adults in the control diet and the diets treated with different concentrations of VPE during 60 days of observations. It is evident from the data that there was no significant mortality in pupal and adult stages except in a few cases. However, larval stage was found to be more vulnerable and was showing higher percentage mortality. They were found to become scrump, motionless, dried up and greyish coloured. The maximum mortality in the case of control was 33% and was observed on the 25th day. There was a sharp decline afterwards (column 3, Table VI.2; Fig. 6.4). In the case of diets treated with VPE, there was a higher percentage of mortality that persisted for longer duration. In the case of diets treated with 2.5% and 5% VPE, maximum occurred on 25 day of observation (50 and 42% respectively), which was similar to that the control. However, in the case of 10% VPE, the percentage of mortality gradually increased from 25th day to 40th day to reach the maximum of 35% mortality (Table VI.2; Fig. 6.4).

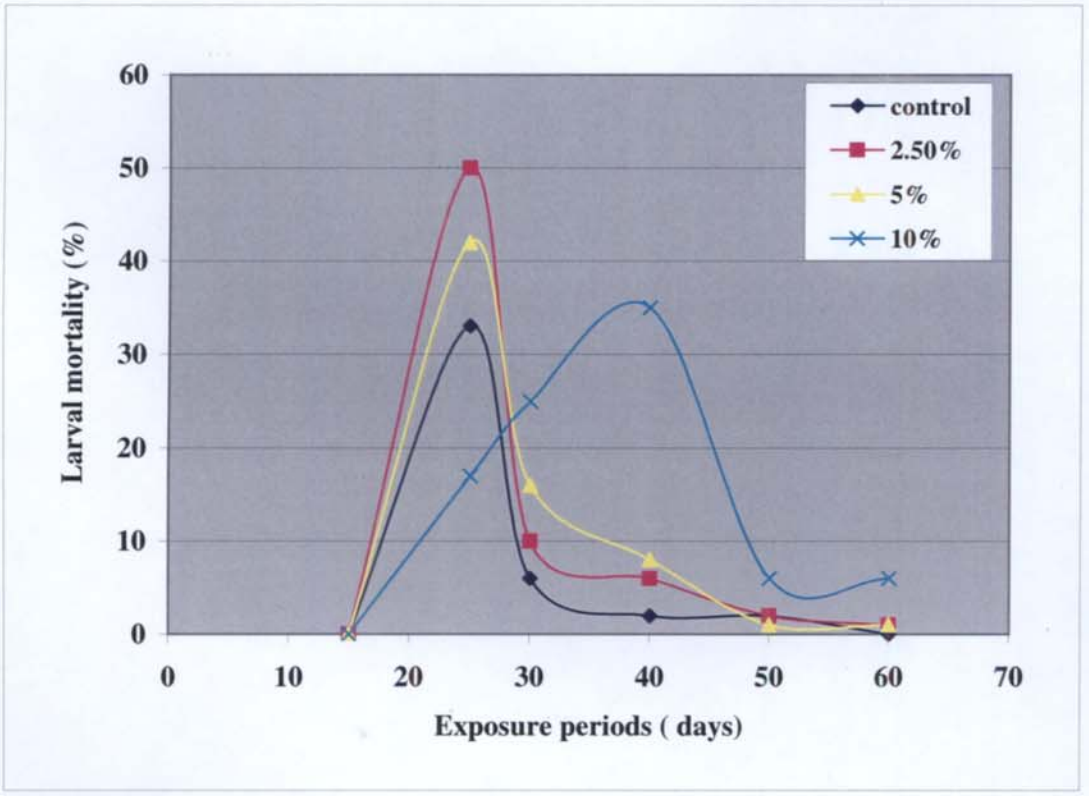
TABLE VI.2

Effects of various concentrations of VPE on mortality of larvae, pupae and adults of *T. castaneum* during different periods

1	2	Mean % mortality of different stages of *T. castaneum*																	
		3 LARVAE						4 PUPAE						5 ADULTS					
Conc. of VPE (%)	Initial no. of larvae	Exposure periods (days)						Exposure periods (days)						Exposure periods (days)					
		15	25	30	40	50	60	15	25	30	40	50	60	15	25	30	40	50	60
Control	415	0	33	6	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0
2.5	440	0	50	10	6	2	1	0	0	0	1	1	0	0	0	0	1	0	0
5	407	0	42	16	8	1	1	0	0	0	1	1	0	0	0	0	0	0	0
10	431	0	17	25	35	6	6	0	0	1	0	0	0	0	0	0	0	1	0

All values are means rounded off to the nearest whole number (n=4).

Fig. 6.4. Effects of various concentrations of VPE on larval mortality of *T. castaneum* during different periods



6.3.2. Effect of VME on emergence, development and progeny reduction in *T. castaneum*

The results of this study indicated that like VPE, VME also delayed the emergence and development of larvae, pupae and adults of *T. castaneum* when exposed to the extract. Table VI.3 and Figures 6.5-7 provide the effect of VME on the emergence and development of larvae, pupae and adults at different periods of exposure to the treated diets. Very small number of pupae were seen to be formed during 15 days (control=2%; 2.5%= 0%; 5%=1%; 10% = 0%). No adults were found during this period (column 3, Table VI.3).

From observation on the 25th day, it was seen that there were still more larvae remaining in all the cases of treated diets and control. However, in the control, pupation also occurred considerably and the highest number of pupae was observed in control (41%). Number of adults formed during this period were very small (column 4, Table VI.3).

On the 30th day, on an assessment of larvae present in the medium, it was seen that in the diets treated with VME, relatively large number of larvae were present compared to the number of larvae present in the control. In the case of control, there was a concomitant higher rate of pupation, which was low in the case of the treated diets. Again in the control, number of adults emerged were also found to be high (nearly 3-8 times) than the number of adults emerged from the treated diets (column 5, Table VI.3).

Further, observations made on the 40th day revealed that in all the cases there have been more adults present than larvae and pupae. In the case of the treated

TABLE VI.3

Effects of various concentrations of VME on emergence and development of larvae, pupae and adults of *T. castaneum* during different periods of exposure

1	2	Mean % progeny transformation during different periods																	
		3			4			5			6			7			8		
Conc. of VME (%)	Initial no. of larvae	15 days			25 days			30 days			40 days			50 days			60 days		
		L	P	A	L	P	A	L	P	A	L	P	A	L	P	A	L	P	A
Control	380	98	2	0	40	41	3	14	31	32	4	3	30	1	1	3	1	0	0
2.5	278	100	0	0	51	22	1	26	15	13	8	3	14	3	1	2	1	0	1
5	375	99	1	0	61	11	1	30	21	4	5	6	19	2	1	5	1	1	1
10	268	100	0	0	57	22	3	26	21	12	6	3	19	2	0	2	1	0	0

All values are means rounded off to the nearest whole number (n=4).
L = Larva; P = Pupa; A = Adult.

Fig. 6.5. Effects of various concentrations of VME on emergence and development of *T. castaneum* larvae exposed for different durations

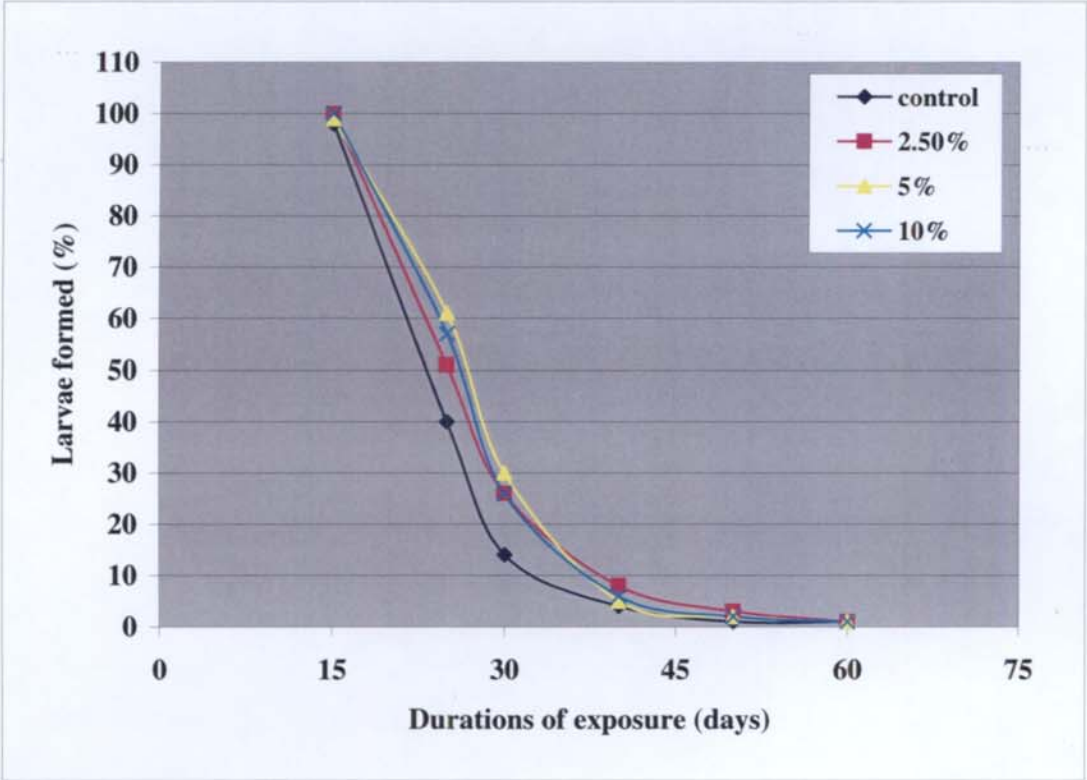


Fig. 6.6. Effects of various concentrations of VME on pupation in *T. castaneum* exposed for different durations

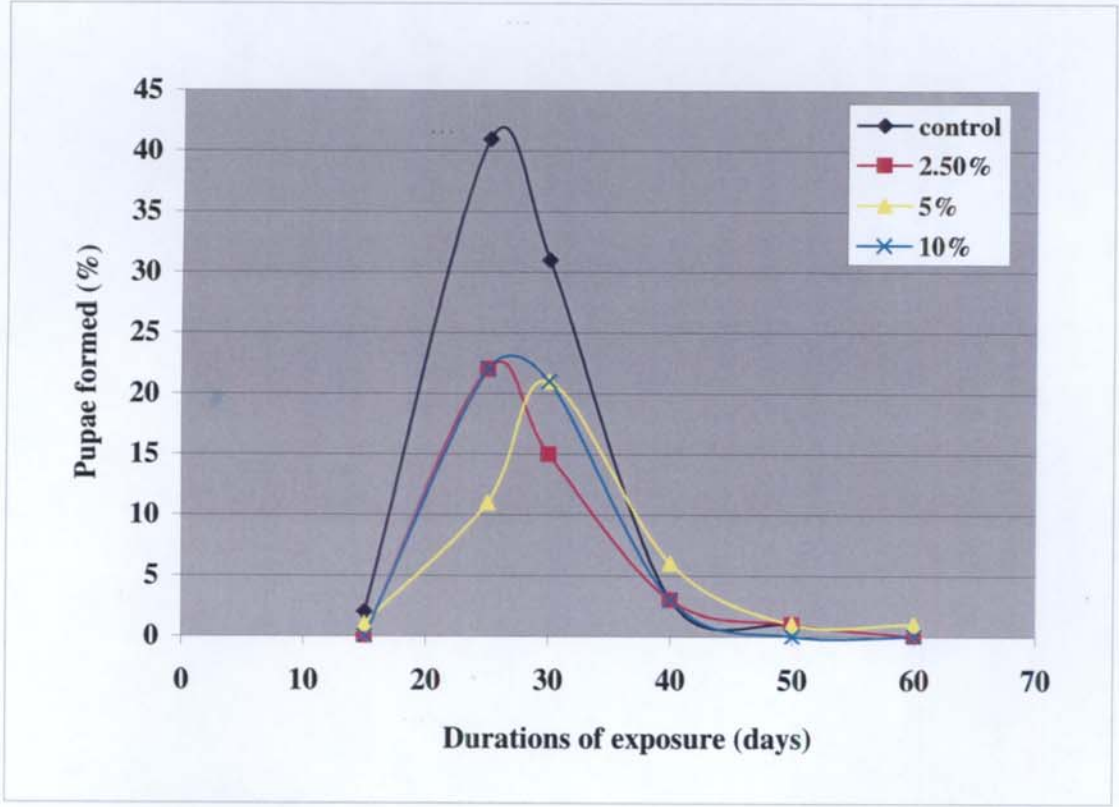
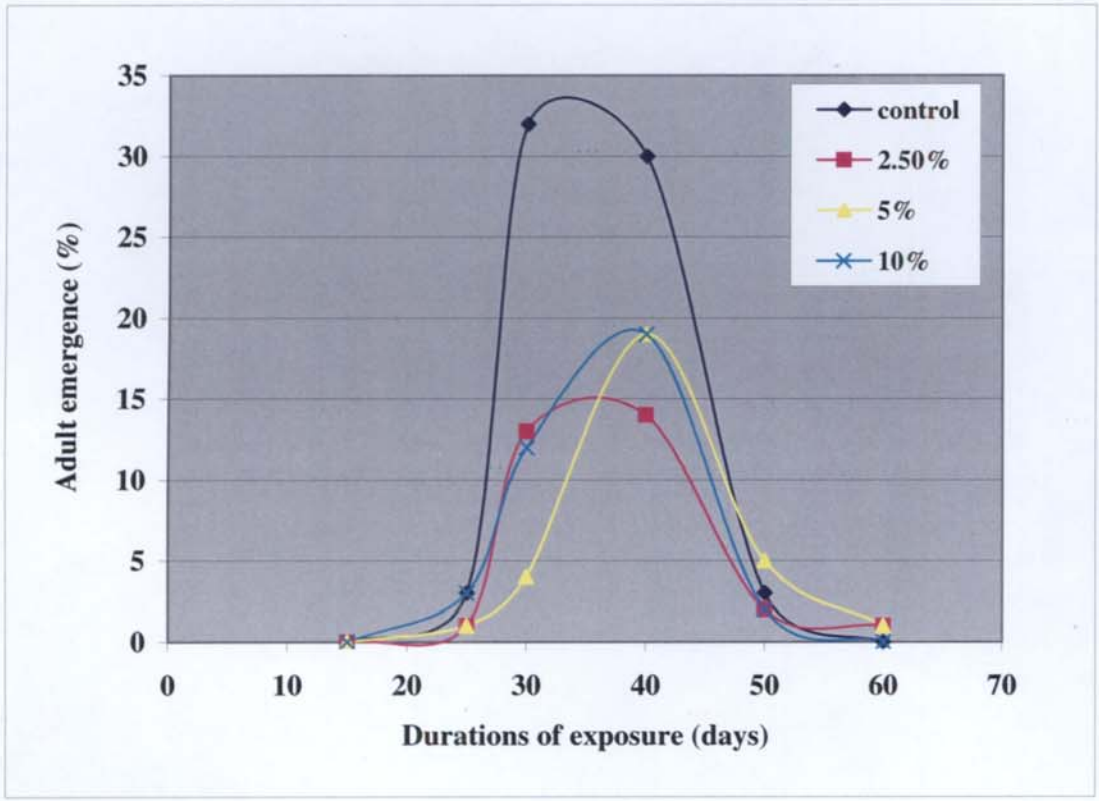


Fig.6.7. Effects of various concentrations of VME on adult emergence of *T. castaneum* exposed for different durations



diets, maximum number of adults were formed during this period. However, control diet still had large number of adults (2-3 times than the treated diets). The number of larvae and pupae found in all the diets were seen to be declining during this period (column 6, Table VI.3).

Determinations made on the number of insects on the 50th day showed that there were more adults than larvae and pupae (column 7, Table VI.3). Observations made on the 60th day showed the presence of some larvae and very few adults in the different diets (column 8, Table VI.3).

Table VI.4 shows the mortality of larvae, pupae and adults of *T. castaneum* during different periods of exposure to diets treated with VME. On the 15th day, mortality of larvae and pupae were not significant (less than 0.5%). On the 25th day, mortality of larvae in control was 16%, which sharply declined during further observations. Mortality of larvae in the diet treated with 2.5% of VME reached maximum (15%) on the 30th day of observation and showed gradual decline afterwards. Maximum mortality of 26% found in the diet treated with 5% VME on 25th day showed a gradual decline during the 60 days of observation. The percentage of mortality of larvae in the diet treated with 10% VME maintained a plateau up to 40 days observation with 18-21% of mortality (column 3, Table VI.4).

Pupal mortality occurred only after 30 days of exposure. The mortality rate was very low in the control and VME treated diets (about 1%) (column 4, Table VI.4). In the case of adults, there was no mortality when exposed to different concentrations VME as well as in the control (column 5, TableVI.4).

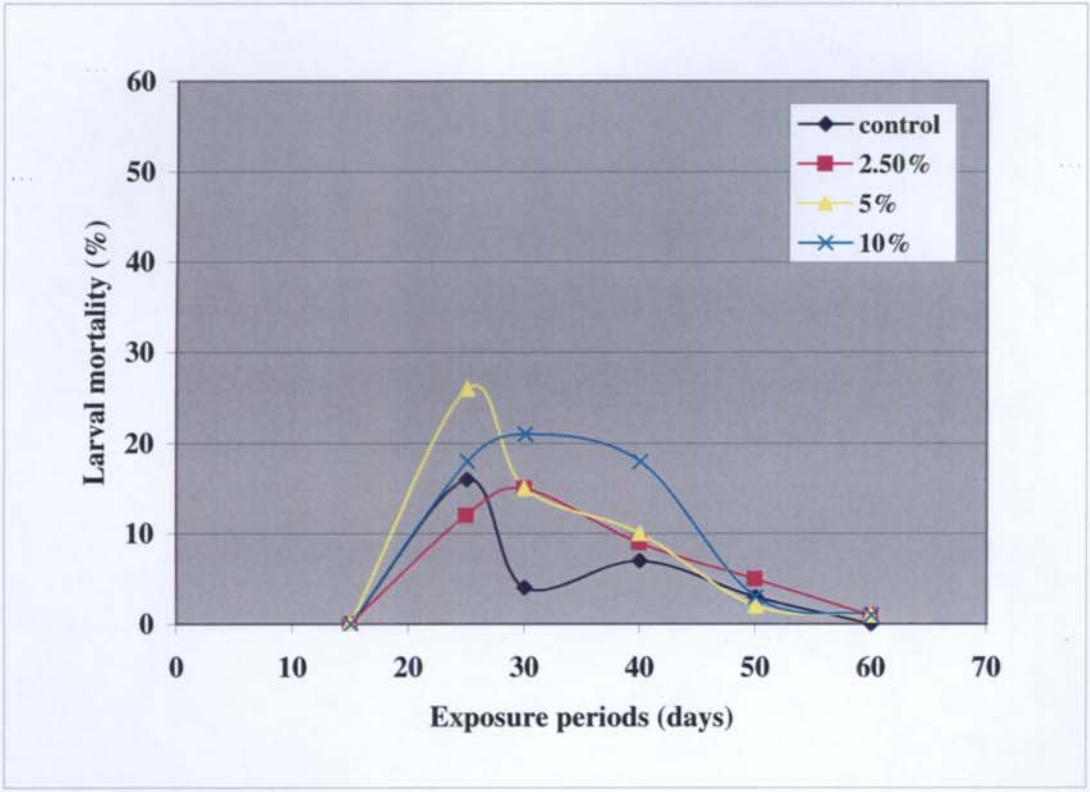
TABLE VI.4

Effects of various concentrations of VME on mortality of larvae, pupae and adults of *T. castaneum* during different periods

1	2	Mean % mortality of different stages of *T. castaneum*																	
		3 LARVAE						4 PUPAE						5 ADULTS					
Conc. of VME (%)	Initial no. of larvae	Exposure periods (days)						Exposure periods (days)						Exposure periods (days)					
		15	25	30	40	50	60	15	25	30	40	50	60	15	25	30	40	50	60
Control	380	0	16	4	7	3	0	0	0	0	0	0	0	0	0	0	0	0	0
2.5	278	0	12	15	9	5	1	0	0	0	1	1	0	0	0	0	0	0	0
5	375	0	26	15	10	2	1	0	0	0	1	1	1	0	0	0	0	0	0
10	268	0	18	21	18	3	1	0	0	0	1	1	0	0	0	0	0	0	0

All values are means rounded off to the nearest whole number (n=4).

Fig. 6.8. Effects of various concentrations of VME on larval mortality of *T. castaneum* during different periods



6.3.3. Statistical analysis of data

The data obtained from the studies on emergence, development and mortality of larvae, pupae and adults of *T. castaneum,* exposed to VPE and VME were subjected to ANOVA (Tables VI.5 & 6).

ANOVA showed that difference between the effects of VPE and VME on emergence and development of larvae of *T. castaneum* was highly significant ($P<0.001$; $F=14.482$). However, difference between the effects of the extracts on the formation and development of pupae ($P=0.1$; $F=2.674$) and adults ($P=0.56$; $F=0.343$) were not significant.

It was also seen that difference among the effects of different concentrations of the two extracts on emergence and development of larvae ($F=6.215$), pupae ($F=10.724$) and adults ($F=19.316$) were highly significant ($P<0.001$). Similarly, difference between the effects during different durations for larvae ($F=254.133$), pupae ($F=57.552$) and adults ($F=58.522$) were also highly significant ($P<0.001$) (Table VI.5).

The data for the effects of VPE and VME on the mortality of larvae, pupae and adults were also subjected to ANOVA. ANOVA revealed that difference between the effects of these two extracts on larval mortality ($F=34.836$) were highly significant ($P<0.001$). However, difference in the effects on mortality of pupae ($F=0.631$; $P=0.43$) and adults ($F=0.2$; $P=0.66$) were not significant. Difference among the effects of various concentrations ($F=5.499$; $P<0.01$) of the

TABLE VI.5

ANOVA of data showing the effects of VPE and VME on emergence and development of larvae, pupae and adults of *T. castaneum* during different periods

Parameters	Source of variables	df	MS	df Error	MS Error	F	P level
Larvae	Extracts	1	37157.5	144	2565.804	14.482	<0.001**
	Conc.	3	15947.4	144	2565.804	6.215	<0.001**
	Days	5	652056.1	144	2565.804	254.133	<0.001**
Pupae	Extracts	1	1587	144	593.427	2.674	0.1 (NS)
	Conc.	3	6363.91	144	593.427	10.724	<0.001**
	Days	5	34153.21	144	593.427	57.552	<0.001**
Adults	Extracts	1	143.52	144	418.056	0.343	0.56 (NS)
	Conc.	3	8075.29	144	418.056	19.316	<0.001**
	Days	5	24465.62	144	418.056	58.522	<0.001**

** Shows very high significance
NS : Not significant

TABLE VI.6

ANOVA of data showing the effects of VPE and VME on mortality of larvae, pupae and adults of *T. castaneum* during different periods

Parameters	Source of variables	df	MS	df Error	MS Error	F	P level
Larvae	Extracts	1	25093.88	144	720.339	34.836	< 0.001**
	Conc.	3	3961.67	144	720.339	5.499	< 0.01*
	Days	5	51112.77	144	720.339	70.957	< 0.001**
Pupae	Extracts	1	3.521	144	5.579	0.631	0.43 (NS)
	Conc.	3	11.361	144	5.579	2.036	0.11 (NS)
	Days	5	27.546	144	5.579	4.937	< 0.001**
Adults	Extracts	1	0.005	144	0.026	0.2	0.66 (NS)
	Conc.	3	0.047	144	0.026	1.8	0.15 (NS)
	Days	5	0.047	144	0.026	1.8	0.12 (NS)

** Shows very high significance
* Shows high significance
NS : Not significant

extracts and also the treatment duration (F=70.957; P<0.001) on the larval mortality were also highly significant.

In the case of pupal mortality, differences among the effects of the various concentrations of the extract (F=2.036; P=0.11) were not significant. However, difference in the effects between durations (F=4.937) were highly significant (P<0.001). Here, it is also seen that difference in the effects among concentrations (F=1.8; P=0.15) and periods (F=1.8; P=0.12) on adult mortality were not significant (Table VI.6).

6.4. Discussion

The results of the present study revealed that extracts of *V. negundo* in petroleum ether and methanol when mixed with the diets adversely affected the total number of insects formed during different stages (larva, pupa and adult), their moulting and development. As a result there was a significant reduction in the number of adults emerged.

It was observed that most of the larvae that hatched out until the 15th day in both the treated diets, transformed into pupae during 25-40 days (with a maximum during 25-30 days) and emerged as adults within 30-40 days (with a maximum at 40 days). In the control, formation of pupae and adult emergence mostly happened during 30-40 days. It was thus observed that these processes were delayed in the case of treated diets. This was more prominent in the diets treated with higher concentrations (5 and 10%) whereas it was less pronounced in the diet treated with 2.5% of the extracts (Tables VI.1 & 3). During the period between 50 and 60

days, the number of progeny were very much less for the reasons that (i) that in the control and in the diets treated with lower concentrations, most of the progeny became adults by about 40 days and (ii) that in the diets treated with higher concentrations, mortality rate during the early days (25 to 40 days) was higher, especially in the larval stage (Tables VI.2 & 4).

The pattern of the effects of different concentrations of VPE and VME on the development of the larvae of *T. castaneum* are represented in Figures 6.1 and 6.5. There has been considerable delay in the development and transformation of larvae to pupae in treated diets compared to the controls. It was found that 10% of the VPE was highly efficient in delaying transformation of larvae compared to other concentrations of this extracts. Failure of larvae to form pupae due to the administration of VPE and VME was prominent during 25-30 days where they were prevented from moulting to the next instars. Some larvae remained without pupation even during 40-60 days especially at higher concentrations of the two extracts.

The delay observed in the development of larvae of *T. castaneum* due to VPE and VME treatment is in agreement with studies of some earlier workers. Ladd *et al.* (1984) reported that azadirachtin from the seeds of *A. indica* caused significantly longer larval periods in the Japanese beetle, *Popillia japonica* and it completely disrupted subsequent normal development to the adult stage. Similarly, significant lengthening of larval period of *T. castaneum* by *A. squamosa* seed oil (Malek and Wilkins, 1995) and extension of larval and pupal durations and reduction in longevity, fecundity, development, growth, reproduction and feeding

in *H. armigera* by neem limonoids (Jeyabalan and Murugan, 1997) had been reported. Prolonged development, growth inhibition, developmental derangements and mortality of *C. cephalonica* brought about by neem oil had also been reported (Chanda and Chakravorty, 1998). Mahla *et al.* (2002) reported that *M. azedarach* prolonged the larval and pupal durations and affected pupation, hatching of eggs, adult emergence and longevity of *Earias vittella* larvae.

Besides the delay in development, significant reduction in the number of larval progeny due to mortality was also observed in all of the treatment. Figures 6.4 and 6.8 show the pattern of larval mortality resulted from treatment with the extracts. The reduction of larval progeny due to mortality was higher in diets treated with VPE compared to VME at different periods. It was seen that with both the extracts, with 2.5 and 5%, there were higher mortality compared to control during 30 days of treatment. The decline observed afterwards may be because of the transformation of most of the larvae into pupae and adults. The activity of 10% of VPE and VME appeared to be lagging and persistent until after 40 days. Larvae treated with 10% extracts appeared extremely small, relatively more sclerotised and for this reason more resistant to the toxic components. However, it was revealed that treatment with 10% extracts resulted in higher rate of total mortality. Here, maximum mortality occurred during 30-40 days (Tables VI.2 & 4).

It has generally been observed that insects are more susceptible to toxic materials during early days of intermoult periods. During these periods, the cuticle will be less sclerotised and less chitinised and thus more penetrable. It is possible in our experiment that lower concentrations of the extract do not inhibit moulting to

any appreciable extent, so that more susceptible stages in the intermoult periods are made available for the toxic substances to act upon unlike the 10% extract treatment where mortality results from a high toxic content.

It is also possible that lower concentrations of the extracts had higher toxic effect, which reflects high volatility of the toxic components. This effect is slightly different in the case of VME where the mortality with the lowest concentration (2.5%) was almost equal to the mortality observed in control. This may be due to the difference in the toxic materials extracted by the two solvents.

Mortality observed with 10% of both the extracts exhibit a slow and sustained activity, although maximum mortality rate was obtained. The delayed activity of the extract may be due to the low volatility of the concentrated extract (10%). However, higher total mortality observed during 30-40 days with this extract may also be due to starvation caused by antifeedant materials present in the extracts (Antifeedancy of *V. negundo* has been demonstrated in other experiments – Chapter V). The delay in toxic effects of 10% extracts might also be due to the interrupted moulting caused by the extracts resulting in the absence of the most susceptible early intermoult stages of the larva. This has been justified by the observations that in diets treated with higher concentrations, the larvae persisted beyond 25-30 days exhibited unusually smaller size characteristic of dauer larvae.

Effects of VPE and VME on larval mortality of *T. castaneum* is in agreement with the earlier reports of various authors. Rani and Jamil (1989) reported that crude extract of water hyacinth (*E. crassipes*) included in the diet retarded development and caused mortality in the 4th instar larvae of *T. castaneum*

and *C. cephalonica*. Singh and Rao (2000) found that topical application of stem extract of *Ageratum conyzoides* caused larval mortality and reduced adult emergence of *S. litura*. Similarly, 13 essential oil-vapours from various plants have been reported to increase neonate larval mortality, influence offspring emergence, reduce fecundity and decrease egg hatchability in addition to repellent and toxic effects in *Acanthoscelides obtectus* (Papachristos and Stamopoulos, 2002).

The extracts also affected the formation of pupae and pupal duration. Both VPE and VME exhibited almost similar patterns of activity (Figs. 6.2 & 6.6). Due to higher rates of larval mortality in the treated diets, the number of pupae formed in them were far less than those in the controls. Both the extracts delayed pupation to various degrees. Pupation was more or less completed by 30 days in the case of VPE whereas it was completed by 40 days in the case of VME (Tables VI.1 & 3). The extracts were also found to cause certain amount of mortality in the pupal population, which was not observed in the controls.

The reduction of pupal progeny of *T. castaneum* in both the treated diets is in agreement with some of the earlier reports. Jilani *et al.* (1988) reported that turmeric oil, sweetflag oil, neem oil and Margosan-O produced significantly fewer larvae, pupae and adults of *T. castaneum* than in the control. Similarly, some vegetable oils (oils of ground nut, traditional coconut, industrial coconut, palm and shark liver) when treated on dried trout, significantly reduced the development of the progeny of the larvae and adults of *D. maculatus* (Don-Pedro, 1989 a). Schmidt *et al.* (1991) reported that the number of offsprings of *S. granarius*, *S. oryzae* and *C. chinensis* emerging from food treated with *A. calamus* oil vapours were

considerably lower than those emerging from the respective controls and the effect was found to be dose-dependent.

Similarly, adult emergence was also found to be affected by the extracts. The peak of adult emergence at 30 days in the case of controls was shifted to 40 days in the case of the treated diets (Figs. 6.3 & 6.7). The effect of the extracts on the larval mortality is reflected in the number of adults emerged which was very much less in the experimentals than the number of adults emerged in the controls. Interestingly, unlike in the controls, few adults were seen emerging out from the treated diets during 50- 60 days (Tables VI.1 & 3).

The results thus suggest the presence of compounds in the extracts that interfere with the normal growth, development and moulting of *T. castaneum*.

The reduction of adult emergence observed is consistent with studies of various authors. Sukumaran *et al.* (1987) reported that dried leaf powder of *V. negundo,* neem or a mixture of both, when mixed with paddy grains reduced adult emergence of *Sitotroga cerealella*. Similarly, *V. negundo* leaf powder reduced oviposition and adult emergence of *C. chinensis* (Miah *et al.*, 1992, 1993). It was also reported that the extracts of some plants including *V. negundo* reduced the fecundity and adult emergence and prolonged the duration for the completion of one generation in *R. dominica* (Singh *et al.*, 1996 a). Chander *et al.* (1992) reported that turmeric powder and mustard oil reduced adult emergence of *T. castaneum*.

The delayed development of larvae, pupae and adults of *T. castaneum* and their reduction with VPE and VME may presumably be due to several constituents

such as monoterpenes, sesquiterpenes, diterpenes, essential oils etc. present in *V. negundo* leaf extracts which adversely affected the development of this insect. This is in agreement with the effects of various plant constituents on several insect species. Powell and Raffa (1999) reported that selected terpenoids of *Larix laricina* affected growth and development of the gypsy moth, *Lymantria dispar*. Similarly, essential oils from *Elletaria cardamomum* reduced hatching of eggs, survival of larvae and adult emergence of *T. castaneum* (Huang *et al.*, 2000). Leaf essential oils of *C. longa* caused oviposition deterrence, ovicidal action, reduction of egg hatching and suppression of progeny production in *T. castaneum*, *S. oryzae* and *R. dominica* (Tripathi *et al.*, 2002).

The chemical constituents (terpenes, essential oils etc.) present in *V. negundo* may act as antifeedants and result in a low rate of feeding in the larvae, consequently resulting in a prolonged larval stage. The results of the action of these chemicals suggest an effect on the endocrine mechanism that regulate moulting and metamorphosis of the insect. This is in tune with the finding in *C. cephalonica* that neem caused adverse effects on the development (Pandey *et al.*, 1985) and in tobacco hornworm , *Manduca sexta* where azadirachtin was found to affect growth and other endocrine events (Schluter *et al.*, 1985).

In conclusion, it is found that both the petroleum ether and methanol extracts of *V. negundo* delayed the development and caused reduction of the progeny of *T. castaneum*. Hence, the results of the present study points to the possibility of using *V. negundo* leaf extracts as a potential material that could be suggested for use to suppress the population of *T. castaneum* in stores. Further, research on the

isolation, identification and mechanism of action of the active substances and ways to exploit the major bioactive constituents are promising approaches for the design and development of stored grain protectants.

Chapter VII

DYNAMICS OF ADULT EMERGENCE

7.1. Introduction

Indigenous plant products and plant secondary metabolites are receiving increasing attention in stored product pest management to minimise the storage losses due to insects. The discovery of the presence of antifeedants insect growth regulators and other behaviour modifying chemicals in plants has assumed much importance in insect pest management. Information on botanicals suggests that they can intervene at several stages of insects' life cycle including the emergence and development of larvae, pupae and adult insects. Several botanicals have been reported to inhibit the adult emergence of various stored product insects.

Chander and Ahmed (1986) reported the efficacy of powdered rhizomes of *A. calamus*, leaves of *Clerodendrum inerma, Tylophora asthmatica, Justicia botanica* and *Cestrum nocturnum* in the reduction of adult emergence of *C. cephalonica*. Turmeric powder and mustard oil in different combinations used as protectants for milled rice against *T. castaneum* infestation, suppressed the progeny of this insect (Chander *et al.*, 1992). Similarly, reduction of adult emergence in *R. dominica* by some indigenous plant products (Sosamma and Sheila, 1993) and *T. castaneum* by leaf powders of *Cassia occidentalis, Tephrosia appolinea, Calotropis procera, Datura metel* and *Croton bonplandianum* (Maheshwari and Dwivedi, 1996) have been reported. Rajapakse (1996) reported that powders of *P. nigrum, A. reticulata, Dillenia retusa* and *O. sanctum* acted as protectants against

C. maculatus where emergence of adult beetles were reduced. Wongo (1998) reported the reduction of adult emergence of *S. oryzae, S. cerealella* and *T. castaneum* by tannin extracts of *Sorghum bicolor.* Neem products such as neem seed kernel powder (NSKP), neem leaf powder (NLP) and neem oil were used as protectants of maize which reduce the emergence of F_1 and F_2 progeny of *S. oryzae, S. cerealella, R. dominica, T. granarium* and *T. castaneum* (Sharma, 1999). Mukherjee and Joseph (2000) reported that commercially available extracts of *A. calamus, Rauwolfia serpentina, Sapindus trifoliatus* and *Commiphora mukul* adversely affected the adult emergence of *T. castaneum.* Similarly, *P. harmala* extracts were found to reduce the adult emergence of *C. chinensis* (Srivastava and Mann, 2002).

Oils from several plants have been reported to reduce the adult emergence in insects. Ten vegetable oils treated on pigeon pea (Khaire *et al.,* 1992), pongam oil treated on green gram (Negi *et al.,* 1994) and crucifer oil treated on pigeon pea (Sharma *et al.,* 1999) were reported to reduce the adult emergence of *C. chinensis.* Similarly, edible oils of cotton seed, sunflower, ground nut, soy bean and mustard mixed with cowpea (Ramzan, 1994) and volatile oils of *Ageratum conyzoides* treated on beans (Gbolade *et al.,* 1999) inhibited the adult emergence of *C. maculatus.*

Studies described in this chapter are to evaluate the emergence of adult progeny during different periods, total number of adults emerged during 50 days, their adult emergence index and the inhibition or reduction of the progeny of *T. castaneum* exposed to VPE and VME.

7.2. Methods

The methods adopted for the study in an adult emergence of *T. castaneum* are mentioned in the section 3.2.6.

7.3. Results

7.3.1. Effect of VPE on adult emergence of *T. castaneum*

Results of the studies on the number of adults of *T. castaneum* emerged in diets treated VPE during different periods (days) are presented in Table VII.1 and Figure 7.1. A perusal of the Table reveals that there was a significant reduction of adult progeny emerged during 25-40 days in all the diets treated compared to control except for 5% VPE on 40th day. At the same time, it was seen that the number of days required for emergence of adults were increased in treated diets (to about 40-50 days).

On the 25th day, more number of adults emerged in control (32.75) whereas in 2.5, 5 and 10% VPE, emergence of adults decreased to 4.01, 1.02 and 0.24 respectively. Number of adults emerged in the control diet increased to about 112 on the 30th day. However, number of adults emerged in the treated diets were considerably reduced. This reduction was also found to increase with an increase in the concentration of the VPE. With 2.5% of VPE, the number of adult emerged was 42.44 and with 5% VPE, it was 23.71. However, there was no adult emergence with 10% VPE. On the 40th day, the number of adults emerged in control was 77.25, whereas with 2.5 and 5% VPE, the number of adults emerged were 70.03 and 91 respectively. However, very few adults (5.54) emerged in diet

TABLE VII.1

Inhibition of adult emergence in *T. castaneum* by VPE during different periods of exposure

Conc. of VPE (%)	Mean number of adults emerged during different periods (days)			
	25	30	40	50
Control	32.75	112	77.25	3.5
2.5	4.01	42.44	70.03	3.54
5	1.02	23.71	91.0	10.71
10	0.24	0	5.54	16.37

All values are means of 4 replicates.
Number of adults in each treatment was normalised with the number in the control.

Fig. 7.1. Effects of various concentrations of VPE on adult emergence of *T. castaneum* during different periods of exposure

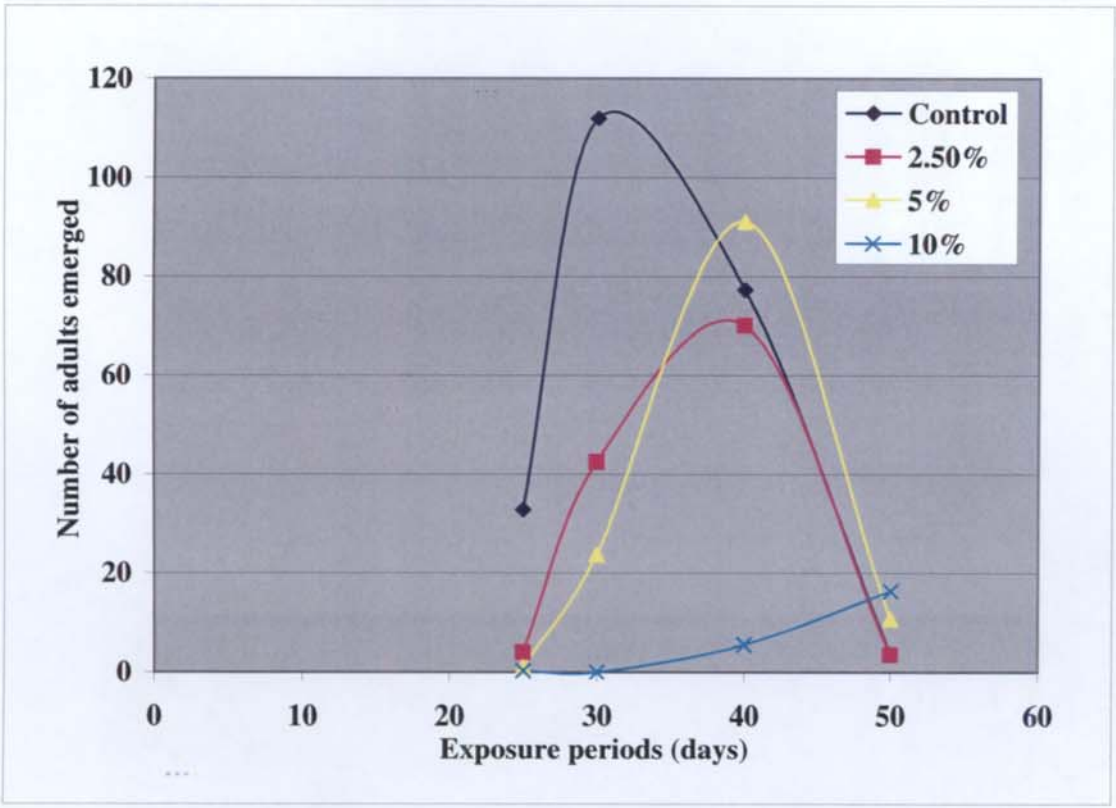


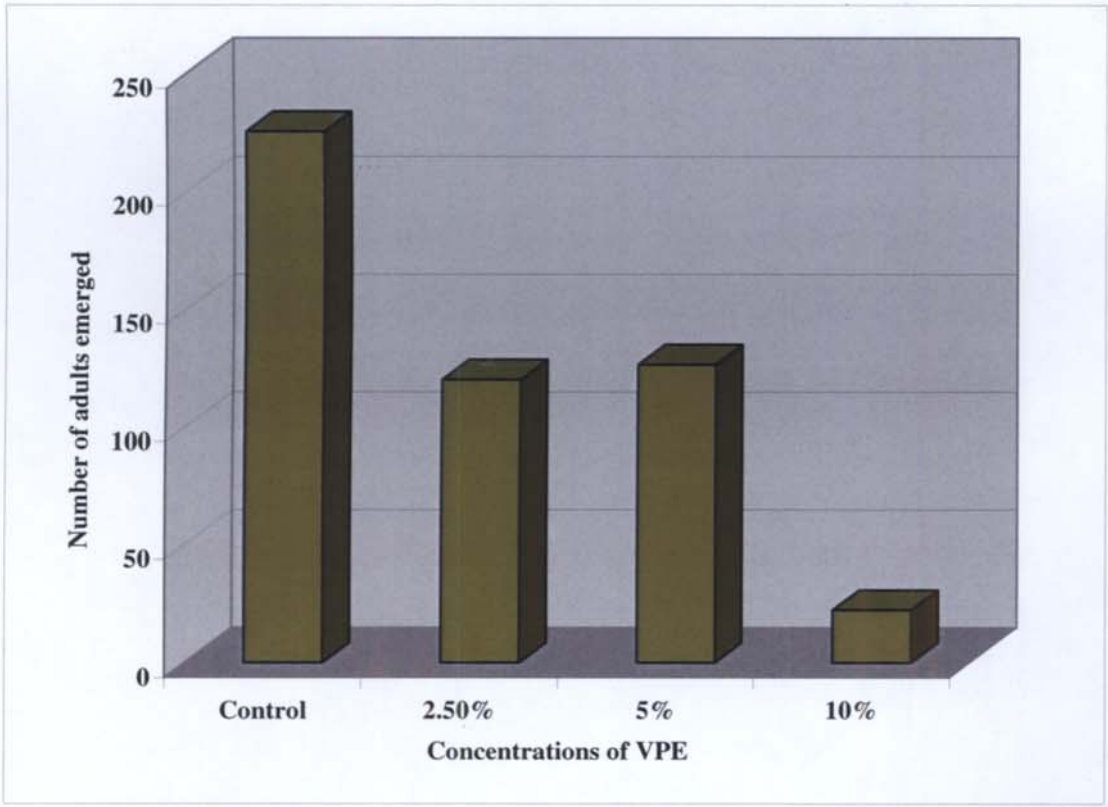
TABLE VII.2

Effect of VPE on adult emergence of *T. castaneum* during 50 days of exposure

Conc. of VPE (%)	Total number of adults emerged during 50 days	Adult Emergence Index (AEI)	% Inhibition of progeny
Control	225.5	--	--
2.5	120.02	0.5322	46.78
5	126.44	0.5607	43.93
10	22.15	0.0982	90.18

All values are means of 4 replicates.
Calculated as described in section 3.2.6 of Chapter III.

Fig. 7.2. Inhibition of adult emergence in *T. castaneum* during 50 days of exposure to VPE



treated with 10% VPE. On the 50th day, a very small number of adults emerged in the control (3.5) and in 2.5% VPE (3.54). Number of adults emerged in the diets treated with 5% and 10% VPE (10.71 and 16.37 respectively) were found to be on the increase at these durations which was interestingly more than the number emerged in the control and in the diet treated with 2.5% (Table VII.1).

Table VII.2 shows the total (mean number) adults emerged, adult emergence index and per cent inhibition of the progeny of *T. castaneum* in VPE treated diets during 50 days of exposure period. Here, it was observed that the total number of adults emerged in diets treated with different concentrations of the extract, decreased considerably compared to control. In the control diet, there were more adults (225.5) than the experimentals. With 2.5 and 5% VPE, the numbers were reduced to 120.02 and 126.44 respectively. With 10% VPE, the number further reduced to 22.15 (Fig. 7.2).

Here, it becomes obvious that the lowest adult emergence index was obtained with 10% VPE (0.0982), Adult emergence indices with 2.5 and 5% VPE were 0.5322 and 0.5607 respectively (Table VII.2). Per cent inhibition of the progeny was showing the highest value of 90.18 with 10% VPE. At the same time, per cent inhibition of progeny with 2.5 and 5% were found to be 46.78 and 43.93 respectively.

7.3.2. Effect of VME on adult emergence of *T. castaneum*.

Diets treated with VME also adversely affected the number of *T. castaneum* adults emerged during different periods of exposure. Table VII.3 and Figure 7.3 provide details of adult emergence of *T. castaneum* in VME treated diets. The data

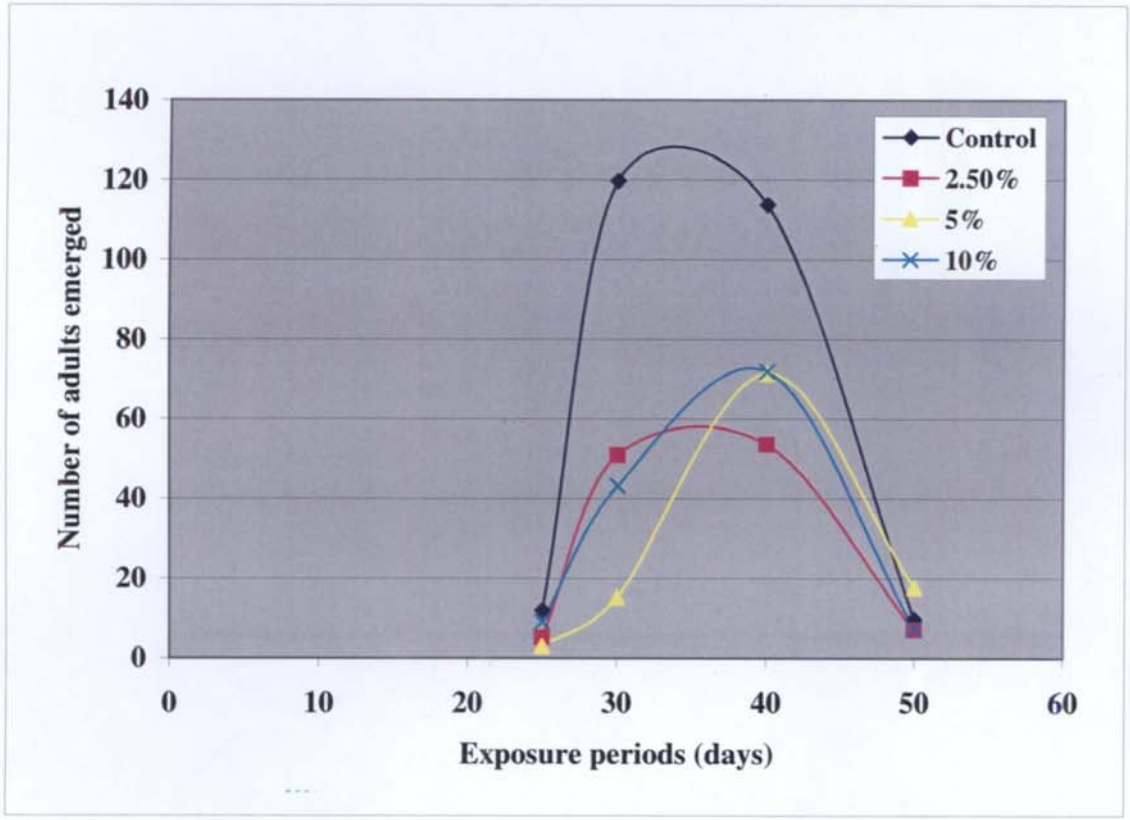
TABLE VII.3

Inhibition of adult emergence in *T. castaneum* by VME during different periods of exposure

Conc. of VME (%)	Mean number of adults emerged during different periods (days)			
	25	30	40	50
Control	12	119.75	113.75	9.75
2.5	5.13	50.92	53.65	7.18
5	3.04	15.2	71.44	17.73
10	9.22	43.25	71.96	7.44

All values are means of 4 replicates.
Number of adults emerged in each treatment was normalised with the number in the control.

Fig. 7.3. Effects of various concentrations of VME on adult emergence of *T. castaneum* during different periods of exposure



revealed that there was significant reduction in the number of adults emerged in treated diets during different periods up to 50 days compared to control. Here, it was also seen that number of days taken for the emergence of adults increased in treated diets compared to that for the control.

On the 25th day, relatively small number of adults (12) emerged in the control. With 2.5, 5 and 10% VME, the adults emerged were 5.13, 3.04 and 9.22 respectively. Observations made on the 30th day revealed that maximum number of adults emerged in the control diet (119.75). Adult emergence with 2.5% and 5% VME were 50.92 and 15.2 respectively. With 10% VME, a reduced activity resulting in a slightly higher number of adults similar to the trend found on the 25th day has been noticed. On the 40th day, there were still more adults (113.75) in the control diet. Here, a reduction in the number of adults was observed in different treated diets compared to the control (Table VII.3). However, the number of adult beetles were more than the corresponding number of adults observed during the 30th day (Table VII.3). With 5 and 10% VME treated diets, considerable number of adults emerged (71.44 and 71.96 respectively) compared to 30th day indicating that there has been some delay in development brought about by VME. On the 50th day, number of adults present in the control was only 9.75. Here, a slight reduction in the number of adult emerged was observed with 2.5% (7.18) and 10% VME (7.44). However, adults emerged with the diet treated with 5% VME (17.73) was slightly more (Table VII.3; Fig. 7.3).

Table VII.4 provides the data on the total number of adults (mean number) emerged, adult emergence index and per cent inhibition of the progeny of *T.*

TABLE VII.4

Effect of VME on adult emergence of *T. castaneum* during 50 days of exposure

Conc. of VME (%)	Total number of adults emerged during 50 days	Adult Emergence Index (AEI)	% Inhibition of progeny
Control	255.25	--	--
2.5	116.88	0.4579	54.21
5	107.41	0.4208	57.92
10	131.87	0.5166	48.34

All values are means of 4 replicates.
Calculated as described in 3.6.6 of Chapter III.

Fig. 7.4. Inhibition of adult emergence in *T. castaneum* during 50 days of exposure to VME

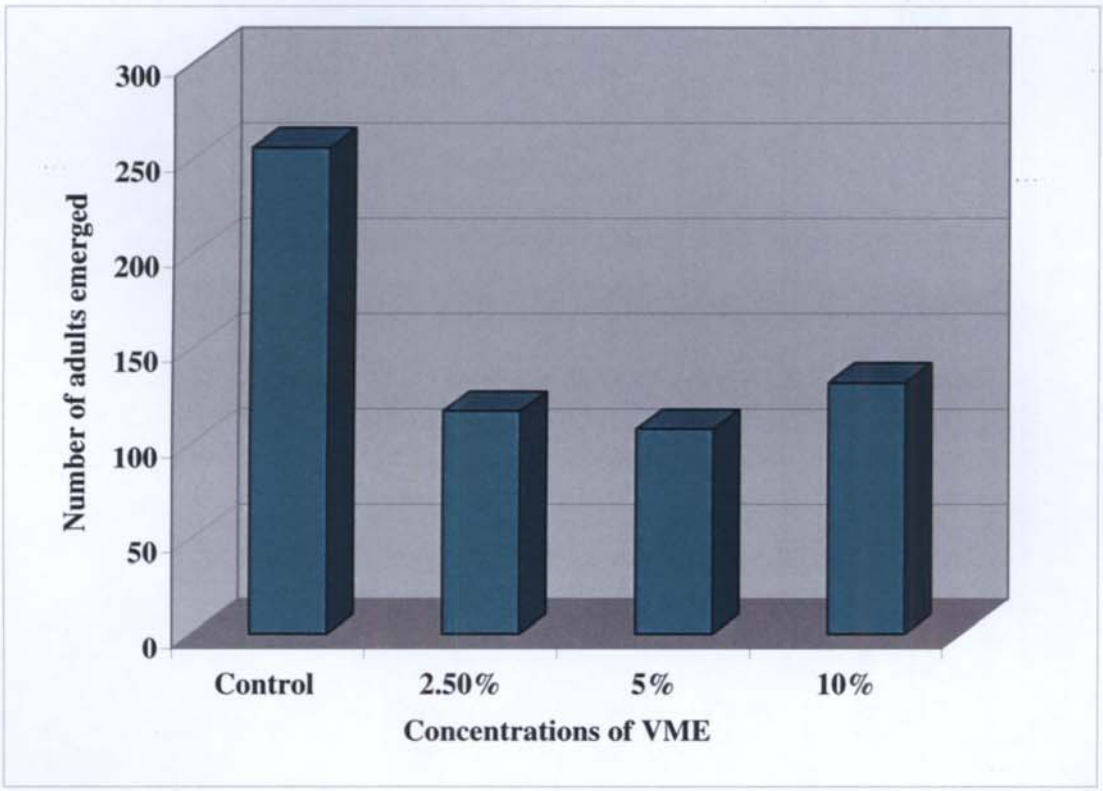


TABLE VII.5

ANOVA of data showing the effects of VPE and VME on adult emergence of *T. castaneum*

Source of variables	df	MS	df Error	MS Error	F	P level
Extracts	1	200	96	626.4739	0.3192	0.57
Conc.	3	12099.84	96	626.4739	19.3142	<0.001
Days	3	26137.76	96	626.4739	41.7220	<0.001

castaneum during 50 days of exposure to VME treated diets. From this Table, it is obvious that the number of adults emerged in diets treated with various concentrations of VME were reduced compared that in the control. Here, it is seen that the maximum number of adults were found in the control diet (255.25), whereas total number of adults emerged were 116.88, 107.41 and 131.87 respectively in diets treated with 2.5, 5 and 10% VME (Fig. 7.4).

In the case of treated diets, the lowest adult emergence index (AEI) of 0.4208 was seen with 5% VME. Adult emergence indices with 2.5 and 10% VME were 0.4579 and 0.5166 respectively. The per cent inhibition of adult emergence by the extracts were 54.21%, 57.92% and 48.34 respectively.

7.3.3. Statistical analysis of data

The data obtained from the studies on emergence of *T. castaneum* adults after exposure to VPE and VME for different periods were subjected to ANOVA and it was seen that the difference between the effects of these two extracts on adult emergence of the insect was not significant ($P=0.57$; $F=0.3192$). However, difference among the effects of different concentrations ($F=19.3142$) of each extract and different durations ($F=41.7220$) were highly significant ($P<0.001$) (Table VII.5).

7.4. Discussion

The present study reveals that the number of *T. castaneum* adults emerged from the diets treated with VPE and VME were showing significant reduction when compared to that in the control during different periods of exposure (Tables VII.1

& 3; Figs. 7.1 & 3). Moreover, it was seen that the total number of adults emerged during 50 days from the diets treated with the plant extracts were always lesser than those from the control (Tables VII.2 & 4; Figs. 7.2 & 4).

When the number of adult insects emerged in the diets treated with different concentrations of VPE and VME were compared, it was found that of all the treatments, 10% VPE showed the lowest adult emergence index. When looked at the per cent inhibition of the progeny, highest value (90.18) for 10% VPE (Tables VII.2 and 4).

It was evident from the result that the peak of adult emergence at 30 days in the case of control was shifted to 40 days in the case of treated diets (Figs. 7.1 & 3).

The results obtained for the reduction of adult emergence of *T. castaneum* by VPE and VME are in agreement with the results obtained by some other investigators using this plant materials against some other insect species. Sukumaran *et al.* (1987) reported the reduction of adult emergence of *S. cerealella* by the dried powder of *V. negundo*, neem or a mixture of both, mixed with paddy grains. Similarly, *V. negundo* leaf powder was found to reduce oviposition and adult emergence (Miah, *et al.*, 1992,1993). Extracts of various plants including *V. negundo,* reduced the fecundity and adult emergence and also caused prolongation of life cycle in *R. dominica* (Singh *et al.*, 1996 a). Raja *et al.* (2000 b) reported that extracts of *V. negundo* and *Cassia fistula* leaves significantly reduced the egg laying and adult emergence of *C. maculatus*.

Reduction of adult emergence in *T. castaneum* by other botanicals have also been reported. *T. castaneum* was showing a reduction in adult emergence in response to dry leaf powders of *C. occidentalis, T. appolinea, D. metel* and *C. bonplandianum* (Maheshwari and Dwivedi, 1996) and to extracts of *A. calamus, R. serpentina, S. trifoliatus* and *C. mukul* (Mukherjee and Joseph, 2000). Wongo (1998) reported that Sorghum tannin extracts were found to reduce the adult progeny of *S. oryzae S. cerealella* and *T. castaneum* and delayed adult emergence.

When the adult emergence indices obtained from the experiments were compared, it was seen that 2.5 and 5% VPE exhibited relatively higher values. On the other hand, 10% VPE was showing the highest per cent inhibition of progeny. Adult emergence index measured by Muralibhaskaran and Janarthanan (2000) for plant oils, provided similar results indicating that *V. negundo* extracts are promising inhibitors of adult emergence of *T. castaneum*. Similar inhibition adult emergence of by several plant materials have been reported by Chander and Ahmed (1986).

The reduction of adult emergence of *T. castaneum* in diets heated with the two extracts was due to mainly the larval mortality caused by the extracts. Pupal mortality also affected the emergence of adults. It was also seen that there was a considerable delay in the adult emergence that caused by the extracts. This delay resulted from a corresponding delay in the larval-pupal-adult transformations brought in by the extracts. Here, most of the larvae failed to pupate and they died as a result of delayed development. Hence, in experimentals, only a few larvae and pupae survived which developed in to adults.

Similar results have been reported earlier. Malek and Wilkins (1995) reported that *A. squamosa* seed oil significantly lengthened the larval period of *T. castaneum*. Crude extract of *E. crassipes* included in the diet of insects retarded development and caused mortality in the 4th instar larvae of *T. castaneum* and *C. cephalonica* (Rani and Jamil, 1989). Similarly, wheat flour diets treated with turmeric oil, sweetflag oil, neem oil and Margosan-O produced significantly fewer larvae, pupae and adults of *T. castaneum* than control (Jilani *et al.,* 1988). It was also reported that *M. azedarach* prolonged the larval and pupal durations, affected pupation, hatching of eggs, reduced adult emergence and longevity of *Earias vitella* larvae (Mahla *et al.,* 2002).

Several chemical constituents (terpenes, essential oils, etc.) present in both the extracts might have adversely affected the development of the insect and caused consequent reduction of *T. castaneum* progeny in the treated diets. These chemicals may act variously as oviposition deterrents, ovicidals, inhibitors of egg hatching, growth inhibitors, antifeedants and/or toxicants. Moreover, these chemicals may have adverse effects on endocrine mechanisms that regulate moulting and metamorphosis of the insect.

The results of this study thus reveal that VPE and VME have considerable adverse effects on the development of *T. castaneum* and hence reduce the emergence of adult insects. Thus, these extracts may be useful in keeping the insect population below economic injury level. The optimum concentration of the extract in specific situations and conditions of storage of the grains is to be determined by trials.

Chapter VIII

EVALUATION OF TOXICITY

8.1. Introduction

Protection of agricultural stored products against insect pests is of utmost importance to ensure a continuous and safe food supply all over the world. Conventional chemical treatments have been used for this purpose but now-a-days other ecologically sound methods based on natural compounds are needed for an integrated approach to pest management. A wide variety of higher plants provide new sources of natural pesticides and antifeedants (Grainge and Ahmed, 1988; Arnason *et al.*, 1989; Ananthakrishnan, 1992). The interaction between plants and insects is mediated by chemical compounds from plants as a result of joint evolution (Strebler, 1989). Therefore, the study of the effects of plant materials and extracts on pests will provide chances for finding alternative insecticides. The main advantage of botanicals is that they are easily produced by farmers and small-scale industries are potentially less expensive and are virtually harmless to other animals. A large number of plant extracts and secondary metabolites having insecticidal, repellent, antifeedant and other biological properties have been screened for their activities against stored product insects.

Plant extracts are reported to possess insecticidal activities against many stored product insects. Insecticidal properties of extract of *Piper nigrum* against *S. oryzae* and *C. maculatus* (Su, 1977) and *P. guineense* and *Capsicum* sp. against *C. maculatus* (Ivbijaro and Agbaje, 1986) had been reported. Rani and Jamil (1989)

evaluated petroleum ether extract of *E. crassipes* for its contact toxicity against *T. castaneum*, *S. oryzae*, *C. maculatus,* and *C. cephalonica*. Similarly, insecticidal effects of *Datura metel* (Khalequizzaman and Islam, 1992) and *A. polystachya* (Talukder and Howse, 1995) on *T. castaneum* and *Calotropis procera* on *T. confusum* (Jahan *et al.*, 1991) have been reported. Several such reports on the toxic/insecticidal properties of a variety of plants against stored product insects appeared in the past few decades (Saxena *et al.*, 1992; El-Lakwah *et al.*, 1994, 1996, 1997; Mbata *et al.*, 1995; Malek *et al.*, 1996; Pemonge *et al.*, 1997; Perveen *et al.*, 1998; Pascual, 1998; Babu *et al.*, 1999; Adedire and Lajide, 1999; Umoetok, 2000; Bright *et al.*, 2001; Jaswanth *et al.*, 2002; Srivastava and Mann, 2002).

Some isolated phytochemical components from the plants were also reported to possess toxic effects. Prakash and Mathur (1985) in their review reported the use of plant products for insect pest management in stored grains and emphasised the need to explore the active principles of plant products showing promising grain protection against insect infestation. Alkaloid-containing fraction isolated from the leaves of *N. tabacum* exhibited insecticidal activity against larvae of *T. castaneum* (Archana *et al.*, 1995). Ho *et al.* (1997 b) reported that trans-anethole, the principal constituent of *Illicium verum* was showing fumigant and contact toxicity against *T. castaneum* and *S. zeamais*.

Oils extracted from plants are also reported to possess toxic/insecticidal activities against some stored product insects. Insecticidal/toxic activities of essential oils of *Cedrus deodara* against *C. chinensis* had been reported by (Singh and Rao, 1985). Don-Pedro (1996) reported the fumigant toxicity of six citrus peel

oils against adults and immature stages of *C. maculatus, S. zeamais* and *D. maculatus*. Other reports of insecticidal activities of plant oils against stored product insects include Su, (1991), HanHong *et al.* (1994, 1996 a, 1996 b) and Talukder *et al.* (1998).

Essential oils/volatile oils from plants act as toxicants/insecticides in the control of stored product insects (Haubruge *et al.*, 1989; Ansari and Mishra, 1990; Shaaya *et al.*, 1997; Obeng-Ofori and Reichmuth, 1997; Obeng-Ofori *et al.*, 1997 a, 1997 b, 1998; Namrata *et al.*, 1997; Prates *et al.*, 1998; Chiam *et al.*, 1999; Gbolade *et al.*, 1999; Verma *et al.*, 2000; Bouda *et al* 2001; Keita, 2001 a, 2001 b; Tripathi *et al.*, 2000 a, 2001 a, 2001 b, 2002).

Similarly, *T. castaneum* and *S. zeamais* were shown to exhibit contact and fumigant toxicity in response to cinnamaldehyde from *C. aromaticum* (Huang and Ho, 1998), to essential oil from nutmeg seeds (Huang *et al.*, 1997) and to essential oils from *E. cardamomum* (Huang *et al.*, 2000). Lee (2002) evaluated fumigant toxicity of lavender and ylang-ylang essential oils against *O. surinamensis*. Lee *et al.* (2001) have also reported the fumigant toxicity of volatile constituents of essential oil extracted from a number of Korean spices and medicinal plants against *S. oryzae*.

The experiments described in this chapter are to evaluate the toxicity of petroleum ether and methanol extracts of *V. negundo* against the flour beetle, *T. castaneum*.

8.2. Methods

Methods adopted for the evaluation of toxicity of the extracts against *T. castaneum* are described in sections 3.2.7.1, 3.2.7.2, 3.2.7.3 and 3.2.7.4.

8.3. Results

8.3.1. Contact toxicity of VPE against *T. castaneum* adults

A. Film Residue Method (FRM)

Toxic effects of various concentrations of VPE on *T. castaneum* adults at 24 h intervals for 7 days by the film residue method are recorded in Table VIII.1. From the result, it is evident that film residue of VPE was not highly toxic to the adults of *T. castaneum*. On the first day, the mean percentage mortality of 2.5, 5 and 10% concentrations of the extract were 2, 2 and 12 respectively. There were no mortality with 0.625 and 1.25% VPE. On the second day, there were no mortality with 0.625, 1.25 and 5% extract whereas 2.5 and 10% concentrations afforded 1 and 2% mortality respectively. On the 3rd day, % mortality observed with 1.25, 5 and 10% were 1, 1 and 2 respectively but in other treatments there were no mortality. Similarly, on the 4th day, 2.5, 5 and 10% VPE were showing mortality of 1, 1 and 2% respectively whereas 0.625 and 1.25% concentrations afforded no mortality. On the 5th day, mortality was not observed in any of the treatments except for 10% VPE which afforded only 1% mortality. On the 6th day, only 1% mortality was observed in 2.5 and 10% VPE whereas there were no mortality in other treatments. On the 7th day, the % mortality were 1, 3, 1, 1 and 4

TABLE VIII.1

Mortality of *T. castaneum* adults exposed to VPE for different periods (FRM)

Conc. of VPE (%)	Mean percentage mortality during different periods (days)							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
Control	0	0	0	0	0	0	0	0
0.625	0	0	0	0	0	0	1	1
1.25	0	0	1	0	0	0	3	4
2.5	2	1	0	1	0	1	1	6
5	2	0	1	1	0	0	1	5
10	12	2	2	2	1	1	4	24

Values are means of 10 replications.
Sample: 10 insects x 6 treatments x 10 replicates = 600 insects.

respectively for the different concentrations. There were no mortality of insects in the case of control during the entire period of observations (7 days).

Here, 10% extract was showing the maximum mortality (24%) whereas 5, 2.5 and 1.25% VPE had lesser toxicity (5, 6 and 4% mortality respectively) and 0.625% concentration had the least toxicity (1%) during the period of observation of mortality (7 days).

B. Topical Application Method (TAM)

The petroleum ether extract of *V. negundo* when applied topically was found to be more effective as a toxicant to *T. castaneum* adults. Table VIII.2. shows the corrected percentage mortality of *T. castaneum* adults to the different doses (applied topically) of VPE during different exposure periods. From the table, it is evident that at 1.5 h all of the concentrations of the extract were showing strong toxicity in a dose-dependent manner. Here, the corrected % mortality of 0.625, 1.25, 2.5, 5 and 10% VPE were 50.77, 58.46, 73.85, 89.23 and 95.38 respectively. At 3 h, none of the concentrations of the extract were found to be effective except 1.25%, which had 1.01% mortality, and 5% had negative value (-1.01). At 6 h, 1.25 and 2.5% afforded the mortality of 2.02 and 1.01 respectively, whereas 0.625% brought no further mortality. At 24 h, 2% mortality was observed in 1.25 and 10% VPE and 1% mortality with 2.5 and 5% VPE. Here, 0.625% did not afford any further mortality. At 48 h, mortality for 0.625, 1.25 and 2.5% of the extract were 2, 2 and 4% respectively and there were no more mortality with 5 and 10% VPE. At 72 h, 0.625, 1.25 and 5% afforded 1, 2 and 2% mortality respectively whereas there were no further mortality in 2.5 and 10% extract. At 96

TABLE VIII.2

Mortality (corrected percentage) of *T. castaneum* adults exposed to VPE for different periods (TAM)

Conc. of VPE (%)	Mean percentage mortality during different periods (hours)						
	1.5	3	6	24	48	72	96
Control	-- (35.00)	-- (1.00)	-- (1.00)	-- (0.00)	-- (0.00)	-- (0.00)	-- (1.00)
0.625	50.77 (68.00)	0.00 (1.00)	0.00 (1.00)	0.00 (0.00)	2.00 (2.00)	1.00 (1.00)	0.00 (1.00)
1.25	58.46 (73.00)	1.01 (2.00)	2.02 (3.00)	2.00 (2.00)	2.00 (2.00)	2.00 (2.00)	1.01 (2.00)
2.5	73.85 (83.00)	0.00 (1.00)	1.01 (2.00)	1.00 (1.00)	4.00 (4.00)	0.00 (0.00)	0.00 (1.00)
5	89.23 (93.00)	-1.01 (0.00)	-1.01 (0.00)	1.00 (1.00)	0.00 (0.00)	2.00 (2.00)	-1.01 (0.00)
10	95.38 (97.00)	0.00 (1.00)	-1.01 (0.00)	2.00 (2.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

All figures are corrected % mortality after transformation of the observed % mortality by Abbot's formula.
Figures in parentheses are observed % mortality.
Sample: values are means of 10 replicates (10 insects x 6 treatments x 10 replicates 600 insects.
Calculated as described in section 3.2.7.

h, none of the concentrations of the extract were showing mortality except for 1.25%, which had 1.01% mortality.

In the case of control, it was observed that petroleum ether when applied on the insect caused contact toxicity. Hence, corrected % mortality of the insects was obtained by transforming the observed % mortality using Abbot's formula.

8.3.2. Contact toxicity of VME against *T. castaneum* adults

A. Film Residue Method (FRM)

Table VIII.3 provides the mean % mortality of *T. castaneum* adults exposed to different doses of VME for different periods by FRM. This result indicates that the extract has strong toxicity against *T. castaneum* adults, which is found to be dose-dependent. The highest toxicity of the extract was observed on the first day of treatment, especially for 2.5, 5 and 10% VME, which afforded 20, 42 and 71% mortality respectively. Here, VME 1.25% was showing the least toxicity (2%) whereas 0.625% afforded no toxic effect on insects. On the second day, 6, 16, 25 and 21% mortality were observed for 1.25, 2.5, 5 and 10% extract respectively, whereas there was still no mortality with 0.625%. Similarly, mortality percentage of 0.625, 1.25, 2.5, 5 and 10% of VME on the 3rd day were 2, 6, 14, 14 and 8 respectively. It was also seen that 10% extract showed 100% mortality of the insects within three days. On the 4th day, the mortality % were 2, 6, 9, 7 with the different concentrations up to 5% extract. But on the 5th day, 3, 6, 12 and 9% mortality were observed with the different doses of the extract. On 6th day, 0.625, 1.25 and 2.5% extract afforded mortality of 2, 10 and 9% respectively, whereas

TABLE VIII.3

Mortality of *T. castaneum* adults exposed to VME for different periods (FRM)

Conc. of VME (%)	Mean percentage mortality during different periods (days)							Total
	1	2	3	4	5	6	7	
Control	0	0	0	0	0	0	0	0
0.625	0	0	2	2	3	2	6	15
1.25	2	6	6	6	6	10	8	44
2.5	20	16	14	9	12	9	3	83
5	42	25	14	7	9	0	3	100
10	71	21	8	0	0	0	0	100

Values are means of 10 replicates.
Sample: 10 insects x 6 treatments x 10 replicates = 600 insects.

there was no further mortality in 5% VME. On the 7th day, mortality % were 6, 8, 3 and 3 with the different concentrations. It was also seen that 100% of mortality observed for 5% extract within 7 days. No mortality of insects were observed in the control during the entire period of observations.

Hence, it is apparent that mortality of insects exhibited a dose- dependent pattern up to 7 days. Here, the maximum toxicity (100%) occurred with 10% extract within 3 days and with 5% extract within 7 days. In the film residue method employed in the case of VME, toxicity was found to persist for longer periods; i.e., in this case for more than 7 days (Table VIII.3). In lower concentrations, the mortality was on the increase and appeared to be active even after 7 days of observation. Higher concentrations were effective in bringing 100% mortality much faster.

B. Topical Application Method (TAM)

Mean percentage mortality of *T. castaneum* adults with different doses of VME by TAM are presented in Table VIII.4. From the data, it is evident that the extract was showing strong toxicity, which was more or less immediate.

At 1.5 h, the highest percentage mortality of the adult insects was observed. With 0.625, 1.25, 2.5, 5 and 10% VME, mortality of 23, 50, 64, 87 and 94% respectively were observed which followed a dose-dependent pattern. At 3 h, 0.625, 1.25, 2.5 and 5% afforded the mortality of 5, 10, 5 and 1% respectively and 10% extract did not show any mortality. After 6 h, the percentage mortality were 6, 4, 3, 3 and 1 with the different concentrations and the mortality recorded after 24 h

TABLE VIII.4

Mortality of *T. castaneum* adults exposed to VME for different periods (TAM)

Conc. of VME (%)	Mean percentage mortality during different periods (hours)							Total
	1.5	3	6	24	48	72	96	
Control	0	0	0	0	0	0	0	0
0.625	23	5	6	4	4	5	3	50
1.25	50	10	4	1	5	2	4	76
2.5	64	5	3	2	1	1	0	76
5	87	1	3	2	2	0	1	96
10	94	0	1	3	2	0	0	100

Values are means of 10 replicates.
Sample: 10 insects x 6 treatments x 10 replicates = 600 insects.

were 4, 1, 2, 2 and 3%. Similarly at 48 h, the respective concentrations of the extract afforded the mortality of 4, 5, 1, 2 and 2. It was thus seen that 100% mortality of insects occurred within 48 h in the case of 10% VME. At 72 h, 0.625, 1.25 and 2.5% VME afforded very low mortality such as 5, 2 and 1% respectively whereas 5% VME was not showing any mortality. Similarly at 96 h, 0.625, 1.25 and 5% afforded mortality of 3, 4 and 1% respectively whereas 2.5% VME did not show any mortality.

8.3.3. Statistical analysis of data

The data obtained from the toxicity studies on *T. castaneum* adults by topical application of VPE and VME, and film residue of VME were subjected to Probit analysis for calculating the regression equation and LC values of the extracts (Table VIII.5).

Toxicity by topical application of VPE on the insects showed a regression coefficient (R) of 0.7302 and LC_{80} and LC_{95} values of 1.88 and 7.14 respectively. Here, toxic effect of VPE on the insect is highly significant ($P<0.001$). Toxicity by film residue of VME on insect has an R value of 0.9089, LC_{50} of 5.78 and LC_{95} of 22.57. Here, also the toxic effect is highly significant ($P<0.001$). Similarly, toxicity by topical application of VME shows the value of 0.8404 (R), 1.48 (LC_{50}) and 8.43 (LC_{95}). Here also toxic effect is highly significant ($P<0.001$) (Table VIII.5).

Figure 8.1 shows the toxicity of VPE against *T. castaneum* adults by TAM. This graph shows the regression equation which represent the probit mortality (%)

TABLE VIII.5

Probit analysis of the data on contact toxicity of VPE and VME against *T. castaneum* adults

Extracts	Testing methods	Regression equation	Regression coefficient		Slope	LC_{50}	LC_{80}	LC_{95}	P values
			R	R^2					
VPE	Topical application	y = 11.253 x +72.489	0.7302	0.5333	11.253	--	1.88	7.14	<0.001
VME	Film residue	y=33.038 x -7.972	0.9089	0.8262	33.038	5.78	--	22.57	<0.001
VME	Topical application	y = 25.824 x + 39.937	0.8404	0.7065	25.824	1.48	--	8.43	<0.001

Sample of 10 insects x 6 treatments x 10 replicates (600 insects) were used for each of the toxicity testing methods using the extracts.
y = Probit mortality, x = Log concentration.
LC_{50} (%), LC_{80} (%) and LC_{95} (%) = concentration calculated to give 50, 80 and 95 per cent mortality respectively.
LC_{80} and LC_{95} for *T. castaneum* adults exposed to the topical application of VPE for 1.5 h were taken as unity.
In TAM, the concentration of VPE required for % kill of < 80% can not be estimated, since the observation starts 0.625%. concentration which kill more than 75% insects.
LC_{50} and LC_{95} for *T. castaneum* adults exposed to the film residue of VME for 24 h were taken as unity.
LC_{50} and LC_{95} for *T. castaneum* adults exposed to the topical application of VME for 1.5 h were taken as unity.

Fig. 8.1. Toxicity of VPE against *T. castaneum* adults (TAM)

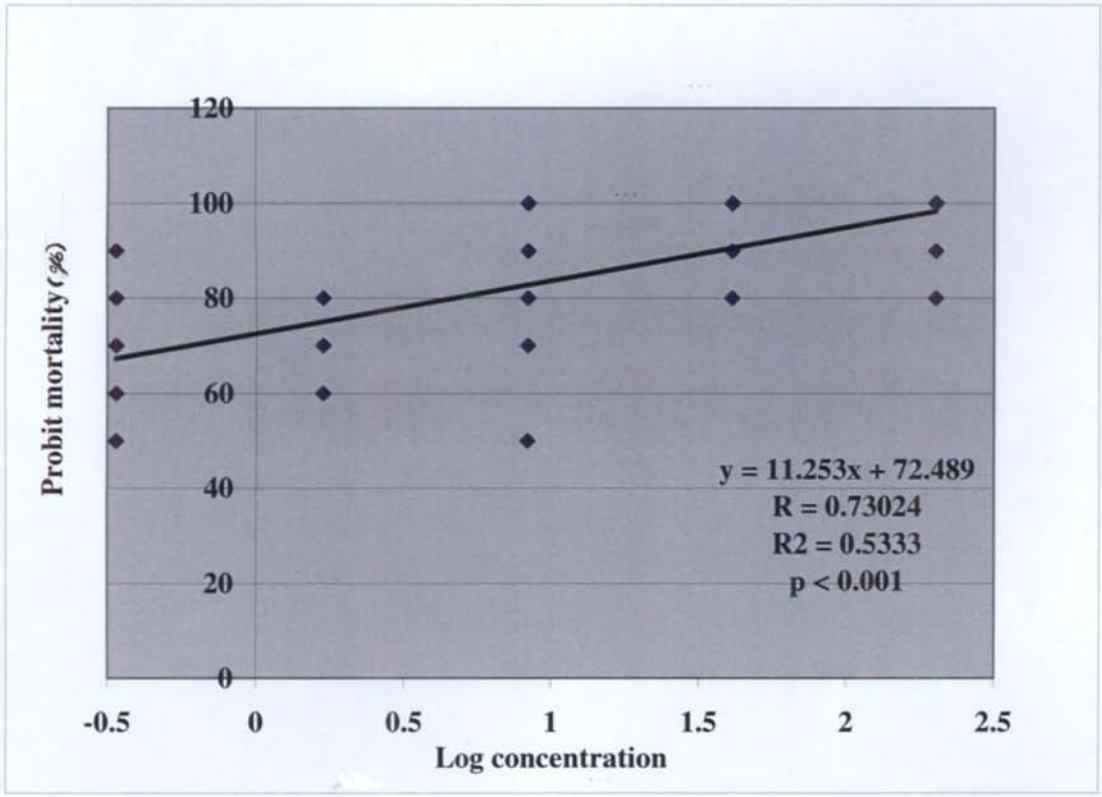


Fig. 8.2. Toxicity of VME against *T. castaneum* adults (FRM)

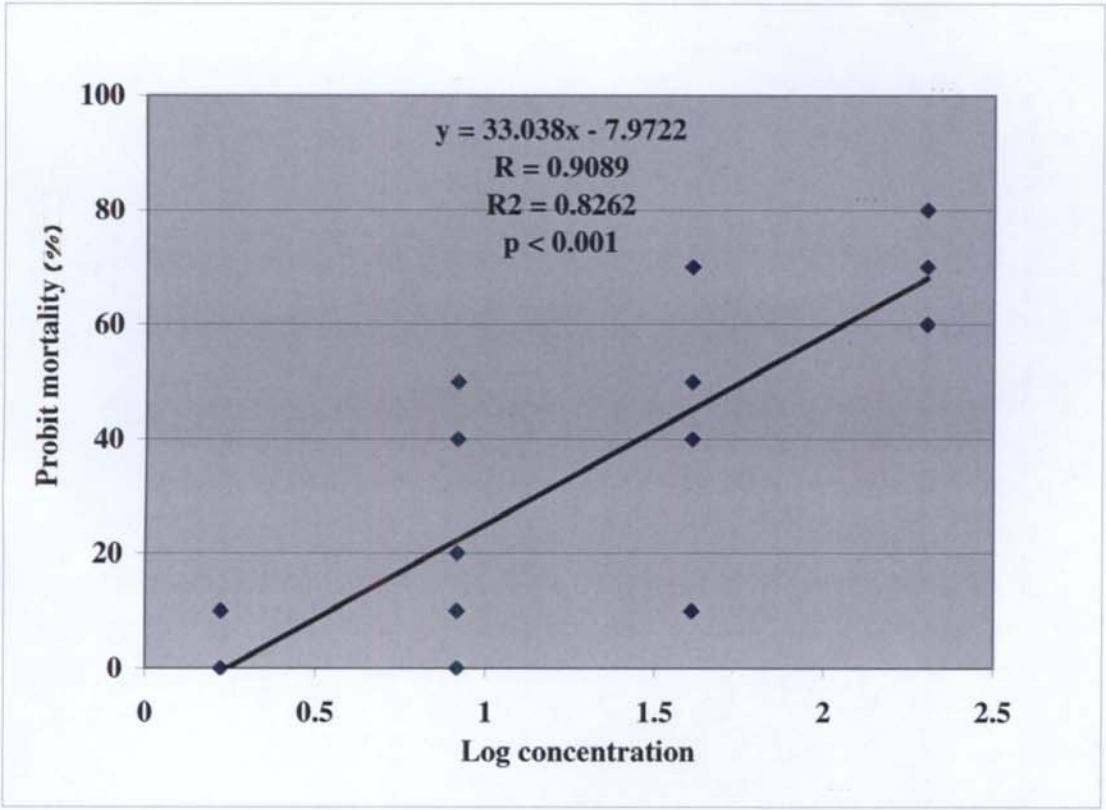
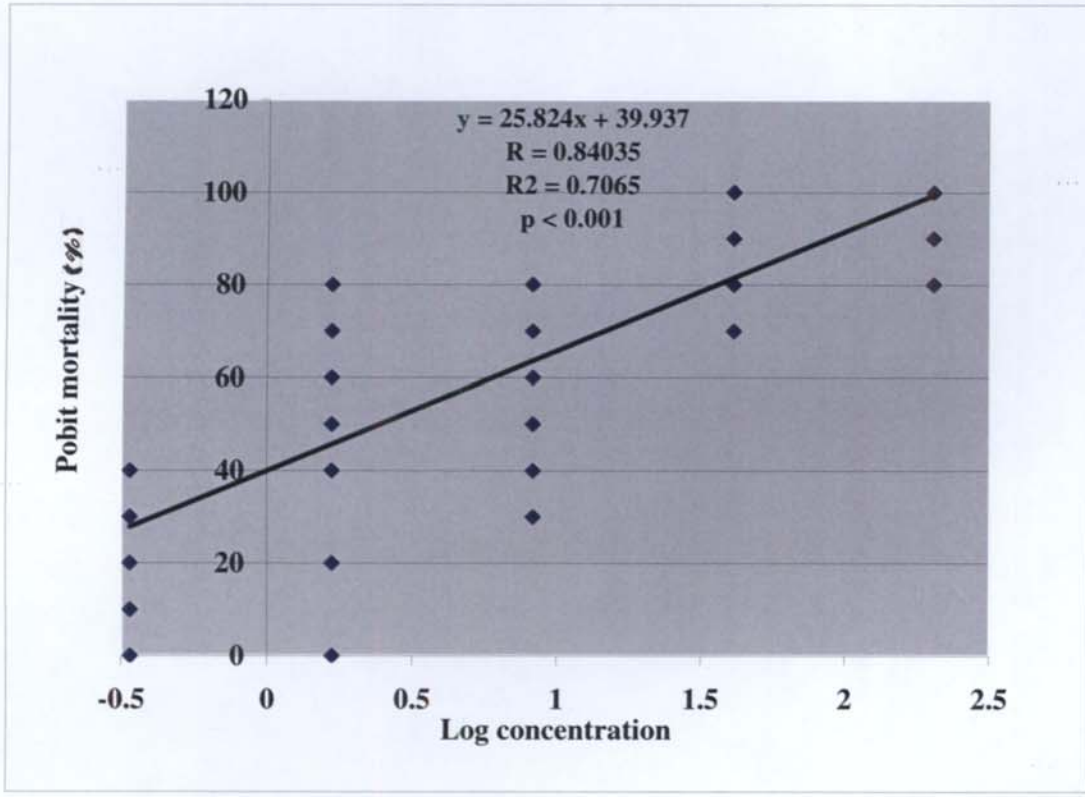


Fig. 8.3. Toxicity of VME against *T. castaneum* adults (TAM)



of y=11.253 and log concentration of x+72.489. The slope of the regression line (11.253) indicates that for unit increases in the log concentration of VPE (dose), there was a corresponding increase in the mortality of adult insects.

Figure 8.2 provides the toxicity of VME against *T. castaneum* adults by FRM. This graph provides the regression equation which shows the probit mortality (%) of y=33.038 and log concentration of x-7.972. The regression line shows a slope of 33.038 indicating high significance. Here also for unit increase in the log concentration of the VME, there was a corresponding increase in the mortality of the insects.

Figure 8.3 presents the toxic effect of VME against *T. castaneum* adults by TAM. The graph shows the regression equation with a probit mortality (%) of y=25.824 and log concentration of x+39.937. The regression line shows a slope of 25.824 and indicates that the unit increase in the log concentration of the VME resulted in a corresponding increase in the mortality of the insects.

8.4. Discussion

The toxicity studies by topical application of VPE and VME for a period from 1.5 to 96 h and by film residue of VME for 1-7 days were showing strong contact toxicity against *T. castaneum* adults. The film residue of VPE was found to be less toxic against this insect (Tables VIII.1-5; Figs. 8.1-3). It was clearly seen that toxic effects of these two extracts were more obvious and instant during early periods of treatments (1.5 h) in the case of TAM. However, in the FRM, toxic effect was prolonged with maximum effect during the early periods of observation

(1 day). For example, toxicity is seen even on the 7th day of observation in the case of VME at lower concentrations although 10% of the extract showed 100% mortality by 3 days. VPE on the other hand, was active only at the highest concentration, which however, was not showing any instant action as in the case of VME.

The data obtained from the toxicity studies indicate that toxic materials are present in *V. negundo* which are extracted by both methanol and petroleum ether. Administration by TAM was found to be more effective than FRM. However, film residue of VPE was found to be less effective than VME. Probit analysis of the data revealed that the toxicity by film residue of VME and topical application of both VPE and VME were highly significant ($P<0.001$).

In the case of topical application of VPE, it was seen that the control (petroleum ether alone) also afforded mortality in insects. This is possibly because petroleum ether is a highly nonpolar solvent which when applied topically on the insects will spread over and penetrate more rapidly through the cuticle causing death of the insects.

The contact toxicity by film residue of VPE and VME was found to be time-dependent. Film residue of VME at various concentrations were showing higher mortality rate than VPE and the effect was immediate. Toxicity persisted over a long period of exposure time of 7 days. This time-dependent toxicity of the extract against the insect is found to be in conformity with the reports of some other plant materials. Toxicity of citrus peel oils against *C. maculatus, S. zeamais* and *D. maculatus* (Don-Pedro, 1996), azadirachtin and neem concentrates against *C.*

ferrugineus, S. oryzae and *T. castaneum* (Xie *et al.*, 1995 a) have been reported. Similarly, neem oil was found to be toxic against 3rd instar grubs of *O. rhinoceros* (Padmasheela and Delvi, 2002).

Topical application of VPE and VME were showing higher toxicity against *T. castaneum* adults than film residue of these extracts and the effect was more prominent during early periods of administration (Tables VIII.2 & 4). These results are in agreement with the toxicity studies using *Trigonella foenum-graecum* against *T. castaneum* and *Acanthoscelides obtectus* (Pemonge *et al.*, 1997) and extracts of plants from the families of Solanaceae and Compositae against *T. castaneum* (Pascual, 1998).

Toxicity was found to increase in a dose-dependent manner when insects exposed to the different concentrations of both the extracts using the two methods. Film residue of VME and topical application of VPE and VME exhibited better mortality rates as shown in the regression line (Table VIII.5; Figs. 8.1-3). The slope of the regression line indicates the unit increases in the concentration of the extracts corresponding increase in the probit mortality of the insects. Hence, film residue of VME shows highest slope of regression line (33.038) followed by topical application of VME (25.824) and VPE (11.253) of (Tables VIII.2-5; Figs. 8.1-3).

Dose-dependent toxicity of plant extracts and oils etc. have been reported by various authors. El-Lakwah *et al.* (1997) reported that mortality of some stored product insects (*S. oryzae, R. dominica* and *T. castaneum*) increased with increasing concentrations of the extract of *Withania somnifera* leaves and fruits. Similarly, activity of 1,8- cineole from the essential oils of *Ocimum kenyense*

(Obeng-Ofori *et al.*, 1997 b), and camphor from the essential oil of *Ocimum kilimandscharicum* on *S. granarius, S. zeamais, T. castaneum* and *Prostephanus truncatus* (Obeng-Ofori *et al.*, 1998) have been reported to possess dose-dependent toxicity.

From the results obtained, it is seen that topical application of VME shows low LC_{50} (1.48) and LC_{95} (8.43) values than the corresponding values obtained for the film residue of the same extract (LC_{50} = 5.78; LC_{95} = 22.57) (Table VIII.5). In the case of topical application of VPE also low value for LC was obtained . For example, LC_{80} and LC_{95} values were 1.88 and 7.14 respectively. However, here LC_{50} can not be estimated because even low concentration of the extract killed more than 50% of the insects. On an overall comparison of the two extracts using the two methods, it was seen that TAM of VPE produced a low LC_{95} value of 7.14 which kills 95% of the insects with minimum quantity of the toxic phytochemical principles (Table VIII.5). However, LC of film residue of VPE cannot be estimated due to the lack of sufficient mortality of the insects.

The present results show low LC_{50} value in the TAM than the FRM. This is in conformity with the data obtained in an evaluation of different bioassay techniques (direct spray and film residue) for the measurement of deltamethrin–resistance level in *T. castaneum* (Sinha and Saxena, 2000). Similarly, low LC_{50} and LC_{95} values were obtained for topical application of essential oils from *E. rutaecarpa* (Liu and Ho, 1999) and allyl disulphide from *A. sativum* (Chiam *et al.*, 1999) against *T. castaneum* and *S. zeamais*.

Several studies on the toxic effects of *V. negundo* against insects have been reported. Larvicidal properties of the extracts of *V. negundo* and some indigenous plants against *C. quinquefasciatus* (Kalyanasundaram and Babu, 1982; Arivoli *et al.*, 2000) and *S. litura* (More *et al.*, 1989) have been evaluated. Khanam *et al.* (1990) investigated the insecticidal properties of some indigenous plants against *T. confusum* and found that extracts of *V. negundo* caused maximum mortality to this insect than other botanicals. Similarly, toxicity of the extracts of *V. negundo* against *Earias vittella, Diaphania indica,* and *Epilachna septima* have been reported (Kalavathi *et al.*, 1991). *V. negundo* and some other indigenous plant species were also found to be toxic to the potato tuber moth, *Phthorimaea operculella* (Kashyap *et al.*, 1992). Dayrit *et al.* (1995) reported that antipest compound, beta-eudesmol isolated from the volatile oils of *V. negundo* leaves had ovicidal and contact toxicity against *P. xylostella.* Similarly, toxic effects of *V. negundo* and other plant extracts on the larvae of *S. litura* (Sahayaraj and Sekhar, 1996) and on different life stages of a reduviid predator, *Rhynocoris marginatus* (Sahayaraj and Paulraj, 1999) have also been reported. Pandian *et al.* (1994) found that smoke of *V. regundo* leaves had toxic effect on *C. quinquefasciatus.*

Among the two methods employed in toxicity studies, it is found that topical application of the extracts was more toxic to the insects than their film residue. Contact toxicity to the insects caused by the film residue of the extracts is less intense than topical application presumably because of the relatively low area for the direct contact of the body with treated area of the Petri dish in the FRM. But in TAM, the extracts have direct contact with the insects. Here, the main route of

entry of toxic chemicals is by means of penetration through the cuticle, partitioning into the haemolymph, and then acting on the central nervous system. The toxicants may also penetrate through the spiracles and tracheoles or they may act on the peripheral nervous system, rather than the central nervous system (Richards and Weygandt, 1945; Gerolt, 1969).

The contact toxicity of the extracts of *V. negundo* on *T. castaneum* is found to be similar to the results obtained by Santos *et al.* (1997). They reported that essential oil components (cineol and limonene) of *Eucalyptus camadulensis*, *E. cameroni* and *Citrus aurantium* have shown significant insecticidal action, being lethal to *R. dominica* and *T. castaneum*. These substances cause toxicity to the insect when they penetrate through the cuticle (contact effect). Similarly, contact toxicity by cinnamaldehyde from *C. aromaticum* (Huang and Ho, 1998), by allyl disulphide from *A. sativum* against *T. castaneum* and *S. zeamais* (Chiam *et al.*, 1999) and by essential oils from *C. longa* against *R. dominica*, *S. oryzae* and *T. castaneum* (Tripathi *et al.*, 2002) have been reported.

The present study thus indicates that both VPE and VME show dose-dependent increase in contact toxicity against *T. castaneum* adults by TAM and FRM and an immediate with effect in TAM. At the same time toxic effect was more prominent during early period of exposure, which lasted for long periods in FRM especially by using VME. On the other hand, film residue of VPE was showing the least toxicity. Thus, it has been shown that topical application of VPE and VME and film residue of VME show strong contact toxicity against *T. castaneum* adults.

The effects of petroleum ether and methanol extracts of *V. negundo* are found to be the promising alternative for chemical insecticide that could be used in insect pest management. The leaf extracts of the plant have shown considerable insecticidal activity and they can act as a potential grain protectant due to their contact toxicity against *T. castaneum* adults. This contact toxicity is brought about by several chemical constituents present in the plant, which may act singly, additively or synergistically on the insects. Hence, the plant offers a new source of biopesticide in stored product pest management. These studies suggest that further investigations on the fumigant properties of this plant against insects are desirable. Moreover, it would be worthwhile if the commercial production of the isolated and identified toxic components of *V. negundo* is considered, so that their potential in stored product pest management can be fully exploited.

Chapter IX

ANALYSIS OF LEAF EXTRACTS OF *V. NEGUNDO* FOR THE CHEMICAL CONSTITUENTS AND THE TOXICITY ON *T. CASTANEUM*

9.1. Introduction

Plant chemicals can be classified as primary or secondary constituents, depending on whether or not they have an essential role in plant metabolism and are universally present in all plants. Primary constituents include the common sugars, proteins, purines and pyrimidines of nucleic acids, chlorophylls and so on. Secondary constituents make up all the remaining plant chemicals from alkaloids to terpenoids and acetogenins to phenolics and represent substances which do not appear to have an essential role in metabolism and which vary in their distribution from plant to plant. However, secondary metabolites have a key role in protecting the plant from environmental pressures or in controlling plant growth. Some of the secondary metabolites derived from plants have diverse biological effects on insects and other organisms.

Bioassay for the toxicity of phytochemicals has generally been found to be most effective with the crude extracts. This has been explained, mainly as a result of synergistic activity of the various chemical components present in the crude extract. However, for a better understanding of the nature of these various components, their structure, class and bioefficacy as toxic materials need to be explored. Various analytical methods have been employed for separating the

extracts for detailed analysis. Probably, the most important and crucial among these is chromatographic separation.

Among various chromatographic techniques, Column Chromatography (CC), Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) are widely used for the separation of the plant chemicals. The phytochemical constituents are analysed by other advanced techniques such as Flame Ionization Detector (FID), Mass Spectrometry (MS), NMR, IR and UV Spectroscopy.

Separation and analysis of various phytochemicals using different techniques as mentioned, have been carried out by several workers and their biological activities reported. Sharma and Dawra (1991) isolated lantadene A, B, C and D from the heptato toxic plant *L. camara* var. *aculeata* by TLC. Similarly, two sesquiterpenes, angulatueoid G and H were isolated from the seeds of *Celastrus angulatus*, of which angulatueoid G was found to be antifeedant against *Aulacophora femoralis* and *Plutella xylostella* (Wu *et al.*, 1992). Jagan *et al.* (1993) reported the structure of insect growth inhibitory and antifeedant, neo-clerodane diterpenes, clerodendrin B and C, isolated from the leaves of *Clerodendrum inerme* and their structures were established by NMR spectral analysis. Yun *et al.* (1994) by their GC/MS analytical studies revealed the identity of phytotoxic substances from *Artemisia princeps* var. *orientalis*. Benzoic acid, sixteen phenolic compounds and nearly 35 terpenoids were identified from the essential oils of this plant. Similarly, Ulubelen *et al.* (1996) reported that some diterpenoid alkaloids were isolated from *Consolida oliveriana* and their structures

were established on the basis of their physical and spectroscopic data including detailed NMR studies.

Rastrelli *et al.* (1998) isolated seven triterpenoid saponins and three ionol-derived glycosides from the leaf extract of *Amaranthus caudatus*. Similarly, germacrene D and caryophyllene were the chemical constituents isolated from essential oils of *Lantana trifolia* (Muhayimana *et al.*, 1998) and eugenol, beta-elemene and beta-caryophyllene were the major essential oil constituents of *Ocimum tenuiflorum* analysed by GC and GC/MS (Pino *et al.*, 1998). Martin *et al.* (1998) isolated and identified the phenolic compounds such as kaempferol and kaempferol-3-0-glucoside from the methanol extract of leaves of *Cassia alata*. Similarly, Jaryis *et al.* (1999) separated the triterpenoids from seeds of *A. indica* using a system of flash chromatography and the fractions were analysed by TLC and HPLC for the determination of eleven tetranortriterpenoids including azadirachtin and salannin. Tsoukatou *et al.* (2000) analysed oils obtained by hydrodistillation and the head space volatiles from the aerial parts of *Bombycilaena erecta* and *Otanthus maritimus* by GC and GC/MS. The major components of the oils of *B. erecta* were camphor, yomogi alcohol and artemisyl acetate while those from *O. maritimus* were cis-chrysanthenyl acetate, camphor and artemisea alcohol. These components were found to have repellent effect on *Monomerius pharaonis*.

Seeds of *V. negundo* have been found to contain various chemical constituents. The unsaponifiable matter from the oils of *V. negundo* seeds contained an anti-inflammatory diterpene, 5β-hydro-8,11,13-abietatrien-6α-ol, a triterpene, lanostan-8,25-dien-3β-ol, a flavonoid, artemetin and a lignan

characterised as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-9-2-naphthaldehyde by spectroscopic methods (Chawla *et al.,* 1991, 1992 a). Similarly, the chemical composition of the volatile oils from the flowering twigs of *V. negundo* was determined by GC-MS and was found to contain compounds such as viridiflorol, β-caryophyllene, 4-terpineol, linalool, globulol, elemol, β-farnesene and aromadendrene (Singh *et al.* (2000).

Several of the phytochemicals described in the aforesaid section have been found to act variously as toxicants, antifeedants, ovicidals etc. Several long chain fatty acid esters such as hexadecanoate, heptadecane, dodecanoate and octadecanoate analysed by GC from three weed species (*Chenopodium ambrosioides, Conyza dioscoridis* and *Convolvulus arvensis* have insecticidal activity against *T. castaneum* and *S. granarius* (Abdallah *et al.,* 1988). Klocke *et al.* (1991) isolated and identified the three grayanoid diterpenes, Rhodojaponin III, grayanotoxin III and kalmanol from *Rhododendron molle* of which Rhodojaponin, the major compound, exhibited antifeedant, growth inhibitory and insecticidal activities against the larvae of *L. decemlineata* and *S. frugiperda*. Similarly, ten natural ryanoids analysed by HPLC and 1H NMR (Jefferies *et al.*, 1992) and several monoterpenoids (Rice and Coats, 1994 b) were found to have insecticidal properties against *M. domestica* and *T. castaneum.* Weaver *et al.* (1994 b) reported that floral, foliar and root extracts of *Tagetes minuta*, a source of naturally occurring insecticidal compounds identified by gas chromatography/mass spectroscopy, were effective against *Zabrotes subfasciatus.*

Kelm and Nair (1998) reported mosquitocidal compounds and a triglyceride, 1,3-dilinoleneoyl-2-palmitin from *O. sanctum.* Effect of some terpenoids of *Larix laricina* on the growth and development of *L. dispar* have been studied by Powell and Raffa (1999). Collins *et al.* (2000) determined the structure of four novel isomeric sesquiterpenoids, viz., caprariolides A, B, C and D by NMR spectroscopy experiments, two of which were found to have insecticidal activity against adult *C. formicarius elegantulus.* Tripathi *et al.* (2002) observed that essential oils extracted from the leaves of *C. longa* were showing contact and fumigant toxicity against *R. dominica, S. oryzae* and *T. castaneum.* Besides, it reduced oviposition and egg hatching, and totally suppressed progeny production of these insects.

The studies described in this chapter were designed to separate and evaluate the toxicity of the various fractions of VPE and VME against *T. castaneum* adults and to identify the toxic chemical constituents in the extracts by thin layer chromatography.

9. 2. Methods

Methods used for the separation of VPE and VME are described in section 3.2.8. Evaluation of toxicity of the TLC fractions of VPE and VME against *T. castaneum* adults were carried out by the methods given in section 3.2.9.

Methods described in section 3.2.10 were employed for the separation and identification of the toxic chemical constituents of VPE and VME.

9.3. Results

9.3.1. Fractions of VPE separated by TLC

Fractions separated from VPE by TLC are provided in the Table IX.1 and Plate IX.1 a. Number of fractions (appeared as bands), their colour under visible light and their R_f values are given in the Table. Fourteen fractions were identified on the basis of visual colours and marked as F_1 to F_{13} and F_0 on the TLC plate. These fractions were used for toxicity determinations against *T. castaneum*.

9.3.2. Contact toxicity of TLC fractions of VPE against *T. castaneum*

Figure 9.1 shows the toxicity of various fractions of VPE against *T. castaneum* adults after treatment for 4 days of exposure (TAM). Here, the mortality % were 16, 10, 12, 12, 14, 10, 6, 6, 6, 14, 8, 16, 4 and 6 respectively for the fractions from F_1 to F_{13} and F_0. Maximum mortality of 16% was shown by fractions F_1 and F_{12} and other fractions showed varying toxic effect. Minimum mortality of 4% was shown by F_{13}.

9.3.3. Fractions of VME separated by TLC

Table IX.2 and Plate IX.I b. provide details of fractions separated from VME by TLC. These provide the number of fractions separated from the extract, their colour under visible light and their R_f values. Here, a total of 18 fractions were identified on the TLC, which were labeled from F_1 to F_{17} and F_0. These fractions of the methanol extract were eluted in acetone and used for evaluating their toxic effects on *T. castaneum* adults.

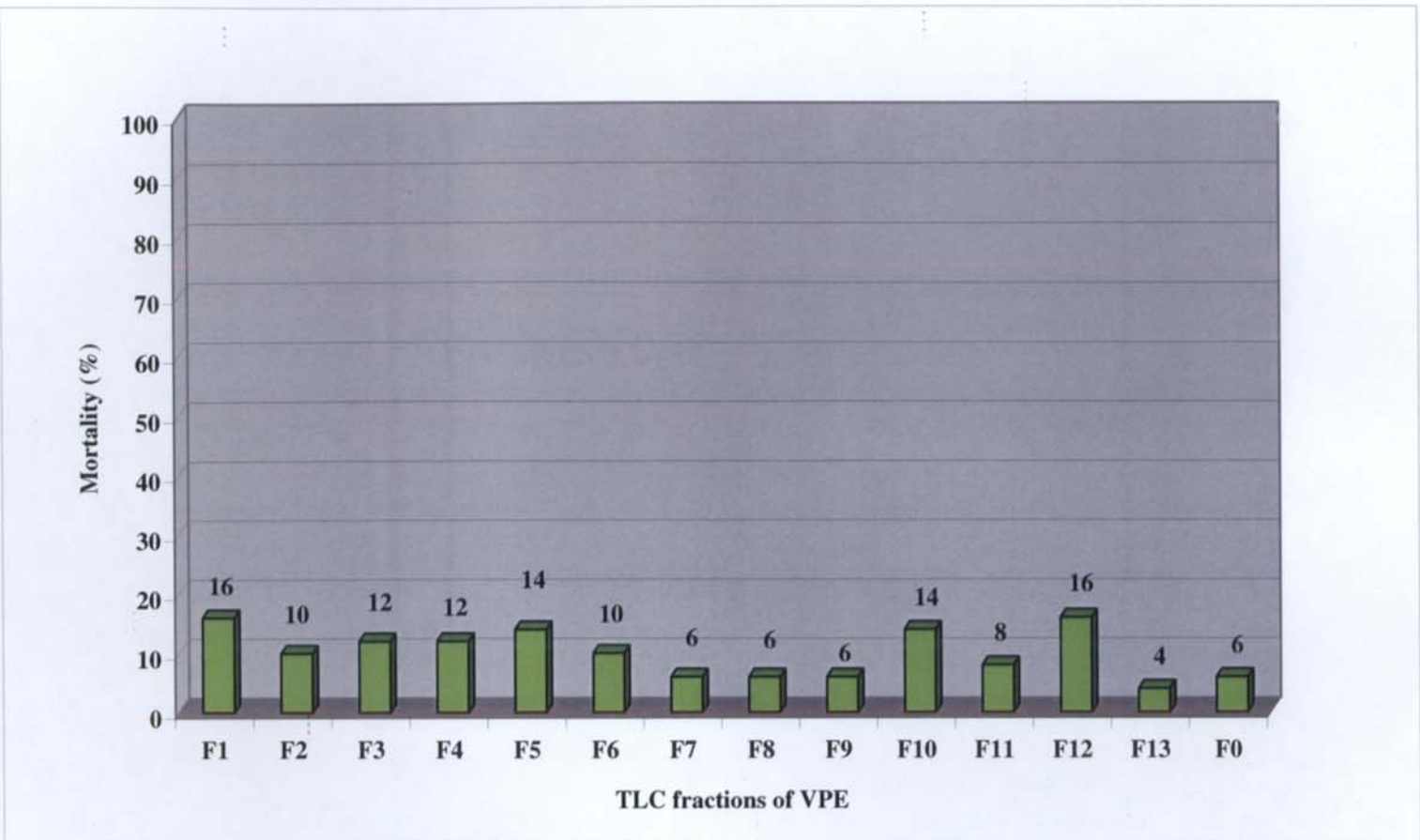
TABLE IX.1

Characteristics of TLC fractions of VPE

No. of bands/ fractions	Colour of the bands/fractions	R_f values
F_1	Orange	0.91
F_2	Colourless	0.82
F_3	Light orange	0.74
F_4	Colourless	0.64
F_5	Faint brown	0.57
F_6	Grey	0.51
F_7	Colourless	0.44
F_8	Greenish dark grey	0.38
F_9	Colourless	0.32
F_{10}	Greenish yellow	0.25
F_{11}	Colourless	0.18
F_{12}	Light yellow	0.12
F_{13}	Colourless	0.06
F_O	Brown	0

Developing solvent : Ethyl acetate: Petroleum ether (1:4).
R_f values are means of 10 replicates of thin layer chromatogram.

Fig. 9.1. Toxicity of TLC fractions of VPE against *T. castaneum* adults after 4 days of exposure (TAM)

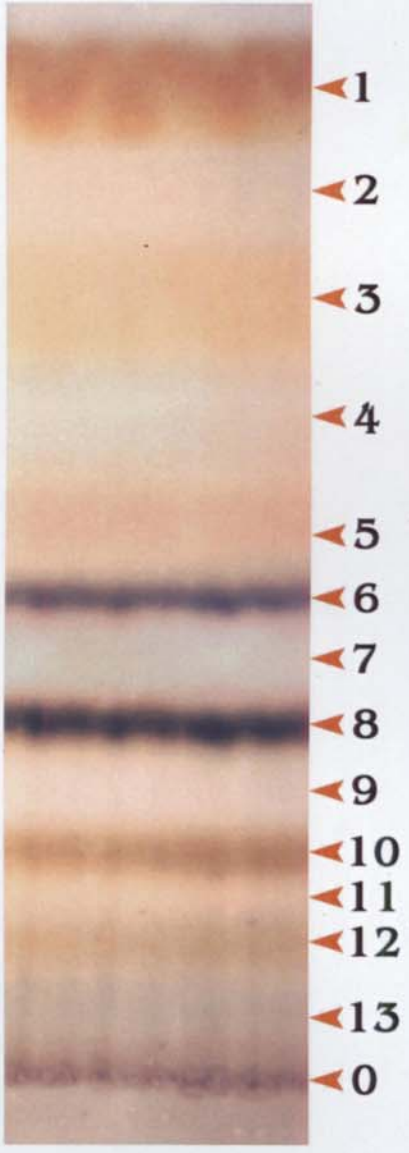


158B

PLATE IX. 1

TLC SEPARATION OF *V. NEGUNDO* EXTRACTS

a



TLC fractions (F_1 to F_{13} & F_0) of VPE before spraying visualizing reagents

b

TLC fractions (F_1 to F_{17} & F_0) of VME before spraying visualizing reagents

TABLE IX.2

Characteristics of TLC fractions of VME

No. of bands/ fractions	Colour of the bands/fractions	R_f values
F_1	Colourless	0.82
F_2	Colourless	0.7
F_3	Light grey	0.54
F_4	Light brown	0.48
F_5	Colourless	0.47
F_6	Greenish grey	0.43
F_7	Colourless	0.4
F_8	Light yellow	0.36
F_9	Colourless	0.32
F_{10}	Yellow	0.29
F_{11}	Colourless	0.26
F_{12}	Grey	0.23
F_{13}	Dark grey	0.19
F_{14}	Greenish yellow	0.15
F_{15}	Dark green	0.12
F_{16}	Light brownish green	0.1
F_{17}	Light green	0.05
F_O	Coffee brown	0

Developing solvent: Ethyl acetate: Petroleum ether (1:4).
R_f values are means of 10 replicates of thin layer chromatogram.

Fig. 9.2. Toxicity of TLC fractions of VME against *T. castaneum* adults after 4 days of exposure (TAM)

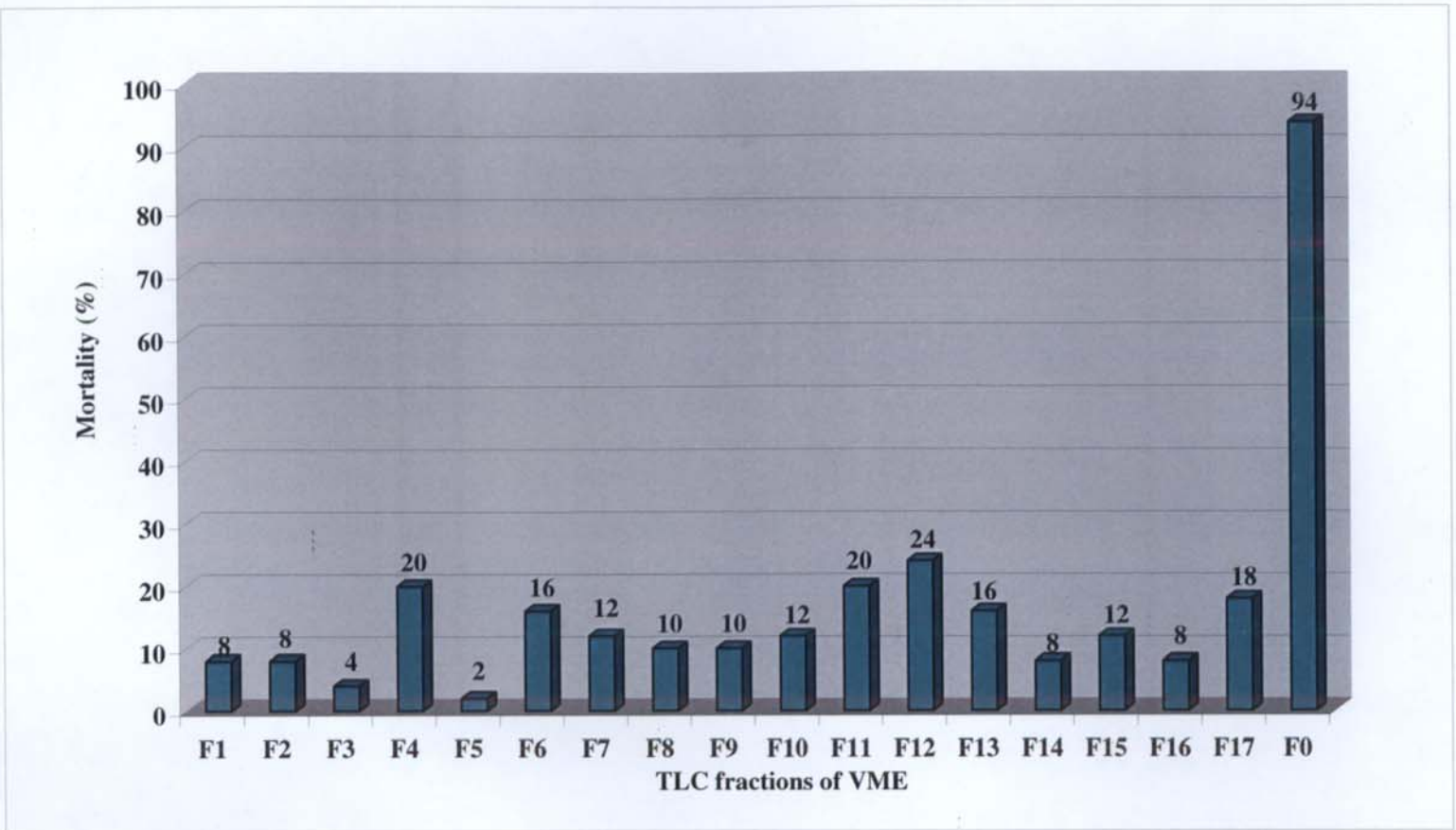


TABLE IX.3

Pearson Chi-square of the data on contact toxicity of TLC fractions of VPE and VME against *T. castaneum* adults

Source	Pearson Chi-square	df	P level
TLC fractions of VPE (F_1 to F_{13} & F_O)	41.06	39	0.38
TLC fractions of VME (F_1 to F_{17} & F_O)	221.075	85	<0.001
TLC fractions of VME (F_1 to F_{17})	72.793	64	0.21

9.3.4. Contact toxicity of TLC fractions of VME against *T. castaneum*

Toxicity of TLC fractions of VME against *T. castaneum* adults after 4 days of exposure (TAM) is represented in the Figure 9.2. Here, the mortality % for F_1 to F_{17} and F_O (18 fractions) were 8, 8, 4, 20, 2, 16, 12, 10, 10, 12, 20, 24, 16, 8, 12, 8, 18 and 94 respectively. Fraction F_O representing the origin, was found to have very high toxic activity on the insects. All the other fractions exhibited more or less a uniform mortality ranging from 2 to 24%.

9.3.5. Statistical analysis of data

The data obtained from the toxicity studies on *T. castaneum* adults by the topical application of TLC fractions of VPE were subjected to Pearson Chi-square test. Chi-square analysis of the data ($\chi^2 = 41.06$; df = 39) revealed that difference among the toxic effects of the fractions on insects was not significant (P = 0.38).

Similarly, data on the toxic effects of the fractions of VME on *T. castaneum* adults were also subjected to Pearson Chi-square test. Chi-square analysis of the data showed that the difference among the toxic effects of the fractions on *T. castaneum* adults ($\chi^2 = 221.075$; df=85) was highly significant (P<0.001). However, when discounting the effect of F_O, there was no significant difference among the toxic effects of the remaining fractions on *T. castaneum* (P = 0.21; $\chi^2 = 72.793$; df = 64) (Table IX.3).

9.3.6. Identity of toxic chemical constituents in the TLC fractions of VPE

Results of the TLC of VPE carried out and subsequent spraying with various analytical reagents are provided in the Tables IX.4-11 and Plates IX.2-4.

TABLE IX.4

Identity of terpenes and steroids in VPE separated on TLC
(*Spray reagent*: Anisaldehyde-sulphuric acid)

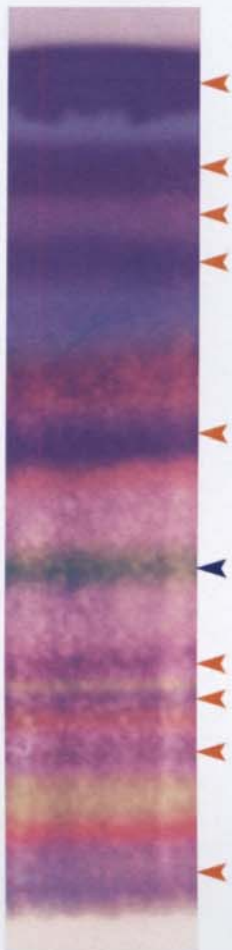
Fractions	R_f I	R_f II	Colour of the bands/ fractions	Identity of the constituents
F_1	0.85	0.85	Deep blue violet	Terpenes
F_2	0.79	--	--	--
	-	0.76	Blue violet	Terpenes
F_3	0.74	--	--	--
	--	0.67	Pinkish blue violet	Terpenes
F_4	0.61	0.61	Blue violet	Terpenes
F_5	0.58	--	--	--
	--	0.56	Brownish violent	--
F_6	0.48	0.48	Deep blue violet	Terpenes
	--	0.46	Light pink	-
F_7	0.41	0.41	Light blue violet zone	--
F_8	0.35	0.35	Greenish grey	Steroids
	--	0.33	Blue violet	Terpenes
F_9	0.29	0.29	Yellow	--
	--	0.26	Blue violet	Terpenes
F_{10}	0.24	--	--	--
F_{11}	0.22	0.22	Brownish red	-
F_{12}	0.19	--	--	--
	--	0.15	Light blue violet	Terpenes
F_{13}	0.12	0.12	Light yellow	--
	--	0.09	Pinkish red	--
	--	0.07	Light blue violet	Terpenes
F_O	0	0	Brown	--

Solvent system: Ethyl acetate : Petroleum ether (1:4).
R_f I : R_f values of the fractions before spraying visualizing reagent.
R_f II : R_f values of the fractions after spraying visualizing reagent.

PLATE IX.2

TLC OF VPE SHOWING CHEMICAL CONSTITUENTS

a

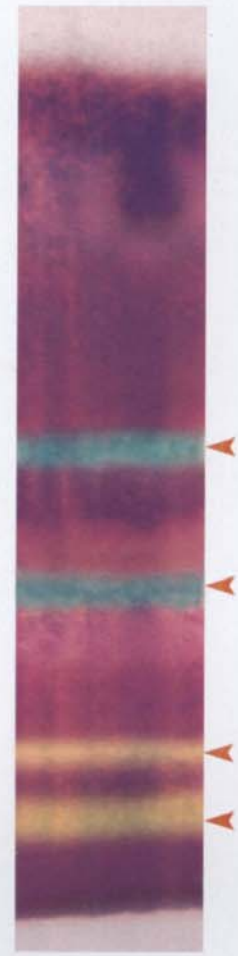


◀: Terpenes

◀: Steroids

(After spraying anisaldehyde-sulphuric acid)

b



◀: Triterpene glycosides & steroids

(After spraying acetic anhydride-sulphuric acid)

Qualitative analysis using specific reagent sprays showed the identity of the different bands. Anisaldehyde-sulphuric acid reagent when sprayed on the chromatogram developed specific colours for terpenes (bluish violet) and steroids (greenish grey). Terpenes were detected in the regions of the chromatogram with R_f values of 0.85 (F_1), 0.76 (F_3), 0.67 & 0.61 (F_4), 0.48 (F_6), 0.33 (F_9), 0.26 (F_{10}) and 0.15 & 0.07 (F_{13}). Steroids were detected in the area with R_f of 0.35 (F_8). It has been found that some of the fractions identified by the colours of the bands before spraying chemical reagents actually were comprised of more than one component when specific identifying chemicals were sprayed (Table IX.4; Plate IX.2 a).

Spraying with Liebermann–Burchard reagent (acetic anhydride-sulphuric acid) followed by heating at 100°C resulted in the appearance of fluorescing bands in different areas on the chromatogram. The fluorescence was characteristic of triterpene glycosides and steroids. At R_f values of 0.44 (F_6) and 0.34 (F_8), green fluorescence were observed where as at R_f values 0.18 (F_{12}) and 0.09 (F_{13}), light yellow fluorescence was seen. Colours other than the fluorescence as mentioned, were seen in the entire run area (as detailed in Table IX.5 and Plate IX.2 b) of the chromatogram were not characteristic of triterpene glycosides and steroids.

From Table IX.6 and Plate IX.3 a it is seen that spraying with perchloric acid produced bands with brown and dark brown colours on the chromatogram characteristic of steroids present in the extract. The steroid components were represented by areas with R_f values of 0.74 (F_1), 0.57 (F_4), 0.41 (F_6), 0.32 (F_8), 0.21 (F_{10}), 0.09 (F_{13}) and 0 (F_O). Coloured bands other than those of steroids found on the chromatogram as seen in the Table were ignored.

TABLE IX.5

Identity of triterpene glycosides and steroids in VPE separated on TLC

(*Spray reagent*: Acetic anhydride-sulphuric acid)
(Liebermann – Burchard reagent) (LB)

Fractions	R_f I	R_f II	Colour of the fractions/bands	Identity of the constituents
F_1	0.82	0.82	Dark brownish violet	--
F_2	0.71	0.71	Light Brownish violet	--
F_3	0.65	--	--	--
	--	0.61	Brownish violet	--
F_4	0.6	--	--	--
F_5	0.53	0.53	Brownish violet	--
F_6	0.44	0.44	Green flourescence	Triterpene glycosides and steroids
F_7	0.38	0.38	Brownish violet	--
F_8	0.34	0.34	Green fluorescence	Triterpene glycosides and steroids
F_9	0.28	0.28	Light brownish violet	-
F_{10}	0.22	0.22	Brownish violet	--
F_{11}	0.2	--	--	--
F_{12}	0.18	0.18	Light yellow fluorescence	Triterpene glycosides and steroids
	-	0.13	Brownish violet	--
F_{13}	0.09	0.09	Light yellow fluorescence	Triterpene glycosides and steroids
	--	0.07	Brownish violet	--
F_O	0	0	Dark brown	-

Solvent system: Ethyl acetate: Petroleum ether (1:4).
R_f I : R_f values of the fractions before spraying visualizing reagent.
R_f II : R_f values of the fractions after spraying visualizing reagent.

TABLE IX.6

Identity of steroids in VPE separated on TLC
(*Spray reagent*: Perchloric acid)

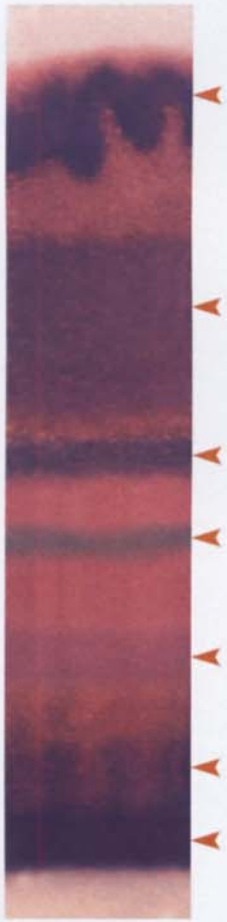
Fractions	R_f II	R_f II	Colour of the bands/ fractions	Identity of the constituents
F_1	0.74	0.74	Dark brown with violet tinge	Steroids
F_2	0.66	--	--	--
	--	0.65	Brown zone	--
F_3	0.61	--	--	--
	--	0.57	Brown with violet tinge	Steroids
F_4	0.54	--	--	--
F_5	0.49	--	--	--
	--	0.44	Green	--
F_6	0.41	0.41	Dark brown	Sterioids
F_7	0.36	--	--	--
	--	0.35	Brown zone	--
F_8	0.32	0.32	Green	Steroids
F_9	0.26	0.26	Brown zone	--
F_{10}	0.21	0.21	Brown	Steroids
F_{11}	0.16	--	--	--
	--	0.15	Yellowish brown	--
F_{12}	0.14	--	--	--
F_{13}	0.09	0.09	Brown zone	Steroids
F_O	0	0	Dark brown	Steroids

Solvent System : Ethyl acetate : Petroleum ether (1:4)
R_f I : R_f values of the fractions before spraying visualizing reagent.
R_f II : R_f values of the fractions after spraying visualizing reagent.

PLATE IX.3

TLC OF VPE SHOWING CHEMICAL CONSTITUENTS (contd.)

a



◄: Steroids
(After spraying perchloric acid)

b

◄: Essential oils, higher alcohols, phenols & steroids
(After spraying vanillin-sulphuric acid)

Spraying with vanillin-sulphuric acid reagent followed by heating at 120°C produced several bands with intense colour. The fractions with R_f values 0.79 (F_1), 0.65 (F_2), 0.62 (F_3), 0.56 (F_4), 0.53 (F_5), 0.39 (F_7), 0.29 (F_9), 0.24 (F_{10}), 0.18 & 0.14 (F_{12}), 0.09 & 0.05(F_{13}) and 0 (F_O), indicated the presence of essential oils, higher alcohols, phenols and steroids (Table IX.7; Plate IX.3 b). It has been found that besides the well defined coloured bands specific to these compounds, the entire gel track was found coloured with various intensity at different places.

Table IX.8 and Plate IX.4 a reveal the results of TLC for the detection of phenols and amines in VPE. Spraying with the reagent, potassium ferricyanide-ferric chloride gave blue or bluish green bands characteristic of phenols and amines which were intensified by subsequent spraying with 2N HCl. The R_f values of the characteristically coloured fractions were 0.71&0.69 (F_1), 0.59 (F_3), 0.51 (F_4), 0.31 (F_8), 0.26 (F_9), 0.21 (F_{10}), 0.16 (F_{12}), 0.11& 0.06, (F_{13}) and 0 (F_O).

Similarly, spraying with aluminium chloride resulted in yellow fluorescence under UV light characteristic of flavonoids. Fluorescent bands detected on the chromatogram had R_f values of 0.77 (F_1), 0.62 (F_3) and 0.24 (F_{10}) (Table IX.9; Plate IX.4 b).

Spraying chromatogram with ceric ammonium nitrate followed by heating produced brown areas on yellow background. The regions above R_f of about 0.35 specific bands were visible. However, below this R_f, the entire area of the gel was found to turn to brown colour. Different bands (as identified from the unsprayed TLC plates) R_f values of 0.9 (F_1), 0.71 & 0.65 (F_4), 0.45 (F_8), 0.34 (F_9), 0.24 & 0.19

TABLE IX.7

Identity of essential oils, higher alcohols, phenols and steroids in VPE separated on TLC

(*Spray reagent*: Vanillin-sulphuric acid)

Fractions	R_f I	R_f II	Colour of the fractions/bands	Identity of the constituents
F_1	0.79	0.79	Blueish violet zone	Essential oils, higher alcohols, phenols and steroids
F_2	0.65	0.65	Light pink zone	Essential oils, higher alcohols, phenols and steroids
F_3	0.62	0.62	Deep pink zone	Essential oils, higher alcohols, phenols and steroids
F_4	0.56	0.56	Pink zone	Essential oils, higher alcohols, phenols and steroids
F_5	0.53	0.53	Pink zone	Essential oils, higher alcohols, phenols and steroids
	--	0.52	Light greyish violet	--
F_6	0.44	0.44	Greyish violet	--
F_7	0.39	0.39	Pink	Essential oils, higher alcohols, phenols and steroids
F_8	0.35	0.35	Greyish violet	-
F_9	0.29	0.29	Light pink	Essential oils, higher alcohols, phenols and steroids
F_{10}	0.24	0.24	Pink	Essential oils, higher alcohols, phenols and steroids
F_{11}	0.21	--	--	--
F_{12}	0.18	0.18	Reddish pink	Essential oils, higher alcohols, phenols and steroids
	--	0.14	Deep pink	Essential oils, higher alcohols, phenols and steroids
F_{13}	0.09	0.09	Reddish pink	Essential oils, higher alcohols, phenols and steroids
	--	0.05	Pink zone	Essential oils, higher alcohols, phenols and steroids
F_0	0	0	Deep pink	Essential oils, higher alcohols, phenols and steroids.

Solvent system : Ethylacetate : Petroleum ether (1:4).
R_f I : R_f values of the fractions before spraying visualizing reagent.
R_f II : R_f values of the fractions after spraying visualizing reagent.

TABLE IX.8

Identity of phenols and amines in VPE separated on TLC

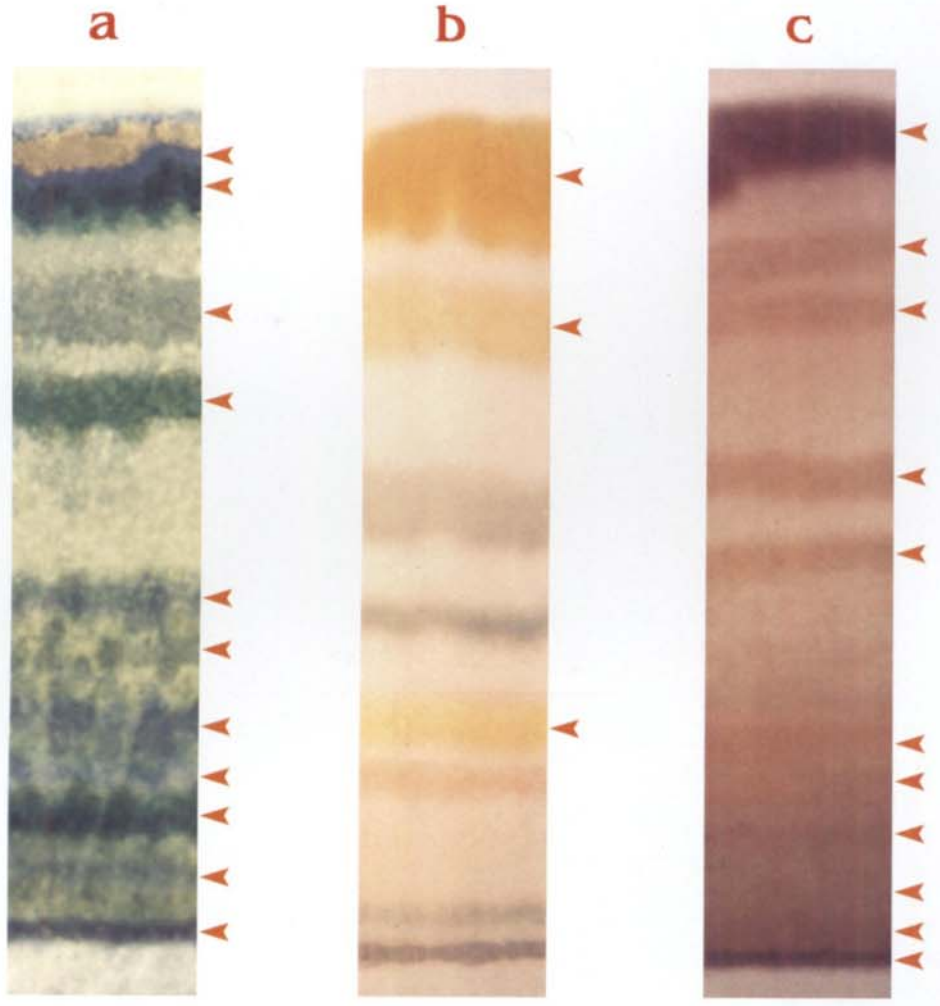
(*Spray reagent*: Potassium ferricyanide-ferric chloride)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constituents
	--	0.78	Blue	--
	-	0.75	Yellowish orange tinge	--
F_1	0.72	--	--	--
	--	0.71	Deep blue	Phenols & amines
	--	0.69	Bluish green	Phenols & amines
F_2	0.6	--	--	--
F_3	0.59	0.59	Light blue zone	Phenols & amines
F_4	0.51	0.51	Deep bluish green	Phenols & amines
F_5	0.47	--	--	--
F_6	0.41	--	--	--
F_7	0.36	--	--	--
F_8	0.31	0.31	Light blue	Phenols & amine
F_9	0.26	0.26	Light bluish green	Phenols & amines
F_{10}	0.21	0.21	Light blue	Phenols & amines
F_{11}	0.18	-	-	-
F_{12}	0.16	0.16	Light blue zone	Phenols & amines
	-	0.11	Bluish green	Phenols & amines
F_{13}	0.08	-	-	-
	-	0.06	Light blue	Phenols & amines
F_O	0	0	Blue	Phenols & amine

Solvent system : Ethyl acetate : Petroleum ether (1:4).
R_f I : R_f values of the fractions before spraying visualizing reagent.
R_f II : R_f values of the fractions after spraying visualizing reagent.

PLATE IX.4

TLC OF VPE SHOWING CHEMICAL CONSTITUENTS (contd.)



◀:Phenols & amines (After spraying potassium ferricyanide-ferric chloride)

◀:Flavonoids (After spraying aluminium chloride)

◀:Alcohols (Polyalcohols) (After spraying ceric ammonium nitrate)

TABLE IX.9

Identity of flavonoids in VPE separated on TLC

(*Spray reagent*: Aluminium chloride)

Fractions	Rf I	Rf II	Colour of the fractions	Identity of the constituents
F_1	0.77	0.77	Yellow fluorescence	Flavonoids
F_2	0.68	--	--	--
F_3	0.62	0.62	Light yellow fluorescence	Flavonoids
F_4	0.55	--	--	--
F_5	0.51	--	--	--
F_6	0.44	--	--	--
F_7	0.38	--	--	--
F_8	0.33	--	--	--
F_9	0.27	--	--	--
F_{10}	0.24	0.24	Light yellow fluorescence	Flavonoids
F_{11}	0.21	--	--	--
F_{12}	0.18	--	--	--
F_{13}	0.09	--	--	--
F_O	0	0	--	--

Solvent system : Ethylacetate : Petroleum ether (1:4).
R_f I : R_f values of the fractions before spraying visualizing reagent.
R_f II : R_f values of the fractions after spraying visualizing reagent.

TABLE IX.10

Identity of alcohols (polyalcohols) in VPE separated on TLC

(*Spray reagent*: Ceric ammonium nitrate)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constitutents
F_1	0.9	0.9	Dark Brown	Alcohols (Polyalcohols)
F_2	0.82	--	--	--
F_3	0.76	0.76	Light brown zone	--
	--	0.71	Light brown	Alcohols (Polyalcohols)
F_4	0.68	--	--	--
	-	0.65	Light brown	Alcohols (Polyalcohols)
F_5	0.59	--	--	--
F_6	0.56	--	--	--
	--	0.53	Light brown zone	--
F_7	0.5	--	--	--
	--	0.45	Light brown	Alcohols (Polyalcohols)
F_8	0.44	--	--	--
F_9	0.38	--	--	--
	--	0.34	Light brown	Alcohols (Polyalcohols)
F_{10}	0.31	--	--	--
F_{11}	0.28	--	--	--
F_{12}	0.24	0.24	Light brown	Alcohols (Polyalcohols)
	--	0.19	Light brown	Alcohols (Polyalcohols)
	--	0.14	Light brown	Alcohols (Polyalcohols)
F_{13}	0.12	--	--	--
	--	0.05	Light brown	Alcohols (Polyalcohols)
	--	0.03	Light brown	Alcohols (Polyalcohols)
F_0	0	0	Brown	Alcohols (Polyalcohols)

Solvent system : Ethyl acetate : Petroleum ether (1:4).

R_f I : R_f values of the fractions before spraying visualizing reagent.

R_f II : R_f values of the fractions after spraying visualizing reagent.

TABLE IX.11

Phytochemical constituents present in the TLC fractions of VPE

Fractions	Colour of the fractions/ bands	Identity of the phytochemical constitutents
F_1	Orange	Terpenes, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines, flavonoids
F_2	Colourless	Essential oils, higher alcohols, phenols, steroids
F_3	Light orange	Terpenes, essential oils, higher alcohols, steroids, phenols, amines, flavonoids
F_4	Colourless	Terpenes, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines
F_5	Faint brown	Essential oils, higher alcohols, phenols, steroids
F_6	Grey	Terpenes, triterpene glycosides, steroids.
F_7	Colourless	Essential oils, higher alcohols, phenols, steroids
F_8	Greenish dark Grey	Steroids, triterpene glycosides, polyalcohols, phenols, amines.
F_9	Colourless	Terpenes, essential oils, higher alcohols, poly alcohols, steroids, phenols, amines.
F_{10}	Greenish yellow	Terpenes, essential oils, higher alcohols, steroids, phenols, amines, flavonoids.
F_{11}	Colourless	--
F_{12}	Light yellow	Triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines.
F_{13}	Colourless	Terpenes triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines.
F_O	Brown	Steroids, essential oils, higher alcohols, polyalcohols, phenols, amines.

(F_{12}), 0.14, 0.05 & 0.03 (F_{13}) and 0 (F_O), which indicated alcohols (polyalcohols) (Table IX.10; Plate IX.4 c).

Table IX.11 summarises the various chemical constituent compounds present in the fractions (F_1– F_{13} and F_O) separated from VPE by TLC and identified by specific spraying reagents. It has been found that the composition of almost all fractions (marked initially before spraying as F_1 to F_{13} and F_O) were more or less same (Tables IX.4-10). Important chemical classes of compounds among them were terpenes, triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines and flavonoids.

9.3.7. Identity of toxic chemical constituents in the TLC fractions of VME

Chromatography of VME showed relatively by large number of coloured bands along the entire track of running and the colours mainly ranged from yellow to green to brown. On spraying with various chemical reagents specific to various chemcial compounds, characteristic colours were developed and were used to identify the components (Tables IX.12-19; Plates IX.5-7). Table IX.12 and Plate IX 5 a provide characteristics of the various fractions (bands) detected as terpenes and steroids using their specific chemical spray reagent, anisaldehyde-sulphuric acid followed by heating, to about 100-105°C. The compounds exhibited characteristic colours of blue-violet for terpenes and green/grey for steroids. Thus, it was seen that the area of the fractions with the R_f values of 0.97, 0.9 & 0.82 (F_1), 0.75 (F_2), 0.62 (F_3), 0.33 (F_9) and 0.06 (F_{17}) represented terpenes. Similarly, steroids were detected on the chromatogram at the R_f values of 0.42 (F_7), 0.21 (F_{13}), 0.18 (F_{14}), 0.15 (F_{15}) and 0 (F_O). Other bands with colours that were not characteristic of

TABLE IX.12

Identity of terpenes and steroids in VME separated on TLC

(*Spray reagent*: Anisaldehyde-sulphuric acid)

Fractions	R_f I	R_f II	Colour of the fractions/ bands	Identity of the constitutents
	--	0.97	Deep blue violet	Terpenes
	--	0.94	Brownish green	--
	--	0.9	Light blue violet	Terpenes
F_1	0.82	0.82	Deep blue violet	Terpenes
	--	0.78	Light blue violet zone	--
	--	0.75	Deep blue violet	Terpenes
F_2	0.71	--	--	--
	--	0.68	Brown	--
	--	0.62	Deep blue violet	Terpenes
F_3	0.61	--	--	--
F_4	0.57	--	--	--
	--	0.53	Light brown	--
F_5	0.51	--	--	--
	--	0.47	Light green	--
F_6	0.46	--	--	--
F_7	0.42	0.42	Grey	Steroids
	--	0.38	Brownish green	--
F_8	0.36	--	--	--
F_9	0.33	0.33	Blue	Terpenes
F_{10}	0.31	--	--	--
F_{11}	0.29	0.29	Brownish green	--
F_{12}	0.27	--	--	--
	--	0.25	Light violet	--
F_{13}	0.21	0.21	Greenish dark grey	Steroids
F_{14}	0.18	0.18	Light grey	Steroids
	--	0.15	Light green	Steroids
F_{15}	0.13	--	--	--
	--	0.11	Red	--
F_{16}	0.09	--	--	--
	--	0.06	Light blue violet	Terpenes
F_{17}	0.05	--	--	--
F_O	0	0	Light green	Steroids

R_fI : R_f values of the fractions before spraying visualizing reagent.
R_fII : R_f values of the fractions after spraying visualizing reagent.
Solvent system : Ethyl acetate : Petroleum ether (1:4).

PLATE IX.5

TLC OF VME SHOWING CHEMICAL CONSTITUENTS

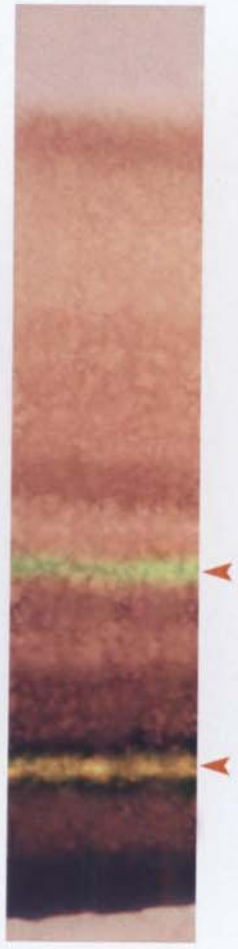
a

◀: Terpenes

◀: Steroids

(After spraying anisaldehyde-sulphuric acid)

b



◀: Triterpene glycosides & steroids

(After spraying acetic anhydride-sulphuric acid)

terpenes and steroids appeared at various places on the chromatogram and were not considered here.

Spraying with Liebermann-Burchard reagent and subsequent heating produced fluorescing bands for triterpene glycosides and steroids in the extract. The fractions with R_f value of 0.35 (F_6) produced light green fluorescence and R_f value of 0.13 (F_{14}) gave yellow fluorescence indicating the presence of triterpene glycosides and steroids (Table IX.13; Plate IX.5 b). There were several coloured bands with various R_f values but are not characteristic for triterpene glycosides and steroids.

Results of TLC carried out for the detection of steroids in the extract by spraying with perchloric acid is presented in the Table IX.14 and Plate IX.6 a. Characteristic colours for steroids, i.e., brown, grey, and violet were obtained after heating for about 10 minutes at 150°C and were found to be present in the fractions with R_f values of 0.94 (F_1), 0.76 (F_2), 0.65 (F_4), 0.45 (F_8), 0.32 (F_{11}), 0.31 (F_{12}), 0.24 (F_{13}), 0.18 (F_{15}), 0.12 (F_{16}), and 0 (F_0). Region below R_f values of 0.32 appeared dark brownish without clear distinction of bands (as identified before chemical spraying) compared to the region above this R_f value, where bands were clearly identifiable.

For the detection of essential oils, higher alcohols, phenols and steroids in VME, vanillin-sulphuric acid reagent was sprayed on thin layer chromatogram and subsequently heated at 120°C to attain maximum colour intensity of the bands. Bands containing the above compounds, appeared pink or violet. Here, the fractions with R_f values of 0.89 (F_1), 0.69 (F_2), 0.56 (F_3), 0.51 (F_4), 0.38 (F_8), 0.35

TABLE IX.13

Identity of triterpene glycosides and steroids in VME separated on TLC

(*Spray reagent*: Acetic anhydride -sulphuric acid (Liebermann-Burchard reagent) (LB)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constitutents
F_1	0.79	0.79	Brown	--
F_2	0.6	0.6	Brown	--
	--	0.53	Brown	--
	--	0.48	Brown	--
F_3	0.47	--	--	--
F_4	0.42	0.42	Brownish grey	--
F_5	0.38	0.38	Light brown zone	--
F_6	0.35	0.35	Light green fluorescence	Triterpene glycosides & steroids
F_7	0.32	--	--	--
	--	0.31	Brownish grey zone	--
F_8	0.29	--	--	--
F_9	0.26	0.26	Grey zone	--
F_{10}	0.23	--	--	--
F_{11}	0.21	0.21	Brownish grey zone	--
F_{12}	0.19	--	--	--
F_{13}	0.16	0.16	Dark greenish grey	--
F_{14}	0.13	0.13	Yellow fluorescence	Triterpene glycosides & steroids
F_{15}	0.11	0.11	Greenish grey	--
F_{16}	0.08	0.08	Brownish grey zone	--
F_{17}	0.04	--	--	--
F_0	0	0	Brown	--

R_fI : R_f values of the fractions before spraying visualizing reagent.
R_fII : R_f values of the fractions after spraying visualizing reagent.
Solvent system : Ethyl acetate : Petroleum ether (1:4).

TABLE IX.14

Identity of steroids in VME separated on TLC

(*Spray reagent*: Perchloric acid)

Fractions	R_f I	R_f II	Colour of the bands	Identity of the constitutents
	--	0.94	Violet	Steroids
F_1	0.85	--	--	--
F_2	0.76	0.76	Brownish violet	Steroids
F_3	0.68	--	--	--
	--	0.65	Deep violet	Steroids
F_4	0.63	--	--	--
F_5	0.58	--	--	--
F_6	0.53	0.53	Green	--
F_7	0.48	--	--	--
F_8	0.45	0.45	Brownish violet	Steroids
F_9	0.41	--	--	--
F_{10}	0.35	--	--	--
F_{11}	0.32	0.32	Brown	Steroids
	--	0.31	Brown	Steroids
F_{12}	0.30	--	--	--
F_{13}	0.24	0.24	Dark grey	Steroids
F_{14}	0.21	0.21	Yellow	--
	--	0.18	Greenish grey	Steroids
F_{15}	0.16	--	--	--
	--	0.12	Light grey	Steroids
F_{16}	0.11	--	--	--
	--	0.07	Light violet	--
F_{17}	0.05	--	--	--
F_O	0	0	Dark brown	Steroids

R_fI : R_f values of the fractions before spraying visualizing reagent.
R_fII : R_f values of the fractions after spraying visualizing reagent.
Solvent system : Ethyl acctate : Petroleum ether (1:4).

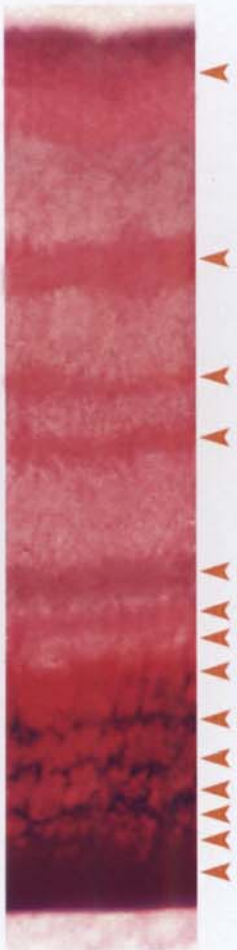
PLATE IX.6

TLC OF VME SHOWING CHEMICAL CONSTITUENTS (contd.)

a

◄: Steroids
(After spraying perchloric acid)

b



◄: Essential oils, higher alcohols, phenols & steroids
(After spraying vanillin-sulphuric acid)

(F_9), 0.31 (F_{10}), 0.26 (F_{12}), 0.22 (F_{13}), 0.19 & 0.18 (F_{14}), 0.16 (F_{15}), 0.09, 0.07, & 0.06 (F_{16}), and 0 (F_0), were found to contain the aforesaid chemical onstituents (Table IX.15; Plate IX.6 b).

Spraying with potassium ferricyanide-ferric chloride reagent produced bluish green or blue bands characteristic of phenols and amines. Fractions with R_f values 0.94 (F_1), 0.85 (F_2), 0.61 (F_4), 0.55 (F_6), 0.35 (F_{12}), 0.31 (F_{13}), 0.27 (F_{14}), 0.24 & 21 (F_{15}), 0.15, (F_{16}), 0.12 & 0.09 (F_{17}), and 0 (F_O) were found to contain chemical constituents such as phenols and amines (Table IX.16; Plate IX.7 a).

Flavonoids were identified in the fractions with R_f values 0.18 (F_{14}) and 0(F_O) by the greenish yellow fluorescence yielded with the spraying of aluminium chloride (Table IX.17; Plate IX.7 b). Other non-fluorescing bands were also detected with R_f values of 0.56, 0.52, 0.45, 0.38, 0.14, 0.09 and 0.05.

Likewise, the TLC when sprayed with ceric ammonium nitrate and subsequent heating, produced brown areas on yellow background typical for alcohols (polyalcohols). Fractions of the extract with R_f values of 0.76 (F_1), 0.51 (F_2), 0.29 (F_8), 0.21 (F_{11}), 0.07 (F_{16}), 0.05 (F_{17}), and 0 (F_0) (Table IX.18; Plate IX.7 c) were found to contain polyalcohols by this method.

Using several spray reagents specific for various phytochemical constituents, it was found that VME contained several constituents that were separated on the TLC. Major constituents separated were terpenes, triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines and flavonoids. A perusal of Tables IX.12-19 indicate that VPE and VME

TABLE IX.15

Identity of essential oils, higher alcohols, phenols and steroids in VME separated on TLC

(*Spray reagent*: Vanillin-sulphuric acid)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constituents
	--	0.94	Violet	--
	--	0.89	Pink	Essential oils, higher alcohols, phenols & steroids
F_1	0.83	--	--	--
	--	0.79	Light pink zone	--
	--	0.69	Pink	Essential oils, higher alcohols, phenols & steroids
F_2	0.67	--	--	--
	--	0.62	Violet	--
F_3	0.56	0.56	Light pink	Essential oils, higher alcohols, phenols & steroids
F_4	0.51	0.51	Pink	Essential oils, higher alcohols, phenols & steroids
F_5	0.47	--	--	--
F_6	0.44	0.44	Light grey zone	--
F_7	0.4	--	--	--
F_8	0.38	0.38	Pinkish violet	Essential oils, higher alcohols, phenols & steroids
F_9	0.35	0.35	Pink	Essential oils, higher alcohols, phenols & steroids
F_{10}	0.31	0.31	Light pink	Essential oils, higher alcohols, phenols & steroids
F_{11}	0.28	0.28	Light grey	--
F_{12}	0.26	0.26	Reddish pink	Essential oils, higher alcohols, phenols & steroids
F_{13}	0.22	0.22	Deep pink	Essential oils, higher alcohols, phenols & steroids
	--	0.19	Dep pink	Essential oils, higher alcohols, phenols & steroids
F_{14}	0.18	0.18	Pink	Essential oils, higher alcohols, phenols & steroids
F_{15}	0.16	0.16	Reddish pink	Essential oils, higher alcohols, phenols & steroids
	--	0.09	Reddish pink	Essential oils, higher alcohols phenols & steroids
F_{16}	0.07	0.07	Pink zone	Essential oils, higher alcohols, phenols & steroids
	--	0.06	Pink zone	Essential oils, higher alcohols, phenols & steroids
F_{17}	0.04	--	--	--
F_O	0	0	Deep pink	Essential oils, higher alcohols, phenols & steroids

R_f I : R_f values of the fractions before spraying visualizing reagent.

R_f II : R_f values of the fractions after spraying visualizing reagent.

Solvent system : Ethyl acetate : Petroleum ether (1:4).

TABLE IX.16

Identity of phenols and amines in VME separated on TLC

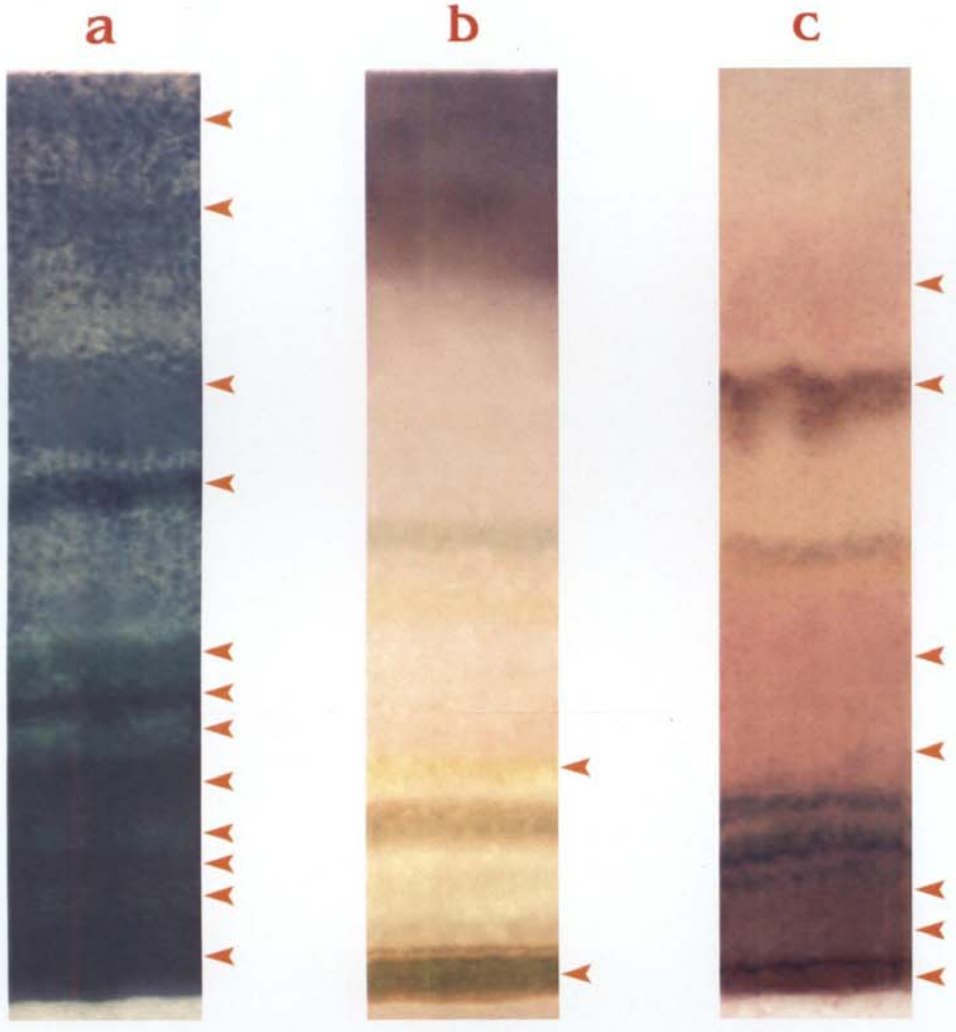
(*Spray reagent*: Potassium ferricyanide-ferric chloride)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constituents
	--	0.94	Light blue	Phenols & amines
F_1	0.91	--	--	--
	--	0.85	Light blue	Phenols & amines
F_2	0.82	--	--	--
	--	0.66	Light blue zone	--
F_3	0.64	--	--	--
F_4	0.61	0.61	Light blue	Phenols & amines
F_5	0.59	--	--	--
F_6	0.55	0.55	Deep blue	Phenols & amines
F_7	0.52	--	--	--
F_8	0.49	--	--	--
F_9	0.45	--	--	--
F_{10}	0.42	-	-	-
F_{11}	0.39	-	-	-
F_{12}	0.35	0.35	Bluish green	Phenols & amines
F_{13}	0.31	0.31	Deep blue	Phenols & amines
F_{14}	0.27	0.27	Bluish green zone	Phenol & amines
F_{15}	0.24	0.24	Bluish green zone	Phenols & amines
	--	0.21	Deep blue	Phenols & amines
F_{16}	0.18	--	--	--
	--	0.15	Blue zone	Phenols & amines
	--	0.12	Deep blue	Phenols & amines
F_{17}	0.09	0.09	Blue zone	Phenols & amines
F_O	0	0	Deep blue	Phenols & amines

R_fI : R_f values of the fractions before spraying visualizing reagent.
R_fII : R_f values of the fractions after spraying visualizing reagent.
Solvent system : Ethyl acetate : Petroleum ether (1:4).

PLATE IX.7

TLC OF VME SHOWING CHEMICAL CONSTITUENTS (contd.)



◀ :Phenols & amines (After spraying potassium ferricyanide-ferric chloride)

◀ :Flavonoids (After spraying aluminium chloride)

◀ :Alcohols (Polyalcohols) (After spraying ceric ammonium nitrate)

TABLE IX.17

Identity of flavonoids in VME separated on TLC

(*Spray reagent*: Aluminium chloride)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constituents
F_1	0.89	--	--	--
F_2	0.78	--	--	--
F_3	0.56	0.56	Grey	--
F_4	0.52	0.52	Brown	--
F_5	0.49	--	--	--
F_6	0.45	0.45	Greenish grey	--
F_7	0.41	--	--	--
F_8	0.38	0.38	Light yellow	--
F_9	0.35	--	--	--
F_{10}	0.29	--	--	--
F_{11}	0.27	--	--	--
F_{12}	0.25	--	--	--
F_{13}	0.21	--	--	--
F_{14}	0.18	0.18	Greenish yellow fluorescence	Flavonoids
F_{15}	0.14	0.14	Green	--
F_{16}	0.09	0.09	Light green	--
F_{17}	0.05	0.05	Light green	--
F_O	0	0	Greenish yellow fluorescence	Flavonoids

R_fI : R_f values of the fractions before spraying visualizing reagent.
R_fII : R_f values of the fractions after spraying visualizing reagent.
Solvent system : Ethyl acetate : Petroleum ether (1:4).

TABLE IX.18

Identity of alcohols (polyalcohols) in VME separated on TLC

(*Spray reagent*: Ceric ammonium nitrate)

Fractions	R_f I	R_f II	Colour of the fractions	Identity of the constituents
F_1	0.79	--	--	--
	--	0.76	Light brown zone	Alcohols (Polyalcohols)
F_2	0.59	--	--	--
	--	0.51	Brown	Alcohols (Polyalcohols)
F_3	0.45	--	--	--
	--	0.42	Light greyish brown	--
F_4	0.41	--	--	--
F_5	0.38	--	--	--
F_6	0.35	--	--	--
F_7	0.32	--	--	--
F_8	0.29	0.29	Light brown zone	Alcohols (Polyalcohols)
F_9	0.26	-	-	-
F_{10}	0.24	--	--	--
F_{11}	0.21	0.21	Light brown zone	Alcohols (Polyalcohols)
F_{12}	0.19	-	-	-
F_{13}	0.15	--	--	--
F_{14}	0.13	--	--	--
F_{15}	0.11	--	--	--
F_{16}	0.07	0.07	Light brown	Alcohols (Polyalcohols)
F_{17}	0.05	0.05	Light brown zone	Alcohols (Polyalcohols)
F_O	0	0	Deep brown	Alcohols (Polyalcohols)

R_fI : R_f values of the fractions before spraying visualizing reagent.
R_fII : R_f values of the fractions after spraying visualizing reagent.
Solvent system : Ethyl acetate : Petroleum ether (1:4).

TABLE IX.19

Phytochemical constituents present in the TLC fractions of VME

Fractions	Colour of the bands	Identity of the phytochemical constituents
F_1	Colourless	Terpenes, essential oils, higher alcohols, polyalcohols, steroids, phenols, amines.
F_2	Colourless	Terpenes, essential oils, higher alcohols, polyalcohols, steroids, phenols, amines
F_3	Light grey	Terpenes, essential oils, higher alcohols, phenols, steroids.
F_4	Light brown	Essential oils, higher alcohols, steroids, phenols, amines
F_5	Colourless	--
F_6	Greenish grey	Triterpene glycosides, steroids, phenols, amines.
F_7	Colourless	--
F_8	Light yellow	Essential oils, higher alcohols, polyalcohols, phenols, steroids
F_9	Colourless	Terpenes, essential oils, higher alcohols, phenols, steroids
F_{10}	Yellow	Essential oils, higher alcohols, phenols, steroids
F_{11}	Colourless	Steroids, polyalcohols, flavonoids
F_{12}	Grey	Essential oils, higher alcohols, steroids, phenols, amines.
F_{13}	Dark grey	Steroids, essential oils, higher alcohols, phenols, amines.
F_{14}	Greenish yellow	Triterpene glycosides, steroids, essential oils, higher alcohols, phenols, amines, flavonoids
F_{15}	Dark green	Essential oils, higher alcohols, steroids, phenols, amines
F_{16}	Light brownish green	Essential oils, higher alcohols, polyalcohols, steroids, phenols, amines.
F_{17}	Light green	Terpenes, essential oils, higher alcohols, polyalcohols, steroids, phenols, amines
F_O	Coffee brown	Steroids, essential oils, higher alcohols, poly alcohols, phenols, amines, flavonoids.

contained almost similar compounds although they were separated into several compounds with either similar or different R_f values. The variation in the intensity of colours of the bands also indicated remarkable differences in the concentration of the materials in the extract.

9.3.8. Identity of toxic chemical constituents in F_O of VME

Further separation of F_O from TLC of VME using another solvent system (ethyl acetate) resolved the fraction further into a few constituents that gave characteristic colours of compounds when sprayed with various chemical reagents. Terpenes, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines and flavonoids were identified by this procedure. Comparing the colours obtained for the bands from the original TLC and the fractions obtained from the rerun of F_0, it was found that both the chromatograms contained similar compounds that reacted with the spray reagents in a similar fashion.

9.4. Discussion

Testing the various fractions from TLC separation of VPE and VME for their toxic effect on *T. castaneum* revealed the presence of several toxic components in the two extracts (Figs. 9.1 & 2). Comparing the two extracts for their toxicity, VME was found to have more toxic principles which corresponded to some extent, with the number of bands observed before any chemical treatment (Plate IX.1 a, b; Tables IX.1 & 2). Methanol seemed to be a better solvent, which extracted more components especially in the low R_f region. Out of 18 fractions separated on TLC, 15 were having R_f values less than 0.5. Among all the

fractions observed, F_0 appeared to have very high and significant toxicity than all the other fractions. In the case of VPE, out of 14 fractions 8 fractions had R_f values below 0.5. However, all the fractions exhibited more or less similar toxic effect.

Chemical analysis for the identity of the fractions revealed their chemical nature. Active compounds included terpenes, triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines and flavonoids. These compounds were seen separated out into different regions on the TLC. It is possible that these compounds exist in either simple or complex forms giving them various chromatographic mobilities. It is also suggested that the polarity of the various compounds extracted by the solvents contribute to their partitioning on the TLC plate. This explains the abundance of materials on the TLC of VME and also the low R_f values of majority of these compounds. In the case of VPE, extraction and separation were greatly affected by the polarity of the solvent.

It was also seen that toxicity more or less concentrated in the region of R_f values of 0.5 and less. In the case of VME, intense staining was observed at F_0, both before and after chemical spraying indicating the presence of many components of biological activity. In fact, the activity was many folds more here than other fractions. F_0 on further seperation proved to contain the constituents of other fractions. Major constituents detected in the low R_f area were relatively polar materials such as phenols, polyalcohols and some steroids.

Several phytochemical constituents and their activities on insects have been reported earlier by many workers. For example, column chromatographic fractions of oils from *V. negundo* leaves contained a mixture of monoterpenes,

sesquiterpenes and their alcohols and showed repellent activity on *A. aegypti* (Hebbalkar *et al.* (1992). Chawla *et al.*, (1991) reported that unsaponifiable matter from the seed oil of *V. negundo* contained a diterpene, 5β-hydro-8,11,13-abietatrien-6α-ol, a triterpene, lanostan-8,25-dien-3β-ol and a flavonoid, artemetin. Terpenoids from plants show toxicity against various insects. Rice and Coats (1994) reported that several monoterpenoids from plants possess insecticidal properties against *M. domestica* and *T. castaneum.* Similarly, the toxicity of monoterpenoids (citral, geraniol and eugenol) against *Coptotermes formosanus* (Cornelius *et al.,* 1997) and monocyclic monoterpenes (1,8-cineole) from *Eucalyptus* spp. and limonene from *Citrus* spp. against *R. dominica* and *T. castaneum* (Prates *et al.,* 1998) have been evaluated. Likewise, toxic effects of the sesquiterpenoids, caprariolides A and B isolated from the aerial parts of *Capraria biflora* on adult *C. formicarius elegantulus* (Collins *et al.,* 2000) and of totarol, a diterpenoid phenol, isolated from *Podocarpus totaria* on *Culex pipiens* larva (Lee *et al.,* 2000) have been reported. Chawla *et al.,* (1992 a) reported the presence of four triterpenoids and Das and Das (1994) reported glycosides and terpenoids from *V. negundo.* It is possible that the TLC fractions that showed toxicity on *T. castaneum* in our experiments also contained related compounds.

Essential oils were also found to be one of the usual components of *V. negundo* leaf extracts as revealed by TLC analysis. Mallavarappu *et al.,* (1994) reported GC and GC-MS analysis of the essential oils of *V. negundo* and found that the oils contained 65 known compounds. Similarly, analysis of the essential oils from the leaves of *V. negundo* var. *negundo and V. negundo purpursescens* by

Jirovetz *et al.,* (1998) had revealed that monoterpenes (terpinen-4-ol, p-cymene, a-terpineol and sabinene) as well as sesquiterpenes (b-caryophellene, globulol, spathulenol, b-farnesene and bis (1-1-dimethyl) methyl phenol) were the main essential oil constituents of this plant species. Singh *et al.,* (1999) studied the volatile constituents of *V. negundo* leaves by GC-MS and revealed the presence of 66 compounds.

The fractions of VPE, VME and F_O of VME, all of which contained essential oils, showed topical toxicity against *T. castaneum* adults, which is in agreement with the results of other authors. Dayrit *et al.* (1995) reported that volatile oils from the leaves of *V. negundo* caused up to 83% mortality of the eggs in ovicidal tests and 91% mortality in 3rd instar larvae of *Plutella xylostella* in topical toxicity tests. Fractionation of the volatile oils and bioassay testing using *P. xylostella* led to the isolation and identification of beta-eudesmol (by GC-MS) as one of the components responsible for its ovicidal and topical toxicity. Similarly, contact and fumigant toxicity of essential oils extracted from *E. rutaecarpa* (Liu and Ho, 1999), *Elletaria cardamomum* (Huang *et al.,* 2000) against *S. zeamais* and *T. castaneum* have been reported. Tripathi *et al.,* (2002) also reported the toxic effect of the leaf essential oils of *C. longa* on *S. oryzae, T. castaneum* and *R. dominica.*

Some other chemical constituents such as phenols, alcohols (higher alcohols and polyalcohols) and steroids are also present in the TLC fractions may also be toxic to *T. castaneum.*

Chemical systematics of *Vitex* species such as *V. altissima, V. negundo, V. peduncularis, V. pinnata* and *V. trifolia* have revealed that all of them contained p-coumaric, benzoic, ellagic and vanillic acids, vanillin and an unidentified phenolic compound (Reddy and Radhakrishnan, 1992). The topical toxicity of phenolic constituents in the TLC fractions of both the extracts of *V. negundo* against *T. castaneum* are in agreement with the studies of Isman and Duffey (1982). They evaluated the toxic effect of phenolic compounds as well as phenolic-rich aqueous extract of tomato foliage on *Heliothis zea.* Similarly, Singh and Upadhyay (1993) in their study revealed that alcoholic and phenolic constituents of a variety of essential oils from some plants such as *Mentha piperita, A. calamus, Anethum. sowa, P. nigrum, P. glabra* and *A. indica* were found to have grain protectant activity. It was also found that these constituents also showed considerable ovicidal activity on *A. aegypti.* Amines, triterpene glycosides and steroids present in the TLC fractions presumably cause toxic effect on *T. castaneum.*

Isolation of flavonoids from *V. negundo* have been reported earlier. Phytochemical studies on the leaves of *V. negundo* by Dayrit *et al.,* (1987) revealed that casticin, chrysoplenol D, luteolin, p-hydroxybenzoic acid, D-fructose, isoorientin and flavonoids were the constituents present in this plant. Chandra and Babber (1987) isolated flavone and two new isomeric pentaoxygenated flavanones from *V. negundo.* Phytochemical analysis of VPE and VME by TLC described here also provided positive results for flavonoids. These compounds were also found to be toxic against *T. castaneum* adults in our experiments.

Experiments described in this chapter revealed the chemical mature of the biologically active constituents of the plant, *V. negundo,* extracted by two solvents (VPE and VME). Chemical identification was not very specific due to the complexity and vastness of the phytochemicals present. However, the broad classes of compounds were recognised. The testing of toxicity of the extracts (crude) and the TLC fractions gave good results although the dosage of the material was not precisely estimated. It was found that crude extract had much better activity than the fractions as evidenced by their toxicity on the insect, *T. castaneum*, tested by topical application method. This was true with both the VPE and VME. Within 96 h treatment, there was nearly 96-100% mortality with crude extract whereas an equivalent concentration of the TLC fractions brought about by 2-24% mortality (with different fractions). The only exception was VME F_0, where there was 94% mortality. This fraction, however, on rerun on TLC was shown to contain many constituents that was separated from the crude extract. Therefore, the F_0 acted more or less like the crude extract. This fact again supports our assumption that the various constituent components of the extract act either individually, additively or synergistically as toxicant against the insect.

To conclude, it is found that *V. negundo* contain toxic constituents that can act against stored product pests such as *T. castaneum* and that the plant materials extracted and formulated in suitable ways and produced in commercial scales, may be ideal in future for maintaining insecticide-free bioecosystems.

Chapter X

GENERAL DISCUSSION

India, with a geographic area of around 330 million ha is endowed with a rich diversity of flora and fauna. Among the 800 plant species of ethnobotanic origin, at least 160 are domestic crops of which 20 are grown predominantly as arable crops over an estimated area of 142 million ha. The cereals and pulse crops are grown in 123.6 million ha to meet food demand of our current population of over one billion. Approximately one third of the food production is lost through damage caused during pre and post harvest stages. Sustenance of food production to meet the growing demands of our future generations with minimum health hazards to man and his domestic animals is therefore, a gigantic task. Pesticides, over the years, have given a tremendous boost to our food and fiber production as these ensure maximum and quick control of pests in minimum possible time.

India is one of the countries in the world consuming very low amount of insecticides (around 300-400 g/ha compared to 10 kg/ha in Japan). The heavy reliance on synthetic pesticides in agriculture, has led to several unforeseen problems, such as rapid development of resistance by pests, increasing insect outbreaks (resurgence), agrochemical pollution, suppression of parasitoids and predators, environmental and food chain contamination (residue), and adverse effects on non-target organisms. More recently, there are reports about public health risks from pesticide-induced suppression of hormonal and immune systems and foetus damage. The environmental considerations in integrated pest management have posed new challenges to scientists for developing

environmentally benign pest control chemicals which are effective against the target species but create minimal adverse effects on non-target species. Protection of agricultural crops from competing plants, insects and pathogens has been a major issue before the scientists engaged in research.

Plants, during their long evolution, have synthesized a diverse array of chemicals to prevent their colonization by insects and other herbivores. More than 4,00,000 chemicals are estimated to be produced by plants. Of these, about 10,000 are secondary metabolites whose major role is to provide defense to the plants against insect pests. These naturally occurring phytochemicals, most of which have now been identified, belong to various chemical groups (terpenoids, alkaloids, glycosides, phenols, polyacetylene, etc.) and exert a wide range of behavioural and physiological effects on insects. These chemicals repel approaching insects and deter feeding and oviposition on the plants. They also disrupt behaviour and physiology of insects in various ways and even prove toxic to different developmental stages of many insects. Such natural defense chemicals are far superior to the synthetic pesticides. The isolation of these protective chemicals from the plants and determination of chemical structure would provide a valuable pointer to the development of new pesticide and novel methods of pest control.

As many as 2400 plant species have been reported to possess pest control properties. Plant origin pesticides have distinct advantages over synthetic pesticides. They are safe, effective, renewable, available, bio-degradable and compatible with nature.

Among the pests of stored grains or products, the rust-red flour beetle, *T. castaneum* is one of the major pests causing severe damage and economic loss. Various plant products have been tried recently against this insect with a good degree of success.

During the present investigation, extracts made from *V. negundo* leaves in petroleum ether and methanol were evaluated for their various effects on *T. castaneum*. Repellency bioassay revealed that different concentrations of VPE and VME were having strong repellent activity on *T. castaneum* during different periods of exposure. The repellency observed in the above investigation was found to be dose-dependent. It was found that the activity increased with increase in concentrations of both the extracts. Similar observations were made by Jilani *et al.* (1988) on the repellency of sweetflag oil, neem oil and Margosan-O and by Tripathi *et al.* (1997) of *P. retrofractum* fruit oil against *T. castaneum*. The repellency was also found to be time-dependent for both the extracts. The higher repellency observed at 1.5 to 3 h duration was reduced considerably during 6 h treatment. It is possible that the extracts contain several volatile compounds having various degrees of volatility. The initial high repellency during early periods of treatment (1.5 to 3 h) may be due to the presence of highly volatile low molecular weight compounds, which escape soon from the experimental chamber and also from the insect receptors. This result in the insects' return to the treated parts of the filter paper. Between 6 and 24 h, insects get further doses of low volatile high molecular weight compounds that are characteristically persistent. This repellency that has been found in the 24-72 h period of treatment thus last long. The slow

decrease in repellency in the high activity plateau may probably be due to the slow acclimatisation of insects to the volatile compounds. Similar decrease in repellent activity of oils from plants on stored product insects for prolonged treatment have been reported (Jilani and Saxena, 1990; Liu and Ho, 1999).

The repellent effect *V. negundo* of on *T. castaneum* is in conformity with the results of Hebbalkar *et al.* (1992) where they found that chromatographic fractions of oil of this plant had repellent activity against *A. aegypti.* Similar observation was also made with extracts of *V. negundo* on *T. confusum* (Pradeep and Radhakrishnan, 1998). It is presumed that the repellent effects of the two extracts in our experiments are presumably due to the volatile oils, monotorpenes, sesquiterpenes, mixture of monoterpene and sesqueterpene alcohols and rotundial etc. which act either individually or synergistically in a dose-dependent manner.

Nutritional studies on *T. castaneum* revealed that both VPE and VME adversely affected growth, food consumption and utilization and feeding behaviour of this insect. Here, both the extracts brought about reduction of RGR and RCR. Such results have been reported earlier by Chiam *et al.* (1999) and Tripathi *et al.* (2001 b) in *T. castaneum.* The reduction of growth and food consumption of *T. castaneum* in this study may possibly be due to the antifeedant action of the extract incorporated into the diets. The study also revealed that the quantity of food material converted into body matter was reduced and that the consumption of lesser quantity of the treated food is likely to be the main cause of growth inhibition. Another observation made in this investigation was the dose-dependent increase of ECI in *T. castaneum* with VPE and VME. Here, the difference between the

activities of the two extracts was not significant. Significantly higher ECI values over control, obtained with all concentrations of the extracts in this study, is thus in agreement with results obtained by Joseph (2000) for the effect of neem seed kernel extract on *Eligma narcissus indica*. Higher ECI values obtained in the present studies presumably reflect the compensation for antifeedant effect of the extracts. Another important observation was that VPE and VME treated diets exhibited a dose-dependent increase of antifeedant activity against *T. castaneum*. The difference between the antifeedant activities of the two extracts was not significant. This result is thus in agreement with the antifeedant activities of extracts (Sahayaraj, 1998) and essential oils (Sharma *et al.*, 2000) of *Vitex negundo* against *S. litura*. The mechanism of perception of inhibitory chemicals in insects were reviewed by Chapman (1974) and Schoonhoven (1982). The mechanism of antifeedant action of inhibitory chemicals present in plant extracts could be explained in a similar way in the case of *V. negundo* against *T. castaneum*. Various chemical components of the extracts of this plant probably inhibit the feeding of the insect and these chemicals are presumably perceived through the receptors like sensilla on the antennae, labrum and maxillary and labial palps of *T. castaneum*. These chemicals may either directly inhibit the receptor input or the signals may be interpreted at the central nervous system (Ma, 1969, 1972; Schoonhoven, 1973; Ishikawa *et al.*, 1969).

Studies on emergence and development of *T. castaneum* revealed that larval-pupal-adult transformations of the insects were prolonged or delayed in diets treated with the two extracts compared to control during different periods of

exposure. The delay in development was more prominent with higher concentrations of the extracts. Retention of larval stages was higher during 25-30 days in all of the diets treated with different concentrations of the extracts and this was more prominent with 10% of VPE. The activities of the two extracts were found to be significantly different ($P<0.001$). Similar delay in development were reported in *P. japonica* with *A. indica* (Ladd *et al.,* 1984) and in *T. castaneum* with *A. squamosa* in (Malek and Wilkins , 1995).

Another observation in our present investigation was the significant reduction of larval progeny due to their mortality. This activity was found to differ significantly between the extracts. The observed decline also may be due to the transformation of most of the larvae into pupae and adults. It has generally been observed that insects were more susceptible to toxic materials during early larval days especially the intermoult periods. During this periods, cuticle will be less sclerotised and chitinised and thus more penetrable. It is possible in our experiments that lower concentrations of the extracts do not inhibit moulting to any appreciable extent, so that more susceptible stages in the intermoult periods are made available for the toxic substances to act upon unlike the 10% treated diets. It appears that lower concentrations of the extracts exhibited higher toxic effect, which reflects high volatility of the toxic components. On the other hand, mortality observed with 10% of extracts exhibit a slow and sustained activity, although maximum mortality was obtained. Here, the delayed activity of the extract may be due to the low volatility of the concentrated extracts (10%). However, higher total mortality observed during 30-40 days with this extract may also be attributed to

starvation caused by antifeedant materials present in the extracts. The delayed toxic effects of 10% extracts might also be due to the interrupted moulting caused by the extracts resulting in the absence of the most susceptible early intermoult stages of the larva. Larval mortality of *T. castaneum* is consistent with the observations of Rani and Jamil (1989), Singh and Rao (2000) and Papachristos and Stamopoulos (2002).

Another observation of this experiment is that these two extracts delayed pupation and adult emergence and caused a reduction in the number of progeny. The difference between the activities of the two extracts was found to be not significant. Pupal and adult mortality were found to be negligible in diets treated with both the extracts. However, it was seen that there was some delay in the emergence of adults in both the treated diets. It was also seen that the number of adults emerged in treated diets were less compared to control. The reduction of adult emergence in treated diets might be largely due to the larval mortality caused by the extracts. Similar observations were made by Jilani *et al.* (1988) for the effects of turmeric oil, sweetflag oil, neem oil and Margosan-O on *T. castaneum* and by Sukumaran *et al.* (1987) on *S. cerealella* by using dried leaf powder of *V. negundo.* The adverse effects on emergence and development of *T. castaneum* may presumably be due to several chemical constituents such as monoterpenes, sesquiterpenes, diterpenes, essential oils etc. present in the extracts of *V. negundo.* These chemical constituents act as antifeedants consequently resulting in a prolonged larval stage. These chemicals might be affecting the endrocrine mechanisms that regulate metamorphosis of the insect. This is in tune with the

reported findings in *C. cephalonica* (Pandey *et al.*, 1985) and *M. sexta* (Schluter *et al.*, 1985).

Studies on adult emergence of *T. castaneum* revealed that there was significant reduction of adult emergence in the treated diets and that the activities of the two extracts were not significantly different. The total number of adults emerged during 50 days in the diets were also reduced. Peak of adult emergence at 30 days in the case of control, was shifted to 40 days in the case of treated diets. The results of our experiments agree with the effects of some botanicals observed in *T. castaneum* by (Maheshwari and Dwivedi (1996) and Mukherjee and Joseph (2000). The reduction of adult emergence in *T. castaneum* is due to the larval mortality and developmental inhibition in response to the extracts. Several chemical constituents (terpenes, essential oils, etc.) in the extracts of this plant adversely affect the development of the insect and they may act variously as oviposition deterrents, ovicidals, inhibitors of egg hatching, growth inhibitors, antifeedants and toxicants.

From the toxicity studies, it was seen that the extracts are potent contact poisons. They brought about significant mortality in *T. castaneum* as shown in figures 8.1-3. However, film residue of VPE was found to have less toxic effect on this insect. In general, TAM was found to be more effective than FRM. This may due to the fact that this method provides more chance for close contact of the extracts with the insects resulting in low LC_{50}/LC_{95} values. Contact toxicity by film residue of VPE and VME was found to be time-dependent. The activities of the extracts were higher and immediate at the early period of exposure, which extended

over a long period when the exposure time was increased. These results are in conformity with the findings of Don-Pedro (1996) on the effects of citrus peel oils, and of Padmasheela and Delvi (2002) of neem oils on some stored product insects. El-Lakwah *et al.* (1997) and Obeng-Ofori *et al.* (1998) reported the dose-dependent increase of toxicity of essential oil constituents in stored product insects. Similar results were obtained in our investigations where a dose-dependent effect was obtained. The slope of the regression line indicates the significance of the effect of unit concentration of the extracts on probit mortality of the insects. Toxic effect of *V. negundo* on *T. castaneum* has been similar to such effects of some indigenous plants including *V. negundo* reported against *C. quinquefasciatus* (Kalyanasundaram and Babu, 1982; Arivoli *et al.*, 2000). The mortality of this insect was due to the various chemical constituents in the extracts of *V. negundo*, which are active either individually or synergistically.

It is presumed that the mode of action of the phytochemicals in the extracts of this plant on *T. castaneum* is similar to the mode of action of some other commonly used botanical pesticides (pyrethroids, rotenoids and nicotinoids) already reported. Pyrethrins, obtained mainly from the flowers of *Chrysanthemum cinerariaefolium*, stimulate the central nervous system as well as peripheral nerve fibres, producing repetitive discharges, which are followed by paralysis. Pyrethrin and allethrin (synthetic compound) first stimulate the nerve cells and nerve fibres and then paralyse them. They block nerve conduction, which in turn causes paralysis.

Synergism is a striking phenomenon for pyrethroids. Synergists (eg. methylenedioxyphenyl compounds) inhibit *in vivo* detoxification of pyrethroids, and consequently the insecticides increase in persistence and toxicity. Pyrethrins are highly toxic as contact insecticide, but generally weakly toxic when fed to insects. Pyrethroids are only weakly toxic to mammals.

Rotenoids are another group of plant pesticides which contain the chief toxic principle, rotenone derived from roots and seeds of *Derris* sp. and *Lonchocarpus* sp. The major effect of rotenoids in insects (and in fishes) is the remarkable decrease in the oxygen uptake, which finally causes death.

Nicotine and other tobacco alkaloids obtained from *Nicotiana* sp. and some other plant species of Solanaceae family, also act as botanical insecticides. Nicotine is known to penetrate the insect body directly through the cuticle as well as through the spiracles and tracheae. In the latter instance, the central nervous system, richly supplied with tracheoles becomes readily accessible to nicotine. Insect nerve ganglia and synapses which are the sites of nicotine action. Nicotine stimulates the action of acetyl choline at nicotinic receptors in the central nervous system, autonomic ganglia and some peripheral nerves. Nicotine is not subjected to hydrolysis by acetylcholine esterase and therefore, even at low concentrations causes symptoms of excitation, depression, paralysis and eventually death at higher concentration.

Identification of active principles of *V. negundo*, which act as repellents antifeedants, toxicants, disruptants of development etc. on *T. castaneum* is very important. Attempts were made for the identification of toxic principles of this

plant by separation of the extracts by TLC, evaluation of toxicity of different fractions of the extracts on *T. castaneum* and by chemical analysis of the fractions (on the TLC) using specific visualizing regents for identifying the classes of chemical constituents. Results of TLC showed that the solvents used for the extraction of the plant materials affected the components extracted. More component fractions (18) were found to be separated on TLC from VME than from VPE where there were only 14 fractions. Toxicity bioassay of these fractions revealed that VME F_0 fraction had the highest degree of contact toxicity (94%) on *T. castaneum* compared to other fractions of VME and all the fractions of VPE. It was also found that all fractions except VME F_0, had more or less similar toxic effects. Phytochemical analysis of the extracts of this plant by TLC indicated that the major constituents such as terpenes, triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines and flavonoids acted as toxic principles against *T. castaneum*. These chemical constituents might act individually or synergistically on this insect causing toxicity.

From the present investigation, it becomes clear that crude extracts of the locally available plant *V. negundo* can act as effective repellents, antifeedants and toxicants and that the extracts have adverse effects on emergence and development of *T. castaneum*. In our experiment, different concentrations of the extracts were shown to have promising activity. However, 5% of VPE and VME appeared to be more effective and thus more suitable for the formulation of insecticide as repellents and toxicants and effective for the insect pest management programme of *T. castaneum*.

For those who are engaged in insecticide research, these natural agents (botanicals) offer a continual source of inspiration and challenge. Investigations can be carried out on various methods of isolation and characterisation of toxic phytochemical constituents as well as other bioactive constituents by techniques such as HPLC, HPLC-NMR, MS, GC-MS coupled with on line data analysis and databases. Identification of these novel biologically active metabolites and their structures serve for the synthesis of optimised marketable analogues or commercial products for IPM. These active principles can be tested against various pests of stored grains and agriculture and insect vectors for their control. Moreover, the binding of the phytochemicals on the cell membrane receptors and the signalling cascade that take place in the cytosol leading to the regulation of various metabolic pathways underlying biological expressions are to be explored in detail.

Inspite of their great potential, much works need to be done before large-scale utilization of botanical pesticides in IPM becomes a reality. After identification of potentially useful species, intensive breeding and selection work will have to be undertaken for economic production of high quality raw materials required for insecticide production. Simple and suitable formulation technology will have to be developed for use by poor and marginal farmers in developing countries so that ready-to-use pesticides can be produced at the local level helping to save money spent on costly synthetic agrochemicals. The fact revealed from our study that crude extracts of the plants showed better activity against insects than their separated fractions, proves to be more beneficial to the common farmers, in that they can directly go for locally prepared decoctions of fresh plants for pest

control. For field application, speedy degradation can be checked to a large extent by incorporation of carriers like flour paste, gums or clay etc. with the extracts while formulating, so as to make them more efficacious to control insect pests.

Some limitations are encountered in the possible commercialization of botanical pesticides. The major ones being the thermal and photolability, poor shelf life, limited availability of raw materials, and standardization. Such problems can be overcome by incorporating suitable antioxidants, UV stabilizers and synergists before developing the commercial products/formulations. Another problem relates to their slow action, which requires serious attention and necessitates a thorough investigation regarding the formulation for the selection of optimum dosage so that such safe pesticides could be suitably incorporated with other pest control strategies in the IPM framework.

This study therefore opens a new line of research towards the management of the stored product insect, *T. castaneum* with *V. negundo* and for that matter, the use of several other such plants against any phytophagous/agricultural/stored product pests in a very safe and cost-effective way avoiding operational and residual hazards that are usually involved in the use of organic and inorganic insecticides.

Summary

SUMMARY

In the present investigation, extracts of *Vitex negundo* leaves, made in petroleum ether and methanol were evaluated for repellent, antifeedant and toxic effects on the stored product pest, *Tribolium castaneum.* Studies were also carried out on the effects of these extracts on emergence, development, reduction of progeny and adult emergence of the insect. Attempts were also made for the separation and identification of toxic chemical constituents in the extracts by TLC.

Bioassay for repellent activity conducted using a 'choice chamber' revealed that different concentrations (0.625, 1.25, 2.5 and 5%) of both *Vitex negundo* Petroleum ether Extract (VPE) and *Vitex negundo* Methanol Extract (VME) were showing strong repellent effects on this insect within a range of 55 to 100% during different time intervals. The difference between the activities of the two extracts was not statistically significant. The repellent activity was found to be dose-dependent, having increased activity with increasing concentrations of the extracts. The repellency observed was also found to be time-dependent. The initial high repellency observed during early periods was reduced considerably by 6 h, then suddenly increased within 24 h and then slightly dropped during 72 h.

Nutritional studies ('no-choice' test) on *T. castaneum* revealed that different concentrations (1.25, 2.5 and 5%) of both VPE and VME reduced growth (RGR) and food consumption (RCR) of insects compared to control. These activities were found to be dose-dependent. Here, activities of the two extracts were not significantly different. Efficiency of conversion of ingested food (ECI) was,

however, found to increase in a dose-dependent manner with both the extracts. Though there was no significant difference between the two extracts, there was a significant difference among the different concentrations.

Another important observation was that these two extracts showed a dose-dependent increase of antifeedant activity. The activity of the two extracts showed more or less a similar pattern.

Studies on emergence and development of *T. castaneum* revealed that larval-pupal-adult transformations were prolonged or delayed in diets treated with both the extracts (VPE and VME) at all the concentrations (2.5, 5 and 10%). The effect was more prominent in 5 and 10% of VPE and VME. Retention of larval stages was more during 25-30 days in all the diets treated with different concentrations of VPE and VME, compared to control. Among these, more number of larvae were retained in diet treated with 10% of VPE. The delay caused on the development of larvae by the two extracts were significantly different between them.

Another observation made in this investigation was the reduction of larval progeny in treated diets due to the mortality caused by the extracts. The activity was found to be significantly different between the two extracts. The larval mortality was higher in diets treated with VPE than VME. During 25-30 days, higher larval mortality was observed with 2.5 and 5% of both the extracts compared to control. The decline observed afterwards might be mainly due to the transformation of most of the surviving larvae into pupae and adults and also due to their mortality. Here, the extent of activities of 10% VPE and VME were found to be lower but persistent until after 40 days. However, 10% extracts exhibited higher

rate of total mortality during 30-40 days. Pupation and pupal duration were also found to be adversely affected by the two extracts. Eclosion was delayed significantly by them. Here also, there was no significant difference between the effects of the two extracts.

Studies on adult emergence of the insect revealed that there was significant reduction or inhibition of emergence of adults during different periods of treatment and the total number of adults emerged during 50 days in treated diets were very much less than the number in the control. This activity was found to be similar in both the cases. It was also seen that the peak of adult emergence at 30 days in the case of controls were shifted to 40 days in the case of treated diets. When the extracts were compared, 10% VPE exhibited the highest per cent inhibition of the progeny.

Toxicity studies (Chapter VIII) indicated that TAM of VPE and VME and FRM of VME were showing strong contact toxicity against *T. castaneum* and the activity was found to be highly significant. Lesser toxic effect was also observed for VPE with FRM. Toxic effects of the two extracts were more obvious and instant during early periods (1.5 h) in TAM. However, toxic effect was extended over a long period with higher activity during the early period of observation (1 day) in the case of FRM. TAM was found to be more effective than FRM. Effect of both the extracts, when used in FRM was found to be time-dependent. The activity of the extracts were higher and immediate at the early period of exposure which diminished during further exposure time. In both the methods, toxicity of the extracts on insects increased in a dose-dependent manner. Another observation

was that TAM of VME produced low LC_{50} (1.48) and LC_{95} (8.43) values than corresponding values obtained for FRM of the same extract.

Separation and identification of toxic principles in VPE and VME were done by TLC. There were 14 and 18 fractions respectively for the extracts. Bioassay of the eluted fractions, indicated that F_0 of VME exhibited considerable toxic effect (94%). All the remaining fractions of both the extracts were showing less toxic effect and the mortality were found to be between 2 and 24%. Chemical analysis of the fractions on TLC indicated that the major constituents were terpenes, triterpene glycosides, steroids, essential oils, higher alcohols, polyalcohols, phenols, amines and flavonoids. These chemical constituents which caused contact toxicity in the insect, had either individual, additive or synergistic effects.

From this investigation, it becomes evident that the locally available plant *V. negundo* contain some active components which show repellent, antifeedant and toxic effects on *T. castaneum*. Moreover, these constituents cause some adverse effects on emergence, development and reduction of progeny of the insect. From the dose-response experiments conducted, it was found that 5% of both VPE and VME provided optimum control of the insects. Therefore, it is recommended that either crude extract at the above-mentioned concentration or any other commercial formulation prepared with an equivalent concentration, may be used as eco-friendly insecticide for the insect pest management of the stored product pest, *T. castaneum*.

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