EFFICACY OF EXTRACTS OF SOME LOCAL PLANTS WITH SPECIAL EMPHASIS ON *CLERODENDRUM INFORTUNATUM* IN THE CONTROL OF THE STORED GRAIN PEST, *CALLOSOBRUCHUS CHINENSIS* LINNAEUS (BRUCHIDAE: COLEOPTERA)

THESIS

Submitted to the University of Calicut
in partial fulfillment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY *in Zoology*

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AUGUST 2004

To my whole family,

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

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24th August 2004

CERTIFICATE

Certified that this dissertation entitled "Efficacy of Extracts of Some Local Plants with Special Emphasis on Clerodendrum infortunatum in the Control of the Stored Grain Pest, Callosobruchus chinensis Linnaeus (Bruchidae : Coleoptera)" is a bonafide record of work done by Ms. Valsala Kizhakke Karmmal in the Laboratory of Insect Physiology of this Department, under my supervision and guidance. Further certified that no part of this dissertation 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 under the guidance and supervision of Dr. M. Gokuldas, Professor, Department of Zoology, University of Calicut and this work has not been submitted elsewhere for any other degree or diploma or other similar titles.

Valsala Kizhakke Karmmal

ACKNOWLEDGEMENT

This thesis owes its culmination in the present form to the guidance, help and support I received from different quarters. I would be failing in my duties if I do not duly acknowledge them.

First and foremost, though it can not be put in writing, I express my heart-felt gratitude to my supervising teacher Dr. M. Gokuldas, Professor of Zoology, University of Calicut, for his invaluable guidance, creative suggestions and exhaustive discussions and also for monitoring the day- to- day progress of my research work.

Secondly, I express my indebtedness to Dr. V. S. Krishnan Nair, Professor and Head of the Department of Zoology, University of Calicut for providing me necessary facilities.

With immense pleasure, I express my profound sense of gratitude and indebtedness to Dr. U. V. K. Mohamed and Dr. T. C. Narendran, Professors and former Heads of the Department of Zoology, University of Calicut, for providing me necessary facilities and also for the constant encouragement given to me.

I feel happy to record my sincere thanks to Dr. N. Raju, Reader, Department of Statistics, University of Calicut for his laborious and painstaking effort in helping me with the statistical analysis of the data.

I am deeply indebted to Dr. P. Mohamed Shafi and Dr. K. K. Vijayan (Professors of Chemistry, University of Calicut) for their motivating suggestions and guidance provided during the phytochemical studies. My special thanks are also due to Sr. Mable and Mr. Ranjith, Research fellows, Department of Chemistry, for their valuable suggestions given during the phytochemical studies.

My sincere thanks are also due to Dr. K.V. Lazar, Lecturer in Zoology, University of Calicut, who has extended valuable suggestions during my work. My reverential thanks are also due to Dr. A. Babu, Entomologist, Upasi, Idukki and Sri. P. Radhakrishnan, Lecturer in Zoology, Governmen Arts and Science College, Calicut, for their encouragement and sustained interest in this research programme.

Financial assistance provided by the University Grants Commission by awarding the teacher fellowship under the Faculty Improvement Programme, is gratefully acknowledged.

My thanks are also due to the University of Calicut for selecting me for the Teacher Fellowship and Principal, Government College, Madappally for deputing me for this course.

I am happy to record my sincere thanks to all my friends and colleagues in the Insect Physiology Laboratory. The help rendered by my friends and well-wishers outside the Department, especially Sri. Vijayan Panicker, HSST, Vatakara; Sri. Sheriff, Librarian, IISR, Chelavoor; Sri. Santhosh, Art and Photography, University of Calicut and Dr. John Periera, Scientist, CFTRI, Mysore are also acknowledged.

I owe my gratitude to my father and mother-in law for the moral support, affection and understanding shown to me during this period. I am very happy to record here my deep felt appreciation and indebtedness for the help and support of my younger sisters Mrs. Latha and Sreeja provided during this period.

With due pride I acknowledge and appreciate my Sivettan, who was a source of constant inspiration and moral support in this endeavour.

With fondness I remember my mother and grandmother, who had evinced keen interest in my work and had always lent a helping hand.

Valsala

CONTENTS

ABBREVIATIONS

g	Gram
mg	Milligram
ml	Milliliter
min	Minutes
° C	Degree Celsius
h	Hour
µl	Microliter
LC_{50}	Medion lethal concentration
RH	Relative humidity
sp	Species
TLC	Thin layer chromatography
CC	Column chromatography
GC-MS	Gas chromatographic mass spectro photometric
KD	Knock down
DQ	Discrimination quotient
DMRT	Duncan multiple range test

Chapter 1

GENERAL INTRODUCTION

1. Early attempts of pest control

Insects are the only animals giving man a real battle for supremacy. They have been upon this earth for about 300 million years and have developed special adaptations to live under various environmental conditions. Insects become "pests" of man when their existence conflicts with his profit, convenience and welfare. Arthropod pests are responsible for global pre- and post harvest crop losses of approximately 20-50 % of potential production and for transmitting a number of the world's most important plant and animal diseases.

There are many weapons for insect control found in today's arsenal, but no method is without drawbacks. Insects can be controlled by legislative methods, physical methods (direct killing of insects using heat, light, X-rays and so on, reduce reproductive capacity or to attract them to something that will kill them), genetic method (sterile male method), ecological methods (involve removal, destruction, modification or isolation of materials that might favour the survival of an insect pest), biological methods (regulation of pests using predators, parasitoids, nematodes and microbial agents) and chemical methods (using natural or synthetic chemicals that act as insecticides, repellents, attractants, antifeedants and chemosterilants). Genetically modified crops have been developed that have resistance against insect attack. This high-tech science is still in its seminal stages and whether this technology truly revolutionizes pest control or whether it

becomes a part of Integrated Pest Management (IPM) paradigm remains to be seen.

Chemical tools for insect control have evolved rapidly in the last 100 years, starting with inorganic agents such as lime, sulfur and arsenicals, and natural products such as pyrethrum, rotenone and nicotine. These chemicals are considered as first generation pesticides. A series of dramatic discoveries during the late 1930s provided new synthetic insecticides of unprecedented power and range of activity that further shifted the emphasis to the chemical approach of insect control. The wonder insecticide DDT was the first of the second generation pesticides (organochlorine, organophosphate and carbamate), which revolutionized insect control.

As a result of the many fold problems that arose from the wide spread use of pesticides, increasing interest was generated in Integrated Pest Management (IPM). IPM is a multidisciplinary, ecological approach to the management of pest populations, which utilizes a variety of control tactics compatibly in a co-ordinated pest management system. This includes biological control, microbial control and also alternative chemicals and natural insecticides (botanicals obtained from plant sources). The biological approach includes the entomopathogenic microorganisms, parasites and predators, while alternative chemicals include semiochemicals (chemicals like allomones, kairomones and pheromones that deliver behavioural messages) and insect growth regulators (IGRs). IGR is a class of insecticides, which interfere with the normal growth (moulting and metamorphosis) and reproduction of insects. A major group of

IGRs is the juvenile hormone analogues, which could be used as insect-specific control agents to which the species may be unable to develop resistance. This class of compounds was referred to as "third generation pesticides" (Williams, 1967). The metamorphic hormones, ecdysone and JH, and their analogues have been found very effective in bringing about a suicidal type of control in insects. Instead of killing, the JH only derails the normal mechanism of development and causes the insects to kill themselves.

The conventional pesticides, many of which are basically neuro-toxicants, which are widely used for the control of insect pests, are highly toxic, non-selective and persistent in nature (Carson, 1962). The reckless use of these broad-spectrum second-generation pesticides on a large scale could constitute an ecological disaster of the first rank. Their continued use has led to the development of insecticide resistance (emergence of multi-resistant strains of pests), resurgence of pest populations, change in the pest status, destruction of beneficial insects, bio-magnification of residues and hazards to the health of higher animals including human beings. Pesticides have thus been responsible in causing a considerable deterioration of environmental quality.

This complicated situation has compelled us to change our attitude and to search for other safer alternatives and eco-friendly technologies, ultimately leading to the birth of bio-pesticides.

2. Botanicals in Pest Management

The use of plant extracts as insecticides can be dated back to at least 4000 years. Rig Veda (2000 BC) makes reference on to the use of poisonous plant for pest control. There are more than 3,08,000 plant species on our earth. Among these, approximately 2400 plant species have been recorded as being useful for pest control. Among the plants evaluated, neem has emerged as number one and most effective source of pesticide. In India alone, neem has been evaluated against 105 species of insects, 12 species of nematodes and 9 species of fungi (Singh and Kataria, 1991). Schmutterer and Singh (1995) listed 417 species of insects susceptible to neem the world over. These include almost all the key pests of agriculture.

Though a number of plants were screened in the first half of 20^{th} century and various reviews published, only three insecticides, viz. nicotine from *Nicotiana tabacum*, rotenone from *Derris* sp. and pyrethrum from *Chrysanthemum cinerariaefolium* were commercialized and only pyrethrum survived in the second half of the 20^{th} century. The other two were gradually pushed out from the market by synthetic insecticides. Pyrethrum is still being used and is considered as an ideal insecticide because it is much safer, non-persistent and at the same time exhibits high toxicity against insect pests. The pesticidal plants remained neglected ever since the powerful synthetic pesticides entered the market. These plants were not systematically screened for activity and many plants, which may have biological activities, could not come to light.

Bio-pesticides of plant origin being indigenous resources with insecticidal, repellent, antifeedant and insect growth regulatory action, are in use for over a century to minimise losses due to pests in storage commodities. Generally these pesticides do not possess a quick knockdown effect unlike synthetic insecticides, which are currently being used in IPM. However, these pesticides have many advantages over synthetic insecticides.

- pesticidal plants possess least or no mammalian toxicity and thus cause no health hazards;
- no environmental pollution and minimum risk of development of insect resistance to these pesticides;
- surface persistence for a longer period of time;
- no adverse effect on grain viability, cooking quality and milling recovery;
- unlike synthetic pesticides, which have only one active compound and exhibit only one type of biological effect, plant derived compounds may have more than one biological effect;
- because of renewability, they fit in well in sustainable agriculture and
- less expensive and easily available.

Role of plant secondary metabolites in pest control

Insects and plants originated almost simultaneously about 300 million years ago, and since then insects and plants have been fighting for their survival. During this long evolutionary history of attacker (insects) and attacked (plants), plants have developed ways and means to combat the attacker. Of the various

defensive mechanisms developed, chemicals elaborated by the plants are the most important one. Plants are nature's 'chemical factories' providing the richest source of chemicals on earth.

These chemicals that plants produce to protect themselves against insect attack are known as secondary plant substances. These are produced as by-products of major biochemical pathways. Chemically, they include alkaloids, terpenoids, phenolics as well as many other compounds. Many of these chemicals have been successfully exploited by humanity for the control of arthropod pests (Swain, 1977). These chemicals deter feeding, disrupt development, provide barrier to attack, assist with wound healing, disrupt digestion and many are neurotoxic to herbivorous pests.

Role of plants in stored grain pest control

DDT and several other synthetic insecticides were successfully used to control insect pests of stored food grains. However, their residual toxicity affected the non-target animals including man. Moreover, insects developed resistance to some of these insecticides. Thus search for natural and non-toxic grain protectant became essential. This search of entomologists and toxicologists for materials that effectively protect stored produce, that are readily available, affordable, relatively less poisonous and less detrimental to the environment, brought the attention to folk medicinal plants, grown locally.

Although several plants were screened in the past, only neem, turmeric (*Curcuma longa*) and sweet flag (*Acorus calamus*) are reportedly promising

traditionally used plants for protection of stored products in developing countries. In addition, farmers in Tamil Nadu and Karnataka use *Vitex negundo* and Karanj (*Pongamia glabra*) leaves for protection of stored rice (Ahmed and Koppel, 1987). Though, grain protecting property of neem leaves was known since time immemorial, it was in 1965 that the neem seed kernel powder (NSKP) was systematically evaluated against the Khapra beetle, *Trogoderma granarium*, the lesser grain borer, *Rhyzopertha dominica* and the rice weevil, *Sitophilus oryzae* (Jotwani and Sircar, 1965). Extensive works have been carried out in this field and is reviewed in the next chapter (Chapter II).

3. Importance of grain conservation and pest management

For centuries man has been striving hard to find some safe means for storing grains ensuring full protection from various pests. By trial and error he has progressed far but none of the methods adopted has been fool proof. If it were possible to prevent wastage of crops caused by birds, rodents, insects, mites and fungi, much of the production efforts could have been minimised. Insect pests constitute the largest group of agents causing loss to the growing crops and stored products. It was estimated in 1961 that storage pests destroyed over 96 million metric tons of cereal grains and if this could be saved, it would have been sufficient for 375 million people for one entire year (Dobrovsky, 1965).

Factors like warm temperature and high humidity are ideal for the rapid multiplication of insects. The damage done to growing crops by insects are immediately noticed, but damage during storage is usually insidious and may escape detection until it is too late. Deterioration may set in even before harvest,

before the crop ripens. Losses in stored products are manifested in several ways including losses in weight, quality and quantity.

The Expert Committee of Government of India on Losses recorded 9.33 per cent loss of food grains during storage (Krishnamurthy, 1975). The United Nations reported that due to ravages of insects and fungi in 1974, India lost 10 million tons of food grains, which if saved, would have been more than enough to make up the world food shortage, which has been estimated to be 7.5 million tons that year. It is recognized by all the grain handlers that when grain is left unattended for even a short while, it is invaded by different types of insects, mites and fungi. This is more common in prolonged period of storage where deterioration is caused by biotic and abiotic factors.

In the food industry today, what is required for insect problem in stored grains is the control of insect infestation. Sanitation by means of avoidance of insect pests, their excrement and other materials that contaminate food go a long way in curbing the losses in quality and quantity of food grains. Protection against insect infestation of food grains going into storage must start with the raw commodity and be continued through processing, packaging, handling, transportation and storage. Once an insect dead or alive is present in the processed commodity, the food is considered contaminated. The first step to achieve the goal of protection is to identify the insect causing contamination and damage.

4. Relevance of the study

Pulses play an important role in Indian diet and are the major source of proteins. India produces around 12.65 million tons of different pulses per year but nearly 8.5 per cent of the same is lost during post harvest handling and storage (Agarwal *et al.*, 1988). Among the pests of stored pulses, the pulse beetle, *Callosobruchus chinensis* is the major pest causing severe damage and economic loss. The infestation of pulses by species of this genus starts in the field. Soon after harvesting, approximately 4% of the seeds offered for sale show the typical emergence hole made by the bruchid. Giles (1964) and Caswell (1974) reported 92.3% damage of cowpea seed stored for 12 months without insect control. Various plant products have been tried recently with a good degree of success against the number of stored grain pests (Gill and Lewis, 1971; Teotia and Tewari, 1971; Ketkar, 1976; Pandey *et al.*, 1976, Pandey and Pandey, 1986; Atri and Prasad, 1980; Yadav and Bhatnagar, 1987; Ayyangar and Rao, 1989; Dixit and Saxena, 1990; Rajapakse, 1996; Chander *et al.*, 2000). The frequency of application or residual efficacy of the natural products applied to the stored grains, however, has not been thoroughly investigated. This is important because many of the plant products act as repellent, mild insecticides or chemosterilants.

The present study has been undertaken to investigate in detail, the efficacy of extracts of certain locally available plants for their insecticidal, oviposition deterrent, repellent and ovicidal effects against the pulse beetle, *C. chinensis*. Though much work has been done in the laboratory and in the field on these aspects, very little has been done in the understanding of the mechanism of action,

isolation and identification of active compounds responsible for biological effects. In the present study, attempts are made to isolate and identify the effective components extracted from the most effective plant from among eight plants screened.

5. Objectives of the present investigation

This study has the following objectives:

- to test the extracts of a few local plants for their effects on the stored grain pest, *C. chinensis*
- to find out the most efficient plant as the control agent
- to estimate the efficiency of the plant extracts (by quantitative studies)
- to separate the active components of the plants using suitable biochemical techniques and
- to study the chemical nature of the active components.

Chapter II

REVIEW OF LITERATURE

1. Introduction

The ultimate practical objective of insect control is to lessen the extent of insect damage to man's possessions or health by the suppression or prevention of insect outbreaks. From the records of efforts and successes during a century of the practice of economic entomology, it seems highly unlikely that it will be possible to eradicate or exterminate any species of insects. Though many major pests have been brought down to minor status by the use of chemicals, cultural practices and sterilization by radiation and chemicals, constant and massive use of synthetic pesticides in certain crops has led to the development of resistant strains of insects. There have been several reports of insecticides inducing the production of detoxifying enzymes that contribute to the development of resistance in insects (Nigam and Misra, 1998).

Like pest management in field crops, insect pest management in stored grains also involve the use of synthetic insecticides, bio-control agents, sex pheromones etc. For stored grains, there is also the possibility of using fumigants and manipulating storage atmosphere with relatively more ease. Practically none of these approaches have individually been proved satisfactory to overcome the problem of insects in stored grains. The necessity of employing various compatible approaches in an integrated manner is now being increasingly felt (Prakash *et al.*, 1987 a).

Grains treated with emulsifiable concentrate of synthetic insecticides protect them from insect attack for varying periods of time. The effective dose of different insecticides tested/ recommended varies greatly with type of grains, type of storage structures, type of insect pests and storage period. Effective concentration of the insecticide like malathion which is considered as a safe grain protectant has to be constantly increased because of the problem of resistance. Presently, the concentration that proves effective in controlling insect pests in stored commodities are too high and may lead to health hazards. It is therefore, desirable to use synthetic insecticides as direct grain protectants exclusively on grains that are used only as seeds (Prakash *et al.*, 1987 b).

Phyto-products are promising alternatives to synthetic insecticides for protection of seed grains during storage. Mixing of dried neem leaves with stored grains and keeping them between folds of clothes to ward off insects are rather well known practices still followed in many developing countries. In Sri Lanka, farmers burn neem leaves to generate smoke for fumigation of stored paddy to kill insects. Plant products being liberally available, these indigenous sources of insecticides and insect repellents have been in use for more than a century in India. The insecticidal property of plants is not very quick as compared to that of synthetic insecticides and fumigants. A number of plant products have been in use against insect pests in stored grains including rice to minimize storage losses due to insects. However, only a few products are generally adopted at the farmers level (Prakash *et al.*, 1986). Majority of farmers are not fully aware of the utility of plant products for the purpose of grain protection.

Prakash *et al.* (1981) reviewed the literature on insect pest management in stored grains using plant products and conclusively emphasized the exploration of active principles of plant products showing promising grain protection against insect infestation. A number of active principles/ components have been isolated, identified and successfully evaluated against storage insects. Most of them have been found to be very effective (Urs, 1990).

Present review covers different storage pests with special emphasis on *Callosobruchus* sp. and role of various plants as repellent, feeding deterrent, oviposition deterrent and ovicidal agents to control these pests. This review also stresses on secondary metabolites, their activity and role in pest management.

2. Stored grain pests

Many factors are involved in the deterioration of produce after harvest. The composition and behavioural characteristics (internal forces) of food grains vary, and grains are constantly being exposed to external forces including physical factors such as temperature and humidity, chemical factors such as oxygen supply, biological agencies such as bacteria, fungi, insects, mites, rodents and man with his method of handling, storing, transporting and disinfesting products.

Biological agents of deterioration differ in their rate of development and ability to cause damage under different conditions of temperature and moisture. Golumbic (1965) has estimated that annual losses due to microorganisms in stored commodities run to millions of dollars. The importance of fungal attack depends on the use to which the produce will be put, mould contamination of cocoa and

coffee imparts a tainted flavour; mould on barley affect malting; mould on wheat can reduce germination; and mould in palm oil and copra can accelerate the liberation of free fatty acids. During growth some fungi produce chemicals which can be toxic to man and/or domestic animals. *Aspergillus flavus*, produce aflatoxin on groundnuts, is a recently well-documented example.

A number of mites associated with stored products are important pests. Under suitable conditions of temperature and moisture, they multiply rapidly to form dense populations that can cause serious damage or loss. Some mites are not known to feed upon stored food and therefore are not considered to be pests. Many, however, are predacious upon stored product mites and upon the eggs and young stages of certain insect pests; others are known to feed on moulds. At present, it is advocated that inspection of stored products should include examination for, and recording of mites. They can serve as a valuable guide to the condition of the stored material since their presence usually means that the product is too moist for prolonged storage (Hall, 1970).

There are thousands of species of insects associated directly or indirectly with stored grains and grain products. Of these, some cause enormous loss and are mainly responsible for most of the damage to grain in farm, transportation and storage. In the tropics, beetles and moths are the main insect pests causing loss and deterioration of stored food grains. Cockroaches, termites and ants are also troublesome in some circumstances. As a result of the feeding activities, the quality of the grain is lowered, germination is reduced or abnormalities occur during germination. The effect of insect attack on germination of dicotyledonous

seeds such as pulses is greater than that on the germination of monocotyledon seeds such as maize. Pingale (1953) has recorded that green gram when holed by bruchid beetles failed to germinate, while wheat damaged by weevils, germinated. Insects do not breed successfully in an environment where the relative humidity is maintained below 40% and temperature below 10°C. Under normal conditions of temperature and relative humidity, insects breed very quickly, the life cycle from egg to adult being completed in a few weeks with each adult female insect laying large number of eggs.

In paddy storage, *Sitotroga cerealella* and *S. oryzae* were placed at the top of the list of major insect pests (Douglas, 1925; 1941). *S. cerealella* infest the grains in both storage and field conditions. Prevett (1959) noted that significant infestation of paddy by this species was possible only in the early stages of drying of the grains. Breese (1961) considered losses caused by *S. cerealella* to be less in comparison with those due to *Rhizopertha dominica* and *S. oryzae*.

Tribolium castaneum was found to be the most abundant of the minor pests in most of the paddy as well as in milled grain samples and its infestation could start only after infestation by *R. dominica* and *S. oryzae* (Prakash *et al.*, 1984). However, it is found to be the number one pest of powdered rice or suji and its products. El-Kashlan *et al.* (1997) reported that wheat flour infested with *T. confusum, T. castaneum* and *Tenebrio molitor* were toxic to mice feeding on it. Morphological and histopathological studies indicated that toxic symptoms were probably due to the defensive secretions containing benzoquinone compounds discharged by the insects. Lale and Yusuf (2000) conducted studies on the insect

pests, *T. castaneum, Cryptolestes ferrugineus* and *Liposcelis bostrychophilus* infesting stored pearl millet and their damage potential. In a survey of stored grain lots, the most commonly encountered pest was *Cryptolestes* species, which often occurred in large populations. Less frequent and with small population size were *R. dominica* and *S. oryzae* (Hamel *et al.*, 2000).

Seck (1993) described the biology and damage caused by *C. maculatus* on stored cowpea and *Sitophilus zeamais, S. oryzae, R. dominica, T. castaneum, Sitotroga cerealella, Ephestia cautella* and *Corcyra cephalonica* on stored cereals. He also described the various control measures such as the use of insecticides, use of neem extracts and biological control.

According to Osuji (1975), *Dermestes maculatus* and *Necrobia rufipes* are the dominant pests on dried fish. The study showed that the techniques adopted in processing and handling, and the conditions of warehouse storage and produce evacuation were important contributory factors in the initiation and subsequent propagation of Dermestid and Clerid infestation.

3. The pulse beetle, *Callosobruchus* species

Beetles of the family Bruchidae are important pests of stored pulses throughout the world. In India *C. maculatus* and *C. chinensis* are the most serious pests of pulses (Lefroy, 1909; Fletcher, 1916; Pruthi and Singh, 1950). Severe losses due to these pests have been reported in several tropical and sub tropical countries. Many insecticides and storage containers have been found effective

for protecting cowpeas from the cowpea weevil (Singh and Benazet, 1974) but at the farm level such inputs are expensive or difficult to obtain.

The life cycle of *C. chinensis* have been studied by many workers in pulse crops. Being cosmopolitan in distribution, the beetle is reported to exhibit polymorphism (Utidas, 1980) and multivoltinism (Southgate, 1980). All varieties of pulses have been reported to be infested by this beetle (Singh *et al.*, 1980). Adults were found to mate within an hour of their emergence from the seeds. Kunhikannan (1919) reported that the adults prefer to come on surface layer of grains in order to get free area for mating. Egg laying by female started from 7-8 hours after mating. Pandey and Singh (1997 b) observed that maximum eggs are laid within the first 24 h and the number gradually dropped till the last day of oviposition. Howe and Currie (1964) noted an average of 45 eggs with a range of 20-64 at optimum conditions (30 ± 2°C and 70 ± 5% RH). Rajak and Pandey (1965) reported a range of 50-103 and Takasugi (1924) between 70-80 eggs at the same atmospheric conditions.

Mourad and Zaghloul (1997) studied the effect of some environmental factors on *C. chinensis* infesting mung bean in Egypt. According to their report, egg parasitism, egg disappearance and infertility were the limiting factors for the development of *C. Chinensis.* Sex pheromone production by *C. maculatus* was studied by Qiang *et al.* (1996). This study revealed that sex pheromone production increased immediately after the adult emergence up to 3rd day of adult life, and then decreased, as female grew older. Mating induced a reduction in post-coital sex pheromone production. Mated females, however, regained

attractiveness to males and this attractiveness increased from 1st day to 3rd day. The role of chemical cues in host finding and acceptance by *C. chinensis* was reported by Ignacimuthu *et al.* (2000). The olfactometer assays revealed that weevils discriminate between seeds containing different stages of developing bruchids on the basis of olfactory cues. The results indicate that *C. chinensis* female use chemical information during both host searching and host acceptance. Volatiles from un-infested or egg carrying seeds act as attractants, while deterrent effect increases as development of bruchids progresses.

Utida (1954) made observation on the polymorphism of *C. maculatus.* There are two adult forms called non-flight and flight forms, normal form and active forms (Caswell, 1960). The active form laid very few eggs and they differ from normal form in their body weight, water content of their body and in the quantity and nature of their fats. When the rearing temperature was kept high or changed to a high temperature at a certain definite larval stage, the percentage of active form in the emerging adult was high, even in the absence of crowding (Sano, 1967; Utida, 1972). The developmental rates and corresponding changes in the biomass of life stages of *C. maculatus* were studied with respect to the functions of food type and temperature (Chandrakantha and Mathavan, 1986). The growth rate of *C. maculatus* was faster when reared on stored seeds of *Vigna unguiculata* (cowpea) followed by *V. radiata* (green gram) and *Dolichos lablab* (bean). Credland and Dick (1987) reported that females always consumed more food than males.

Observations by Credland *et al.* (1986) and Giga and Smith (1991) indicated that crowding and competition in *C. maculatus* reduce survival and fitness of individuals. On the other hand, observations of Ofuya and Reichmuth (1994) revealed that larval and pupal mortality was generally higher in seeds with low infestation than in seeds with high infestation.

Generally, infestation of legumes by *Callosobruchus* occurs both in the field and during storage. In the field, infestation is characterised by its insidious nature (Taylor, 1981). Eggs are usually glued to the maturing or drying pods, the young first instar larvae bore into the seeds and at threshing, seeds either show slight or no apparent damage (Booker, 1967; Caswell, 1968; Southgate, 1978). Although, infestation and damage in the field is generally low, such infestation has serious implications. This is because the insects multiply very rapidly, with very high consequent damage, once the infested seeds are stored (Taylor, 1981).

4. Pesticides used in stored grains

There are two main types of chemicals used in the control of insect pests of stored products. They are contact insecticides and respiratory poisons or fumigants. A contact insecticide is a poison, which is able to penetrate the insect cuticle and thereby enter the body tissues. A fumigant is a gas or vapour, which is taken into the body of the insect through its respiratory system. Contact insecticides can confer long-term protection (usually referred to as the residual effect), but often tend to be somewhat specific in their effect upon insect species and to produce more resistance than the respiratory poisons. Fumigants provide no residual effect, but unlike contact insecticides, have the power to penetrate

throughout stacks or bulks and to become absorbed into individual grains or kernels, killing all stages of insect life within (Page and Lubatti, 1963).

Among the contact insecticides, pyrethrum is the best-known natural insecticide, the important constituents of which are known as pyrethrins. Their toxicity to insect can be greatly increased through addition of a synergist, such as piperonyl butoxide. Pyrethrum is virtually non-toxic to man and is therefore very safe to be used on food. The efficacy of seed treatment with five synthetic pyrethroids along with malathion was studied against *C. maculatus* (Patil *et al.*, 1994) on pigeon pea seed at 4, 8 and 12 weeks after treatment. Decamethrin, fenvalerate and cyfloxylate were the most effective insecticides, recording higher adult mortality, lower egg laying and adult emergence. Permethrin, cypermethrin or fenvalerate resulted in high mortality against *C. chinensis* (Gupta *et al.*, 1995 a) and deltamethrin is found to be most toxic to *C. maculatus* and *C. chinensis* than cypermethrin, permethrin and fenvalerate (Ramzan, 1995). Rahman and Yadav (1987) suggested that toxicity due to dust was higher than that with solutions. Most of the insect species were initially susceptible to all the insecticides, but mortality declined with time (Chakanyaka and Giga, 1993).

Pathak and Jha (1999) tested four insecticides namely deltamethrin, chloropyrifos-methyl, etrimfos and malathion against *Sitophilus* sp. and *Sitotroga cerealella* and found that deltamethrin was the most toxic insecticide. Bioactivity and residual toxicity of deltamethrin, mixed with rice bran and rice husk when used were reported by Usharani (1996). Food grains mixed with these chemicals were offered to the pest insects like *T. castaneum, S. oryzae, R. dominica,*

C. maculatus and *C. cephalonica.* Hundred per cent mortality was reported. It was also noted that a single application of deltamethrin prevented insect infestation for a period up to 20 months.

Combined action of methyl quinone, aggregation pheromone and pirimiphos methyl on *T. castaneum* larvae was studied by Mondal (1993). Results showed that methyl quinone acted as a synergist, increasing larval mortality. When food grains were treated with sodium tetraborate and boric acid, a significant reduction in oviposition and adult emergence of *C. analis* was noticed (Khan *et al.,* 1998). The relative toxicity of seven fumigants on the four life cycle stages of *C. chinensis* were studied by Adu and Muthu (1985). The results revealed that phosphine followed by methyl iodide were the most toxic to the insect stages, while methyl formate and ethyl formate were the least potent. The general trend of susceptibility of life cycle stages of the insect to the fumigant was egg > adults > larva > pupa.

The organochlorines, DDT and BHC were used prior to 1954 as grain protectant but gradually malathion was adopted as prophylactic spray at 50 mg/m^2. The continuous use of this resulted in a gradual rise in the dose up to 150mg $/m^2$ to reach the level of effective kill (Yadav, 1987). Larvae of *T. granarium, C. maculatus, Lasioderma serricorne* and *S. oryzae* among beetles and *E. cautella* and *C. cephalonica* from moths were found least susceptible to most of the insecticides.

Persistent toxicity of some insecticides namely malathion, DDVP (dichlorovos), methoxychlor, thanite, pyrethrin and pyrethrin with PBO

(piperonyl butoxide) were studied against *C. chinensis* by Dhari *et al.* (1977). They suggested that for long and safe storage of gram grains, more than 24 ppm dose of malathion and methoxychlor were sufficient. Pyrethrin alone and used with PBO, DDVP and thanite provide contact toxicity for a very short period. Singh and Yadava (2001) have reported that malathion and cypermethrin were highly effective against *C. maculatus*. Daglish *et al.* (1993) investigated the efficacy of chemical protectants viz., organophosphorus compounds, chloropyrifos-methyl, fenitrothion, methacrifos and pirimiphos-methyl, synthetic pyrethroids and insect growth regulators against *C. phaseoli* and *C. maculatus* in mung beans (*Vigna radiata*). It was found that during storage, the organophosphorus compounds lost activity against *C. phaseoli* much faster than synthetic pyrethroids and IGR. Malathion has been used as a contact insecticide in storage since 1954, but survey indicated that many strains of stored product insects have developed resistance to malathion (Champ and Dyte, 1976). Besides, it is less effective and require high dose against moth pests (Yadav, *et al.*, 1979). So pyrethroids are reported to be effective against stored product insects (Bengston, 1978).

Though, the mixing of insecticides with food grains is prohibited, according to the existing rules for prevention of adulteration in food by Ministry of Health, Government of India, insecticides may be utilized to save the grain for sowing purpose and for long term storage (Dhari *et al.*, 1977). The direct mixing of persistent and toxic dusts of organochlorine and organophosphorus insecticides with seeds may prove hazardous during handling and many storage pests have

developed resistance against these insecticides (Dyte, 1974). This stresses the need to develop safe and potent substitutes against storage pests.

5. Role of botanicals in stored grain pest management

During the development of organochlorine, organophosphorus and carbamate insecticides, the toxicological studies conducted were restricted to the immediate mammalian toxicity, which was found to be within safe limits. However, the indiscriminate long-term use resulted in bio-accumulation (because of their non degradability) and have caused serious health hazards to human beings and have upset the ecosystem. Moreover, it has been observed that insects have developed resistance to a number of these chemicals (Ayyangar and Nagasampagi, 1990). This has prompted the scientists to turn towards nature and a search for safer insect control agents from botanicals, i.e., plant products all over the world.

During the co-evolution of plants and insects, plants have biosynthesized a number of secondary metabolites to serve as defence chemicals against insect attack. These defence chemicals may serve as insecticides, antifeedants, oviposition deterrents, growth inhibitors, juvenile hormone mimics, anti-juvenile hormones, moulting hormones, anti-moulting hormones, attractants and repellents. This understanding of co-evolution by chemists and biologists has been responsible for evolving a new strategy that envisages the use of plant defence chemicals against insects.

In India there are several plants known for their insecticidal property and are popularly used as pesticides. The most important and popular among these is neem, which has international recognition. Probably no plant is known to possess such a diverse biological activity as neem. Neem has been evaluated against 105 species of insects, 12 species of nematodes, and at least 9 species of fungi (Singh, 1990). Neem, unlike synthetic insecticides, has diverse behavioural and physiological effects on insects and these effects are governed by about 30 compounds. A majority of phyotochemical work has been conducted on plants of the genera *Azadirachta* and *Melia*. From these plants more than one hundred limonoid type triterpenes have been isolated many of which have insect growth regulatory or antifeedant activities. Though, the insect managing quality of neem was known to Indians since time immemorial, a breakthrough in its use was made at Indian Agricultural Research Institute, New Delhi, in 1960 and was subsequently confirmed under field conditions (Jotwani and Sircar, 1967; Butterworth and Morgan, 1971; Yadava and Bhatnagar, 1987). Several reports have appeared on the insecticidal, antifeedant, growth regulatory, oviposition deterring, anti-hormonal and anti-fertility activities of neem against a broad spectrum of insects. The above mentioned activities for the oil expelled from seeds and leaves, leaf extracts, seed extracts, neem cake, fruit extracts and various isolated compounds (*viz.*, azardirachtin, nimbin, nimbidin etc.) have been demonstrated. Feeding deterrency and repellency of neem oil (Saxena *et al.*, 1981); oviposition deterrency of neem seed kernel (Islam, 1984 and Ayyangar and Rao, 1989) and antifeedant property of neem leaf extract (Sosamma and Shiela, 1994) were reported on various agricultural pests.

Efficacy of neem products to prevent damage on stored grains were reported by various workers. Devi and Mohandas (1982) and Pereira (1983) reported that neem oil at 1% and 0.8% applied on red gram and cowpea respectively, acted as a good protectant against *C. chinensis*. Jhansi Rani (1984) evaluated the de-oiled neem kernel powder (DNKP) against *C. cephalonica* and *T. castaneum* of wheat flour. The study revealed that DNKP arrested the growth and development of larvae of both the insects. In laboratory trials, 1-2% powdered neem kernel mixed with wheat seed protected the grains against the rice weevil *S. oryzae, R. dominica* and *T. granarium* (Pruthi, 1937). Yadav (1973) reported that even after 12 months no progeny developed when *C. maculatus* and *C. chinensis* adults were released in lentil seeds treated with neem kernel extract. Egg laying by *C. chinensis* was prevented up to 8 months (Singal and Chauhan, 2000). Neem bark powder contains insect growth regulating and insecticidal materials that effectively control the increase of *C. chinensis* population (Pandey and Singh, 1997 a). Powder and ash from neem seed, seed shells and leaves were much lethal to *C. maculatus* (Buraimoh *et al.*, 2000).

Plants traditionally used in the tropics to protect the stored products from various insect pests had been reviewed by Golob and Webley (1980). Rice and wheat are traditionally mixed with 2% turmeric powder during storage in some Asian countries (Chatterjee, 1980). People in some parts of India and Pakistan apply turmeric powder along with mustard oil and salt to basmathi rice. This provides protection of rice against storage insect and also fungal infection (Jilani, 1985). *Ocimum suave, O. kilimandscharium* and *O. kenyense* are medicinal plants

widely grown in many parts of the tropics, especially in India and Africa (Kokwaro, 1976; Paton, 1991) which are used as effective protectant of maize and sorghum against the attack of *S. zeamais*, *R. dominica* and *S. cerealella* in storage (Bekele *et al.*, 1997). Prakash *et al.* (1990) have screened 20 plant products, which are indigenously available and known for possessing bio-pesticidal properties against *S. oryzae*. Among these, seven plant products were found to significantly reduce the adult population and loss of the grain caused by this weevil. Kardinan *et al.* (1996) have investigated the insecticidal activities of various plants against *C. analis*. Their study revealed that extract from *Chrysanthemum* flowers, *Pachyrhizus crosus* seeds and *A. squamosa* seed powder at 1% (w/w) concentration affected the mortality and oviposition of this insect. Keita *et al.* (2000) have screened various essential oils of different plant species (*Tagetus minuta, Hyptis suaveolens, Ocimum canum* and *O. basilicum*) against *C. maculatus*. The results suggested that plants of the genus *Ocimum* could be used as an alternative to synthetic insecticides.

The bulb of garlic exhibits many promising properties for control of stored product pests (Debkritaniya *et al.*, 1980). Insecticidal activity of garlic oil was tested against the Khapra beetle, *Trogoderma granarium* by Bhatnagar *et al.* (1974); it exhibited repellency on *C. chinensis* (Pandey *et al.*, 1976) and *S. oryzae* (Chatterjee, *et al.*, 1980).

Lale (1993) studied the biological effects of three essential oils such as citronella, clove and lemon on *C. maculatus*. The volatile essential oil of *Murraya koenigii* leaves was evaluated for its contact and fumigant toxicity

against *C. chinensis* (Pathak *et al.*, 1997). Gakuru and Foua-Bi (1996) compared the effect of essential oils of four plants against cowpea weevil, *C. maculatus* and rice weevil *S. oryzae*. Results proved that essential oils had no effect on *S. oryzae*, however, the essential oils of *Eucalyptus citriodora* and *O. basilicum* were more potent against *C. maculatus*. Insecticidal, larvicidal and antibiotic effects of essential oils from clove (*Syzygium aromaticum*), West African black pepper, (*Piper guineenze*) and ginger (*Zingiber officinale*) were evaluated against *T. castaneum* (Lale and Ajayi, 2000). Their study revealed that clove oil was significantly more toxic to adults and larvae of *T. castaneum* than other oils tested. The period of exposure appeared to be the most important factor to determine the efficiency of extracts rather than dosage (El- Nahal *et al.*, 1989). Similar results were reported by Su (1991) for the contact toxicity of *A. calamus* to adults of *C. maculatus*, *S. oryzae* and *Lasioderma serricorne* and adults and eggs of *C. phaseoli* by Rahman and Schmidt (1999).

Pacheco *et al.* (1995) evaluated the efficacy of soybean and castor oil in the control of *C. maculatus* and *C. phaseoli*. Both oils inhibited population growth of the two insect species as compared to untreated seeds. Castor oil was more effective than soybean oil. No harmful effect was observed on the germination of oil treated seeds. Ali *et al.* (1983) reported that seeds treated with neem, coconut, mahua, sesame and palm oil did not permit adult beetles (*C. maculatus*) to lay eggs and thus inhibited the development of subsequent population. Seed oils of *Cassia occidentalis* induced high mortality of bruchid eggs and first instar larvae than the fresh and dry leaves or ground seeds (Lienard *et al.*, 1993). Several fatty

acids (linoleic, oleic and stearic) present in the oil were responsible for this toxicity. Gupta *et al.* (2000) evaluated the efficacy of different vegetable oils such as castor, mustard, linseed, soybean, coconut, groundnut and sesame against *S. oryzae.* It was observed that all the oils afforded protection over a period of around 120 days. Among these, mustard and linseed oils were significantly superior in comparison to other oils. According to Shaaya *et al.* (1997), edible oils are potential control agents against stored grain pests like *C. maculatus, S. zeamais, S. oryzae* and *S. cerealella* on the farm level itself.

Richa *et al.* (1995) have evaluated the effectiveness of essential oils of some plants (basil, geranium, rue, lemon grass, citronella, eucalyptus and lemon) in protecting faba beans from *C. chinensis.* According to their findings, essential oils of basil and geranium had the greatest insecticidal effect, while oils of lemon grass and eucalyptus were not toxic to adults but showed some effect on oviposition. Insecticidal effect of volatile oils of *Lippia adoensis, Cymbopogon citratus, Lantana camara* and *Chromolaena odorata* against *C. maculatus* was studied by Gbolade and Adebaye *(1995).* Essential oils of *Diplophium africanum* exhibited insecticidal activity against *C. maculatus* and *C. subinnotatus* (Koumagle *et al.*, 1995).

Mbata and Ekpendu (1993) investigated the insecticidal action of four botanicals against *C. maculatus, C. subinnotatus* and *S. oryzae.* Among the four plants tested, *Piper guineense* was found effective in causing high adult mortality of three beetle sp. even at low concentrations. Effectiveness of two indigenous plant extracts, *Calotropis gigantea* and *Ipomoea nil* were examined for their

toxicity against the adults of *S. oryzae*, *T. castaneum* and *Cryptolestes ferrugineus* (Perveen *et al.*, 2000). *Capsicum* powder and extract cause toxic effect on *C. maculatus* (El-Lakwah *et al.*, 1996). According to their findings, insect mortality was found to increase with increasing concentration of the extracts used.

Usharani and Jamil (1989) tested the effectiveness of petroleum ether extract of *Eichhornia crassipes* on the biological activities of *T. castaneum, S. oryzae, C. maculatus* and *C. cephalonica* and observed the cuticle melanization in *C. cephalonica* larvae and high mortality in *C. maculatus.* Some plant extracts inhibit egg laying and progeny development for a long period of time. *A. calamus* rhizome powder inhibit the egg laying and progeny development of *S. oryzae* and *T. castaneum* in stored milled rice for about 3-6 months (Chander *et al.*, 1990). Karanj oil and neem oil have been found to prevent oviposition of *C. chinensis* up to 100 days (Khair *et al.*, 1992). Kamakshi *et al.* (2000) also supported this. When the leaf extracts of five edible plants (*O. sanctum, Sesbania grandiflora, Mentha arvensis, Murraya koenigii* and *Coriandrum sativum*) were tested against *C. maculates*, it was found that leaf extract of *M. arvensis, S. grandiflora* and *O. sanctum* treatment reduced the number of eggs laid, adult emergence and loss of seed weight of cowpea than control. 'Embelin' isolated from berries of *Embetia ribes* acts as an efficient grain protectant against *C. cephalonica* and *Ephestia cautella* larvae (Chander and Ahmed, 1987).

Don-Pedro (1989 a) suggested the possibility of using oils in combination with synthetic insecticides in simple mixtures making their use more attractive and effective. Plant oils (coconut, sunflower, sesame and mustard) in combination

with 1,8 cineole, eugenol or camphor when applied on *T. castaneum, S. granarius* and *Prostephanus truncatus* resulted in significant mortality compared with untreated grain (Ofori and Reichmuth, 1999). Likewise, Mohamed (1996) have studied the effectiveness of *Datura* leaf extracts and their mixtures with malathion against the cowpea beetle, *C. maculatus*. It was proved that mixtures of plant extracts plus malathion exhibited a synergistic effect at higher concentration and an additive effect at low concentration. 'Asaron' (obtained from the rhizome of *A. calamus*) and its dichlorocyclopropyl analogues and three alkyl oxime ethers were tested as insecticides against eggs and adults of *C. chinensis*. The results showed that all derivatives were toxic to eggs and the adult fecundity was reduced to about 20-60 % (Pajni *et al.*, 1996).

Dhingra (1996) and Murugappan *et al.* (1998) demonstrated the synergistic effect of different vegetable oils with synthetic insecticides on the blister beetle, *Mylabris pustulata.* Their study revealed that mortality ratio was higher in mixed formulation than insecticide alone. A higher degree of synergism was obtained with sumicidin and neem leaf extract. Sridevi and Dhingra (1999) conducted experiment with four non-toxic vegetable oils, viz., sesame oil, karanj, neem and citronella, in combination with piperonyl butoxide and deltamethrin against the adults of susceptible (S) and resistant (R) strains of *T. castaneum*. All vegetable oils were proved additive when combined with deltamethrin except neem oil which showed antagonistic effect against the S-strain of *T. castaneum*. Irrespective of the method of application, karanj and piperonyl butoxide exhibited synergism.

Ulrich and Mewis (2000) proposed a method for controlling the stored product pests, *S. oryzae* and *T. castaneum* by mixing the rice with neem and diatomaceous earth. This treatment was more effective than the single treatment in reducing the number of surviving beetles. Neem leaf powder and cob ash were tested to find out their efficacy against *R. dominica* on stored maize (Sharma, 1995). Both the test compounds at different dosages were effective in reducing the per cent of grain damage compared to control. A similar result was obtained when cow dung ash was applied on green gram against *C. chinensis* (Chiranjeevi, 1991).

Botanical pesticides are also used to prevent the agricultural and other field pest populations. For example, leaf extracts of *A. indica, Vitex negundo, Pongamia glabra* and *Calotropis gigantia* were effectively used against the life stages of the reduviid predator, *Rhynocoris marginatus* (Sahayaraj and Paulraj, 1999). Similarly, *Uvaria narum* and *U. hookeri* extracts in ethyl acetate were used to control the sweet potato weevl, *Cylas formicarius* (Padmaja *et al.,* 1995). Hiremath *et al.* (1996) studied the insecticidal activity of methanol extracts of 49 Indian plants against *Nilaparvata lugans*. Extracts from the root of *Eclipta alba* and leaves of *A. indica* were also found to cause high mortality of brown plant hopper (Rao and Rao, 1979). Sharma and Srivastava (1998) have evaluated the efficacy of some plant extract (*Artemisia annua, Carica papaya, Cuscuta reflexa* and *Lantana indica*) against *Culex* larvae. Their findings showed that *C. reflexa* was the most toxic to *Culex* larvae irrespective of the exposure period, followed by the extract of *A. annua, C. papaya* and *L. indica.* Dose-dependent larvicidal

and pupicidal activities of *A. squamosa* was reported by Mehra and Hirdhar (2000) on *Culex quinquefasciatus*. Their results showed that 80-84% death occurred in 3rd instar larvae and pupae within 24 h after application.

6. Botanicals as repellents

Dethier (1956) has defined repellents as 'those substances, which elicit avoiding reactions'. These responses are behavioural and arise through the stimulation of chemoreceptors, olfactory receptors and gustatory receptors. Repellents are usually volatile chemicals that express their activity in the vapour phase. A strong repellent will be sensed by insects a few centimeters distance and causing them to fly or crawl away. Less active repellents may allow insects to alight or touch the surface before being repelled. The advantages of using repellents are two fold. They often have low toxicity and therefore can be used safely on humans, plants and domestic animals. Insect repellent based on natural substances such as wood smoke, oils, pitches, tars, various earths and camel urine were used by humans hundred years ago to ward off insects and other arthropods. Repellent for human possessions include chemicals to protect fabrics and wood products (Pedigo, 2002).

The products of plant origin or botanical insecticides as they are called, are being used as insect repellents in our country from time immemorial. Dried and powdered leaves of neem against wool moths, *Calotropis* against white ants and turmeric against mosquitoes are some of the classical examples. Several plant constituents with strong pungent odour make them unattractive or unpalatable or offensive. Essential oils of citronella, camphor and cedar wood oil and other plant

compounds like terpenes, quinines, phenol etc. repel insects away from host plants and thus protect them (Rao, 1990).

In the folk tradition, the use of plants, plant extracts and other substances for pest control was rampant because, they were strong smelling, odoriferous and pungent. Earlier workers have reported the repellent action of neem leaves, cake and seed powder against the insect pests of stored wheat (Pruthi, 1937; Jotwani and Sircar, 1965; 1967). The insect repellent and antifeedant action of neem has been attributed to the triterpenoid azadirachtin and other related compounds (Butterworth and Morgan, 1968; Nakanishi, 1975; Zanno *et al.*, 1975). Ant repellent property of turmeric was reported by Murthy and Murthy (1959) and Watt and Brandwijk, (1962). Ether extracts of custard apple, (*A. squamosa*) and neem, exhibited gustatory repellency against *S. oryzae* and it also had repellent effect on *S. oryzae* and *T. castaneum* (Quadri, 1973). Plant oils obtained from cotton seeds, soybean, maize and peanuts act as very good repellents against *S. granarius* (Yun and Burkholder, 1981). Jilani and Su (1983) have demonstrated the repellent effect of turmeric (*Curcuma longa*), neem (*A. indica*) and fenugreek (*Trigonella foenumgracum*) against three species of stored product insects, *T. castaneum, R. dominica* and *S. granarius*. Results showed that turmeric powder was the most effective against *S. granarius* and *R. dominica* while only solvent extract was effective against *T. castaneum*.

Pandey *et al.* (1976) reported that petroleum ether extract of garlic repels *C. chinensis*. Similarly, the bulb of garlic exhibited strong repellent property against many stored product pests (Deb-Kritaniya *et al.*, 1980); chopped garlic

and ethyl acetate extracts of garlic are highly repellent to *T. castaneum* and *S. zeamais* (Ho *et al.*, 1996). Repellent and growth-inhibitory effects of turmeric oil, sweet flag oil, neem oil and Margosan-O on red flour beetle, *T. castaneum* were reported by Jilani *et al.* (1988 b). Their report indicated that repellency increased with increasing concentration of the oils and Margosan.

Singh and Singh (1991) screened 31 essential oils of plant origin for repellent and insecticidal properties against the housefly, *Musca domestica.* The essential oils obtained from *Ocimum gratissimum, Thymus serpyllum, Illicium verum, Myristica fragrans,* and *Curcuma amada* showed 100% repellent activity, and *A. calamus* showed 40% activity. Biochemical studies of these promising oils may emerge as new leads in developing future pesticides. Malik and Naqvi (1984) have screened seven plant species for their repellent activity against *T. castaneum* and antifeedant activity against *R. dominica.* The best repellent activity was for the rhizomes of *Saussurea lappa* and antifeedant activity for the leaves of *Chenopodium ambrosioides* and for azadirachtin isolated from neem kernel.

Repellent, toxic and food protectant effect of pithraj, *Aphanamixis polystachya* extracts against the pulse beetle, *C. chinensis* was studied by Talukder and Howse (1995). Their study showed that seed extract had poor repellent effect, while it had high contact toxicity to adults at 72 h after application. Eucalyptus leaf extracts were tested for their effects on adult *C. chinensis* and *S. oryzae* by Khan and Shajahan (1998). It was found that *S. oryzae* was repelled and *C. chinensis* was attracted by these extracts. Behal

(1998) have screened 12 plant oils for their repellent effect against the rice moth, *C. cephalonica*. Study showed that there was a complete repellency for larvae with sweet flag, (*A. calamus*) oil irrespective of its concentration, while with other oils, a concentration dependent repellency was noticed. Petroleum ether extracts of *Cassia tora, C. fistula* and *C. articulata* seeds exhibited more than 80% repellency against *T. castaneum* (Pradeep and Radhakrishnan, 1999).

Recently, Chander *et al.* (2000) have conducted experiments to study the repellency of a few plant extracts (*A. calamus, Saussurea lappa, Murraya* sp., *Citrus deliciosa, Curcuma longa* and crude mustard oil), two commercial neem formulations (nimbicidin and repelin) and one synthetic pyrethroid (cypermethrin) at 1% level as bag treatments for the control of *T. castaneum*. Based on the insect count per 100 g grains, cypermethrin and repelin were found to be most effective. Treatment with mustard oil and *C. deliciosa* were the least effective. Egwunyenga *et al.* (1998) evaluated the repellent effect of *Dennettia tripetala* extracts on the larvae of the leather beetle, *Dermestes maculatus* and compared the effect with that of a pyrethrum standard. *Dennettia* seed powder showed higher repellency than pyrethrum. Acetone and ethanol extracts were good repellents to *D. maculatus* up to 4 weeks after treatment.

Recently, experiments conducted by Sahayaraj and Paulraj (2001) on *Helicoverpa armigera* by using various plant extracts, have shown that repellent property increased as the concentration of extracts increased. Similar observations have been made by Tripathi *et al.* (2000). When the essential oil of *Artemisia annua* was tested for its toxic and development inhibiting activities against

T. castaneum and *C. maculatus*, it was found that increase in dose caused decrease in survival and adult emergence of both the insects. A dose-dependent effect of sweet flag oil, sowa oil, clove oil and cedar wood oil along with citronella and eucalyptus at higher concentration (5%) significantly repelled the rice moth *C. cephalonica* (Behal, 1998). Jilani *et al.* (1988 b) also obtained similar results. Sahayraj and Paulraj (2000) observed that *Spodoptera litura* larva was repelled by groundnut leaves treated with *Tridax procumbens* leaf extract and the repellency increased as the concentration of leaf extract increased.

The per cent repellency was found to decrease over a long interval of time (Urs and Srilatha, 1990). When essential oil of eucalyptus was used against the rice weevil, *S. oryzae*, 80% repellency was shown after 10 min, 50% after 30 min and after 60 min it decreased to 20% (Ahmed and Eapen, 1986). Malik and Naqvi (1984) also reported a similar time-dependent decrease of repellent property in the case of *T. castaneum*. According to Jilani and Su (1983) this type of decrease of repellency with time is due to the loss of some highly volatile unstable active components in the extracts.

7. Botanicals as antifeedants

Plant derivatives possessing insect repellent and antifeedant properties offer a class of compounds that could be employed in a novel approach in the management of crop and stored grain insect pests and insect transmitted viral diseases. Antifeedants are chemicals, which prevent insects from feeding on grains or other products. This results in their starvation and subsequent death. Efficacy of insect repellents and antifeedants can be enhanced by improved

extraction procedures, use of additives, synergists, other botanicals, better timing and improved application methods (Saxena, 1987).

One of the important sources of insect feeding deterrent is the neem tree. A mention has already been made about the age-old practice of protecting clothes and grains by neem leaves. Azadirachtin isolated from *A. indica* is found suppressing appetite, besides disrupting growth and upsetting mating in dreaded pests like locusts, bollworms etc. (Rao, 1990). The antifeedant potency of NSKE (neem seed kernel extract) can be significantly improved when combined with sesame oil at 4 and 6% concentrations (Rajasekharan and Kumaraswami, 1985). Kulkarni *et al.* (1996) have tested the bioactivity of neem against the 5th instar larvae of *Eutectona machaeralis.* They found that methanolic extract of neem seed was very effective as antifeedant at 2 and 1% concentrations. Antifeedant property of azadirachtin against *Spodoptera litura* were reported by Meisner *et al.* (1981) and Raffa (1987) and neem seed kernel suspension against *S. litura* (Joshi *et al.*, 1984). Jayarajan *et al.* (1990) reported the antifeedant property of azadirachtin rich fraction against *S. litura.* Gujar and Mehrotra (1985) have reported the effect of neem seed oil on consumption, digestion and utilization of maize leaves by 5th instar nymphs of the desert locust, *Schistocerca gregaria.* Sosamma and Sheila (1994) conducted studies on the antifeedant activity of datura *(Datura alba),* neem *(A. indica)* AK plant *(Calotropis procera)* and Croften weed (*Chromolaena odoratum*) against the leaf caterpillar *Selepa docilis.* Out of the four plants tested for antifeedant property against *P. ricini*, neem ranked first followed by *Calotropis.*

Investigations on the phago-deterrent effect of various plants other than neem on insects have also been carried out. Earlier, Banerji *et al.* (1982) have reported the feeding deterrent efficacy of six botanical extracts (*Derris indica, A. calamus, Cyperus rotundus, Abelmoschus moschatus, Actinodaphne hookerii* and *Allium sativum*) against mustard saw fly *Athalia proxima.* Of these, *A. calamus* and *A. sativum* showed 100% protection compared to the control. Feeding deterrent and growth inhibitory effect of calamus oil on *S. litura* was reported by Koul (1987). Due to the antifeedant effect of this plant (*A. calamus*) the larval and adult period of *T. castaneum* was extended for several months (Chandel *et al.*, 2000). Koul and Isman (1990) noticed that essential oils of *A. calamus* decreased food consumption and larval growth in a dose-dependent manner in 1st and 2nd instar larvae of *Peridroma saucia.*

Antifeedant properties of *A. squamosa, Argemone mexicana, Calotropis gigantea* and *Ricinus communis* against 2nd instar grubs of *Henosepilachna vigintioctopunctata* at different concentrations were tested by Rao *et al.* (1990 b). The study revealed that petroleum ether extract of plant products exhibited higher antifeedant activity than the aqueous extracts and among the aqueous extracts higher concentrations were more effective in their antifeedant activity against this grub. Similarly, Facknath and Kawal (1993) investigated the antifeedant and insecticidal effect of five plant extracts, namely *Argemone mexicana, Artemisia absinthium, Cassia occidentalis, Cymbopogon citratus* and *Siegesbekia orientalis*, against 3rd instar larvae of cabbage worm, *Crocidolomia binotalis.* On comparing

the activities of the extracts it was found that *A. mexicana* exhibited the maximum antifeedant effect followed by *C. citratus.*

Petroleum ether extract of three plants viz., *Ageratum conyzoides, L. camara* and *Eupatorium* were evaluated for their antifeedant action against brinjal hadda beetle, *H. vigintioctopunctata* (Mehta *et al.,* 1995). One per cent concentration of each of *Ageratum* and *Lantana* extracts gave complete protection to brinjal leaves from 1st, 2nd and 3rd instar grubs. In the case of adults, maximum antifeedant effect was exhibited by the extract of *Lantana* (1%) followed by *Eupatorium* (1%). According to Shah (1996), earlier larval instars are more susceptible to antifeedants than later larval instars. Tripathi and Singh (1994) have evaluated the antifeedant activity of 37 plants belonging to 21 families collected from different parts of India. Methanolic extracts of all these plants were tested for feeding deterrency against *Spilosoma obliqua,* of which eight plants were found to have antifeedant activity.

In addition, a large number of plant extracts and secondary metabolites have been screened for their antifeedant, repellent and insecticidal properties against stored product insects (Grainge and Ahmed, 1988; Arnason *et al.*, 1989; Jacobson, 1989). Antifeedant activity of nutmeg oil against *T. castaneum* and *Sitophilus zeamais* revealed that it could act as a valuable weapon against grain feeding insects, particularly against the destructive *S. zeamais* (Huang *et al.,* 1998). Nutritional studies showed that nutmeg oil significantly affected the growth rate and food consumption of both insect species depending on the

concentration used, but the antifeedant activity was more pronounced against *S. zeamais* than *T. castaneum.*

Urs and Srilatha (1990) observed the antifeedant and repellent properties of certain plant extracts viz. neem, mustard, datura, eucalyptus and lemon grass against the rice moth, *C. cephalonica.* Among these, the most effective antifeedant was neem followed by eucalyptus. Huang and Ho (1998) studied the toxicity and antifeedant activities of cinnamaldehyde against *T. castaneum* and *S. zeamais.* Antifeedant action was observed to increase with increasing cinnamaldehyde concentration. Koshiya and Ghelani (1990) made similar observations with the antifeedant activity of different plant derivatives against *S. litura* on groundnut. It was found that leaf and seed extract of neem and the seed extract of karanj showed a dose dependent antifeedant property against this insect. According to their findings, percent feeding decreased with increase in the concentration of the antifeedants.

Besides total extracts, fractions isolated from the extract were also tested for their antifeedant effect. Simmond *et al.* (1989) isolated clerodane diterpenoid compounds from *Teucrium,* whose antifeedant activity against larvae of *S. litura* and *Heliothis armigera* were tested. Isopongaflavone and tephrosin have been isolated from *Tephrosia elata,* which were shown to be very active antifeedant materials on *Maruca testulalis* and *Eldana saccharina* (Bentley *et al.,* 1987). However, Deshpande *et al.* (1990) stated that total extracts were more effective than fractions.

Torto *et al.* (1992) have isolated six amide alkaloids of *Piper guineense,* which were tested for the antifeedant activity against 5th instar larvae of *Chilo partellus.* The amide piperine and its di-hydro saturated derivatives exhibited the most potent antifeedant activity. Their results suggested that the presence of methylene dioxy benzene and salicylic amide group in the compound might be crucial for high antifeedant activity.

Many plants have thus been shown to possess chemicals exhibiting feeding deterrence against insects (Hedin *et al.*, 1974) and some plants also exhibited antifeedant and insecticidal activity (Srimannarayana and Rao, 1984). Antifeedant action can result from adverse effect on orientation/ attraction, biting and swallowing responses (Pradhan and Jotwani, 1968). An array of chemicals present in a plant act concertedly on both behavioural and physiological processes. Thus the use of natural antifeedants offers a harmonious approach to pest management (Saxena, 1983).

8. Botanicals as oviposition deterrents

The pulse beetle shows a definite intra-varietal response for oviposition (Chavan, *et al.,* 1997). The cowpea lines with rough seed surfaces were less preferred for oviposition, where the percentage of grains infested with eggs and the number of eggs laid per grain were lower compared to the lines with smooth grain surfaces. Lan *et al.* (1999) reported the effect of male interference on oviposition behaviour of the azuki bean weevil *C. chinensis.* It was found that females pairing with more males can cause reduction in both longevity and the total number of eggs laid. Therefore, excessive male solicitation behaviour can

cause interference, which can decrease female fitness. The adult females have the ability to distinguish their own oviposition markers and appear to ignore the oviposition markers deposited by other females in further oviposition on egg laden seeds (Wijeratne and Smith, 1998). Ofuya (1989) reported that the presence of larvae in grains affect the number of eggs laid and seed choice for oviposition by *C. maculatus*. Seeds bearing no larvae or few larvae were preferred for oviposition to those bearing greater number of larvae. In addition, seeds bearing younger larvae were preferred for oviposition to those bearing older larvae. Lale (1998) studied the effect of solar heat on oviposition, development and adult mortality of the cowpea bruchid, *C. maculatus* in the Nigerian savanna. Their study revealed that oviposition decreased with increased exposure of mated females to sun.

Extensive works have been undertaken to control the oviposition of stored grain pests by using plant materials. In earlier days, various oils have been used for the control of *Callosobruchus* species, which included neem oil (Sujatha and Punnaiah, 1985; Yadav, 1985 and Das, 1986). Oil dressing on cowpea seeds exhibited significant oviposition deterrency and complete inhibition of emergence of adult insects (Pereira, 1983). By evaluating the effectiveness of six vegetable oils as protectant of cowpea and Bambara groundnut against *C. maculatus*, it was found that out of the six oils, only neem oil reduced oviposition. Almost a similar result was obtained by Kachare *et al.* (1994), by treating the grains with neem, castor and karanj oils against bruchid attack. A significant repellent action on egg laying by adult beetles and suppression of egg hatching were the results.

According to Singh *et al.* (1994) seed treatment with oils normally delayed the development of the insects. Their study using some plant oils such as taramira, coconut, sunflower, safflower and castor were found to be effective to check the egg laying of the pulse beetle, *C. chinensis* on gram seeds. This result in tune with the results of Varma and Pandey, (1978); Rajapakse and Senanayake (1997); Sharma *et al.* (1999) and Raja *et al.* (2001). Study of Bhatnagar *et al.* (2001) revealed that neem oil has significantly higher repellent, oviposition deterrent and ovicidal effect against the pulse beetle of all the vegetable oils tested, all except neem oil lost their efficacy within 30 days after treatment.

Negi *et al.* (1997) observed that different concentrations of pongam oil (*Pongamia pinnata*) affected egg laying and adult emergence of pulse beetle, *C. chinensis* on green gram (*Vigna radiata*). Oils from *L. camara, C. citratus, Eugenia nerifolia* and *Lippia adoensis* were also found to be reducing or inhibiting oviposition and adult emergence in *C. chinensis* (Gbolade and Adebaye, 1995).

Dried leaf powder, and extracts are also potent oviposition deterrents of various stored pest. Rajapakse *et al.* (1998) found that dried leaf powders of *Piper nigrum, A. reticulata, A. indica, Capsicum annum* and *Citrus lemon* were very effective to inhibit the oviposition and adult emergence of *C. maculatus*. It was shown that *A. indica* caused significant reduction in oviposition (37.5%) and adult emergence (20.3%) of these insects after treatment on the 2nd day and 30th day respectively. Rajapakse *et al.* (1997) screened ten botanicals and four vegetable oils for their oviposition deterrent property against three bruchid

species, *C. chinensis*, *C. maculatus* and *C. rhodesianus*. The number of eggs laid was significantly reduced in treatement with powders of *C. citratus, Cinnamomum camphora, Derris inudata, Monodora myristica, Zingiber spectabile.*

Acetone extracts of various parts of eight indigenous plants, viz., leaves of *Tagetus, Ipomoea, Acacia, Lawsonia, Eucalyptus,* seeds and flowers of *Lantana,* flowers of *Bignonia* and fruits of *Cassia,* were tested for their oviposition deterrent properties against the rice moth, *C. cephalonica* (Dwivedi and Garg, 2000). Leaf extracts of *Tagetus, Ipomoea* and *Acacia* exhibited nearly 50% reduction in oviposition, while the remaining six treatments were less effective. Rouf *et al.* (1996) investigated the individual and combined effects of neem, *V. negundo* and *Poligonum hydropiper* against *C. chinensis*. Four grams of *P. hydropiper* seeds was most effective in reducing the oviposition and adult emergence, and it also reduced pest damage and seed weight loss. *V. negundo* leaf powder was effective in reducing the number of eggs laid, adult emergence and seed weight losses caused by *C. chinensis* (Prakash and Rao , 1989; Miah *et al.,* 1995). The effect of trona (sodium sesquicarbonate) solution on *C. maculatus* (Ofuya and Lagunja, 1998) was also similar.

Root bark powder and extracts of *Zanthoxylum zanthoxyloides* significantly reduced the reproductive fitness of *C. maculatus* (Ogunwolu *et al.,* 1998). Constituents of root bark powder such as terpenoid, phenolics and alkaloids significantly reduced oviposition. Similarly, powdered grape fruit peel and lime peel discouraged oviposition, suppressed emergence of the F_1 generation and subsequently reduced damage to cowpea seed by *C. maculatus* (Onu and

Sulyman, 1996). According to Mulatu and Cebremedhin (2000), *A. indica, Milletia ferruginea* and *Chrysanthemum cinearaefolium* were effective in preventing egg laying and adult emergence of *C. chinensis*. Tabassum *et al.* (1996) have investigated the effect of dimilin on inhibition of egg laying and adult emergence of *C. analis*.

Pandey *et al.* (1986) have reported the oviposition inhibiting effect of *A. indica* (leaves and twigs), *L. camara* (leaves and flowers), *Ageratum conyzoides* (leaves and flower buds), *Thevatia nerifolia* (leaves, twigs and branches) and *Ipomoea carenae* against *C. chinensis*. Their report suggested that all tested plants acted as very good repellent and oviposition deterrents against the insect.

In addition to these, various plants were used to discourage oviposition of field crop pest also. Neem derivatives such as neemark, repelin, wellagra, neem seed kernel suspension, neem rich and nicotine sulphate were found to repel *Chrysopa* from depositing their eggs on cotton (Yadav and Patel, 1990). Raju and Thakur (1995) observed the action of flower extract of *Butea monosperma* on the ovaries of *Dysdercus similis*. Methanol extracts of *B. monosperma* topically applied on 5^{th} instar larvae caused abnormalities in the ovaries. Compound egg chambers were observed in treated adults and the results were poor yolk deposition and unovulated batch of oocytes. At the highest concentration (4%) tested, there was a complete inhibition of oogenesis and vitellogenesis.

Ayyangar and Rao (1989) reported the oviposition deterrent and repellent effect of neem on *S. litura*. They showed that there was no egg laying in the area treated with methanol extract of neem for a period of 5 days. Rao *et al.* (1990 b)

noted that oviposition deterrency of all the botanicals tested was directly proportional to their concentration. Chiranjeevi (1991) and Kachare *et al.* (1994) also supported this. According to Pandey and Khan (1998 b), the decline in oviposition at higher doses was attributed to the interference of plant materials on vitellogenesis and severe damage caused to the egg chambers in the ovaries of *C. chinensis*. Dhar *et al.* (1996) reported that ovipositon seems to be regulated by the absorption of volatile extracts through the cuticle. Raju and Thakur (1995) observed the action of flower extract of *Butea monosperma* on the ovaries of *Dysdercus similis*. Methanol extract of *B. monosperma* when topically applied on 5th instar larvae, caused abnormalities in the ovaries. Compound egg chamber were observed mostly in the ovaries of treated adults. Poor yolk deposition and unovulated batch of oocytes were the major results. At the highest concentration (4%) there was a complete inhibition of oogenesis and vitellogenesis.

9. Role as growth inhibiting and ovicidal agent

In recent years there has been increasing interest in the development of alternative methods for insect pest control. One of the methods being explored is the use of chemicals related to insect hormones that cause disrupting effects on their life cycles (Williams, 1967). Such insect growth regulators have two major advantages over conventional insecticides; they are effective in very small quantities and are specifically active against target species. Therefore, extensive studies are being carried out to develop insect growth regulators from plant sources, which could be cheaper and readily available.

Williams (1967) espoused the concept of using an insects own hormone to control pest populations almost 40 years ago, and he coined the term "third generation pesticides" to describe such agents. The first publication of IGR (insect growth regulators) protection of stored grain was in 1968. It indicated the activity of a juvenile hormone analogue against. *T. castaneum* (Thomas and Thomas, 1968).

Abivardi (1977) observed the effect of camphor on embryonic and post-embryonic development of *C. chinensis.* Exposure of the newly laid eggs of *C. chinensis* to different concentrations of camphor crystals (0, 12, 24, 48, 96 ppm) in airtight containers for one week and four weeks resulted 65% and no (0%) hatch respectively. Earlier studies have shown that the acetone extract of water hyacinth petiole is capable of inhibiting reproduction as well as inducing morphological abnormalities in *Dysdercus cingulatus* and *T. castaneum*; besides producing ovicidal and toxic effects in *D. cingulatus* (Jamil *et al.,* 1984). Malformed elytra and crumpled hind wings were found in the water hyacinth treated larvae of *C. cephalonica.* Such deformities have also been reported in the insect, *Malacosoma californicum* (Wellington, 1969; Das and Gupta, 1974). The symptoms produced on the insect after treatment with water hyacinth resembled those produced by juvenile hormone compounds.

Haque *et al.* (2000) have evaluated the development inhibiting activity of the extracts of the seeds of *Basella alba* and leaves of *Operculina turpethum* and *Calotropis gigantea* against *S. zeamais*. The number of adults that emerged from the grain treated with 0.5% concentration of *B. alba, O. turpethum* and *C.*

gigantea were reduced up to 62, 95 and 75% respectively. Earlier, Gujar and Mehrotra (1983) have reported the juvenilizing effects of azadirachtin on last instar larvae of *S. litura.* Other neem products also showed varying degree of deleterious effects on growth and development. These are presumed to be due to interference with the neuroendocrine system of insect. Isman and Rodriguez (1983) have isolated natural products from various species of parthenium and fed the herbivorous insects, *Heliothis zea* and *Spodoptera exigua* to assess the ability of the compounds to inhibit larval growth. At the dietary concentration of 3 mμ/kg tetraneurin A (parthenium) reduced larval growth of *H. zea* by 88% relative to the control.

Growth disrupting effect of azadirachtin was reported by Koul (1984) in the red cotton bug. It was found that azadirachtin when applied to various stages of development caused non-plasticisation of wing lobes and development of wingless adults. This compound when fed with foliage to early stage larvae of *S. litura,* growth was found disrupted more severely than older larvae (Koul, 1985). Insect growth regulatory properties of petroleum ether extracts of ten indigenous plants were tested against *Culex pipiens fatigans* and *Musca domestica* by continuous exposure and topical application methods (Deshmukh and Renapurker, 1987). Out of the ten plants, *A. calamus* and *A. indica* showed growth regulatory activity against *C. pipiens* and also against *M. domestica.*

Neem oil at sublethal concentrations (0.1 to1.0%) affected nymphal growth suggesting insect growth regulatory effects. It also showed comparatively high morphogenetic activity in the last instar nymphs during the nymph-adult

transformation of red cotton bug, *D. koenigii* (Gujar and Mehrothra, 1990). Gallic acid, a common allelochemical found in various woody plants, act as a deterrent of oviposition, extending the larval and pupal period and adult emergence of *S. litura* (Mukherjee and Sharma, 1990). Investigations showed that with the increasing titer of gallic acid in the diet, the per cent pupation as well as per cent adult emergence declined, suggesting that in the case of *S. litura*, gallic acid reduced survival. Neem products affected the development of the maize stalk borer, *Chilo partellus* larvae (Siddique *et al.*, 1990) and the mosquito larvae (Zebitz, 1986; Murugan *et al.*, 1996). The major limonoids extracted from grape fruit seeds, limonin and nomilin, act as larval growth inhibitors against *H. zea* and *S. frugiperda* (Klocke and Kubo, 1982).

Ipomoea palmata leaf extract was found to be effective ovicide against *C. chinensis* (Dwivedi and Kumari, 2000 a). Citrus clean, a mixture of four plant oils viz. citronella oil, pine oil and natural oils extracted from lemongrass and marigold, exhibited effective ovicidal properties against *C. chinensis* (Dwivedi and Kumari, 2000 b). Dwivedi and Bajaj (2001) studied the effect of acetone extract of some plants on the eggs of *Trogoderma granarium* for their ovicidal activity. Highest ovicidal activity was recorded with *Cassia fistula* (88.6%) followed by *Acacia nilotica*, *L. camara* and *Tagetus indica*. The ovicidal activity was found to due to the interference of the extracts with the normal embryonic development of the eggs by suppressing hormonal and biochemical processes. Lale and Abdulrahman (1999) reported the post-oviposition treatment of cowpeas

with neem seed oil and the significant reduction in adult emergence of cowpea bruchid, *C. maculatus*.

Many indigenous plant products have been reported to possess ovicidal activity against insect pests in the field. Yadav and Patel (1990) reported the ovicidal action of some botanicals against *Chrysopa scelestes.* Among these botanicals (neemark, repelin, wellagro, neem seed kernel suspension, nicotine sulphate and neem rich) nicotine sulphate was found to have strong effect on the hatching of the eggs. Agarwal (1993) conducted experiments to study the ovicidal activity of *A. indica, Calophyllum inophyllum* and *Sweitenia macrophylla* and heartwood extract against *D. koenigii.* It was found that all the four plant extracts were effective ovicides against early stages of the eggs (0 to 3 days old), while hatching in advanced stage of egg (5 days old) could not be prevented significantly. Topical application of different plant extracts on eggs of *D. koenigii* resulted in complete inhibition of hatching (Suryakala *et al.,* 1995). At lower doses even though the larvae hatched, they could not survive for more than two days.

Laurent *et al.* (1996) tested sixty-three essential oils isolated from Bolivian plants for ovicidal and larvicidal properties against *Triatoma infestans*. Among these, twenty oils exhibited toxic effects against eggs and nymphs. The ovicidal activity of non-edible oils (neem, *Pongamia pinnata, Madhuca longifolia* and castor oil) against 1, 2 and 3 day old eggs of *Chilo partellus* was tested by Bhatnagar *et al.* (1996). Neem and *P. pinnata* oils reduced the survival of eggs. It was found that two and three day old eggs were comparatively more susceptible

to the action of these oils than one-day old eggs. Bhatnagar *et al.* (1996) have shown that petroleum ether, chloroform, methanol and distilled water extracts of the same plant had toxic and ovicidal activity against *Chilo partellus.* Methanol extracts of *Melia azadirach* have shown its ovicidal activity against *H. armigera, Earias vitella* and *Plutella xylostella* (Rani *et al.,* 1998). Patel and Patel (1998) reported the ovicidal property of some indigenous plant extracts against *Spodoptera litura.* Ovicidal activity of neem products against *Culex tarsalis* and *C. quinquefasciatus* were studied by Su *et al.* (1998) and *Atriplex canescens* extract against *C. quinquefasciatus* by Ouda *et al.*(1998). Aqueous extracts of *Trichilia pallida* leaves and twigs on *Tula absoluta* have shown high ovicidal activity (Thomazini *et al.,* 2000).

According to Amonker (1973) and Fagoone and Lauge, (1981), the reason for increase in the egg mortality due to plant extracts is the interference of these substances with the normal embryonic development of the eggs by suppressing hormonal and biochemical processes. Su *et al.* (1972) also obtained supporting results. The egg mortality has been attributed to toxic components and also to physical properties, which cause changes in surface tension and oxygen tension with in the egg (Singh *et al.,* 1978). Studies of Dwivedi and Kumar (1999) strongly supported these findings. They found that the ovicidal activity of *Vinca rosea* and *Withania somnifera* was due to the presence of vincain and withanin respectively. These chemicals cause disturbance in embryonic development of *Corcyra cephalonica.* Pandey *et al.* (1981) reported that toxic constituents of the vegetable oils enter the eggs through the micropyles and kill the yolk.

10. Chemistry of plant extracts

The metabolic activity of plants produce not only the food material essential for life but also certain other substances some of which are harmful to animals including insects. The poisonous properties of a plant are due to the presence of these toxic constituents. Our knowledge of plant chemistry has advanced considerably. In recent years, the constituents responsible for the specific physiological actions of the plants have, in many cases, been recognized, isolated, purified and identified as definite chemical compounds. Further, the pharmacological actions of many of these substances have been studied by modern methods of assay. The exact nature of the action of the drugs containing these constituents is by and large, known both in higher animals and insects (Urs, 1990).

The important toxic constituent principles of insecticidal /pesticidal values present in plants were described by Urs (1990). Among the toxic principles, alkaloids, saponins and essential oils are important. Saponins possess a bitter acrid taste and in dry powder are irritating to the eyes and nose, and are particularly toxic to insects and cold-blooded animals like fishes and frogs. Essential oils include terpenes and sesquiterpenes, open-chain alcohols and aldehydes, aromatic alcohols and their ketones. From the nature of the compounds, essential oils would act as antiseptic, disinfectant, insect repellents and insecticides.

Another important toxic constituent is the bitter principles, which impart bitter taste, found in a number of plants especially in the wild members of the

family Cucurbitaceae. The neem plant is unique in that it contains a fairly good number of pesticidal constituents including triterpenoid, vermicilin, salannin, neemol, nimidin, nimbidin, nemicidin and meliantriol. All these compounds have been found to control many pest species including insects, mites and nematodes. Neem cake, enriched with fatty acids like myristic, palmitic, linoleic, capric, caproic, stearic and linolenic acids can be applied as insecticide. Neem products act variously as antifeedants, growth regulators, repellents, hormones, sterilants and pesticides on all stages of insects.

It has been reported that more than 2000 plant species contain toxic principles, which are effective against insects (Devkumar and Sukhdev, 1993). Besides neem, *A. squamosa, Tephrosia villosa, V. negundo, Madhuca butyrica, Cleistanthus collinus, Derris elliptica* etc. are also important sources of botanical pesticides (Srimannarayana, 1990). So far, twenty acetogenins are isolated from *A. squamosa,* which include squamocin, neoanonin and squamostatin (Zafra Polo *et al.,* 1988). Among these, squamocin is important and is characterized as dihydroxy-bis tetrahydrofuran fatty acid containing 30 carbon atoms (Kawazu *et al.,* 1989). They are potent cytotoxic agents, insecticides, fungicides, repellent and anti-tumour agents (Ruprecht *et al.,* 1990) as well as potent mitochondrial inhibitors (Xing-wan and Hong, 1999). Some of the secondary metabolites found in plants and their activities against insects are listed in the Table II. 1.

Koumagle *et al.* (1996) have analysed by GC and GC-MS, the essential oils, steam distilled from leaves of *Lippa multiflora.* Three chemo-types were characterized; a citral type rich in neural and geraniol, a thymol type rich in

thymol and a 1,8 cineole type, rich in 1,8 ceneole. The essential oils exhibited insecticidal activity against *C. maculatus* in a dose dependent manner. A wide range of monoterpenes had insecticidal effect and depressive effects on the reproductive development of several insect species (Weaver *et al.,* 1991). Kumar *et al.* (1989) had succeeded in isolating three new isoflavones from the stems (without bark) of *Milletia recemosa.* All these compounds showed promising insectcidal activity against 5^{th} instar larvae of *S. litura.* The larvae suffered mortality due to stomach poisoning. Toxicity and repellent effects of several pure compounds were tested against *T. castaneum* by Ojimelukwe and Adler (2000).

Singh (1998) have reported the effect of terpenoid lactone on reproduction of *C. maculatus*. A terpenoid lactone, constunolide, isolated from costus root, *Saussurea lappa* drastically reduced fecundity, egg hatch and adult emergence in the pulse beetle *C. maculatus*. Recently, Ulubelen *et al.* (2001) investigated the repellent activity of the diterpenoid and nor-diterpenoid alkaloids against *T. castaneum.* Of the 29 tested alkaloids, 21 compounds showed promising insect repellent activity.

Singh and Agarwal (1988) bio-assayed the insecticidal activity of chromatographic fractions of Himalayan cedar wood oil against the pulse beetle, *C. analis* and the housefly *M. domestica.* Their study revealed that almost all fractions exhibited insecticidal activity against both the pest species. Peterson *et al.* (1989) have isolated and characterized few biologically active compounds from the crude extracts of *Chenopodium ambrosiodas*, *Conyza dioscordisis* and *Convolvulus arvensis* which were found to be active against the stored grain pests,

T. castaneum and *S. granarius.* Su *et al.* (1982) have isolated, purified and identified two insect repellent compounds ar-turmerone and turmerone from *Curcuma longa.* These compounds showed strong repellent effect on *T. castaneum.* Turmeric powder or the crude extract also showed strong repellency on *T. confusum, R. dominica* and *S. granarius* adults and *Attagenus megatoma* larvae (Jillani and Su, 1980).

Gbewonyo and Candy (1992) have separated insecticidal components from the roots of male *Piper guineense* by gas chromatography. Petroleum ether extract of *P. guineense* male roots showed insecticidal activity when tested against *M. domestica.* Gas chromatography of the extract yielded four active fractions. One of these was identified as pellitorine and was responsible for the insecticidal property of the extract. Other components have lost about half of their insecticidal potencies during passage down the GLC column, and much of the residual activity was due to the presence of pellitorine. Acetone extract of *P. nigrum* seeds has been found contain piperidine, piperine, resin and volatile oils, out of which piperine was highly toxic followed by piperdine and both these constituents are mainly responsible for imparting toxic effects of the seed extract (Desai *et al.,* 1998).

11. Phytochemical studies on *Clerodendrum* species

There is a lot of scientific literature available on the medicinal, agricultural as well as the chemical aspects of this plant species. The fresh juice of this plant parts have been used as a vermifuge, fungicide and insecticide traditionally. It contains an array of chemical components having these properties. The essential

oils of leaves and root bark of *C. infortunatum* contain a number of compounds, partially used as drugs, laxative and remedy against skin diseases or tumours (Saidutty, 1999).

Jacke and Rimpler (1983) have examined twelve *Clerodendrum* species for iridoids. Eight of them contain iridoid glycosides. Jirovetz *et al.* (1999) have isolated and analysed various essential oils from the leaves and root bark of *C. infortunatum* by means of gas chromatographic-mass spectrometric and olfactory methods. Among these, linalool showed insecticidal and molluscicidal properties. Sinha *et al.* (1983) isolated a glycoside from the flowers of *C. infortunatum*. It has been identified as acteoside. Akihisa *et al.* (1988) studied the CNMR spectroscopy of 24-ethyl cholesta-5, 22 E - dien- 3 β-ol (clerosterol) isolated from the aerial part of *C. fragrans* and *C. infortunatum.* They also isolated two minor sterol constituents, sitosterol and stigmasterol. Seed fat of the plant is found to be rich in palmitic acid, oleic acid and linoleic acids and root shows the presence of lupeol and 2-sitosterol. Leaves and flowers of *C. infortunatum* contain clerodin, hentri acontane, fumaric acid, ethyl and methyl esters of caffeic acid, 2-sitosterol and its glucoside and new flavone glycoside.

Roy and Pandey (1994) isolated a new chalcone glycoside from the flowers and leaves of *C. phlomidis*. Six new compounds were similarly isolated from *C. indicum* by Tian *et al.* (1996). The structures of these compounds were determined by spectral and chemical analysis. In addition, several neo-clerodane type diterpenoids were isolated from the aerial parts of *C. inerme* by Raha *et al.* (1991) and Rao *et al.* (1993). A new diterpene from the leaves of this plant was

isolated by Achari *et al.* (1992). The structure was elucidated by spectrophotometric method as 15-methoxy-14, 15-dihydro 3-epicaryoptin. Three iridoid glycosides (inermenosides A1, C and D) were isolated by Calis *et al.* (1993); neolignin by Spencer and Anderson (1981) and steroid glycoside by Rahman *et al.* (1997) from this plant.

In addition, a variety of terpenes were also isolated from *Clerodendrum* sp. by various workers. A clerodane diterpene was isolated by Achari *et al.* (1990) and 22, 24-dimethyl 25-dehydrolophenol and 4 K-methyl steroid by Akihisa *et al.* (1990) from *C. inerme*. Two novel abietene diterpene derivatives were isolated from *C. cyrtophyllum* by Tian *et al.* (1993). These compounds have growth inhibitory and antifeedant activity on insects.

Ruchira *et al.* (1996) have isolated flavonoids from 12 spp. of *Clerodendrum.* According to Prasad *et al.* (1995) two basic proteins isolated from *C. inerme* induce systemic anti-viral resistance in susceptible plants. Cabruvin and quercetin isolated from the roots of *C. infortunatum* exhibited inhibitory effect on spore germination in fungus (Roy *et al.*, 1996).

The importance of the study of toxic principles of plants in an over populated and a predominantly agricultural country like India cannot be overlooked. Many of these chemical principles in regulated doses constitute potent and effective remedies against pests and diseases. Some of them while poisonous to insects and other cold-blooded animals are relatively harmless to man and other warm blooded animals, and are being increasingly used for the fight against insect pests. The present investigation is aimed at identifying active

principles in some of the locally available aromatic plants that could be used for controlling stored grain pests. Chemical characterization of components of some extracts is also planned which would help to design alternatives to conventional chemical pesticides that pose great hazards to human beings and other beneficial species of plant and animals.

Table II. 1. Secondary plant metabolites involved in host-plant resistance to insect pests

Sl. No.	Substances	Putative function
1	Alkanes, aldehydes, ketones, long chain waxes	Barrier to insect attack, involved in wound healing
2	Alkaloids	Neurotoxic, antifeedants
3	Lignin and Tannins	Barrier to insect attack, involved in wound healing, affect insect digestion
4	Monoterpenoids	Neurotoxic, deterrent
5	Sesquiterpenoids	Feeding deterrents, toxic, hormonal interference
6	Diterpenoids	Feeding inhibition
7	Triterpenoids	Feeding deterrents, disrupt development toxins and deterrents
8	Steroids	Developmental interference
9	Phenolic compounds	Toxic
10	Flavonoids	Feeding deterrents, toxic
11	Quinones	Feeding deterrents, toxic
12	Non protein amino acids	Antimetabolite, toxins
13	Cyanogenic glycosides	Toxic and feeding deterrents
14	Glucosinolates	Toxic and feeding deterrents
15	Lectins	Toxic

Source: Panda, N. and Khush, G.S.(1995). *In*: **Host Plant Resistance to Insects**. Wallingford: CAB International.

Most of the above substances are found in a range of plant species. The exact mode of action(s) for many of the above chemicals has to be determined.

Chapter III

MATERIALS AND METHODS

A. MATERIALS

1. Experimental animal

For the present study, the pulse beetle *Callosobruchus chinensis* was used. Identification of the species and determination of sexes were done on the basis of the keys published by Southgate *et al.* (1957), Halsted (1963) and Arora (1977). The insect *C. chinensis* can be distinguished from other species by the whitish ivory spots near the middle of the body. Median basal thoracic lobe is elevated, the head is small with a blunt rostrum and short antennae which are often pectinate in male and serrate in female. The elytra do not cover the last abdominal segment. In male a pair of tubercles are present at the base of third and fourth otrias of each elytron. Elytral tubercles are absent in female. The legs are short and the hind femora thickened (Singh and Kumari, 2000) (Plate III.1.a).

Life cycle

The life cycle of *C. chinensis* has been reported by many workers in pulse crops (Dina, 1971; Pandey and Singh, 1997 b; Singh and Kumari, 2000). Adult insects copulate within one hour of their emergence from the seed. Mating last for about 5-8 min at temperature of 30 ± 2° C and 70 ± 5 % relative humidity (Raina, 1970). Females prefer smooth whole seed for oviposition. A single female lays an average of about 78 eggs ranging from 63-90 over a period of 8 days. Eggs laid afresh are oval, plano-convex and broad anteriorly and narrow

posteriorly, translucent, smooth and shining. Later they become pale yellowish or grayish white. (Singh and Kumari, 2000).

Eggs hatch in about 4-5 days. The average incubation period is 3-5 days at 30°C and 70% relative humidity and the percentage of hatching ranges from 94-99. The newly hatched grubs bore into the pod or seed and develop inside them. It bears a large spine on either side of the first abdominal segment and two groups of smaller spines dorsally on the tergal plate of the pronotum. The grub becomes full grown within 14-21 days during summer. It measures about 6 mm in length, cylindrical in shape, fleshy, strongly wrinkled and white in colour except in the region of its mouthparts, which are brown. The larva undergoes four molts before pupation. The larva chews a circular hole near the seed coat till only a thin layer of seed covering is left intact. This is the indication of larva entering pupation (Plate III.1.b).

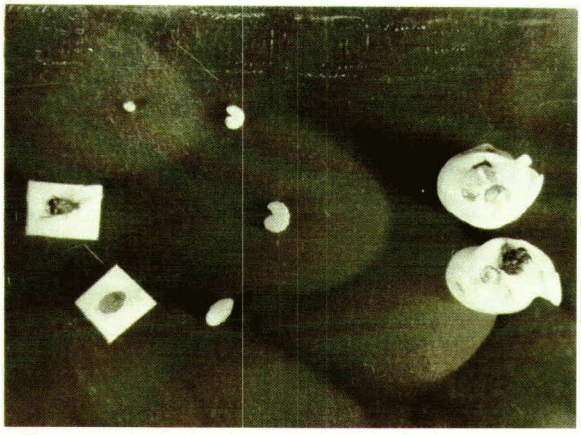
Pupa is brown in colour. Pupation takes place inside the grain. The pupal period is about 4 days in summer and extended up to 28 days in winter. Complete development from egg to adult takes an average of 22-23 days (PlateIII.1.c). Adults move about inside the seeds for some time and get sexually matured when they emerge. Mating and oviposition follow emergence. Males are more active and better fliers than females. Males live for 7-10 days while females live for 5-10 days.

PLATE III-1

a

b

c



a- Adult insect, *Callosobruchus chinensis*
b- Infected grain showing emergence hole
c- Life cycle stages of *Callosobruchus chinensis*

2. Experimental plants

The plants selected for the present study (Plate III.2. a to h) were collected from various parts of Kozhikode District of Kerala, as and when needed, mainly during the period from December to May.

The following plants were collected and were identified from the herbarium of Department of Botany, University of Calicut.

(a) *Achyranthes aspera* Linn.

Family: Amaranthaceae

(Sansk: Apamarga; Hindi: Latigera; Malayalam: Katalati)

An erect herb reaching three feet in height, with velvety tomentose, stiff stem, not much branched. Leaves few, usually thick. The plant possesses valuable medicinal properties and is considered useful in the treatment of dropsy, piles, eruptions of the skin etc. The dried plant is given to children for colic and also as an astringent in gonorrhoea. Every part of the plant is recommended in the treatment of snake bite and scorpion sting, but no part has been found effective in the antidotal and symptomatic treatment of either snake bite or scorpion sting. Ash is rich in potash and might be of value as manure.

(b) *Adathoda vasica* Nees

Family: Acanthaceae

(Sansk: Vasaka; Hindi: Arusha; Mal: Atalotakam)

Adathoda vasica is a small gregarious evergreen shrub, growing throughout the plain of India, and in sub-Himalayan tracts ascending up to 4000

ft. *A. vasica* is a well known drug in the Ayurvedic and Unani system of medicine and is recommended for a variety of ailments such as bronchitis, asthma, fever, jaundice and consumption. Leaf juice is used in diarrohoea, dysentery and glandular tumours.

The leaves contain very small amount of essential oil, a crystalline acid and a white crystalline alkaloid, vasicine ($C_{11}H_{12}N_2O$, m.p. 190 ± 1°C). Leaves are rich in vitamin C (up to 250 mg/100 g) and carotene (4500 μg/100 g) and yield an essential oil; flowers contain essential oils and seeds yield fatty oil. The pharmacological action and therapeutic properties are attributed to vasicine. Ether extract of the leaves yields a resin which is toxic to grain insects, but non-toxic to human beings. Plant is used as a green manure.

(c) *Cassia fistula* Linn.

Family: Caesalpiniaceae

(Sansk: Suvarnaka; Hind: Amaltas; Mal: Kanikkonna)

A tree of 20-30 ft. height. Leaves are pinnate and 12-18 inches long, deciduous. Petioles round, without glands. Racemes are pendulous, simple from 1 to 2 ft long. Flowers large, bright yellow, fragrant and on long slender smooth pedicel. Dried fruit used as a purgative, laxative for habitual constipation. Root bark extract is used for the treatment of black water fever. The pulp of wood contains mucilage, pectin, hydroxyl methyl anthraquinones and a large proportion of sugar.

(d) *Clerodendrum infortunatum* Linn.

Family: Verbanaceae

(Sans: Barhichuda; Hind: Bhantaka; Mal: Peruku)

C. infortunatum is a gregarious shrub common throughout India, Myanmar and Sri Lanka. It grows commonly in waste lands; leaves round, ovate to oblong, hairy, up to 10 inch long and 8 inch broad; flowers in large, terminal, erect panicles, calyx persistent, corolla white or tinged red, drupe black, totally enclosed by the enlarged red calyx.

In the complex folk medicinal system, Ayurveda in India, different plant parts of *C. infortunatum* are used for various applications. The leaves have a disagreeable odour and are used as bitter tonic, antipyretic, vermifuge, laxative, and cholagogue. The leaves and roots are used externally for tumours and certain skin diseases. Fresh juice is used as an injection into the rectum for ascaris. Root extract is effective against hair loss disorders. Some of the chemical components of this plant show insecticidal and molluscicidal properties.

From the petroleum ether extract of the air-dried leaf powder, a bitter principle, clerodin ($C_{13}H_{18}O_3$, m.p.161±2°C) possessing antihelminthic properties has been isolated. Aqueous solution of clerodin has been found to kill earthworm within 30 min, small fishes within half an hour and mosquito larvae in two hours.

(e) *Cymbopogon citratus* Stapf.

(syn. *Andropogan citratus*)

Family: Graminaceae

(Sansk: Bhusrina; Hin: Gandhatrina; Mal: Vasanappullu)

Perennial, densely tufted, often aromatic herbs, leaves flat, often very coarse. Inflorescences are paired racemes and the pedicels filiform. The plants are grown for their aromatic oil. West Indian Lemon grass oil is used for flavoring soups and curries. The oil is obtained by subjecting fresh or partially dried and bruised grass to steam distillation. An infusion of the grass is taken as a beverage. Also used in Java in the preparation of a highly spiced sherbet.

Its essential oil is an ingredient of medicated oil products, anti mosquito and anti-septic aerosols, perfumes and soaps. The West Indian lemon grass oil contains citral as the principal constituent, the percentage of which varies with locality. The oil contains also, citronellal, geraniol and myrcene. Its spent hay containing 40% cellulose, is used as a raw material for the paper industry and ceiling-cover sheets, and as a source of fertilizer because it contains 84.3% organic substance, 0.98% nitrogen, 0.123% P_2O_5 and 1.1% K_2O.

(f) *Glyricidia sepium* Walp.

(syn. *G. maculata*)

Family: Papilionaceae;

(Hin: Wilayati shiris; Eng: Madre tree; Mal: Seemakkonna)

This is a soft-wooded, quick growing tree of recent introduction in India. It is a deciduous tree, grows up to 20 meter. The leaves are compound, consisting

of 7-8 pairs of leaflets with a terminal solitary one. Leaflets are roughly oval in shape, more or less bare of leaves from January onwards; flowers are pink or purplish or lilac in colour, or all these colours may be seen on the same tree, depending on the stage of development of the flowers.

In various parts of America, the bark of this tree is used as a rat poison. In India, the tree is often cultivated as an avenue tree, or for its shade, or as a windbreak at the edges of banana or other crop field. The leaves are very good for use as a green manure.

(g) *Hyptis suaveolens* Poit

Family: Labiatae

(Hindi: Wilayati tulsi); Beng: Wilayati tulsi; Mal: Bilati tulasi)

H. suaveolens is a rigid sweety aromatic herb, sometimes attaining a height of 7 ft; found in Deccan Peninsula, North East India and Andaman and Nicobar Islands. This is a tall, sweet smelling herb with tetragonal hispid stem; ovate, cordate and denticulate leaves and small blue flowers. The fruiting calyx companulate and ribbed with five aristate teeth.

H. suaveolens is considered to be a stimulant carminative, sudorific and lactogogue. Leaf juice is given in colic; shoot tops are edible and are also used as a flavouring agent. Leaves are used in the preparation of a mint-flavoured beverage. The plant yields 0.06% of greenish yellow ethereal oil. Analysis of the oil indicated the presence of 1-sabinene, d-limonene, azulenic sesquiterpene and unidentified sesquiterpene and sesquiterpene alcohols. Seeds contain fatty oil. The root, stem and leaves contain hydrocyanic acid.

PLATE III -2

15

(a-h) Plants used for screening test

(h) *Uvaria narum* Wall.

Family: Annonaceae

(Kan: Kariballi; Tamil: Pulichan; Mal: Naram panel)

U. narum is a large climber. Its leaves are oblong, lanceolate, acute or acuminate, glabrous on both surfaces, reticulately veined, petioles short, flowers reddish, solitary, terminal, sepals orbicular ovate.

The oil obtained from the roots by distillation is used medicinally in various diseases. Decoction of root-bark is given to women at the time of delivery to control fits. This is also used for rheumatism, bowel complaints and eczema. Leaves are prescribed in rheumatism, jaundice, biliousness and fever. The root is fragrant and the leaves smell like cinnamon.

3. Chemicals and Equipments

Chemicals

Laboratory chemicals and organic solvents (analytical/Guaranteed grade) were obtained from local suppliers.

1. Acetone (MERCK)
2. Anisaldehyde (BDH)
3. Antimony trichloride (GLAXO)
4. Aluminum chloride (MERCK)
5. Chloroform (GLAXO)
6. Copper sulphate (BDH)
7. Ethyl acetate (MERCK)

8. Ferric chloride
9. Glacial acetic acid (GLAXO)
10. Iodine
11. Methanol (MERCK)
12. Perchloric acid
13. Petroleum ether (B.P. 40- 60°C) (MERCK)
14. Potassium iodide (BDH)
15. Silica gel G for TLC (Qualigens)
16. Silica gel (50-120 μ mesh size) for column chromatography (Qualigens)
17. Sulphuric acid
18. Vanillin (SRL)

Equipments

1. Automatic shaker
2. Buchner funnel
3. Hamilton syringe (10μl capacity)
4. Micropipette
5. Vacuum pump
6. TLC Apparatus
7. Glass sprayer
8. Hand lens
9. GC-MS- Hewlett Packard 6890 model
10. UV/ Visible Spectrophotometer (Shimadzu 1601)

B. METHODS

1. Insect rearing

Laboratory culture of *Callosobruchus chinensis* was maintained on green gram *Phaseolus radiatus* at optimum conditions of temperature and relative humidity in plastic containers of 500 ml capacity (20 x 14 cm). Green gram seeds were purchased from the local market and 250 g each were taken in a few containers. Introduced 20 pairs each of adult insects in to each container. Covered the mouth of the container with cotton cloth and kept them at 28±2°C and 75±5% relative humidity in a glass cage. Optimum relative humidity was maintained by keeping a small bowl containing water in the cage. Care was taken to protect the containers from ants. After about 45 days, it was observed that the containers had sufficient number of insects.

2. Preparation of experimental grain

Sufficient quantity of green gram *Phaseolus radiatus* was thoroughly washed and sun dried for 3 to 5 hours. This helped to remove any insecticidal residues already applied on it and also to remove the eggs of pests or any other materials attached to the grains. The grain was kept at -18°C for one week to eliminate any prior infestation by insects. Then it was stored in sealed polythene bags at 5°C until required for experiments.

3. Preparation of plant extracts

The plants selected for the experiment were collected from the local habitats during the months from December to May. Leaves were washed thoroughly and

shade dried at the temperature of 28-30°C for about three weeks, by which time they were crisp dry. The dried leaves of each plant species were pulverized into fine powder using domestic grinder and sifted through fine mesh of sieve (sieve size 0.25 mm). The powdered plant materials were then sealed in separate plastic covers and stored at 4°C. Plant extracts were prepared as described by Sheela (1997). Petroleum ether (40-60°C) and methanol were used as the solvents for extraction. Fifty grams of each powdered material was extracted with 500 ml of each solvent. At first 50 g of the powdered material was taken in a conical flask and 250 ml of petroleum ether was added to it. Mouth of the flask was kept tightly closed with aluminium foil and rubber band. The mixture was agitated on an automatic shaker for about 24 h and then the extract was filtered through Whatman No.1 filter paper by negative pressure using a Buchner funnel and vacuum pump. The residue was re-extracted once more using another 250 ml petroleum ether. The combined filtrate was dried at room temperature (29-30°C) to constant weight. Similarly, another 50 g of the powdered material was extracted with methanol to prepare the methanol extract which was also dried to constant weight. From the dried extracts, 10% stock solutions were prepared by dissolving 1.0 g extract in 10 ml acetone. Required concentrations of the extracts were prepared from 10% stock solution by diluting with acetone and were stored in air-tight glass containers.

4. Mode of applications

To test the toxicity of plant extracts, different modes of application were adopted. Since the larvae of *C. chinensis* live deep inside the grain, they were not affected with extracts directly and therefore the tests were confined to adult insects

only. In the present study the following methods were adopted for the application of the extracts.

(a) Food treatment method

In food treatment method, grains of green gram, *Phaseolus radiatus,* were treated with different concentrations of the extract. The extracts were prepared by diluting the stock solution with appropriate volume of acetone. A definite quantity (10 g) of experimental grains were taken in each Petri dish (8 cm diameter) and smeared with a fixed volume (1.0 ml) of each concentration of the extract. Mixed the grains well and kept the Petri dishes open at room temperature until the acetone had completely evaporated off. Same quantity of grains treated with same volume (1.0 ml) of acetone alone was used as control. The solvent free grains were taken in separate sets of glass tubes (10 × 2.5 cm) and introduced required number of newly hatched and healthy insects (*C. chinensis*) in to each sample and covered the mouth of the tubes with cotton wool. The experiments with the different concentrations were replicated ten times. The experimental set up was kept at 28 ± 2°C and 70 ± 5% relative humidity. Mortality counts were made every day until the death of the last insect.

In general practices, food materials are treated with the suitable chemical and the toxicity of the compound is judged on the basis of percentage mortality. In the present project, however, this method was also used to study the oviposition deterrent and ovicidal effect of the plant extract, since in both the cases studies were conducted on eggs, deposited on the surface of the grains.

(b) Residual film method (Surface treatment method)

In the surface treatment method, a residual film was prepared and the insects were exposed to the film. This method is based on the principle that within the limit of the assay, a quantity of insecticide distributed over a small area which results in the same percentage of mortality to insects as the identical quantity of insecticide spread over a large area.

In the present study, a standard volume (1.0 ml) of different concentrations of the test materials were poured into separate glass Petri dishes (8 cm diameter). The extract was allowed to spread evenly on the entire inner surface of the dish and lid by gentle rotation leaving a residual film. Insects (10 numbers) were released into each of the Petri dish. A control set in which the Petri dish was smeared with acetone alone, was also kept. The experiment was replicated ten times.

(c) Topical application method

This method involves the application of small volume of insecticide solution to individual insects using micropipette or micro syringe. In the present study, required number of insects (5 insects /set) were immobilized by chilling them for a few minutes. With the help of a Hamilton microsyringe (10 µl capacity), 5 µl of each of different concentrations of the extracts were applied on the dorsum of each insect. Same volume of acetone was applied to a set of insects taken as control. The insects were observed after 1 h, 3 h, and 24 h intervals. Those that did not move or respond on gentle touch even after 1 h were considered dead and the

insects that were no longer able to upright themselves were considered knocked down. All experiments were replicated ten times.

(d) 'Y' tube method to test the repellency

In order to evaluate the repellent effect of different plant extracts, a 'Y' shaped glass apparatus (locally fabricated) was used (Plate III.3). The 'Y' tube had an inner diameter of 2 cm; each of the paired-arm was 12 cm long and median arm was 14 cm long. One of the paired-arm was used for control test and the other one for experiment. Grains (10 g) treated with 1.0 ml each of plant extracts were taken in experimental arm and the control arm contained grains treated with acetone. After introducing the grains through the open ends, these two arms were plugged with cotton wool. Twenty insects were released through the third arm of the Y tube. The open end of this arm was also then plugged with cotton wool. The experimental set up was kept undisturbed in a white enamel tray at room temperature. The number of insects moved towards the control arm as well as the treated arm were recorded after 30 min, 1 h and 3 h of duration. The experiment was replicated 10 times each with different plant extracts and with different concentrations.

5. Phytochemical analysis

To isolate and identify the chemical components of the extract of *C. infortunatum,* three methods were adopted in the present study. They were: (a). Thin layer chromatographic method (TLC), (b). Column chromatographic method (CC) and (c). Gas chromatographic-Mass Spectrometric method (GC-MS).

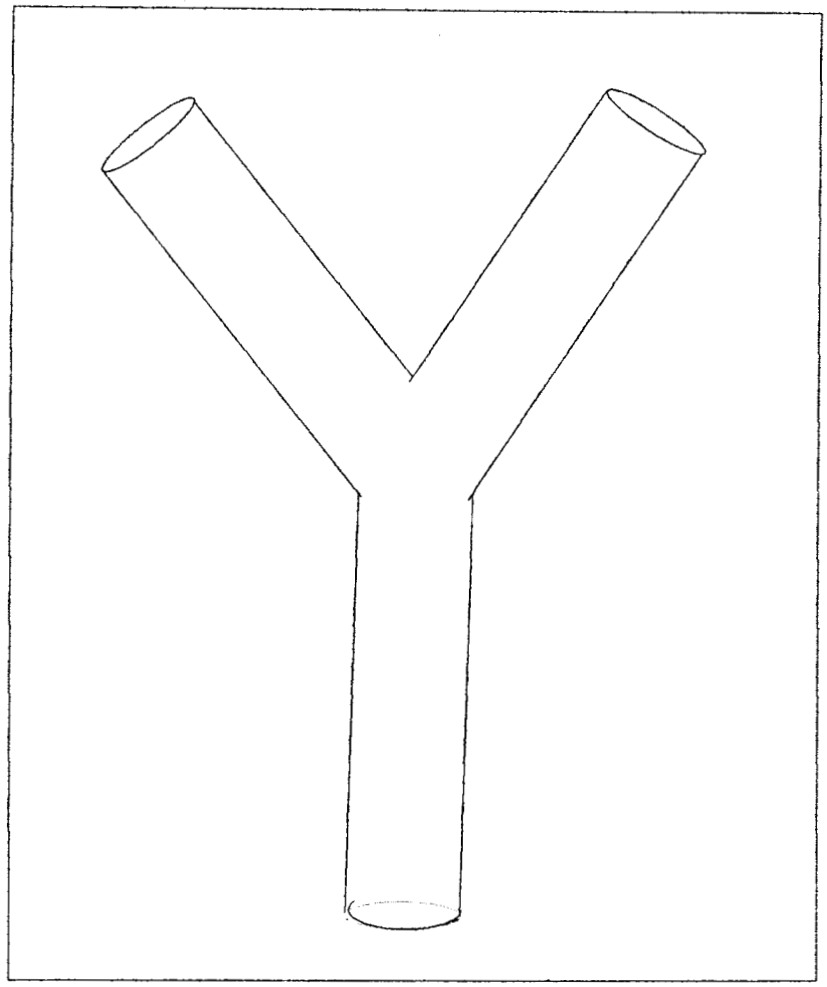


Figure III.3. Diagramatic Representation of Y - Tube.

(a) Thin Layer Chromatographic Technique

For analyzing and isolating the toxic principles present in the extract of *C. infortunatum*, thin layer chromatography (TLC) was adopted. The special advantage of TLC compared to other conventional chromatographic techniques is its versatility, speed and sensitivity. Versatility is due to the fact that a number of different adsorbent may be spread on to a glass plate or other support and employed for chromatography. Sensitivity of TLC is such that separation of less than microgram amounts of materials can be achieved if necessary.

Preparation of TLC plates

Fractionation and isolation techniques followed by Gbewonyo and Candy (1992) with slight modification was used in this experiment. Preparative silica gel chromatographic plates (400 μm thick silica gel in 20 ×20 cm glass plates) were used for separating the various fractions of the extract for bioassays as well as for qualitative analysis.

Chromatographic glass plates (20 × 20 cm) were washed thoroughly and dried. Fifty grams of silica gel G (Merck) was stirred well with 100 ml 10% methanol. The slurry was spread on the glass plates within one minute using a movable spreader (Stahl type). The thickness was adjusted to 400 μm. After drying at room temperature for about 30 min, the plates were dried in a hot air oven at 40°C for 30 min. Then they are cooled and stored in desiccated cabinet. These plates were activated at 110°C for 30 min just before use.

Preparation of developing solvent

For the separation of the various chemical components of *C. infortunatum* extract using TLC, a mixture of ethyl acetate and petroleum ether (1: 4 ratio) was used as the developing solvent. For purifying the most active fraction, a mixture of acetone and petroleum ether (1:4) was used as a developing solvent.

Fractionation of extract

In preparative TLC, samples were applied as streaks. Gel plate was partitioned into three tracks. Plant extracts (0.5 ml) of 6% concentration prepared from 50 g dried leaf powder of *C. infortunatum* were applied on each track of the activated TLC plate with the help of micropipette (10 µl capacity). After drying the extract, the plate was kept in the glass chamber saturated with developing solvent. The chamber was lined with a filter paper soaked with the solvent to maintain the tank saturation. Allowed the chromatogram to develop at room temperature of 28±2° C. When the solvent front reached 2.5 cm below the upper edge, plate was removed from the tank and allowed to dry. Different coloured bands appeared were marked (at the back of the glass using marker pencil). R_f values of the bands were calculated.

Five plates were developed in the same manner. Different bands were recovered by scraping off the appropriate region of silica gel. Identical bands with same R_f value from the five plates were pooled and eluted in 20 ml acetone: petroleum ether (2:1 ratio). The mixtures were centrifuged at 1000 rpm for 15 min,

collected the supernatants and then evaporated down to 3 ml. The materials were stored in airtight glass vials in a refrigerator.

Visualization and identification of chemical components

Two methods were used for visualising components after the development of chromatograms: physical method such as the use of UV light and chemical method such as reaction with chemical substances. Various reagents reacting specifically with phytochemicals were used for detection. The reagents were prepared in appropriate solvents and sprayed using glass sprayers onto the TLC plates. Phytochemicals were identified from their characteristic colours. Details are provided in Chapter X.

(b) Column chromatography (CC)

For the identification of chemical components and its structural studies, large quantities of different fractions present in the extracts had to be separated. For this, column chromatography was adopted. Method followed by Achari *et al.* (1990) and Rahman *et al.* (1997) with slight modification were adopted for this purpose.

Column setting and fractionation of extract:

Glass column (65 cm x 1.5 cm diameter) was filled with the adsorbent, silica gel (50-120 μm for column chromatography, Qualigens) made into slurry with petroleum ether, free of air bubbles was allowed to stand for about 2 h. The height of the gel column was set at 50 cm. Added 7.5 ml of the concentrated extract of *C. infortunatum* (prepared from 200 g of plant powder) on the top of the column and

allowed to seep slowly through the stationary phase. When the extract percolated up to 2 cm in to the gel, the eluting solvent (ethyl acetate and petroleum ether, 1:4 ratio) was poured drop by drop on to the top of the column. The chromatographic column was developed by eluting with the solvent at a flow rate of 1 ml /min. A total of 100 samples of 5.0 ml eluents each, were collected in glass vials.

Samples from the eluents collected in each vial were subjected to TLC in the solvent system ethyl acetate and petroleum ether (1: 4 ratio) mixture to ascertain the purity. The fractions having same R_f value (from the TLC) were pooled and evaporated the solvent by warming in water bath and dried up to 3.0 ml. Eleven groups of such fractions were collected and used for further bioassays. Details are given in Chapters X.

Evaluation of toxicity of fractions

For evaluating the toxicity of different fractions of the extracts (separated by TLC and column chromatography) on *C. chinensis*, topical application method was adopted. The procedure is explained in detail in Chapter X.

(c) Gas Chromatographic-Mass Spectrometric Analysis (GC- MS)

Active fractions isolated from the extract by column chromatography and TLC were further analysed by GC-MS. GC-MS analysis was carried out using a Hewlett- Packard HP 6890 series GC system with Hewlett Packard 5973- Mass selective Detector (MSD) using helium as carrier gas. The column used for analysis was 30 m × 0.32 mm bonded non-polar FSOT- RSL- 200 fused silica (film thickness 0.25mm) made in USA. The samples were kept at 40°C for 5 min and

heated to 290°C at a rate of 5°/min and the flow rate was 2.5 ml/min. Compounds were identified by comparing the GC-MS data with those in the computer database Wiley 275 MS Library. The retention indices of the identified compounds matched with published retention indices data.

6. Statistical analysis

In the present study, different variables (concentrations, time, different plant extracts etc.) had to be compared at a time. For this, a more acceptable method, One Way Analysis of Variance (ANOVA) was used. Significance of differences between different groups were found out by adopting Duncan's post- hoc multiple comparison tests.

In the Chapter IV, different plant extracts were compared for their toxic effects at different time intervals. Similarly, in Chapter V, the repellent effect of different plants at different time interval and different concentrations of one among these plants at different time durations were compared. In chapters IX and X also this type of comparison of extracts and fractions were required. Hence in all these cases ANOVA was found to be appropriate. The ANOVA tables thus computed in each case and comparison of significant differences were presented in the concerned chapters.

In the Chapter VII, to find out the LC_{50} value of *C. infortunatum*, probit analysis was done (Finney, 1969). In the Chapter VI and VIII, to test the significance of data Chi-square test was adopted.

In the ANOVA tables, SS denotes the sum of squares, MSS denotes the mean sum of squares which is MSS / df; df is degree of freedom which is equal to number of cases of the factor minus one (n-1).

Chapter IV

SCREENING SOME LOCAL PLANTS FOR THEIR PESTICIDAL EFFICACY

1. Introduction

The importance of preserving natural equilibrium in ecosystem is now well recognized. Even though, the role of conventional synthetic organic insecticides in the green revolution of our country cannot be underestimated, the side effects and hazards stemming up from indiscriminate and extensive use of these chemicals necessitated a serious revolution in the pest management strategies to be adopted. The concept of IPM advocating suppression of pest populations below levels of economic injury rather than total eradication has emerged. Thus, the search for pest control agents from natural sources has already begun and it needs to be hastened as plant products being naturally evolved ingredients, have an edge over synthetic insecticides in preserving the natural equilibrium in the ecosystem (Chari *et al.*, 1990).

In India, there are several plants known for insecticidal property that are popular as pesticides. Most important and popular is neem, which has international recognition. Probably, no plant is known to possess such a diverse biological activity as neem (Singh, 1990). Today, numerous reports are available describing the insecticidal, antifeedant, oviposition deterrent and repellent effect of many other locally available plants that are used in pest management in field as well as in storage (Gill and Lewis, 1971; Teotia and Pandey, 1979; Ketkar, 1976; Pandey *et al.*, 1986; Yadav and Bhatnagar, 1987; Jilani *et al.*, 1988 a; Ayyangar

and Rao, 1989; Dixit and Saxena, 1990; Chandel *et al.* (2000); Sahayraj and Paulraj (2000); Bhatnagar *et al.* (2001) and Raja *et al.* (2001).

The present experiments described in this chapter were designed to investigate the toxicity of some locally available plants such as *Clerodendrum infortunatum, Cassia fistula, Cymbopogon citratus, Adathoda vasica, Achyranthus aspera, Uvaria narum, Glyricidia sepium* and *Hyptis suaveolens* against the pulse beetle, *Callosobruchus chinensis*.

2. Methods

From the stock extracts prepared from the above- mentioned plants in petroleum ether (as mentioned in the Materials and Methods section), 1% extracts were prepared by diluting with acetone. Samples of experimental grains *Phaseolus radiatus* (10 g) were treated with different concentrations of extracts separately. Food treatment method (as mentioned in the Materials and Methods section) was adopted in this experiment. Data collected from these experiments were subjected to One Way Analysis of Variance (ANOVA) and difference were compared by Duncan's Multiple Range Test (DMRT) (Duncan, 1951).

Percentage of mortality was calculated by using the formula:

$$\text{Percentage of mortality} = \frac{\text{Number of dead insects}}{\text{Total number of treated insects}} \times 100$$

From the data, percentage of corrected mortality was calculated by applying Abbott's formula (1925).

$$\text{Percentage of corrected mortality} = \frac{\%\ \text{killed in treated} - \%\ \text{killed in control}}{100 - \%\ \text{killed in control}}$$

3. Results

Results presented in the Table IV.1 and IV.2 reveal that, all the 8 plants tested, brought about mortality of varying degrees. *A. vasica* was found to be least toxic, killing only 19% of the beetles; extract of *A. aspera, U. narum* and *G. sepium* were moderately toxic. Percentage of mortality of these extracts was 27.8, 36.8 and 49.3 respectively (Table IV.2). Extracts of *H. suaveolens* and *C. citratus* exhibited fairly high mortality (49.8 and 54.8% respectively). The highest bioactivity (64.8 and 79.8%) were manifested by the flower extracts of *C. fistula* and leaf extract of *C. infortunatum*. The order of toxicity of the plant materials was, leaf extract of *C. infortunatum* > flower extract of *C. fistula* > leaf extracts of *C. citratus* > *H. suaveolens* > *G. sepium* > *U. narum* > *A. aspera* > *A. vasica*.

The manifestations of bioactivity by all the extracts have been generally gradual, reaching the maximum on 6th day. On the second day of observation, *C. infortunatum* and *C. fistula* caused 26% and 20% death respectively, while other plants caused mortality between18% and 4%. The least effective extract was *A. vasica* (4%). A gradual increase in the mortality was observed towards the 4th day. Here also *C. infortunatum* and *C. fistula* appeared superior to others (mortality 53% and 42% respectively). *C. citratus* and *H. suaveolens* showed 34% and 30% mortality respectively. In the case of *U. narum*, *A. aspera* and *A. vasica* the mortality were 22%, 19% and 8% respectively.

Table IV. 1. Toxic effect of different plant extracts against *C. chinensis*

Sl. No.	Plants used	Mean ± SD
1	*C. infortunatum*	8.00^{f} ± 0.82
2	*C. citratus*	5.50^{de} ± 1.58
3	*G. sepium*	3.80^{bc} ± 1.32
4	*A. vasica*	1.80^{ab} ± 1.23
5	*A. aspera*	2.80^{bc} ± 0.79
6	*U. narum*	3.70^{bc} ± 0.95
7	*C. fistula*	6.50^{c} ± 1.08
8	*H. suaveolens*	5.00^{d} ± 1.41
9	Control	1.50^{a} ± 0.97

Data are mean number of dead insects in 10 replicates. Mean mortality in the column differ significantly at the indicated significant level of DMRT ($P< 0.05$). Means followed by same superscript letter (a-f) indicate no significant difference at 5% level.

When the data on toxic effect of 1% concentration of different plant extracts against *C. chinensis* was subjected to ANOVA and difference were compared by Duncan's Multiple Range Test at 5% level ($P< 0.05$), it was found that there were a significant differences in the mean number of death due to the different plant extracts. Based on these differences, plants were grouped into six homogenous sets (a to f) (Table IV.1). Extracts from plants belonging to the same group have no significant differences. Subset 'a' included the control and *A. vasica*. The mean mortality was 1.50 and 1.80 respectively. *A. vasica* and *A. aspera* comes under the subset 'b'. Here mean mortality was 1.8 and 2.8 respectively. Subset 'c' consists of *A. aspera, U. narum* and *G. sepium*. The mean numbers of dead of insects were 2.80, 3.70 and 3.80 respectively. Among these plants, *U. narum* and *G. sepium* showed almost equal toxic effect (Table IV.1). Similarly, *H. suaveolens* and *C. citratus* belonged to the same subset (subset 'd'). They exhibited 5.00 and 5.50 mean mortality respectively. *C. citratus* and *C. fistula* belonging to the subset 'e' showed still higher toxic effect. The last group (subset 'f') contained only one plant *C. infortunatum*; it exhibited the highest toxic effect (mean 8.00) compared to all other plants tested. Mortality observed was about 80%.

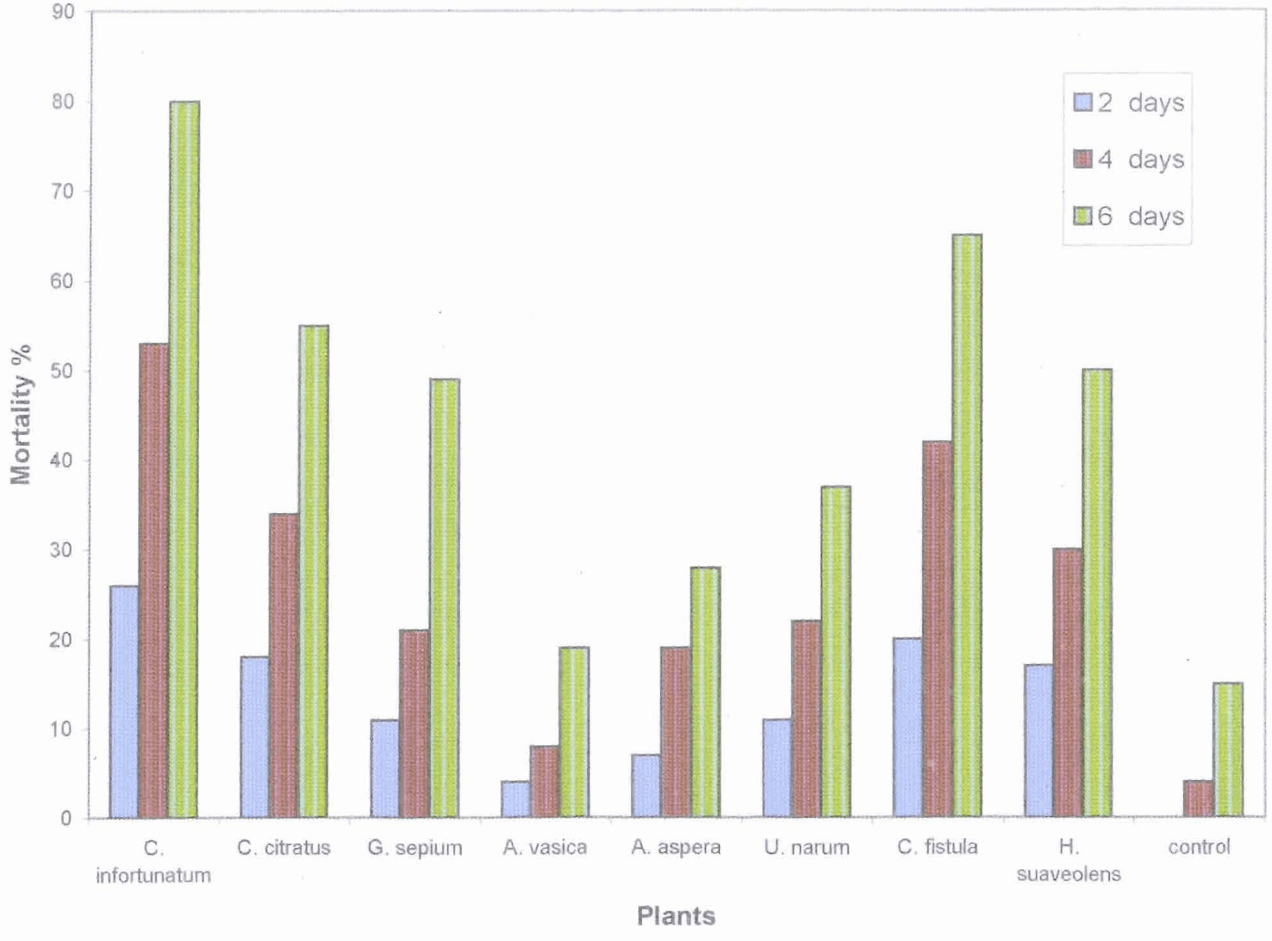
From the above data, it is evident that all these treatments are significantly active ($P< 0.001$) over control. From this screening test, it was, thus, found that *C. infortunatum* was the most toxic and *A. vasica* was identified as the least effective plant against *C. chinensis*.

Table IV. 2. Toxic effect of petroleum ether extract of eight plants on *C. chinensis*

Sl. No.	Plants used	Percentage of mortality			*Corrected mortality (%)
		2nd day	4th day	6th day	
1	*C. infortunatum*	26	53	80	79.8
2	*C. citratus*	18	34	55	54.8
3	*G. sepium*	11	21	49	49.3
4	*A. vasica*	4	8	19	18.8
5	*A. aspera*	7	19	28	27.8
6	*U. narum*	11	22	37	36.8
7	*C. fistula*	20	42	65	64.8
8	*H. suaveolens*	17	30	50	49.8
9	Control	0	6	15	14.8

Data presented in the table are the average of 10 replicates.
* Corrected mortality was calculated using Abbot's formula

Figure IV.1. Toxic effect of different plant extracts on *C. chinensis*



4. Discussion

Eight local plants were tested for their efficacy as toxicant against *C. chinensis*. Extracts of 1% concentrations were prepared. Of the eight plants tested, *C. infortunatum* caused the highest mortality (80%) to *C. chinensis* on 6th day after treatment. *C. fistula* and *C. citratus* caused 65% and 55% mortality respectively. All other plants, viz., *H. suaveolens, G. sepium, U. narum* and *A. aspera* exhibited decreased levels of toxicity to *C. chinensis* (50%, 49%, 37% and 28% respectively). The least effective extract was that of *A. vasica.* It caused only 19% mortality to this insect.

Even though not much work has been reported on the use of *C. infortunatum* for the control of stored grain pest *C. chinensis*, many workers have reported the insecticidal efficacy of this plant on agricultural pests. Earlier, Johnson *et al.* (1979) and Rajamma (1982) have reported the insecticidal and feeding deterrent activity of *C. infortunatum* against *Cylas formicarius*. High insecticidal effect (mortality 70%) was obtained with a 1% extract of *C. infortunatum* against the rice earbug, *Leptocorisa acuta* (Sheela, 1997). Jirovetz *et al.* (1999) analysed the essential oils of leaves and root bark of *C. infortunatum*.

From our investigation, it appeared that plants such as *C. fistula, C. citratus* and *H. suaveolens* caused fairly high mortality against *C. chinensis*. Some of the earlier works also support this. Prakash *et al.* (1989) have reported the effectiveness of *C. fistula* for the protection of stored paddy from *S. cerealella*. Studies conducted by Prakash *et al.* (1990) also confirmed the grain protecting property of *C. fistula* from the attack of *S. oryzae* in milled rice. *C. citratus* have

been reported to have insecticidal activity against *C. formicarius* (Johnson *et al.*, 1979 and Rajamma,1982) and *C. maculatus* (Dharmasena *et al.*,1998).

In our screening test, *H. suaveolens* exhibited 50% mortality against *C. chinensis*. Sheela (1997) has confirmed the insecticidal property of this plant by testing on *L. acuta*. Methanolic and n-hexane extracts of this plant at 1% and 0.5% concentrations caused 20-27.7% mortality in this insect.

Extract prepared from *G. sepium* caused 49% mortality on *C. chinensis* in the present experiment. Lawrence *et al.* (1993) reported fairly high mortality (60-67%) from *G. sepium* in *T. castaneum*. *U. narum* caused 37% mortality in *C. chinensis*. Padmaja *et al.* (1995) confirmed this result. An acetogenin isolated from *U. narum* and *U. hookeri* gave 1 to 50% mortality at 0.002 to 0.005% concentrations. Dead beetles from grains treated with plant oil showed sign of rapid immobilization. Their legs were flexed and they clung to either the grain or the container surface. Ofori and Reichmuth (1999) reported that beetles on the grain treated with essential oil appeared paralysed with their metathoracic wings unfolded and stretched before they die.

Insecticidal efficacy of *A. aspera* has not been reported so far. Our experiment showed a mortality of 28% in *C. chinensis* when the extract of this plant was used. Its toxic effect was slightly greater than *A. vasica*. *A. vasica* is found to be the least effective plant extract. It caused only about 19% mortality of *C. chinensis*. Its activity does not differ much from control (16%). Prakash *et al.* (1990) also reported this poor grain protectant effect of *A. vasica* tested against *S. oryzae*. However, these two results contradict the result obtained by some other

workers. For example, it caused more than 50% larval mortality in groundnut leaf minor *Stomopteryx subsecivella* at higher concentrations (0.75 and 1.0%) (Shah, 1996). Under continuous screening programme of toxic plants for antifeedant activity, Thripathi and Singh (1994) have found that methanolic extracts of *A. vasica* gave 98.91% protection against *Spilosoma obliqua*. In view of these results it is evident that this plant is not effective for the control of stored product insects as it is for other agricultural pests.

Another finding of our experiment is that mortality is increased with increase in time. Of all the plants tested, comparatively higher mortality of insects was obtained 6 days after treatment. Irrespective of plants, there occurred a gradual increase of mortality rate from 2nd day to 6th day. Similar observations were made by Lawrence *et al.* (1993) in *T. castaneum*. The treatment with different plant extracts against *T. castaneum* revealed a gradual increase of mortality from 24 h with a maximum at 72 h. By treating with *Annona*, the percent of mortality of *T. confusum* was 27, 33 and 67 on day 1, 3 and 5 respectively and by neem, 13, 23 and 53% mortality at the same interval (Lawrence *et al.*, 1993). Raman *et al.* (2000), however, reported that mortality rate decreased to some extent with time. He argued that this might be due to their degradation in the field environment. Lemon caused very high mortality at 4 days after treatment and pepper gave significantly high mortality at 6 day after treatment in *C. chinensis* (Rajapakse *et al.*, 1998).

From this screening test, among the eight plants, *C. infortunatum* was found to be the most effective one against *C. chinensis*. Compared to the other

plant extracts, it exhibited the maximum mortality percentage in all the observed time durations (2, 4 and 6 days) of treatment (Fig.IV.1). Even with 1% of the plant extracts used, more than 50% mortality resulted within 4 days. Hence it could be concluded that this plant is a rich source of many active components capable of stored grain protection and deserves a detailed study of various actions (repellent, oviposition deterrent and ovicidal).

Chapter V

BIOASSAY FOR REPELLENCY OF SOME LOCAL PLANTS

1. Introduction

The subject of protection of agricultural animals and plants using repellents is almost a virgin territory as a means of practical pest control. Prior to about 1920, various oils and other natural products were the only repellent in general use, particularly against biting flies, gnats and mosquitoes. Examples are oils of cassia, cedar wood, citronella and pennyroyal and alcoholic solution of camphor. Various smokes as from burning pyrethrum flowers, creosole mixtures and sulfur were also extensively used, often ineffectively to prevent the entrance of flying pests into the room.

Several compounds with strong pungent odour make host plant unattractive or unpalatable or offensive, which repel insects away from host plants and thus protect them (Rao, 1990). Repellents might be used more widely for temporary control in limited areas. They might be applied to some natural breeding grounds to direct insect to other areas for control by insecticides or chemosterilants. Botanical insecticides are broad spectrum in pest control and many are safe to apply, unique in action and can be easily processed and used.

Use of insect repellents offer a hope for protection of stored grains from insect attack because they are relatively specific and may have low mammalian toxicity. For protection of stored products from insect attack, there is an increasing need for the use of repellent that are more effective, more persistent

and more economical than existing synthetics available. Locally available plants and minerals have been widely used in the past to protect stored produce against damage by insect infestation (Golob and Webley, 1980). The effectiveness of many plant derivatives for use against stored grain pests have been reviewed by Jacobson (1958, 1975 & 1983), Ketkar (1976), and Jilani (1984). Various activities of extracts and essential oils of spices against stored product pests were reported by several workers (Nawrot *et al.*, 1986; Sharaby, 1988; Khanam *et al.*, 1990; Serit *et al.*, 1992; Talukder and Howse, 1994). Chopped garlic and garlic extracts are repellents to two species of bruchid beetles (Ho and Ma, 1995); acetone extract of black pepper seeds repel *T. castaneum* (Singhamony *et al.*, 1984); various products of neem, *A. indica* have been used since long for the control of various pests of stored grains as reported by Pruthy (1937), Jotwani and Sircar (1965) and Pandey *et al.* (1976).

In the experiments described in this chapter, attempts have been made to evaluate the repellent property of petroleum ether extract of five plants to protect green gram from the attack of the bruchid pest, *C. chinensis*.

2. Methods

A. Screening of five indigenous plants for repellency

For conducting experiments on bioassay of repellent effect of plant extracts, a 'Y' shaped glass tube as described by Read *et al.* (1970), Ahmed and Eapen (1986) and Appel (1994) was employed. The Y tube had an interior diameter of 2 cm; each of the paired-arm was 12 cm long and median arm was 14

cm long was used for this experiment. Petroleum ether extracts (1%) of leaves of *Adathoda vasica*, *Clerodendrum infortunatum*, *Hyptis suaveolens* and *Cymbopogon citratus* and flowers of *Cassia fistula* were prepared from the stock solutions by diluting with acetone.

Five gram samples of green gram were treated with 1.0 ml each of the extracts separately and the solvent was let to evaporate off. The treated grains were taken in one of the paired arms of the Y-tube. The other arm contained control grains, which was treated with 1.0 ml of acetone alone. Twenty healthy *C. chinensis* were released in to the Y tube through the median arm. The open ends of the tubes were immediately plugged with cotton wool. Number of insects present in the treated grains (experimental), control grains and median arms were counted after 30 min, 1 h, 3 h and 24 h. The experiment was replicated five times.

Per cent repellency was calculated by using the formula made by Gillenwater and Mc Donald (1975).

$$\text{Repellency (\%)} = \frac{\text{No. of insects in the control} - \text{No. of insects in the treated}}{\text{No. of insects in the control} + \text{no. of insects in the treated}} \times 100$$

Average percentage repellency from five replicates was calculated for each time period (30 min, 1 h, 3 h and 24 h) for all the tested plants. Positive (+) values indicated repellency and negative (-) values showed the attractancy. The overall average values with respect to the individual plants were calculated and

assigned a repellency class using the following scale described by Mc Govern *et al.*, (1977).

Class	Repellency Rate (%)
0	<0.1
I	0. 1 - 20
II	20.1- 40
III	40.1- 60
IV	60.1- 80
V	80.1- 100

B. Repellent effect of *Clerodendrum infortunatum*

This study was carried out to evaluate the repellency of extract of *C. infortunatum* at different concentrations against *C. chinensis*. In this experiment exposure period was extended up to 3 days.

Different concentrations (0.5%, 2%, 4% and 6%) of petroleum ether extract of *C. infortunatum* were prepared by diluting the stock solution with acetone. Samples of five grams each of green gram (*Phaseolus radiatus*) were smeared with 1.0 ml each of the extract separately in glass Petri dishes. Allowed the grains to dry by placing the Petri dishes under an electric fan for about 15 min.

The treated grains were placed in the experimental arm of the Y-tube and the control arm contained the grains treated with acetone alone. Introduced 20 insects (*C. chinensis*) through the median arm of the Y-tube. All the three open

ends of the tubes were plugged with cotton wool and kept the experimental set up undisturbed in an enamel tray for about 3 days. Number of insects present in three arms were recorded after 30 min, 1 h, 3 h, 24 h, 48 h and 72 h. Experiments were replicated 5 times.

Percentage repellency was calculated by using the formula mentioned earlier. From these values, average percentage of repellency and overall averages were calculated and classified each concentration based on the scale described by Mc Govern *et al.* (1977).

Number of dead insects was also counted after 24 h onwards. Mortality percentage was calculated using the formula

$$\text{Percentage of mortality} = \frac{\text{No. of dead insects}}{\text{Total no. of insects}} \times 100$$

3. Results

(a) Indigenous plants as repellents

In the present experiment, five plant extracts prepared in petroleum ether were assayed for their repellent property against *C. chinensis* by using Y-tube method. Observations were made after at 30 min, 1 h, 3 h and 24 h exposure of insects to the treated grains. Number of insects present on the grains in each arm of the Y tube was counted and the data obtained were analysed statistically by One Way ANOVA. The results of ANOVA are presented in the Table V. 2. It is

Table V. 1. Repellent effect of different plant extracts on *C. chinensis*

Plants	Percentage of repellency				Overall Average of % repellency	Repellency class
	30 min	1 h	3 h	24 h		
C. infortunatum	85	79.24	54.1	37.82	63.53	IV
C. fistula	55.38	41.46	41.30	28.9	41.76	III
H. suaveolens	66.84	50.1	41.98	32.4	47.83	III
C. citratus	72.46	57.12	43.76	32.52	50.92	III
A. vasica	49.6	42.58	27.1	16.76	34.57	II

Data presented in the table are the percentage of repellency, presented as mean of five replicates. Repellency class assigned by Mc Govern *et al.* (1977) based on the range of percentage of repellency [The class I, 0.1- 20; class II, 20.1- 40; class III, 40.1- 60; class IV, 60.1- 80; class V, 80.1-100].

Table V. 2. Repellent effect of different plant extracts on *C. chinensis* during different exposure periods

Plants	Mean no. of insects present in treated sample after			
	30 min	1 h	3 h	24h
C. infortunatum	0.600^{a}	2.000^{a}	2.800^{a}	5.200^{a}
C. fistula	2.200^{cd}	3.600^{c}	3.800^{ab}	6.400^{a}
H. suaveolens	1.600^{bc}	3.200^{bc}	3.800^{ab}	5.600^{a}
C. citratus	1.000^{ab}	2.600^{ab}	3.600^{ab}	5.400^{a}
A. vasica	2.800^{d}	3.200^{bc}	4.600^{b}	6.400^{a}
P value	0.001 ***	0.001 ***	0.0793 NS	0.245 NS

Data presented in the table are the number of insects presented as means of five replicates. Within the vertical column, means having same superscript are not significantly different at 5% level of Duncan's Multiple Range Test. *** very highly significant ($P< 0.001$); NS- Non-significant. The experiment was conducted with 1%extract of the plants.

Table V. 3. Level of significance of repellent effect of different plant extracts on *C. chinensis* at different durations

Dura-tion	Source	D.F.	Sum of square	Mean square	F ratio	P value
30 m	Between group Within group Total	4 20 24	15.760 8.000 23.760	3.9400 0.4000	9.8500	0.001 ***
1 h	Between group Within group Total	4 20 24	7.840 6.000 13.840	1.9600 0.3000	6.5333	0.0016 ***
3 h	Between group Within group Total	4 20 24	8.240 16.800 25.040	2.060 0.8400	2.4524	0.0793 NS
24 h	Between group Within group Total	4 20 24	6.400 21.600 28.000	1.600 1.080	1.4815	0.2452 NS

*** very highly significant ($P < 0.001$); NS – non significant at 5% level of one way ANOVA.

apparent from the data that repellency at shorter time of exposure (30 min, 1 h) with different plant extracts were significantly different ($P<0.001$). However, when time of exposure was longer (3 h, 24 h), difference between plants become insignificant.

There was a gradual decrease in the percentage repellency as the time of treatment was increased (Fig. V.1.). However, extract of *C. infortunatum* maintained the highest level of activity in all the time period tested. In this case, the activity of 85% when observed at 30 min treatment, decreased to about 38% at the end of 24 h treatment. After 30 min of treatment, percentage of repellency in the case of *C. citratus* was about 72.46% and that of *H. suaveolens* was about 67%. *C. fistula* and *A. vasica* exhibited about 55% and 50% repellency against *C. chinensis* at the duration of exposure of 30 min.

When the results of repellency of extracts of different plants after one hour were compared, *C. infortunatum* and *C. citratus* extracts showed 79.24 and 57.12 % repellency respectively. Percentage of repellency of the other three plants (*H. suaveolens, A. vasica* and *C. fistula*) were 50.1, 42.58 and 41.46 respectively (Table V.1.).

After 3 h and 24 h treatment, repellent effect shown by *C. infortunatum* were 54.1 and 37.82% respectively and that shown by *A. vasica* were 27.1 and16.76% respectively. There was no marked difference observed among the other 3 plants. After 3 h, *C. fistula* and *H. suaveolens* have shown more or less the same repellent effect (41.30 and 41.98 respectively). After 24 h, *C. citratus*

and *H. suaveolens* showed almost similar repellent effect (32.52% and 32.4% respectively) (Table V.1) (Fig. V.1).

Based on the overall averages of percentage of repellency at 30 min, 1 h, 3 h and 24 h, plants were categorized into different repellency classes (Mc Govern *et al.*, 1977), as mentioned in the methods section. The repellency classes are; class I, 0.1 to 20%; class II, 20.1 to 40 %; class III, 40.1 to 60%; class IV, 60.1 to 80%; and class V, 80.1 to 100% of repellency.

Repellency classes for the plants tested are presented in the Table V.1. The plants screened were categorized into three classes. *A. vasica* belongs to the repellency class II (20.1- 40%). It exhibited 34.57% repellency. Other three plants (*C. citratus, H. suaveolens* and *C. fistula*) belong to the repellency class III (40.1-60%). The overall averages of repellency of these plants were 50.92, 47.83 and 41.76% respectively.

From this experiment, it was revealed that within the period of 30 min, all the plant extracts exhibited repellent activity from class III to class V and repellent activity decreased to class III to class II during further exposure. The best repellent activity at 24 h exposure period was exhibited by the extract of *C. infortunatum*.

The results of analysis of variance and the comparison of the repellent effect by DMRT are presented in the Tables V. 2 and V.3. It is apparent from the data that the differences in the effect of extracts of various plants during exposure

for 30 min and 1 h, were significant at 0.1% level ($P< 0.001$). However, repellent effect of the extracts after 3 h and 24 h exposure were found to be non-significant.

The number of insects present in the treated sample indicates the efficiency of plant extract as repellent. At 30 min of exposure, only a minimum number of insects (average 0.60) were found in *C. infortunatum* treated sample. *C. citratus* stand next to this. In this case, an average number of 1.00 insect was found on the grain. Maximum number of insects have been found in grains treated with *A. vasica* and *C. fistula* (2.80 and 2.20 respectively). There is a gradual increase in the mean number of insects on prolonged exposure irrespective of plant extract used (Table V. 2)

To compare the significance of differences among plants at different time intervals, DMRT was done at 5% level. Based on this, plants were categorized into different subsets, represented as a, b, c, etc. This grouping is based on the average number of insects present in each set. Samples with same superscript indicate that they belong to the same group or they have no significant differences between them. When the results of 30 min of observations were compared, *C. infortunatum* and *C. citratus* were found to be in the same subset (subset 'a'); *C. citratus* and *H. suaveolens* belonged to another subset (subset 'b') and *H. suaveolens* and *C. fistula* belonged to the 3rd subset 'c' and, 4th subset 'd' consists of *C. fistula* and *A. vasica*. In most of the cases the subsets were found to be overlapping. The difference in repellent effect between extracts after 30 min of exposure was very highly significant ($P< 0.001$).

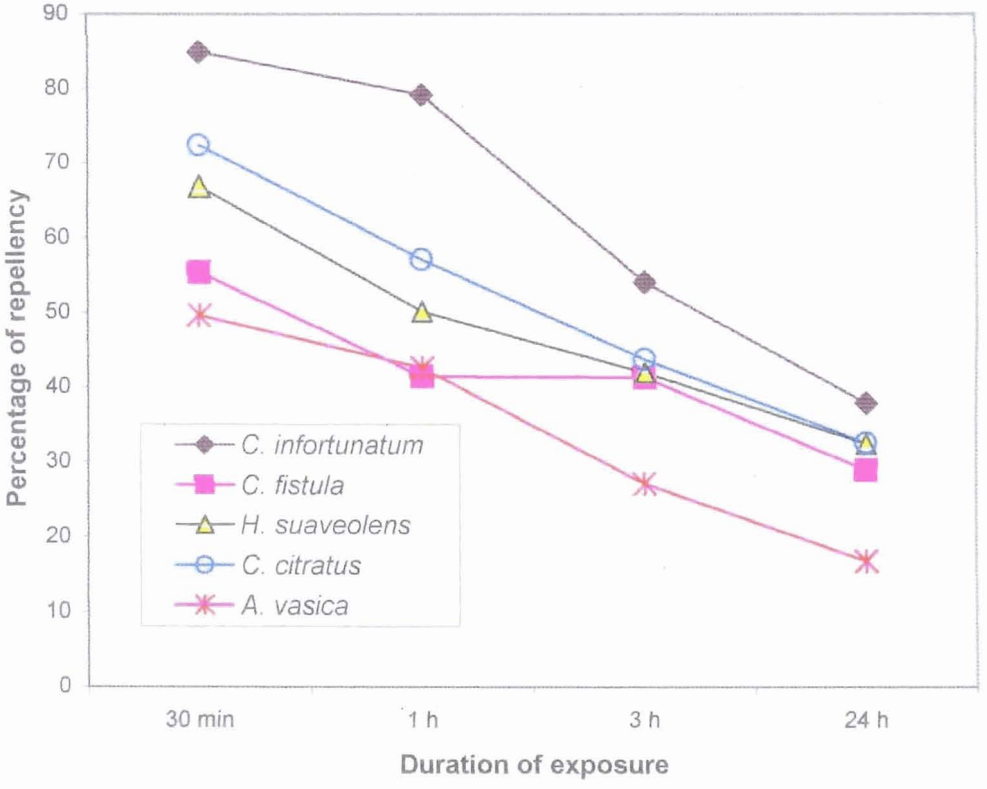
After 1 h exposure also, difference in repellent effect was very highly significant ($P< 0.001$). In this case *C. infortunatum* and *C. citratus* belonged to the same subset (subset 'a'). The mean number of insects present in them were 2.0 and 2.6 respectively. *C. citratus*, *H. suaveolens* and *A. vasica* belonged to the same subset (subset b), and the mean number of insects present in them were 2.6, 3.2 and 3.2 respectively. The 3rd subset, 'c' included *H. suaveolens*, *A. vasica* and *C. fistula*. The mean number of insects present in them were 3.2, 3.2 and 3.6 respectively. Thus it is seen that during 1 h treatment also, minimum number of insects were found in *C. infortunatum* and *C. citratus*.

On the basis of the effect of the extracts after 3 h, plants were grouped into two subsets ('a' and 'b'). Subset 'a' consisted of *C. infortunatum, C. fistla, H. suaveolens* and *C. citratus*, while subset 'b' consisted of all the other four plants except *C. infortunatum*. Differences in repellent effect among plants after 3 h and 24 h were not found to be significant (Table V. 2).

(b) Repellent effect of *C. infortunatum*

In the present investigation different concentrations (0.5%, 2%, 4% and 6%) of petroleum ether extract of *C. infortunatum* were tested against *C. chinensis* to assess their repellent effect. The results revealed that there is a significant difference in repellency between lower (0.5%) and higher (6%) concentrations (Table V. 6). It was also noted that the repellent effect showed a dose response to the concentrations of the plant extracts tested (Fig. V. 2.).

Figure V. 1. Repellent effect of different plant extracts on *C. chinensis*



Percentage of repellency is presented in the Table V. 4. It is apparent that the highest percentage of repellency was observed within 30 min and it gradually decreased with time. After 30 min, even the lower concentration (0.5%) showed more than 50% repellent effect. Based on the percentage of repellency, these samples were grouped into three repellency classes (classes III to V). Maximum repellency (92.57%) was exhibited by 6% concentration and the minimum (58.5%) was by 0.5% concentration. Other two concentrations (2 and 4%) showed 61.60 and 73.20% repellency respectively.

After 1 h and 3 h, a gradual decrease in repellency was observed. In the case of 6% concentration, repellency of 76.2% and 55.5% resulted after 1 h and 3 h respectively. Similar decrease was observed in other concentrations also. In samples treated with 4% concentration, 51.8% insects were repelled after 1 h and 38.65% insects after 3 h. With 2% concentration, the percentage repellency were 46.3 and 32.5 for respectively. Where as, in the case of 0.5% concentration, this was 35.9% and 29.6% respectively (Table V. 4).

After 24 h, repellency further decreased. With 0.5% concentration, only 10.72% insects were repelled while with 2% and 4% concentrations, repellency were 16.45 and 25.02% respectively. Even with 6% concentration of the extract, there was only 45.5% repellency (Table V. 4). Perusal of the Table V.4 reveals that after 48 h, negative value (attractancy?) was observed in some concentrations. In the case of 0.5%, repellency was lost and insects were found to move into the treated samples. About 3.9% more insects moved into the treated grains. Similar effect was also observed in 2% concentration after 72 h exposure. In this case,

Table V. 4. Percentage of repellency and subsequent mortality effect of *C. infortunatum* on *C. chinensis*

Conc.	Treatment after												Mortality %	Overall average	Repellency class
	30 min		1 h		3 h		24 h		48 h		72 h				
	R	M	R	M	R	M	R	M	R	M	R	M			
0.5%	58.05	-	35.9	-	29.6	-	10.72	1.25	-3.9	5	-4.2	7.5	13.8	19.36	I
2.0%	61.60	-	46.3	-	32.5	-	16.45	5	5.0	8.8	-10.8	13.75	27.5	26	II
4.0%	73.20	-	51.8	-	38.6	-	25.02	12.5	14.3	17.5	2.93	21.3	51.3	34.3	II
6.0%	92.57	-	76.2	-	55.5	-	45.5	20.0	20.7	22.5	5.9	25.0	67.5	49.4	III

Data presented in the Table are the percentage of repellency presented as means of 5 replicates. R= repellency; M= mortality; - ve value indicates the % of attractancy; repellency class assigned by Mc Govern *et al.* (1977) based on the range of percentage of repellency. The class I, 0.1- 20; class II, 20.1- 40; class III, 40.1- 60; class IV, 60.1- 80; class V, 80.1-100.

Table V. 5. Repellent effect of different concentrations of *C. infortunatum* against *C. chinensis*

	Mean no. of insects on the treated grains after					
Conc.	**30 min**	**1 h**	**3 h**	**24 h**	**48 h**	**72 h**
0.5%	3.500^{b}	5.500^{c}	6.500^{b}	8.000^{b}	10.750^{c}	12.750^{c}
2.0%	3.500^{b}	4.250^{b}	5.250^{b}	7.750^{b}	9.500^{bc}	9.500^{b}
4.0%	2.500^{ab}	3.500^{b}	5.000^{b}	6.750^{b}	7.500^{ab}	8.000^{a}
6.0%	1.250^{a}	1.500^{a}	2.750^{a}	4.500^{a}	7.250^{a}	7.750^{a}

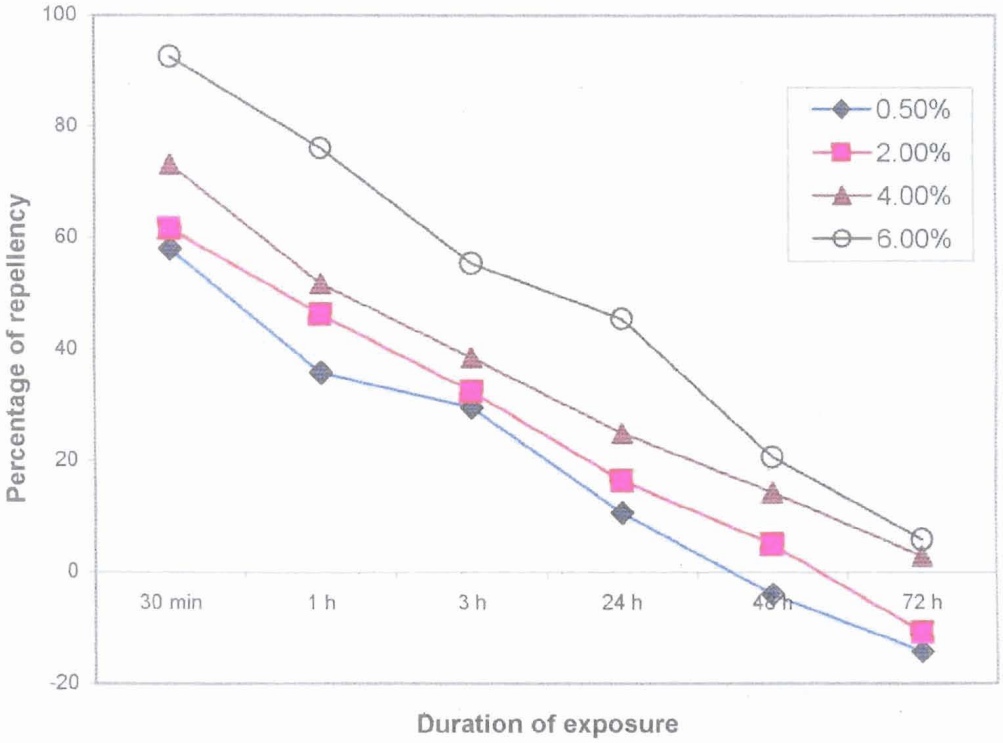
Data presented in the table are the mean number of insects present in 10 replicates. Within the vertical column, means having same superscript are not significantly different at 5% level DMRT.

about 10.8% more insects were found to be coming across to the treated sample. In the case of 0.5% concentration, per cent attractancy has increased up to 14.2. However, a very low level of repellency was still exhibited by 4% and 6% extracts. Their repellency percentage was 2.93 and 5.9% respectively.

Based on the overall averages of repellency, the tested concentrations were categorized into different repellency classes. Among the four concentrations tested, comparatively higher repellency (49.4%) was shown by 6% concentration of extract and it comes under the repellency class III. Lowest repellent effect was shown by 0.5% concentration (19.36%) and it comes under the class I and the other two concentrations (2% and 4%), come under the repellency class II, since their overall average of percentage of repellency were only 26.0% and 34.3% respectively (Table V. 4).

When the results obtained using the four different concentrations (0.5, 2, 4 and 6%) were statistically analysed by ANOVA and differences were compared by DMRT at 5% level, it was seen that the difference between groups (different concentrations) even after 72 h were very highly significant ($P<0.001$)(Table V. 6). Among the four concentrations, the highest concentration (6%) significantly differed from all others. Based on the mean number of insects present in the treated sample, concentrations were categorized into different subsets (Table V. 5.). Observation after 30 min revealed that 6% and 4% concentrations belong to the same subset (subset 'a'). Mean number of insects found in them were 1.25 and 2.50 respectively. The subset 'b' consisted of 0.5, 2 and 4% concentrations. Mean number of insects in them were 3.50, 3.50 and 2.50 respectively.

Figure V. 2. Repellent effect of *C. infortunatum* at different concentrations on *C. chinensis*



After 1 h, concentrations were categorized into three subsets. Subset 'a' consisted of 6% concentration alone and 2% and 4% belonged to subset 'b' and 0.5% concentration fell into the 3rd subset, 'c'. Here also a very highly significant ($P< 0.001$) difference in repellent effect was observed between concentrations (Table V. 6).

After 3 h and 24 h, there were no significant differences between all of the tested concentrations, except 6%. In this case, concentrations were categorized into two subsets (sub set 'a' and 'b'). Subset 'a' consisted of 6% concentration and subset 'b' consisted of all the other concentrations (0.5%, 2%, and 4%). Here also, interaction between groups were highly significant ($P< 0.01$) (Table V. 6).

Analysis of the results for 48 h exposure indicated the highest significant difference ($P< 0.001$) between groups. In this case, 4% and 6% concentrations belonged to the same subset (subset 'a'). The mean number of insects present in them were 7.50 and 7.25 respectively. Overlapping effects were seen between other three concentrations. That is, 4% and 2% belonged to subset 'b' and 2% and 0.5% into subset 'c'. Mean number of insect present in them were 10.75, 9.50, 7.50 and 7.25 for 0.5, 2, 4 and 6% concentrations respectively.

After 72 h, a highly significant ($P< 0.001$) difference in repellent effect between concentrations were observed. In this case, three subsets were categorized. Subset 'a' consists of 4% and 6% concentrations. Mean number of insects present in them were 8.00 and 7.75 respectively. Subset 'b' consisted of 2% concentration (mean 9.50) and subset 'c' consisted of 0.5% concentration (mean 12.75). However, results from this experiment proved that even after 72 h,

Table V. 6. Showing the level of significance of repellent effect of different concentrations of *C. infortunatum* extract on *C. chinensis*

Duration	Source	D.F.	Sum of squares	Mean square	F ratio	P level
30 m	Between group	3	16.7500	5.5833	5.1538	0.0161
	Within group	12	13.000	1.0833		**
	Total	15	29.7500			
1h	Between group	3	33.6875	11.229	23.434	0.000
	Within group	12	5.750	0.479		***
	Total	15	39.437			
3h	Between group	3	29.250	9.750	7.090	0.005
	Within group	12	16.500	1.375		**
	Total	15	45.750	10.166		
24h	Between group	3	30.500		14.352	0.0003
	Within group	12	8.500	0.708		***
	Total	15	39.000			
48h	Between group	3	33.500	11.166	6.232	0.008
	Within group	12	21.500	1.791		**
	Total	15	55.000			
72h	Between group	3	63.500	21.166	39.076	0.000
	Within group	12	6.500	0.541		***
	Total	15	70.000			

*** very highly significant (P< 0.001); ** highly significant (P< 0.01).
Concentrations used are: 0.5%, 2.0%, 4.0% and 6.0%.

difference between groups (concentrations) were highly significant at 5% level by DMRT.

Another observation of this experiment was that after 24 h exposure, a remarkable percentage of insects were found dead with each concentration. The mortality percentage increased with increase in concentration and treatment period (Table V. 4). After 24 h, about 1.25% insects were found dead in 0.5% concentration and 5% and 12.5% mortality resulted in the case of 2% and 4% concentrations respectively. With 6% concentration, an average of 20% insects were found dead, whereas, after 48 h, mortality percentage increased to 5%, 8.8%, 17.5% and 22.5% with 0.5, 2, 4, and 6% concentrations respectively (Table V. 4). Further increase in mortality was observed after 72 h exposure. From 6% concentrated extract about 25% insects and from 0.5% concentration about 7.5% insects were found dead. In the other two concentrations (2 and 4%), mortality percentage was 13.75 and 21.3 % respectively. Total mortality percentage from each concentration during the 72 h of treatment were 13.8%, 27.5%, 51.3% and 67.5% for 0.5, 2, 4, and 6% respectively (Table V. 4).

4. Discussion

(a) Indigenous plants as repellents

In the repellency test using Y-tube, three options were provided to insects to select, i.e., arm with treated grains, arms with untreated grains and median arm without grains. In the case of all the five plant extracts tested, majority of the insects avoided the treated grains even after 24 h. It was observed that a very

good percentage of insects preferred the untreated grains and a few insects were found in the median arm also. In this experiment it was revealed that grains treated with plant extracts cause some behavioural changes in insects, which kept the insects away from the treated grains. This behavioural response was sustained for a few hours. To calculate the percentage of repellency, the insects present in the base arm, referred to as the un-reached group (Jilani *et al.*, 1988 [illegible] were not considered.

Among the five plant extracts tested for their repellent effects on *C. chinensis*, after 30 min of exposure, *C. infortunatum* showed the highest repellency (85%) and *C. citratus* showed next higher activity (72.46%). *C. fistula* and *H. suaveolens* showed repellency of 55.38% and 66.84% respectively. Lowest repellent effect was shown by *A. vasica* (49.6%) (Table V.1.). Results of ANOVA indicated that the significance of differences between repellent effects of different plant extracts at this time duration (30 min) was very high ($P < 0.001$). Results suggested that all the plant extracts tested, did not show similar effect on insect movement during the first 30 min of observation (Table V. 2). Even though, the two plants, *C. infortunatum* and *C. citratus* statistically belong to the same subset (subset 'a'), the mean number of insect present in them varied. Similar were the cases with other plants also (Table V. 2). Observation after 30 min also revealed that there was an overlapping effect in the mean number of insects affected by some plant extracts, i.e., *C. citratus* belonged to both the subsets 'a' and 'b'; *H. suaveolens* came under two subsets 'b' and 'c' and *C. fistula* came under the subsets 'c' and 'd'. This overlapping effect is based on the

comparison of variation in means by DMRT at the corresponding significant levels (5%).

The degrees of repellency of the various plants observed after 30 min of treatment were found to diminish on further exposure. Data provided in Table V. 1. is indicative of this decrease. Although all the plant extracts followed almost a similar pattern in this decrease during the 24 h of treatment, *C. infortunatum* extract still had an appreciable percent repellency (64%). *C. citratus* also exhibited a reasonably good degree of repellency (51%). The order of repellency of the different extracts after 24 h treatment was *C. infortunatum* > *C. citratus* > *H. suaveolens* > *C. fistula* > *A. vasica.*

Statistical analysis of the data on repellency showed that the effects of the different plant extracts during the initial stages of treatment (30 min and 1 h) were highly significant ($P < 0.001$), whereas effects of treatment on further exposure beyond 1 h (3 h and 24 h) were found to be non- significant (Table V. 2 & 3). By comparing the percentage of repellency of different plant extracts tested at different time interval, it was revealed that the percentage of repellency decreased with time (Table V.1.). The pattern of decline in the repellency is suggestive of possible complete loss of activity on continued exposure.

This type of inverse relationship was reported by Ahmed and Eapen (1986) when essential oil of eucalyptus was tried against *S. oryzae*. There was 80% repellency after 10 min; 50% after 30 min and after 60 min, repellency decreased to 20%. It was found that in the case of lemon grass oil (*C. citratus*) also, the loss of activity was found to be similar to that of eucalyptus. Decline in the

percentage of repellency during the increased exposure time was reported by Urs and Srilatha (1990). It was found that eucalyptus showed 100% repellency against the test insect, *Corcyra cephalonica* after 10 min at the highest concentration, and it gradually decreased to 15% after 8 h. Malik and Naqvi (1984) also reported a similar decrease of repellent property of plant extracts with time. However, Singh and Singh (1991) reported that highest repellent activity (100%) of *Ocimum gratissimum* obtained when tested against houseflies persisted even after 5 h. The results obtained in the present work are in agreement with that of some of the earlier published results. It has been shown that the repellent effects of plants vary and it depends on the presence of some highly volatile chemical components in it. Jilani and Su (1983) expressed a similar view based on their results. According to them, decrease of repellency with time is due to the loss of some highly volatile or unstable active components that are present in the extract.

Table V.1. provides details of the various classes assigned to different plant extracts depending on their efficiency as repellent. These classes were found to shift to lower classes as time of treatment increased. Of all the different repellency classes recognized on the basis of the overall average percentage of repellency, *C. infortunatum* occupied the highest position (class IV), whereas *A. vasica* occupied the lowest position. The results thus reveals that various plants contain different constituent phytochemicals in various proportion and also that due to the difference in the volatility of these components, the duration of repellency varies.

Similar observations were made by many workers. Malik and Naqvi (1984) tested seven plants for their repellent activity against *T. castaneum* and found that in the first week most of the plant extracts exhibited repellent activity from class III to class IV, with the maximum being from *Saussurea lappa.* According to their findings, repellent activity decreased subsequently to class III to class II. Ethanol and acetone extracts of *Dennettia* seed powder elicited optimum repellency (class III) against the leather beetle, *Dermestes maculatus* and the activity remained up to 4 weeks after treatment (Egwunyenga *et al.,* 1998). The overall average per cent repellency of turmeric crude extracts and other components (turmeron, ar-turmeron and curcumin) were tested against *T. castaneum* for 8 weeks and was found that the crude extract and ar-turmeron gave an average of 71.5% and 62.9% repellency (class IV) and turmeron gave 43.1% repellency (class III) (Su *et al.,* 1982).

The studies reported in this chapter thus brings out that the plants screened for repellency do have many compounds that are capable of repelling *C. chinensis.* However, this activity varies among the various plants to a great extent. Moreover, the activity had drastically diminished within 24 h. This is attributed to the high volatility of the repellent compounds, which evaporates gradually over time. It is also possible that insect get accustomed to the compounds making the insect non-reactive.

(b) Repellent effect of *C. infortunatum*

From the screening test for repellency of five plants, *C. infortunatum* was identified as the most effective plant against *C. chinensis.* In the present study,

petroleum ether extract of this plant was subjected to further repellency test using various concentrations. Results obtained were analysed statistically by one way ANOVA and differences in repellent effects due to different concentrations were compared by DMRT at 5% level. From this analysis it was revealed that difference in repellent effects due to different concentrations during different time duration were very highly significant ($P<0.001$). However, perusal of the Table V.4 revealed that repellency increased with concentration and decreased with time.

In the present experiment, the exposure period given was up to 72 h. Results presented in Table V. 4 indicates that highest level of repellency occurred at the first 30 min of exposure of the insects. Within 30 min, it was observed that about 92.57% insects were repelled from samples treated with 6% extract. Decrease in the concentrations resulted in decreased level of repellency (Table V. 4). However, all the concentrations tested exhibited more than 50% repellency. High repellency observed may be due to the occurrence of highly volatile components present in the extract. Further treatment for longer period (up to 72 h) with all the concentrations gave similar pattern of response. Similar effects reported by Ahmed and Eapen (1986) in the case of repellency of essential oils of eucalyptus and cineole against *M. domestica* was due to volatilization of the oil. According to their study, these essential oils were highly effective during the first ten minutes of exposure when maximum volatilization of oils took place. Thereafter, the volatilization and also the repellency declined gradually. Malik

and Naqvi (1984) also reported a gradual decrease of repellent property of different plant extracts against *T. castaneum* with the advancement of time.

However, it was found that after 48 h and 72 h, repellent effect of the extract at 0.5% and 2% concentrations were lost and a good number of insects remained in the treated area. This resulted in negative values, which could be designated as attractancy of the extracts (Talukdar and Howse, 1995). Here, the number of insects present in the treated sample is higher than that in the control. In the case of 0.5% concentration, after 48 h, an average of 3.9% insects get attracted to the treated area and it increased to 14.2% after 72 h. These were represented as –3.9% and –14.2% respectively (Table V. 4). From these results, it was proved that repellency is lost with the decreased dose and increased duration.

Another finding of this experiment was that, there was a dose dependent repellency by the extract against *C. chinensis,* irrespective of duration. The difference in repellent effect was very highly significant ($P< 0.001$) in almost all the tested durations (Table V.6). Percentage of repellency increased with increasing concentrations (Table V. 4). Data provided in Table V. 4, reveals that lowest level of repellency was recorded with lower concentration (0.5%) in all time duration and, a progressive increase resulted as the concentration increased. A concentration dependent effect was seen even after 72 h treatment. In this case, with 0.5% concentration, about 4.2% more insects moved in to the grains treated with the plant extract, whereas in 2% concentration it was 10.8%. In the case of 4% and 6% concentrations very low levels of repellent effects (2.93% and 5.9%) were still exhibited. Sahayaraj and Paulraj (2000, 2001) also obtained similar

results from experiment with different plant extracts against *Spodoptera litura* and *Helicoverpa armigera.* Dose dependent repellent effect of sweet flag, sowa, clove and cedar wood oil along with citronella and eucalyptus at a higher concentration (5%) were reported in the rice moth, *C. cephalonica* (Behal, 1998). Similar results were obtained with turmeric oil, sweet flag oil, neem oil and margosan-O against *T. castaneum* by Jilani *et al.* (1988 b) and with essential oils of *Artemisia annua* against *T. castaneum* and *C. maculatus* by Tripathi *et al.* (2000).

Another observation made in our experiment is the occurrence of mortality of the insects after 24 h of treatment. In all the concentrations tested (0.5, 2, 4 and 6%) there was remarkable mortality, which was found to be dose-dependent and time-dependent (Table V. 4). From the observations, it was revealed that repellent effect of *C. infortunatum* persisted only for a few hours (3 h). More than its repellent activity, it exhibited toxic effect. With higher concentrations such as 4% and 6%, more than 50% insects were found dead within 72 h.

With the various concentrations of the extract, promising repellency was exhibited during a period of exposure of 30 min. The highest repellency was exhibited by 6% concentration (92.57%) and it belongs to the class V. At the same duration, other concentrations such as, 0.5%, 2% and 4% exhibited 58.05, 61.60 and 73.20% repellency respectively and they are included in the repellency class IV. After 1 h, repellency declined in the case of all the concentrations and after 48 h, change to negative values were observed in certain cases (0.5%). Based on the overall average of percentage of repellency, the concentrations of

the extracts were categorized in to 3 different repellency classes (Mc Govern *et al.*, 1977). According to this, 6% concentration belongs to repellency class III; 2% and 4% belongs to repellency class II and 0.5% concentration belongs to repellency class I.

From these findings, we come to the conclusion that presence of highly volatile chemical components in the *C. infortunatum* extracts resulted in the immediate repellent effect against the tested insect and loss of this components may lead to the decline of repellent effect. In other words, substances that cause persistent repellency are absent in this extract. However, substances that are more toxic to the insects were in great abundance in this extract. Some of the low volatile essential oils such as terpenes, steroids and glycosides belong to this group. To get a clear picture of such components, further detailed chemical study was required. Sahayaraj and Paulraj (2001) reported that there is no relationship between toxicity and repellency. Present study is in conformity with these findings. Even though, *C. infortunatum* did not act as a persistant repellent against *C. chinensis*, it acted as a very effective insecticide for a long period of time.

Chapter VI

EFFECTS OF DIFFERENT PLANT EXTRACTS ON OVIPOSITION OF *CALLOSOBRUCHUS CHINENSIS*

1. Introduction

Many factors affect the number of eggs laid by the females of the genus *Callosobruchus*. Number of host seeds available to each female (Credland *et al.*, 1986), characteristics of host seeds such as roughness of seed coat (Nwanze and Horber, 1976), seed size and shape (Nwanze *et al.*, 1975), temperature, humidity (Howe and Currie, 1964; Giga and Smith, 1983) and density of adult beetles (Bellows, 1982), all contribute to the variation in oviposition rate. For internally feeding granivorous insects such as the bean weevils, a single ovipositing female will maximize her fitness by dispersing her eggs over the available seeds to minimize the effects of larval competition between her offsprings (Smith and Lessells, 1985; Wilson, 1988).

Egg distribution on cowpea seeds and the influence of the quantity of available seeds on the extent of oviposition by *C. maculatus* were investigated by Ofuya (1987). The study revealed that the number of eggs laid by females increased with increase in the quantity of seeds. Dias and Yadav (1988) suggested that physical and biochemical factors play major roles in the process of oviposition and host selection of pulse beetle. The pulse beetle showed a definite intra-varietal response for oviposition. Seeds with rough surface were less preferred for oviposition, where the percentage of grains infested with eggs and the number of

eggs laid per grain were minimum compared to the grains with smooth surface (Chavan *et al.*, 1997). Influence of some fatty acids on oviposition of the bruchid beetle, was studied by Parr *et al.* (1998). The study revealed that an appropriate mixture of fatty acids in the epicuticular waxes stimulated oviposition. However, an elevated level of oleic acid in conjunction with others acted as deterrents.

Deterrent effect of some botanical products on oviposition of *C. maculatus* was reported by Elhag (2000). Extracts prepared from plants were tested as oviposition deterrent by choice and no-choice methods for *C. maculatus*. Oil of *Azadirachta indica*, *Milletia ferrugenea* and *Crysanthemum cinerariaefolium* were much effective in partially or completely preventing egg laying and subsequent hatching. Uvah and Ishaya (1993) worked out the effect of some vegetable oils like olive oil, ground nut oil etc. on oviposition and longevity of *C. maculatus*. Their results indicated significant reduction in oviposition, progeny emergence and longevity by a single application of ground nut oil or palm oil at the rate of 2.5 ml - 3.0 ml/kg seeds. Chiranjeevi and Sudhakar (1996) reported the effect of some indigenous plant materials on the fecundity, adult emergence and development of *C. chinensis*. They found that the egg laying capacity of the pest and total number of adult emerged decreased with increase in concentration of powder and ashes of the plants.

Leaf extracts of *C. siphonanthes* reduced fertility of *C. chinensis*. The decline in oviposition at higher doses was attributed to their interference with vitellogenesis and also to the damage caused to the egg chambers in the ovaries of *C. chinensis* (Pandey and Khan 1998 a). *A. indica* gave significantly high reduction

in oviposition of *C. maculatus* (Rajapakse *et al.*, 1998). Dwivedi and Maheshwari (1997) screened extracts of ten plants prepared in two different solvents and reported that *Croton bonplandianum* (acetone), *Verbisinia enceliodes* (petroleum ether) and *Cassia occidentalis* (in both solvents) exhibited better oviposition deterrent properties against *C. chinensis*.

In the present investigation, attempts have been made to evaluate the efficacy of petroleum ether extracts of three selected plants, *C. infortunatum* (leaves), *Cymbopogon citratus* (leaves) and *Cassia fistula* (flower) as oviposition deterrent against *C. chinensis*.

2. Methods

From the stock extracts of *C. infortunatum*, *C. citratus* and *C. fistula*, 1% and 2% concentrations of the extracts were prepared by diluting with acetone. To find out the oviposition deterrent properties of these extracts, appropriate bioassays were conducted. In the experiment, 3 samples of 50 grains each of green gram (*Phaseolus radiatus*) were treated with 1 ml each of the plant extracts. After evaporating the solvent, grains were taken in glass Petri dishes and one pair (1 male & 1 female) of *C. chinensis* was introduced in to each set. A control set was included in which seeds were treated with solvent only. Experiment was replicated five times and it was kept at optimum temperature (28 ± 2°C) and relative humidity (75 ± 5%). Total number of eggs laid by the individual females in each set within six days were recorded.

Oviposition deterrence was measured by means of Discrimination Quotient (DQ) adopted by Messina and Renwick (1983).

$$DQ = \frac{\text{No. of eggs in control} - \text{No. of eggs in treated}}{\text{Total no. of eggs in control and treated samples}}$$

DQ ranges from -1 (all eggs in treated vial) to +1 (all eggs in control vial)

Percentage of reduction in oviposition was calculated by the formula:

$$\%\text{ of reduction in oviposition} = \frac{\text{No. of eggs on control} - \text{no. of eggs in treated}}{\text{Total no. of eggs on control}} \times 100$$

Data obtained were subjected to one way Analysis of Variance and level of significance was compared by Duncan's Multiple Range Test (DMRT) at 5% level. Per cent reduction in oviposition and DQ were subjected to chi-square test to compare the level of significance.

3. Results

Oviposition deterrence of three plants, *C. infortunatum*, *C. citratus* and *C. fistula* are presented in the Table VI.1. Perusal of the Table VI.1 reveals that the highest reduction in oviposition was brought about by extracts of *C. infortunatum* at 1% and 2% concentrations (61.82% and 70.91% respectively) followed by *C. citratus* at 2% concentration (32.73%). Among the plants tested, lowest oviposition deterrency was shown by *C. fistula* at 1% concentration (14.25%) (Fig. VI.1).

Table VI. 1. Mean number of eggs laid by *C. chinensis* on green gram treated with different plant extracts

Plants	Conc.	No. of eggs (Mean ± SD)
C. infortunatum	1%	21.20 [a] ± 5.45
	2%	15.80 [a] ± 7. 82
C. citratus	1%	41.60 [b] ± 1.34
	2%	37.20 [b] ± 6.72
C. fistula	1%	47.00 [bc] ± 12.21
	2%	41.20 [b] ± 7.85
Control	-	54.60 [c] ± 11.06

Means followed by same superscript letter indicates no significant difference at 5% level.

Table VI. 2. Oviposition deterrence caused by three plant extracts (petroleum ether) on *C. chinensis*

Plants	Conc.	Average no. of eggs in treated	Average no. of eggs in control	Oviposition reduction (%)	DQ	Chi-square value	P level
C. infortunatum	1%	21	55	61.82	0.452	21.02	0.001
	2%	16	55	70.91	0.549	27.65	0.001
C. citratus	1%	42	55	23.64	0.134	3.07	NS
	2%	37	55	32.71	0.195	5.89	NS
C. fistula	1%	47	55	14.55	0.078	1.16	NS
	2%	41	55	25.45	0.145	3.56	NS

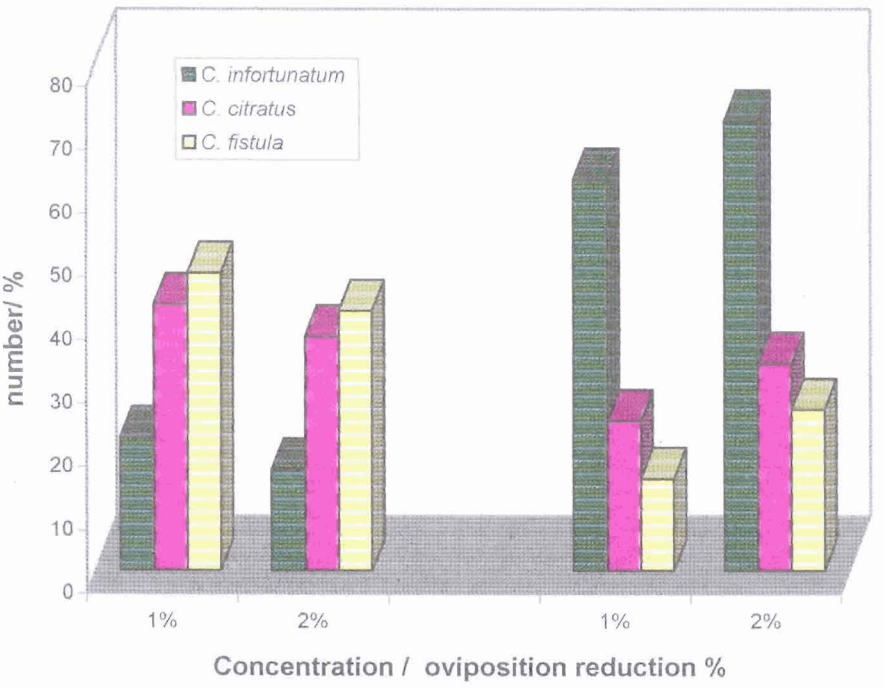
DQ= Discrimination quotient; P= Significant level of chi-square test; NS = not significant.

Data obtained were subjected to ANOVA and the means compared by Duncan's Multiple Range Test at 5% level. It has been found that all the treatments caused significant ($P<0.001$) reduction of oviposition by *C. chinensis* in comparison with control (Table VI. 2). It is quite apparent from the data presented in the table that maximum number of eggs were laid in the control sample (average 54.60), followed by grains treated with 1% *C. fistula* extract (47.00). Grains treated with 1% *C. citratus* extract showed an average of 41.60 eggs, while the grains treated with 2% extract of *C. fistula* showed only 41.20 eggs. Minimum number of eggs was found in the grains treated with 1% and 2% *C. infortunatum* extracts (21.20 and 15.80 respectively) (Table VI.1) and these effects were significantly different from those of all other plant extracts tested.

The DQ values reflect the oviposition deterrent property. Since the values obtained in the present experiment were all positive, it is presumed that the plants tested were having promising oviposition deterrency. However, *C. infortunatum* at the concentrations tested (1% and 2%) were giving better results. Here, deterrency was far better (DQ values 0.452 and 0.549 for 1% and 2% respectively) than other plants tested (Table VI. 1). DQ values of *C. citratus* were 0.134 and 0.195 and *C. fistula* were 0.078 and 0.145 for 1% and 2% concentrations respectively (Table VI.1)

These results were analysed statistically by chi-square test. It was found that effect of *Clerodendrum* extracts were highly significant at $P<0.001$ level while others are statistically non-significant. It is quite apparent from the results that leaf

Figure VI. 1. Oviposition deterrent effect of three plants on *C. chinensis*



extracts of *C. infortunatum* and *C. citrtus* and flower extracts of *C. fistula* possess oviposition deterrent properties against *C. chinensis*.

4. Discussion

In the present experiment, three plants (*C. infortunatum*, *C. citratus*, and *C. fistula*) were selected for evaluating their oviposition deterrent effect against *C. chinensis*. The plants were selected on the basis of the satisfactory results obtained in the screening experiments described in the previous chapters (IV and V) with respect to their insecticidal and repellent properties against this insect.

Among the three plants, *C. infortunatum* exhibited high oviposition deterrent effect against *C. chinensis* than others. Perusal of the Table VI.1 revealed that minimum number of eggs (21.80 and 15.80) were laid on grains treated with *C. infortunatum* extract at 1% and 2% concentrations respectively. Grains treated with *C. citratus* extract showed slightly lower effect. In this case, a mean number of 41.60 and 37.20 eggs were laid in 1% and 2% extracts respectively. While, maximum number of eggs were found in *C. fistula* extract treated samples (47.00 and 41.20 at 1% and 2% concentrations respectively). The number of eggs present on the grains of the control sample was significantly higher than the other treated samples. (54.60)(Fig VI.1).

Percentage of reduction in oviposition and discrimination quotient presented in the Table VI. 2 revealed that per cent reduction in oviposition is maximum (70.91% & 61.82%) when treated with 2% and 1% concentration of *C. infortunatum* extracts and minimum in grains treated with 1% *C. fistula* flower

extract (14.55%) (Figure VI. 1.). About 32.73% reduction in oviposition resulted by treatment with *C. citratus* extracts at 2% concentration. However, neither 1% concentration of *C. citratus* nor 2% concentration of *C. fistula* extracts showed any significant difference between them (TableVI.1and VI.2). Their percentage of reduction in oviposition was 23.64% and 25.45% respectively.

By analyzing the data of mean number of eggs present on the grains by one way ANOVA and means compared by DMRT at 5% level, it has been found that extracts of *C. citratus* (1% and 2%) and *C. fistula* (1% & 2%) have no significant difference between them. They come under same group (group 'b'). All these treatments showed significant ($P < 0.001$) difference with the control except 1% concentration of *C. fistula* extract (Table VI.1). Among the three plants tested, *C. infortunatum* exhibited increased level of oviposition deterrent property against this insect, irrespective of the concentrations. These two extracts (1% and 2%) come under separate group (group 'a'). Only this plant extract exhibited more than 50% reduction in oviposition (Table VI.2).

The DQ values indicated the efficiency of oviposition deterrent property (Messina and Renwick, 1983). Perusal of the Table VI.2 revealed that the DQ value ranges from 0.078 to 0.549 among all the tested samples. Highest value resulted in 2% concentration of *C. infortunatum* (0.549) and 1% concentration of the same plant came next (0.452). Since DQ value of all these treatments showed positive value, it can be concluded that all the tested samples are effective oviposition deterrents against *C. chinensis*. By analyzing the data with chi-square

test, *C. infortunatum* 1% and 2% are found highly significant ($P<0.001$), while others are statistically non-significant.

These findings are in accordance with the observations of Jadhav and Jadhav (1984) and Pandey *et al.* (1986) who observed that extracts of *Jatropha curcas*, *Azadirachta indica*, *Lantana camara*, *Ageratum conyzoids* and *Thevatia nerifolia* have anti-ovipositional properties against stored grain pests. Earlier, Ofuya (1990) reported that very few eggs were laid by *C. maculatus* on cowpea seeds when mixed with bark powder of *Erythrophleum suaveolens* and shoot powder of *Ocimum gratissimum*. It may be possible that the toxic substance present in the plant extracts affect physiological and biochemical processes associated with embryonic development resulting in low egg hatchability.

In the present study, *Cymbopogon citratus* has moderately inhibited the oviposition with the two concentrations (23.64 and 32.73% eggs with 1% and 2% respectively). Rajapakse and Senanayake (1997) reported that *C. citratus* oil inhibited oviposition of *C. chinensis* most effectively at 0.8% concentration (mean 4.20 eggs) in comparison to other oils tested. Rajapakse (1996) also obtained similar results in *C. maculatus*. This finding was in agreement with the report of Sharaby (1988). According to his finding, 1-2% concentrations of lemon grass oil affected the egg masses as well as the newly hatched larvae, and thus protected the cotton crops from being attacked by *S. exigua.*

The volatile oil of *Piper nigrum* completely suppressed oviposition and adult emergence at 0.8% concentration. Among the vegetable oils, neem oil was most effective as oviposition deterrent in which significantly less number of eggs (8.9)

was deposited (Bhatnagar *et al.*, 2001). *Cassia* fruit extract exhibited 8.11% reduction in oviposition by *C. cephalonica* (Dwivedi and Garg, 2000).

The mean number of eggs laid by *C. chinensis* was observed to be very low (27.7%) in grains treated with sweet flag *A. calamus* (Rao *et al.*, 1990 a). This was in agreement with the results of Agarwal *et al.* (1973) who ascribed the effectiveness of sweet flag to its active principle 'osarone' which adversely affected the fecundity. Oils from *L. camara, C. citratus, Eugenia uniflora* and *Lippia adoensis* were more potent than their respective powders in reducing or inhibiting oviposition and adult emergence (Gbolade and Adebaye, 1995). Contrary to these findings, Ban *et al.* (2000) reported that plant oils did not have any significant effect on oviposition behavior or survival of immatures of *C. chinensis*, whereas the beetles significantly avoided the oil- treated area.

Mulatu and Cebremedhin (2000) reported the oviposition deterrent and toxic effect of seven plants on *C. chinensis*. Among this, *A. indica, Milletia ferruginea* and *Chrysanthemum cinearaefolium* were effective in partly or completely preventing egg laying and hatching of eggs.

Leaf extracts of *Clerodendrum siphonanthus* reduced fertility of *C. chinensis*. The decline in oviposition at higher doses of extracts was attributed to their interference in the vitellogenesis and the development of ovaries (Pandey and Khan, 1998 b). Effect of volatile oils on oviposition seems to be brought in by absorption through the cuticle, although passage through the spiracles could not be excluded (Dhar *et al.*, 1996). Flower extract of *Butea monosperma* caused abnormalities in the ovaries of *Dysdercus similes* (Raju and Thakur, 1995).

Compound egg chambers were observed mostly in the ovaries of treated adults. Poor yolk deposition and unovulated batches of oocytes also resulted. At a concentration of 4% there was a complete inhibition of oogenesis and vitellogenesis. Oil dressing on cowpea seeds exhibited significant oviposition deterrence and complete inhibition of emergence of adult insects. Gbolade *et al.* (1999) noticed a similar effect on *C. maculatus* by oil of *Ageratum conyzoids*.

In brief, it is found that oviposition of *C. chinensis* depends on many factors. Besides the seed characteristics, environmental factors and population factors, it has been found that several plant materials such as essential oils and its crude extracts etc. coated on grains also lead to the inhibition of oviposition of insects. In the present investigation, three selected plants were tested for their oviposition deterrent property against *C. chinensis* and it was found that the three plants tested, *C. infortunatum*, *Cymbopogon citratus* and *Cassia fistula* were very effective in inhibiting oviposition of the insect, *C. chinensis*. All of them exhibited significant oviposition deterrent activity ($P < 0.001$). However, among these plants, *C. infortunatum* showed better activity and *C. fistula* was the poorest deterrent.

Chapter VII

TOXIC EFFECT OF *CLERODENDRUM INFORTUNATUM* ON *CALLOSOBRUCHUS CHINENSIS*

1. Introduction

In the complex folk medicinal system of Ayurveda in India, different parts of *C. infortunatum* are used for various ailments (Akihisa *et al.*, 1988). It is used to treat cough, skin diseases and tumours and is used as a vermifuge. Singh and Anand (1996) reported the ethnomedical property of this plant. The essential oils of leaves and root bark of *C. infortunatum* contain an array of chemical components having some of these properties. Cabruvin and quercetin isolated from the root of *C. infortunatum* had good inhibition on spore germination of fungus (Roy *et al.* (1996).

Rao *et al.* (1993) isolated two neoclerodane diterpenes, clerodendrins B and C, which act as insect growth inhibitors and antifeedants. Insecticidal and molluscicidal properties of *C. infortunatum* was observed by Jirovetz *et al.* (1999) and according to them, this property was due to the presence of a chemical component, linalool. Sheela (1997) tested the insecticidal effect of 1% petroleum ether extract of five plants and found that *C. infortunatum* had the highest level of toxic action against the rice ear bug, *Leptocorisa acuta*. Likewise, various studies have been carried out with other species of *Clerodendrum.* Pandey and Khan (2000) reported the antifeedant effect of *C. siphonanthes* leaf extract against *C. chinensis* female by dipping method. Effect of leaf extracts of *C. colebrookianum*

on the blood pressure in rat was studied by Gupta *et al.*(1995 b). An extremely bitter fraction giving a positive test for alkaloids was isolated from the leaves of *C. colebrookianum.* This fraction produced the fall in blood pressure in a dose dependent manner. Similar effect was reported by Lu and Yukimura (1994), using *C. trichotomum.* Pereira and Gurudutt (1990) studied the growth inhibitory effect of clerodane compound, (-)-3 epicaryoptin isolated from *C. inerme* on *Culex quinquefasciatus* larvae.

Although, *Clerodendrum* species have been used for medicinal purpose and for the control of various pest insects, very little work has been reported on the control of stored grain pests. Kumar *et al.* (1990) studied the efficacy of *C. siphonanthes* against *C. chinensis.* The highest adult mortality (73.3%) was attained at one day after treatment, while 100% mortality was obtained after 8 days of treatment.

In view of the results obtained in the screening test and repellency test described in chapters IV and V, it was thought worthwhile to study the toxic effect of *C. infortunatum* against the pulse beetle, *C. chinensis* in a detailed manner. Effort was made to study the insecticidal effect of this plant extract at different concentrations and at different durations of exposure.

2. Methods

In the present experiment, residual film method was adopted. Different concentrations (0.5%, 1%, 2%, 4% and 6%) of the two extracts of *C. infortunatum* (petroleum ether and methanol) were prepared from the stock solution by diluting

with acetone. Glass Petri dishes (10 cm diameter) were layered with the test material by pouring 1.0 ml of each extract and immediately spreading all along the inner side of both the dishes and their lids uniformly and dried by gentle shaking. Introduced 20 healthy adult insects in to each Petri dish. The control sets with 1.0 ml of the carrier solvent, acetone, in the place of extract was also kept. Each concentration was replicated 5 times. Experiment was carried out at 28 ± 2°C and 70 ± 5% relative humidity.

Observations on mortality were recorded after 2, 4, 6, 10 and 15 days of treatment with each of the different formulations. Data were subjected to one way Analysis of Variance (ANOVA) and t- test was used to compare the means.

From the data obtained, mortality percentage was calculated by applying the formula:

$$\text{Percentage mortality} = \frac{\text{No. of dead insects}}{\text{Total no. of treated insects}} \times 100$$

LC_{50} values were obtained for 2, 4 and 6 days of both the petroleum ether and methanol extract treatment by probit analysis (Finney, 1971) by using maximum likelihood programme software.

3. Results

In the present experiment, different concentrations (0.5, 1, 2, 4 and 6%) of petroleum ether and methanol extract of *C. infortunatum* were tested against *C. chinensis* to evaluate their toxic effect. From the data obtained, total percentage of mortality and LC_{50} values at 2, 4 and 6 days were calculated. In addition to

this, average time required to bring 100% mortality with different concentrations were also calculated.

Cumulative mortality percentage after treatment with different concentrations of petroleum ether and methanol extracts are provided Table VII.1. During the second day of observation, 10% mortality was effected by 0.5% concentration of both the extracts. Petroleum ether extracts of 1%, 2%, 4% and 6% concentrations resulted in 19%, 28%, 39% and 54% mortality respectively. While with the same concentrations (0.5, 1, 2, 4 and 6%) of methanol extract, the mortality percentages were 10%, 12%, 20%, 27% and 30% respectively.

Similar dose dependent effect was seen during 4 and 6 days treatment also. In samples treated with 0.5% petroleum ether extract, a cumulative of 35% of insects were found dead after 4 days. In the case of samples treated with 6% petroleum ether extract this was enhanced to 92% and in the case of methanol extract, a maximum of 58% insects were found killed by treating with 6% extract. Percentage of mortality after 4 days of methanol extract at 0.5, 1, 2, 4% concentrations were 27, 28, 41 and 55% respectively and those of petroleum ether extracts, it was 35, 52, 65 and 74% respectively.

After 6 days, it was found that about 66% insects were killed in 0.5% concentration of petroleum ether extract and at the same exposure period, mortality percentage was about 38% in the case of same concentration of methanol extract. Mortality percentage increased to 79%, 94%, 98% and 100% in the case of 1, 2, 4 and 6% of petroleum ether extracts respectively. However, the corresponding mortality were 45%, 61%, 79% and 88% in the case of methanol

Table VII. 1. Cumulative percentage of mortality of petroleum ether and methanol extracts of *C. infortunatum* on *C. chinensis*.

Extracts	Conc.	Percentage of mortality on				
		2nd day	4th day	6th day	10th day	15th day
Petroleum ether	0.5%	10	35	66	88	95
	1%	19	52	79	95	100
	2%	28	65	94	100	-
	4%	39	74	98	100	-
	6%	54	92	100	-	-
Methanol	0.5%	10	27	38	55	71
	1%	12	28	45	67	86
	2%	20	41	61	88	96
	4%	27	55	79	100	-
	6%	30	58	88	100	-
control	-	0	3	7	14	-

Data presented in the table are the mean value of 5 replicates.

Table VII. 2. LC_{50} value of petroleum ether and methanol extracts of *C. infortunatum* at different exposure period.

Duration / Extracts	2nd day	4th day	6th day
Petroleum ether	5.5	2.65	0.68
Methanol	7.77	3.98	2.24

Data presented in the table are the results of the probit analysis (Finney, 1971).

extract (Table VII.1). From these results, it was revealed that 100% mortality was attained by 6% concentration of petroleum ether extract between 4 and 6 days of exposure (Fig. VII.1). A relatively lower level of toxic effect was shown by methanol extract. In this case high mortality was attained only after treatment for a period of more than 6 days. At about 10 days, there was 100% mortality (Table VII.1) (Fig. VII.2).

A considerable number of insects were found dead after 10 days and 15 days of treatment with both the extracts. A 100% mortality was achieved by 2% and 4% concentration of petroleum ether extract and 4% and 6% concentration of methanol extracts after 10 days. With 0.5% concentration of the extracts, mortality were 88% and 55% respectively and with 1% concentration of extracts, mortality were 95% and 67% respectively (Table VII.1).

After 15 days, no insects survived in samples treated with 1% to 6% concentrations of petroleum ether extracts, while about 5% insects were found active in 0.5% extract. Similarly, very few insects were found active in 0.5%, 1% and 2% methanol extract. The mortality caused by these extracts (0.5, 1 and 2%) were 71%, 86% and 96% respectively.

The LC_{50} values of the extracts on 2^{nd}, 4^{th} and 6^{th} days of treatment are presented in the Table VII.2. These results revealed that about 5.5 % concentration of petroleum ether extract was required to kill 50% insects after 2 days treatment. While after 4 days, to attain this target, only about 2.65% concentration of petroleum ether extract was sufficient. Very low (0.68%) concentration of petroleum ether extract was sufficient to kill 50% of insects after

Table VII. 3. Average survival (in days) of insects treated with different concentrations of petroleum ether and methanol extrat of *C. infortunatum*

Extract	0.5%	1.0%	2.0%	4.0%	6.0%
Petroleum Ether	6.64	5.67	4.38	3.82	1.28
Methanol	7.94	7.76	6.60	5.2	4.7

Values in the table are the results of ANOVA of the data at 5% level of significance.

Table VII. 4. ANOVA table showing the effect of petroleum ether and methanol extract of *C. infortunatum* on *C. chinensis*

Extracts	Source	DF	SS	MSS	F- ratio	P value
Petroleum ether	Between group	4	740.96	185.24	28.6114	< 0.01
	Within group	490	3172.422	6.474		
	Total	494	3913.382			
Methanol	Between group	4	753.584	188.36	13.4595	< 0.01
	Within group	448	6270.766	13.997		
	Total	452	7024.35			

Figure VII. 1. Toxic effect of petroleum ether extract of *C. infortunatum* at different concentrations on *C. chinenesis*

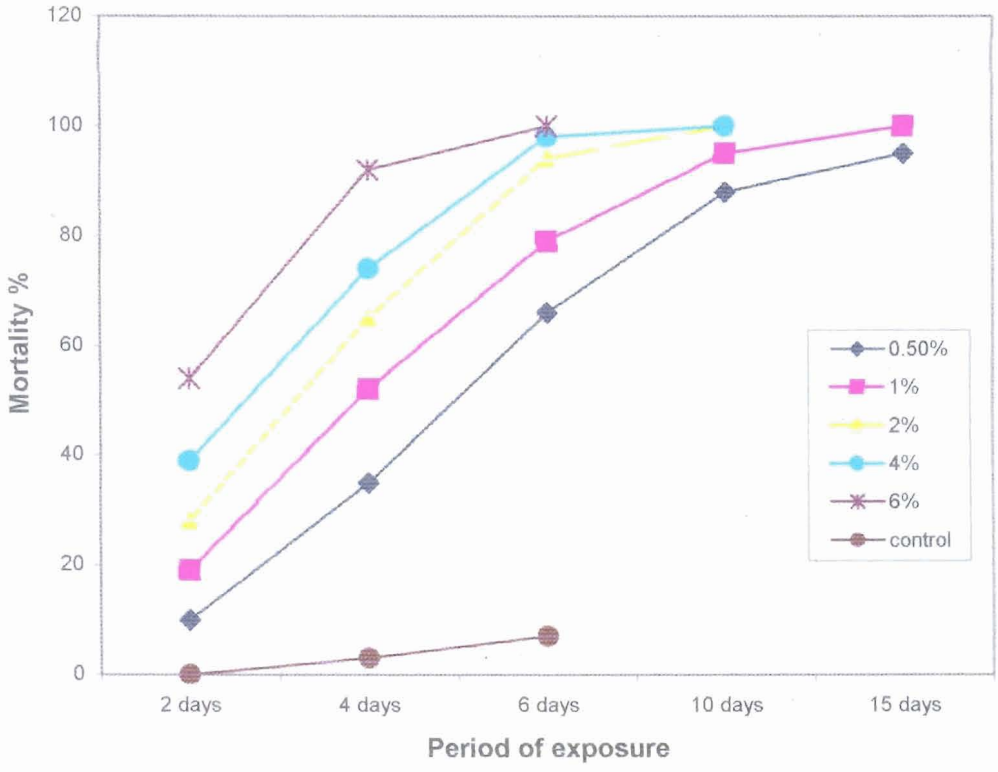
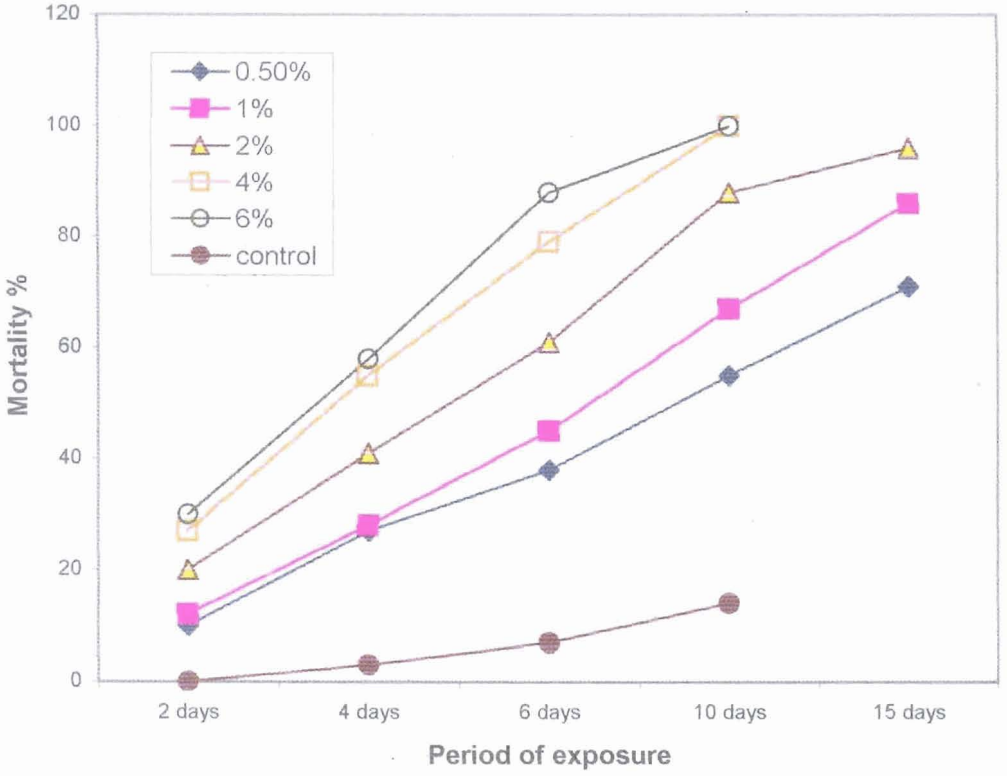


Figure VII. 2. Toxic effect of methanol extract of *C. infortunatum* at different concentrations on *C. chinensis*



6 days. By comparing the LC_{50} values of methanol and petroleum ether extracts, it became clear that petroleum ether extract was far more efficient in its insecticidal efficacy than methanol extract against *C. chinensis*. In the case of methanol extract, about 7.77% extract was required to kill 50% insects after 2 days of treatment. About 3.98% and 2.24% were required to kill 50% insects after 4 and 6 days of treatment respectively.

In the Table VII.3, the average survival of insects (in days), when treated with different concentrations of extracts are provided. When the insects were treated with 0.5% concentration of petroleum ether extract, insects survived up to an average of 6.64 days. With the same concentration of methanol extract, the insects survived up to an average of 7.9 days. Similarly, when treated with 1% petroleum ether and methanol extracts, survival days for insects were 5.67 and 7.76 days respectively. Similarly, when treated with 2, 4, and 6% concentration of petroleum ether extracts, average survival days were 4.38, 3.82 and 1.28 respectively. Whereas, with the same concentrations of methanol extracts, the survival days were 6.6, 5.2 and 4.7 respectively for 2, 4 and 6% concentrations.

In the Table VII.4, the results of analysis of variance of data of two extracts (petroleum ether and methanol) are presented and it was revealed that difference between the toxic effect of two extracts were significant at 5% level.

4. Discussion

From the screening of eight indigenous plants for their insecticidal effect on *C. chinensis, C. infortunatum* was found to be the most effective toxic plant.

The experiments described in this chapter, were conducted to evaluate the insecticidal efficacy of petroleum ether and methanol extracts of this plant at different concentrations against the stored grain pest, *C. chinensis*.

Of the five concentrations (0.5, 1, 2, 4, and 6%) tested, a dose dependent mortality effect was observed. Observations were recorded at 2, 4, 6, 10 and 15 days of exposure. In the case of petroleum ether extract, on the 2nd day the mortality rates were 10%, 19%, 28%, 39% and 54% with the various concentrations tested. A similar dose-dependency was observed with all the other exposure periods tried (4, 6, 10 and 15 days) (Table VII.1). A similar effect was observed in grains treated with methanol extract also. On the 2nd day of observation, a concentration dependent increase in mortality was observed.

On the 4th day of observation also, percentage of mortality of insects with petroleum ether extracts and methanol extracts were dose-dependent. Mortality increased as treatment continued (Fig. VII.1 and 2). The maximum insect mortality (100%) was observed with a 6% concentration of petroleum ether extract after 6 days. However, with 2% and 4% extracts, 100% mortality occurred after 10 days only. In the case of methanol extract, on the other hand, 100% mortality was attained only after 10 days and with 4% and 6% concentrations (Table VII.1).

Thus, in the experiments described, a concentration dependent mortality was observed. In the case of 6% petroleum ether extract, 100% insects were killed with in 6 days, while, in the case of 0.5% extract, only 95% insects were killed even after 15 days. Similar effect was seen in methanol extract also

(Fig.VIII.1& 2). It was observed that in samples treated with 6% methanol extract, 88% insects were found dead with in the period of 6 days. Where as only 38% insects were found dead in samples treated with 0.5% of the extract. At higher concentrations (4% and 6%) in both the cases (petroleum ether and methanol extract), 100% mortality was attained between the period of 6 and 10 days. In the case of 1% and 2% petroleum ether extracts, the percentage of mortality after 10 days were 95% and 100% respectively and in the same concentrations of methanol extracts, the mortality were 67% and 88% respectively (Fig.VII.1 & 2).

From these results, it was revealed that mortality response was directly proportional to the increase in dose and time. Srivasthava and Mann (1999) also obtained similar results when treated the grain, *Vigna radiata* with the plant extract, *Peganum harmala* against *C. chinensis*. In their experiments, mortality was positively correlated with the concentration of the extracts. Similar results were reported by Mathur *et al.* (1985) and Ivabijaro (1990). The adult mortality was attributed to the contact toxicity or to the abrasive effect on the pest cuticle (Mathur *et al.*, 1985). Growth disrupting effect of azadirachtin was also clearly evident with the increasing dosages (Schoonhoven and Jermy, 1977). Chiranjeevi (1991) also confirmed the dose-dependency in his experiments with different plant materials and ashes for their grain protectant effect against *C. chinensis*. The percentage of grain protection increased with the increase in concentrations. A study on dose dependent mortality effect caused by neem oil on the last instar nymph of *Dysdercus koenigii* (Gujar and Mehrotra, 1990) also confirmed this

fact. Sahayaraj and Paulraj (1999) reported the mortality of *Rhynocoris marginatus,* which was found to increase with an enhancement of concentration and time of treatment with the plant extracts.

A concentration and time (duration of exposure) dependent high mortality was observed in *C. chinensis* when the pulse grain was treated with a mixture of tobacco leaves and neem seed powder (Kumari *et al.*, 1988) and neem bark powder (Pandey and Singh, 1997 a). El-Lakwah *et al.* (1996) have also reported the dose dependent mortality of *C. chinensis* with different plant extracts. Bioefficacy of neem-azal (azadirachtin 10,000 ppm) against cotton bollworm, *Helicoverpa armigera* was studied by Rao *et al.*(1995). Of the different concentrations they tried, the highest dose of 0.4% exhibited the most toxic effect by recording 100% mortality.

El-Nahal *et al.,* (1989) proposed that the duration of exposure could be the most important factor to determine the efficiency of extracts rather than dosages. Similar proposals were made by Su (1991) from his results from the contact toxicity of *A. calamus* to adults of *C. maculatus*, *S. oryzae* and *Lasioderma serricorne* and by Rahman and Schmidt, (1999) on adults and eggs of *C. phaseoli.* However, in the present experiment, the LC_{50} value in the case of both the extracts (petroleum ether and methanol), were inversely proportional to the duration of exposure. As the time of exposure increased, a minimum concentration of extracts was required to kill 50% insects (Table VII.2).

Solvents used for the preparation of extract play a significant role in the activity of the plant material. The petroleum ether extracts of *C. infortunatum* at

different concentrations were found to cause higher mortality than different concentrations of methanol extract (Table VII.1). Observations made after different periods of exposure (2, 4, 6, 10, and 15 days), confirmed this superiority of petroleum ether extract over methanol extract. At highest concentration (6%), mortality percentage of petroleum ether after 2, 4, and 6 days were 54%, 92% and 100% respectively, whereas, those of methanol extracts, were 30%, 58%, and 88% respectively. Nearly 100% mortality reached within the period of 4 and 6 days as far as the 6% concentration of petroleum ether extract was concerned, whereas, this is achieved only after 10 days in the case of 6% methanol extract (Fig VII.1&2). These findings are in agreement with the findings of Teotia and Pandey (1979). According to their report, among the extracts of the sweet flag prepared with different solvents, petroleum ether extract was the most toxic followed by ether and alcoholic extracts. However, Mukherjee and Govind (1959) reported that ether extract of *A. calamus* to be 0.8 times more toxic than petroleum ether extract and 0.9 times more toxic than alcoholic extracts. Reports of other workers such as Pandey *et al.*(1976); Usharani and Jamil (1989); Sheela (1997) have also proved the efficiency of petroleum ether extract of different plant materials to control the insect pests better than extracts using five other solvents. Jilani and Su (1983) reported that petroleum ether extract of *Curcuma longa* was more effective than the acetone and ethanol extracts against stored grain pests.

LC_{50} values of both the petroleum ether and methanol extracts on 2, 4 and 6 days are presented in the Table VII. 2. Perusal of the Table reveals that LC_{50} values decreased as the exposure period increased. On the 2nd day, about 5.5%

petroleum ether extract was required to kill 50% of the insects and it decreased up to the level of 0.68% on 6th day. On comparing the two extracts, (petroleum ether and methanol) a significant difference was observed. LC_{50} value of methanol extract on 2nd day was 7.77%, for 4th day it was 3.98% and on the 6th day it decreased to 2.24%. Similar observations were made by Sharma and Srivasthava (1998). According to their findings, LC_{50} values for the extracts of *Artemisia annua, Carica papaya, Cuscuta reflexa* and *Lantana camara* were 0.29, 0.67, 0.15 and 1.19 after 24 hour exposure and it decreased to 0.16, 0.66, 0.01 and 1.05 after 48 h exposure respectively.

Statistical analysis revealed that all treatments were significantly different from control (P< 0 .05%). Among the five concentrations tested, 6% concentration (irrespective of the solvent used) was found to be the most effective and was significantly different from other concentrations. While comparing the two solvents, petroleum ether extract appeared comparatively more toxic than methanol extract (irrespective of concentration).

From these findings, it was revealed that petroleum ether extract of the indigenous shrub, *C. infortunatum* is an effective toxicant that could be recommended for effective control of the stored grain pest *C. chinensis.* Its insecticidal property was found to be time and dose-dependent. However, the optimum dose and time of exposure required are to be determined using a trial and error method, depending on the specific storage conditions.

Chapter VIII

CLERODENDRUM INFORTUNATUM AS OVIPOSITION AND FEEDING DETERRENT AGAINST *CALLOSOBRUCHUS CHINENSIS*

1. Introduction

Insect feeding deterrents have great potential as alternative insect control agents to insecticides in protecting crops from insect damage. One of the important sources of insect feeding deterrents is the neem tree. Several attempts have been made to screen Indian flora for their antifeedant property against several phytophagous insects (Mane, 1968; Wada and Munakata, 1971; Russel *et al.,* 1972). Earlier studies have indicated that antifeedants derived from seeds, flowers, fruits, leaves and roots could be used effectively against the growth and metamorphosis of noxious insects. Antifeedants have been found to bring in molting failure or mortality when fed along with normal diet or applied topically on the integument (Ruscoe, 1972; Osmani and Sighamony, 1980; Mwangi, 1982; Meisner *et al.*, 1983; Adler and Uebel, 1984; Saxena and Harshand, 1992; Gupta and Gupta, 1993; Venkateswarlu *et al.*, 1993).

Even though the role of most of the natural plant products are poorly understood, it is at least well established that many of these plants have secondary metabolites which can function as antifeedants because they are either unpalatable or produce deleterious effects on a variety of animals. These toxic plants, endowed with such compounds, generally constitute a considerable challenge to animals that ingest their tissues. Therefore, screening of such type of plants for

antifeedant activity is important in discovering safe, biodegradable alternative to synthetic insecticides (Tripathi and Singh, 1994). Antifeedants are chemicals which, when perceived, reduce or prevent insect feeding. Consequently, growth, development, survival, and reproduction are adversely affected. Antifeedants, in some plants, constitute important defence barriers against insect attack. The use of insect antifeedants offer a hope for protection of stored grains from insect attack because they are quite specific and may have low mammalian toxicity. To aid in the protection of stored products from insect attack, there is a continuing need for antifeedants that are more effective, more persistent and more economical than existing synthetics available (Wright, 1963).

Oil dressing on cowpea seeds exhibited significant oviposition deterrence and complete inhibition of emergence of adult pulse beetle (Pereira, 1983; Singh *et al.*, 1988; Sharma *et al.*, 1999). However, the precise effect of plant oils on gravid female bruchids is still unclear (Raja *et al.*, 2001).

Objective of the present study was to evaluate the antifeedant and anti-ovipositional action of *Clerodendrum infortunatum* extracts prepared in petroleum ether and methanol on *C. chinensis.* Since the larvae and adults of this insect are internal grain feeders, antifeedant effect could be determined only by comparing the grain weight at the beginning and end of the experiments.

2. Methods

A. Oviposition deterrent effect

To determine the oviposition deterrent effects, five sets of 20 grains each of green gram (*Phaseolus radiatus*), were weighed (weight of the grains were required for the purpose of getting data for feeding deterrence experiment) and grains in four sets were smeared with 0.2 ml each of the different concentrations (0.5%, 2.0%, 4.0% and 6.0%) of petroleum ether extracts of *C. infortunatum*. The fifth set, the control, was treated with acetone alone. Likewise, five sets of grains treated with methanol extract of similar concentrations were also kept. Grains were freed from any trace of solvent. After drying, each lot was taken in individual vials (6 × 3 cm) and introduced a pair each of (1 male + 1 female) newly emerged pulse beetle, *C. chinensis* into it. Covered the mouth of the glass vials with muslin cloth and tied with rubber band. The experiment was replicated ten times. The experimental set up was kept undisturbed at optimum temperature (28 ± 2°C) and relative humidity (75 ± 5%). After 7 days, insects were removed from the vials and the numbers of eggs attached on all the grains of all the sets were counted using a hand lens.

Oviposition deterrence was calculated by using the discrimination quotient (DQ) (Messina and Renwick, 1983).

$$DQ = \frac{\text{No. of eggs on control seeds - no. of eggs on treated seeds}}{\text{Total no.of eggs on control and treated seeds}}$$

DQ ranges from (–1), all eggs on treated seed to (+1) all eggs on control seeds

Percentage of reduction in oviposition was calculated by the formula

$$\% \text{ of reduction in oviposition} = \frac{\text{No. of eggs on control - no. of eggs in treated}}{\text{Total no. of eggs on control}} \times 100$$

B. Feeding deterrent effect and loss of grain weight

Since the larvae and adults of this insect are internal grain feeders, antifeedant effect could be determined only by comparing the grain weight at the beginning and end of the experiments. To determine this, the above experimental set up (after removing the adult pair) was kept undisturbed for about six months at optimum temperature and relative humidity (28 ± 2°C and 75 ± 5% respectively). The number of insects emerged from each set after 2, 3, 4 and 5 months were counted. Newly emerged insects were removed from the sample to prevent the development of F_2 progeny. Final weight of the grains were taken after 6 months and the percentage of weight loss of grains were calculated. Data were analysed statistically by chi-square method.

3. Results

A. Oviposition deterrent effect

Comparing the results obtained from counting the number of eggs laid on the grains of each vial revealed that petroleum ether extract was more effective as an oviposition deterrent than methanol extract. With respect to the mean number

of eggs laid, the differences between the control and the experimentals of both the extracts were significant. Data presented in Tables VIII. 1 and 2 indicate that the number of eggs laid in the samples treated with 6% extracts are minimum. In the case of samples treated with methanol extract, the mean number of eggs laid was 21.4 whereas in the case of petroleum ether extract it was only 1.5. Similar differences were obtained with other concentrations also (Tables VIII.1 & 2). There appears to be a considerable difference between the two extracts and a dose-dependent reduction in mean number of insects was observed (Figures VIII. 3 & 4). Maximum number of eggs (36.9 and 51.4) were found in samples treated with the lowest concentration (0.5%) of the petroleum ether and methanol extracts respectively. In the case of 2% and 4% petroleum extracts, means of 31.2 and 20.7 eggs were found. Whereas with the same concentrations of methanol extract, the mean numbers were 36.6 and 28.4 respectively.

Similar differences were obtained in reproduction control of insects in both the samples treated with the extracts (Figures VIII. 1 & 2). An average of 97.1% reproduction control resulted in sample treated with 6% petroleum ether extract, while in the case of methanol extracts treated sample this was about 65.16% (Table VIII. 1& 2). Reproduction control was minimum where the samples were treated with 0.5% concentration of both the extracts. From the Tables, it is revealed that percentage reduction in oviposition increased with increase in concentrations.

Comparing the DQ values, the highest value (near to +1) was obtained in the case of insects exposed to treated with 6% concentration of petroleum ether

Table VIII.1. Oviposition deterrence of petroleum ether extract of *C. infortunatum* on *C. chinensis*

Conc.	Mean no. of eggs on treated seeds	Reproduction control (%)	DQ	Chi-square	P value
0.5%	36.9	27.6	0.165	3.90	NS
2.0%	31.2	38.4	0.245	7.69	<0.01
4.0%	20.7	59.4	0.430	18.00	<0.001
6.0%	1.5	97.1	0.947	48.04	<0.001
control	51	-	-	-	-

Data presented in the table are mean value of 10 replicates

DQ = discrimination quotient; NS = not significant

Table VIII. 2. Oviposition deterrence of methanol extract of *C. infortunatum* on *C. chinensis*

Conc.	Mean no. of eggs on treated seeds	Reproduction Control (%)	DQ	Chi-square	P value
0.5%	51.4	19.66	0.118	1.659	NS
2.0%	36.6	40.5	0.261	10.08	<0.001
4.0%	28.4	53.8	0.369	17.81	<0.001
6.0%	21.4	65.16	0.490	26.15	<0.001
control	61.5	-	-	-	-

Data presented in the table are mean value of 10 replicates
DQ = discrimination quotient; NS = not significant

Table VIII. 3. Effect of petroleum ether extract of *C. infortunatum* on feeding and adult emergence of *C. chinensis*.

Conc.	Mean no.of eggs	No. of insects emerged after 150 days	Initial grain Wt.	Final grain Wt.	Weight loss	Weight loss (%)
0.5%	36.9	12.15	1.0669	0.64	0.470	44.7
2.0%	31.2	11.4	1.1337	0.77	0.335	33.6
4.0%	20.7	9.3	1.0434	0.74	0.293	29.9
6.0%	1.5	0.5	1.0946	1.06	0.037	3.4
control	51	42.7	1.1118	0.15	0.975	86.7

Data presented in the table are mean value of 10 replicates

Table VIII. 4. Effect of methanol extract of *C. infortunatum* on feeding and adult emergence of *C. chinensis*

Concn.	Mean no. of. eggs	No. of insects emerged after 150 days	Initial grain wt.	Final grain wt.	Weight loss	Weight loss (%)
0.5%	51.4	39.4	2.116	0.649	1.467	69.12
2.0%	36.6	31.6	1.972	0.789	1.186	60.2
4.0%	28.4	23.4	2.134	1.227	0.907	42.3
6.0%	21.4	14.8	1.916	1.170	0.745	38.9
control	61.5	44.25	2.135	0.360	1.775	83.01

Data presented in the table are mean value of 10 replicates

Figure VIII.1. Oviposition deterrence of petroleum ether extract of *C. infortunatum* at different concentrations on *C. chinensis*

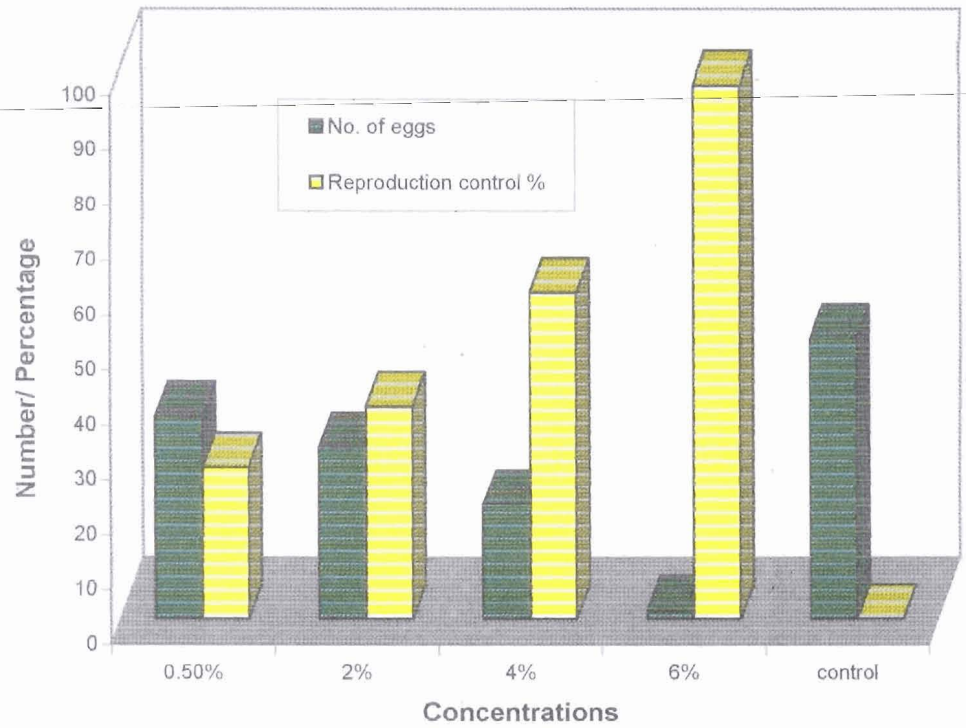
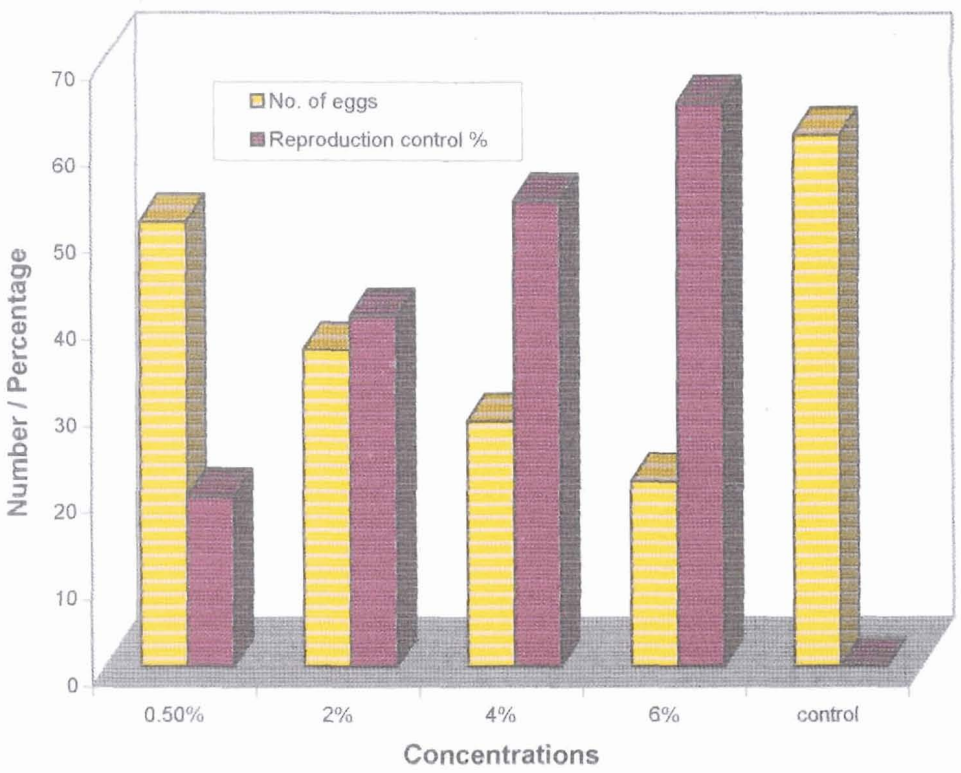


Figure VIII. 2. Oviposition deterrence of methanol extract of *C. infortunatum* at different concentrations on *C. chinensis.*



extract (0.947), whereas for insects exposed to grains treated methanol extract, it was 0.490. The DQ values for samples treated with petroleum ether extract of 0.5%, 2% and 4% were 0.165, 0.245 and 0.430 respectively (Tables VIII. 1& 2). Lower DQ value indicated the inefficiency of oviposition deterrent effect (Messina and Renwick, 1983).

DQ values revealed that there is no marked difference between the two extracts at the lower concentrations (0.5%and 2%). However, the two extracts differed considerably at 6% concentration. Since the 6% concentration of petroleum ether extract of *C. infortunatum* had the highest DQ value (0.947) than all the other concentrations in the case of both the extracts, it appeared that petroleum ether extract of *C. infortunatum* at 6% concentration could be a better choice for inhibiting the oviposition of the bruchid *C. chinensis.*

These results were statistically analysed by chi-square test and determined the level of significance. It was found that the oviposition deterrent effect of higher concentrations (4% and 6%) of both the extracts were significant ($P< 0.001$). In the case of 0.5% concentration of both the extracts (petroleum ether and methanol) the DQ values were 0.165 and 0.118 respectively which were statistically non- significant.

B. Feeding deterrent and emergence of adult insects

There is a remarkable difference in the results obtained for emergence of insects and grain weight after 150 days of treatment between the two extracts (Table VIII. 3 & 4). Here also a concentration dependent effect was observed.

Number of insects emerged was inversely proportional to the concentrations. The average minimum number of insects emerged from grains treated with 6% concentration of petroleum ether and methanol extracts were 0.5 and 14.8 respectively (Figure VIII. 3 & 4). Maximum number of insects emerged from the grains treated with the lowest concentration (0.5%) in the case of both the extracts. Mean numbers of 12.15 and 39.4 insects emerged respectively from the samples treated with petroleum ether and methanol extracts. However, from the control samples, an average of 42.7 and 44.25 insects emerged.

Percentage of weight loss of grain also was related with the concentration (Figure VIII. 3 & 4). In the case of grains treated with the highest concentration (6%) of petroleum ether and methanol extracts, relatively low percentage of grains was spoiled (3.4% and 38.9% respectively) measured on the basis of weight loss, compared to other concentrations and control (Table VIII. 3 & 4). Perusal of the Tables VIII. 3 and 4 reveals that there existed a direct relationship between number of insects emerged and percentage of weight loss of grain. In both the extracts, maximum weight loss of grain occurred in sample from which maximum number of insects emerged. In the case of 0.5% methanol extract an average of 39.4 insects emerged and it showed about 69.12% loss of grain weight. While from 6% petroleum ether extract only an average of 0.5 insects were emerged and a very low level of destruction (3.4%) of grains occurred. In this case also a dose dependent effect was observed.

Figure VIII.3. Effect of petroleum ether extract of *C. infortunatum* on feeding and adult emergence of *C. chinensis*

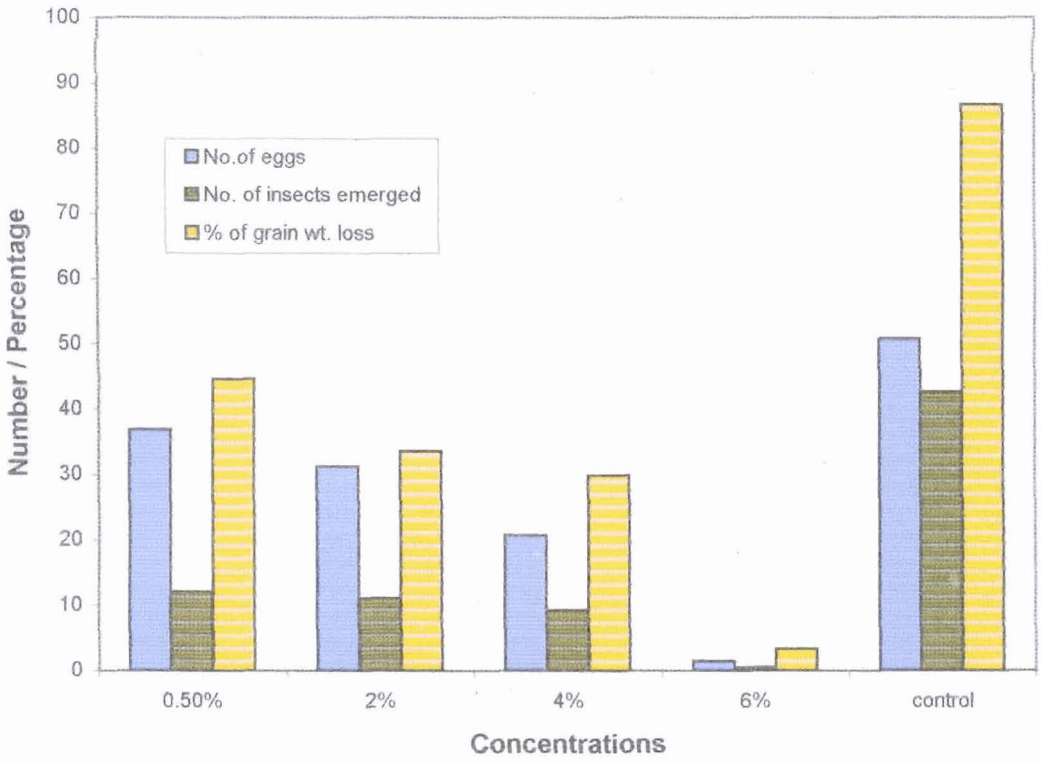
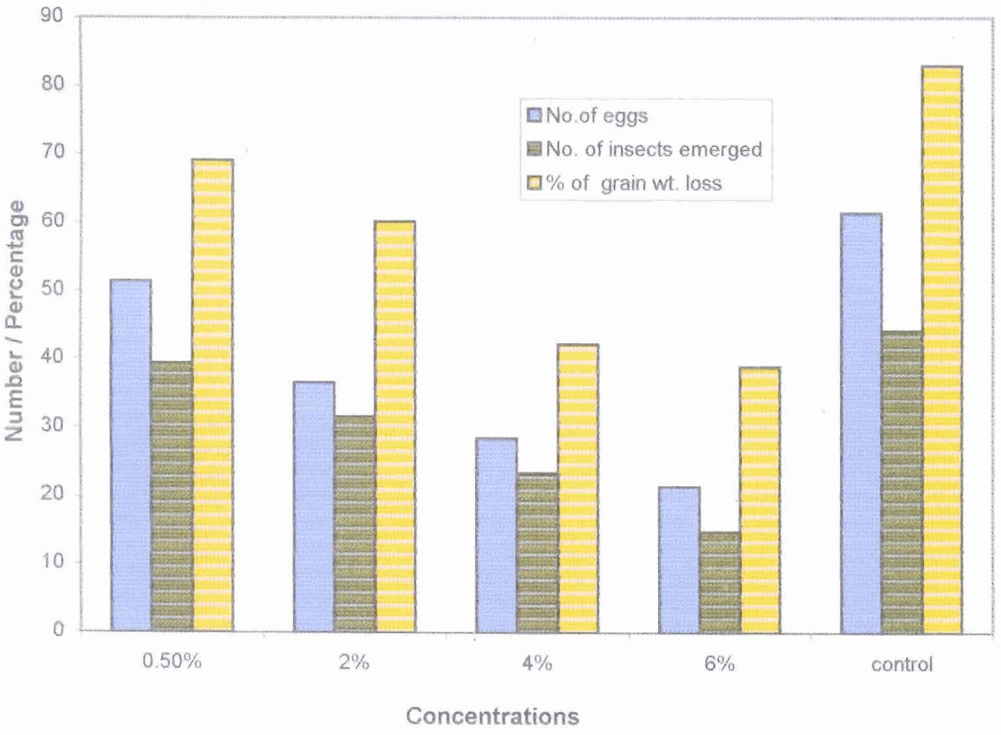


Figure VIII. 4. Effect of methanol extract of *C.infortunatum* on feeding and adult emergence of *C. chinensis*.



3. Discussion

A. Oviposition deterrent effect:

Treatment of green gram seeds with crude extracts of *Clerodendrum infortunatum* prepared in methanol and petroleum ether at various concentrations (0.5, 2, 4 and 6%) significantly reduced the egg deposition, which in turn influenced the number of adults emerged, seed damage and loss in seed weight in comparison to solvent treated control. Among the two extracts (Methanol and Petroleum ether) 6% concentration of *C. infortunatum* prepared in petroleum ether was the most effective oviposition deterrent, in which significantly less number of eggs (1.5) were deposited on treated grains (Figure VIII. 1). The egg deposition on seeds treated with other concentrations of same extract was also significantly less in comparison to the corresponding concentrations of methanol extract and control.

Perusal of the Tables VIII.1 & 2 indicate that in the present investigation there was a considerable decrease in the egg laying by *C. chinensis* with increase in the concentration of *Clerodendrum* extracts. The number of eggs laid by *C. chinensis* on green gram seeds decreased gradually from 36.9 to 31.2, 20.7 and 1.5 at 0.5%, 2%, 4% and 6% concentrations respectively in the case of petroleum ether extracts. A similar trend was observed in methanol extracts, where also a dose dependent decrease in number of eggs was observed (Figure VIII. 2). At 0.5% concentration maximum oviposition occurred (51.4). It gradually decreased to 36.6, 28.4 and 21.4 with increase of concentrations to 2%, 4% and 6% respectively. Sharma *et al.* (1999) have recorded a similar type of dose-dependent

decrease of oviposition of *C. chinensis* when pigeon pea (*Cajanus cajan*) was treated with different concentrations of crucifer oil. Considerable reduction in oviposition of *C. chinensis* was reported by Parsai *et al.* (1994) on pigeon pea seeds treated with mustard, sesame and soybean oils at 0.25, 0.50, 0.75 and 1.00 per cent with maximum effect at 1.00 per cent. Prakash and Rao (1989) also reported similar results. According to their study, *Vitex negundo* at the highest dose (3%) was found to reduce the egg laying of *C. chinensis* to the minimum (6 eggs) when compared with control (1034). Rajapakse (1996) reported that at low concentrations, the powder made from fruits of *Piper nigrum* significantly reduced oviposition and adult emergence. A 100% mortality was obtained at a higher concentration of 42 %.

Lale and Abdulrahman (1999) reported that neem seed oil prepared by soxhlet method and kneading method was significantly effective in reducing oviposition and adult emergence of *C. maculatus*. Reduction or complete inhibition of oviposition by female bruchids with plant oils have been reported by a number of workers (Singh *et al.*, 1978; Sharma *et al.*, 1981; Messina and Renwick, 1983 and Lambert *et al.*, 1985; Rajapakse and Senanayake, 1997; Sharma *et al.*, 1999; Raja *et al.*, 2001).

In the present investigation, the egg laying was found to decline in a dose-dependent manner (Figures VIII. 1 & 2). Average number of eggs laid were 36.9, 31.2, and 20.7 respectively on seeds treated with 0.5%, 2%, and 4% of petroleum ether extract. Further increase in concentration (6%) resulted in a sharp decline. The average number of eggs laid per sample showed a sharp decline to 1.5 from

20.7 (Table VIII. 3). The present results are thus, in conformity with the results of Negi *et al.* (1997). A marked effect of the concentration of pongam oil on egg laying (in terms of number of eggs laid on seeds) was observed by them in *C. chinensis*. Girish *et al.* (1974) observed that the oil treated seeds were not preferred for oviposition in the case of *C. maculatus*. Periera (1983) observed that in cowpea, the neem kernel oil reduced the oviposition when used @ 8 ml/ Kg seed. The per cent protection over control was found to increase with the increase in concentrations in all treatments (Chiranjeevi, 1991). Kachare *et al.* (1994) also reported similar results. By applying different vegetable oils at different concentrations (0.5, 0.75 and 1%) on pigeon pea, it was found that the highest concentration (1%) showed significant deterrent action on egg laying of adult bruchid *C. chinensis*.

The decline in oviposition at higher doses of plant extracts have been attributed to the interruption of vitellogenesis and damage to the egg chambers in the ovaries of *C. chinensis* (Pandey and Khan, 1998 b). Dhar *et al.* (1996) reported that oviposition was possibly regulated by the volatile compounds absorbed through the cuticle. Results obtained by Raju and Thakur (1995) also supported this. Topical application of methanol extract of *Butea monosperma* on the 5^{th} instar larvae of *Dysdercus similis* caused abnormalities in the ovaries. Compound egg chambers were observed mostly in the ovaries of treated adults. Poor yolk deposition and un-ovulated batch of oocytes were the results. At the highest concentration (4%), there was a complete inhibition of oogenesis and vitellogenesis.

Solvents have been found to play a crucial role in the extraction of principal insecticidal constituents from plant materials (Srivasthava and Mann, 1999). This was found to be true during the present investigation also, where the petroleum ether extract of *C. infortunatum* was found to cause high oviposition deterrent property than methanol extract. Srivasthava and Mann (1999) reported that ether extracts of different plant parts were found to cause higher mortality. However, Mukherjee and Govind (1959) reported that diethyl ether extract of *Acorus calamus* to be 0.8 times more toxic than petroleum ether extract and 0.9 times more toxic than alcoholic extracts. Teotia and Pandey (1979) found that *S. oryzae* when treated with *Acorus calamus* extract, prepared in various solvents, petroleum ether extract was the most toxic among them followed by ether and alcoholic extracts. These results are in conformity with the results from the present study. Comparing the oviposition deterrent property of methanol and petroleum ether extract of *C. infortunatum* against *C. chinensis* it was revealed that percentage of reproduction control was maximum in petroleum ether extract treated sample than methanol extract treated sample (Tables VIII. 1 & 2). Sharma *et al.* (1999) reported that petroleum ether alone (control) had no adverse effect on oviposition in insects. They compared the egg laying of *C. chinensis* on untreated and petroleum ether treated control grains and found no significant difference between them.

The results obtained from the present investigation revealed that irrespective of solvent used for extraction, oviposition of stored grain pests were affected by the extracts in a dose dependent manner. However, higher

concentration of petroleum ether extracts showed the highest oviposition deterrent effect than those of methanol extract.

B. Adult Emergence

Number of adults emerged from the treated sample after 150 days of treatment revealed that there is a dose dependent decrease in the emergence of adults in both petroleum ether and methanol treated samples (Figures VIII. 3 & 4). A minimum number of insects emerged from grains treated with the highest concentration (6%) of both the extracts. In the case of *C. infortunatum* in methanol extract, an average of 14.8 insects emerged with in 150 days after treatment. It was only 0.5 in the case of same concentration of petroleum ether extract; but both were significantly different from the control (Tables VIII. 3 & 4).

In the present experiment it was observed that the number of insects emerged was proportionate to the number of eggs deposited on the grains within a period of one week. Srivasthava and Mann (1999) have also observed this type of reduction in adult emergence of *C. chinensis* from grains treated with extract of *Peganum harma.* Main *et al.* (1993) reported the decrease in the emergence of *C. chinensis* from grains treated with different plant extracts.

It has been revealed from the present investigation that the number of adults emerged from the grains treated with different concentrations (0.5, 2, 4 and 6%) of petroleum ether extract of *C. infortunatum* are always lower than the number of adults emerged from grains treated with the corresponding concentrations of methanol extract (Table VIII. 3 & 4). A contradictory results

was obtained by Sharma *et al.* (1999) by conducting an experiment on the effect of different plant oils on adult emergence in *C. chinensis*. Their experiment proved that there was no significant increase or decrease of adult emergence from grains treated with mustard oil with different concentration of the extract.

However, results of many other workers have supported the dose dependent effect on insect emergence. Remarkable decreases in the adult emergence of *C. chinensis*, with increase in the concentrations of extracts of pongam oil was reported by Negi *et al.* (1997). The highest percentage (81.6%) of insect emergence was observed at the lowest concentration of pongam oil (0.1%). Khaire *et al.* (1992) reported that adult emergence of *C. chinensis* was completely prevented by Karanj oil at 0.75% and 1.0% and neem oil at all tested concentrations up to 100 days. Their study proved that as the concentration increased, the emergence of adults also decreased. Prakash and Rao (1989) also reported the dose dependent decrease of adult emergence of *C. chinensis*. Green gram treated with leaf powder of *Vitex negundo* at 3% (w/w) significantly reduced the adult emergence. Ali *et al.* (1983) suggested that 1.5% of pongam oil could be considerd as a good additive in inhibiting egg laying as well as adult emergence of *C. chinensis*. According to Su *et al.* (1972) adult emergence of *C. maculatus* was inhibited by treating the grains with lemon oil. Singh *et al.* (1978) reported that treatment with groundnut oil prevented the emergence of progeny rather than affecting the oviposition or mortality of the adult weevil. Treatment with neem oil prevented the oviposition and adult emergence significantly up to 30 days, while groundnut oil was least effective (Bhatnagar *et al.*, 2001).

In the present study, the results obtained for adult emergence was in accordance with the results of other workers. Even though there are some exceptions, emergence of insects from treated sample is inversely proportional to the concentration of the extract used for the treatment of the grains (Figure VIII.3 & 4). The solvents used for the preparation of the extract also have been found to have an important role. In the present study, percentage of adult emergence is lower from petroleum ether extract treated sample than methanol extract treated sample (Figure VIII.3 & 4).

C. Weight Loss in Grains:

Weight loss indicates the quantitative loss in stored grains due to insect feeding and thus showing a direct relationship between insects population. In the present investigation, a marked decrease occurred in the grain weight within 150 days after treatment with methanol and petroleum ether extract of *C. infortunatum* on green gram, *P. radiatus*. The percentage of grain weight loss is directly related with the number of insects emerged from the samples (Figure VIII. 3 & 4). In the present experiment, a dose dependent loss of grain weight was observed. It was inversely proportional to the increase in the concentration. Maximum grain weight loss was obtained from control samples (Figure VIII.3 & 4). It was recorded to be 86.7% and 83.01% respectively with these samples. Among the different concentrations tested (0.5, 2, 4 and 6%) percentage of weight loss was maximum at 0.5% concentration. In the case of petroleum ether extract, the weight loss was 44.7% and with methanol extract it was about 69.12%. There was a gradual decrease in weight loss with respect to the increase of concentration

of extract. In the present experiment, the weight loss reached the minimum at 6% concentration treated sample. In samples treated with petroleum ether extract, there was 3.4 % grain weight loss, whereas, in methanol extract treated sample it was about 38.9%. The present findings are in agreement with the earlier observations of Ali *et al.* (1981), Kumari *et al.* (1990), Ivbijaro (1990), Chiranjeevi (1991) and Khaire *et al.* (1992). As the concentrations increased, there was a decrease in the loss of grain weight at all time intervals after treatment (Khaire *et al.*, 1992). There was a significant ($P< 0.001$) reduction in weight losses caused by *Sitophilus oryzae* to the wheat grain, treated with various plant powders (Niber, 1994). Their report revealed that losses of grain weight were significantly reduced by increase in the concentration. Observations of Koshiya and Ghelani (1990) have also reported similar results from their experiment with different plant derivatives against *Spodoptera litura.*

Another finding of our experiment is that number of insects emerged and grain weight loss was interrelated. Minimum grain weight loss was recorded for samples from which minimum number of insects emerged. From the grains treated with 0.5%, 2% and 4% methanol extracts, mean number of insects emerged were 39.4, 31.6 and 23.4 respectively (Table VIII. 4), corresponding percentage of weight loss was 69.12, 60.2 and 42.3% respectively. Minimum weight loss was recorded for grains treated with 6% concentration (38.9%) and the number of insects emerged from this was only 14.8. More convincing results were obtained from petroleum ether extract treated samples. Here, number of insects emerged were comparatively less. After 150 days of treatment, 12.15,

11.4, 9.3 and 0.5 insects emerged from grains treated with 0.5, 2, 4 and 6% concentrations of extracts respectively. Here, grain weight loss was minimum (3.4%) at 6% concentration. Moreover, a remarkable difference in weight loss is observed between grains treated with methanol and petroleum ether extracts.

Singh *et al.* (2001) reported the remarkable differences in the grain weight loss caused by the infestation of *C. chinensis* between untreated and plant products treated grains. The loss in weight was as high as 47.40% in untreated grains which was considerably reduced to a level of 0.63, 1.00 and 1.10 per cent by application of neem oil, neem leaf powder and castor oil respectively. Similar results were reported by Chaudhary (1990) who found that oils of neem, groundnut, sesame, linseed, soybean, castor, sunflower and coconut provided significant reduction in seed damage due to *C. chinensis.* Kumar *et al.* (1990) evaluated the oils of mustard, linseed, til, groundnut, neem and mahua at 1.0 % concentration and proved that they were equally effective in the reduction of damage of *C. chinensis.* Sangappa (1977) reported that neem, mustard and castor oils used at a concentration of 1%, protected red gram for 161, 133 and 77 days respectively. Prakash *et al.* (1981, 1989) reported that leaves of *V. negundo* and powder of turmeric, *Curcuma longa* inhibited the population build up of *S. oryzae* and reduced weight loss in milled rice caused by this weevil.

In the present investigation, the difference in grain weight loss between grains treated with petroleum ether extract and methanol extract were estimated. Weight loss of the grains were more in the methanol treated samples. From these findings it is revealed that feeding deterrent property of *C. infortunatum* extracted

with petroleum ether is comparatively better than that extracted with methanol. Both the extracts showed a dose dependent effect with a maximum at 6% concentrations. This experiment was in conformity with the findings of Rao *et al.* (1990 b) in which it was revealed that petroleum ether extracts of plant products exhibited higher feeing deterrent activity than the aqueous extracts against *Henosepilachna vigintioctopunctata.*

In view of the results obtained in the present work, it is revealed that petroleum ether extract of *C. infortunatum* acts as an efficient grain protectant from the infestation of the bruchid *C. chinensis*.

Chapter IX

OVICIDAL ACTIVITY OF *CLERODENDRUM INFORTUNATUM* ON *CALLOSOBRUCHUS CHINENSIS*

1. Introduction

The great majority of seed beetles (Bruchidae) attach their eggs firmly to the pods or seeds of leguminous plants. On hatching, the first instar larvae penetrate into the same seed to which the eggs were attached and further development is completed inside. After eclosion from the pupa, the adult insect emerges from the seed through a 'window' initially demarcated by the final instar larva, which removes most of the underlying plant tissues from a defined area of seed testa before entering pupation.

Both embryonic and larval development of bruchids are temperature dependent, the former takes around 5 days and the latter another 15-20 days at normal temperature. Thus a relatively short period of the total life cycle is accessible to any non-fumigant chemical control agents (Credland, 1992).

Eggs of *Callosobruchus* are oval in shape but taper sharply at one end as the posterior pole of the egg. The most conspicuous feature of the egg is the short funnel at the posterior pole (Credland, 1992). When the eggs are laid, it has a fluid coating, which spreads to a small area on the seed testa as the egg makes contact with it. This fluid (spumaline) contains an adhesive that dries rapidly and makes the characteristic folds around the point of attachment of the eggs to the seeds. The eggs when laid, thus are raised from the surface of the testa of the grain providing large air filled space between the developing embryo and the

testa, which connects with the exterior only through the funnel at the posterior end. The shape of the egg thus resembles an oval inverted saucer with the embryo and yolk in the thickened central portion. The funnel at the posterior end serves as the only route through which exchange of respiratory gases and water loss or uptake take place between the egg and the exterior. When the egg hatches, the first instar larva breaks through the thin chorion, enters the space beneath the egg, and thence burrows into the seed. Thus the larva opens a route into the core of the seed, from the space beneath the remains of the egg. Even after larval eclosion, the funnel therefore provides the primary route of gaseous exchange during the entire life stages within the seeds.

Control of bruchids in stored legume seeds have frequently been attempted at subsistence farmer level by the use of oils, mostly of local origin (Jackai and Daoust, 1986). All these oils are of plant origin and they form a traditional part of agricultural practice in many parts of the world. Oils can reduce oviposition and can be ovicidal, larvicidal or more generally, reduce adult emergence (Messina and Renwick, 1983). One hypothesis explaining the ovicidal and larvicidal effects of oil is that they result in asphyxiation by occluding the funnel and cutting off the air supply to the developing insect (Biement *et al.*, 1982). The activity could be further enhanced by suitable additions or treatments and thereby enhancing their effectiveness for bruchid control. However, the direct metabolic toxicity of some constituents of the oils cannot be discounted (Don-Pedro, 1990). In such a case, penetration of oils into the space beneath the embryo, and reaching the very thin chorion may be important in providing access to the toxins.

Singh *et al.* (1978) reported that groundnut oil entered the eggs of *C. maculatus* through the micropyle and, in 1-2 day old eggs, protoplasmic movement was stopped and the protoplasm coagulated, whereas in larvae, death occurred within minutes of the entry of the oil. Egg mortality has been attributed to toxic components (Su *et al.,* 1972) and also to physical properties, which cause changes in surface tension and oxygen tension within the eggs (Singh *et al.*, 1978). Vegetable oil treatment is a convenient and inexpensive method of protection of stored seeds from insect infestation on small farms and in households. The oil does not affect the germination rate and the small amount of oil in treated seeds does not affect the flavour.

Now-a-days, many reports are available on the use of plant extracts / oils to suppress the stored pest populations. Ethanol extract of cinnamon exhibited ovicidal activity against bean weevil (Garcia, 1990). Dwivedi and Bajaj (2001) conducted experiments with acetone extracts of some plants on the eggs of *Trogoderma granarium* for their ovicidal activity. The reason for increase in the mortality is that these extracts interfere with the normal embryonic development of the eggs by suppressing hormonal and biochemical processes. Jadav and Jadav (1984) reported the ovicidal property of *Jatropha curcas* and *A. indica* against *C. maculatus.*

The present investigation was contemplated to study the ovicidal activity of petroleum ether and methanol extracts of *C. infortunatum* at different concentrations against *C. chinensis.*

2. Methods

To study the ovicidal activity of *C. infortunatum* extracts, six sets of small glass vials (6 cm x 2.5 cm) were taken and 20 numbers of pre- sterilized green gram *P. radiatus* were introduced in to each vial. Three pairs (3 males + 3 females) of newly emerged healthy insects were introduced into each vial and the mouth of the glass vials were covered with muslin cloth and tied with rubber band. The experimental set up were kept at 28 ± 2°C and 75 ± 5% RH. Allowed the insects to lay eggs for 24 h. After 24 h the insects were removed from the vials and the number of eggs attached on the grains were counted. Freshly laid eggs appeared translucent, smooth and shining.

Different concentrations (0.5%, 2%, 4%, and 6%) of petroleum ether and methanol extracts of *C. infortunatum* were prepared from the stock solutions by diluting with acetone. Immersed the experimental grains with attached eggs in 0.5 ml of each extract of different concentrations for about 10 seconds. A control set was also kept in which the grains were treated with 0.5 ml acetone. Allowed these grains to dry in order to get rid of the solvent. After that, covered the mouth of the glass vials with muslin cloth and kept the experimental set up at 28 ± 2°C and 75 ± 5% RH.

After 5 days, the hatched and unhatched eggs were counted separately. Hatched eggs could be easily identified by the white colour and opaque nature of the empty shells. The grains with the grubs inside were then kept undisturbed for about two months to allow their development to complete inside the grains. The

number of adult insects emerged from the grains were recorded after a period of two months.

From the data, percentage of corrected egg mortality was calculated by applying Abbott's formula (1925).

$$\text{Percentage of corrected mortality} = \frac{\%\ \text{killed in treated} - \%\ \text{killed in control}}{100 - \%\ \text{killed in control}}$$

Percentage of adult emergence was calculated by using the formula:

$$\text{Percentage of adult emergence} = \frac{\text{No. of adults emerged}}{\text{Total no. of eggs laid}} \times 100$$

The results were statistically analysed by One Way ANOVA and comparison made based on Duncan's Multiple Range Test (DMRT) at 5% level of significance.

3. Results

Extracts of *C. infortunatum* prepared in petroleum ether and methanol were tested on the eggs of *C. chinensis* to determine their ovicidal activity and the data obtained are presented in the Table IX.1 and 2. The results revealed that the viability of eggs were considerably reduced when the eggs were treated with different concentrations of plant extracts. With both the extracts, mortality of eggs increased with increased concentration of the extracts.

Among the five concentrations tested, the percentage of hatched eggs was maximum on grains treated with 0.5% concentration of the extracts (84.7% and 54% in methanol and petroleum ether respectively). Minimum number of larvae hatched from eggs treated with 6% concentration of both the extracts (21.4 % and 3.5% in methanol and petroleum ether respectively). Percentage of eggs hatched from grains treated with other concentrations were 47.8, 43.2 and 37.6 in 1, 2 and 4% methanol extract respectively and 20.8, 14.9 and 8.2 in similar concentrations of petroleum ether extracts respectively (Table IX. 1 & 2). Here a concentration dependency of egg hatching was observed irrespective of solvent used for extraction (Figure IX.1 & 2). Maximum percentage of egg mortality (96.5%) was obtained with 6% concentration of petroleum ether extract and minimum (15.3%) in 0.5% methanol extract. In the case of petroleum ether extract, the percentage of mortality (corrected) at 0.5%, 1%, 2%, 4% and 6% concentrations were 46.24, 78.56, 84.52, 90.82 and 95.99 respectively and the corresponding values for methanol extracts were 15.3, 52.23, 56.8, 62.35 and 78.60% respectively (Table IX. 1 and 2).

Details regarding the percentage of hatched eggs and the corresponding adult insect emergence are given in Tables IX.1 and 2. The data revealed that the percentage of insects emerged increased with a decrease in the concentration of both the extracts. Maximum number of insects emerged from grains treated with the lowest concentration (0.5%). From 0.5% concentration of petroleum ether extract, 43.3 % insects emerged as adults, whereas, with the same concentration of methanol extract, more adults (61.1%) emerged from the grains. When grains

Table IX. 1. Ovicidal activity of petroleum ether extract of *C. infortunatum* at different concentrations on *C. chinensis.*

Conc.	% of hatched eggs	% of unhatched eggs	% of adult insects emerged	% corrected mortality
0.5%	54.0 ± 18.7	46.0 ± 19.4	43.3^{b} ± 22.9	46.24
1.0%	20.8 ± 16.6	79.2 ± 16.7	16.6^{a} ±11.4	78.56
2.0%	14.9 ± 16.9	85.1 ± 16.9	12.5^{a} ±11.7	82.52
4.0%	8.2 ± 10.6	91.8 ± 10.6	6.2^{a} ± 7.6	90.82
6.0%	3.5 ± 4.6	96.5 ± 4.5	2.09^{a} ± 2.5	95.99
control	88.2 ± 14.2	11.8 ± 15.2	87.9^{c} ± 22.8	-

Data followed by same superscript letter do not differ significantly at 5% level of DMRT ($P < 0.001$). Data presented in the table are the average percentage of 10 replicates.

Table IX. 2. Ovicidal activity of methanol extract of *C. infortunatum* at different concentrations on *C. chinensis.*

Conc.	% of hatched eggs	% of unhatched eggs	% of adult insects emerged	% corrected mortality
0.5%	84.7 ± 4.7	15.3 ± 4.7	61.1^{c} ± 18.3	15.3
1.0%	47.8 ± 8.2	52.2 ± 8.2	27.9^{b} ± 12.6	52.23
2.0%	43.2 ± 8.1	56.8 ± 8.1	21.8^{ab} ± 9.8	56.8
4.0%	37.6 ± 8.5	62.4 ± 8.6	17.5^{a} ± 7.1	62.35
6.0%	21.4 ± 3.8	78.6 ± 3.8	7.8^{a} ± 3.9	78.60
Control	100	0	88.1^{d} ± 5.6	-

Data followed by same superscript letter do not differ significantly at 5% level of DMRT ($P<0.001$). Data presented in the table are the average percentage of 10 replicates.

treated with 1%, 2%, 4% and 6% petroleum ether extracts, adult emergence were 16.6%, 12.5%, 6.2% and 2.09% respectively (Table IX.1). With the same concentrations of methanol extracts, percentage of adult emergence were 27.9%, 21.8%, 17.5% and 7.8% respectively (Table IX.2). From these results, it was revealed that percentage of adult emergence and concentration of extracts are inversely proportional (Figure IX.1 and 2).

Statistical analysis of the result by ANOVA showed that the overall effect of the treatment with respect to the mortality of the eggs and the adult emergence were highly significant compared to the control ($P< 0.001$). In the case of petroleum ether extract, tested samples were found to fall in three groups based on the significance of difference ($P< 0.001$) between them. Here, 1%, 2%, 4% and 6% extracts fell in the same group (group 'a') and 0.5% and control fell in the other two groups, 'b' and 'c' respectively. [The data designated to a particular subset do not differ significantly ($P< 0.05\%$)]. Similarly in the case of methanol extracts, samples were arranged into four groups. Here, group 'a' consisted of 2% and 6% concentrations; group 'b' 1%, 2% and 4% concentrations, whereas 0.5% concentration fell in group 'c' and control in group 'd' (Tables IX.1 & 2).

When the ovicidal effects of the two extracts (petroleum ether and methanol) of *C. infortunatum* were compared, petroleum ether extract appeared to be highly promising. However, in both the cases, more than 50% egg mortality occurred even with 1% concentration. From these results, it is shown that irrespective of the solvent used for extraction, *C. infortunatum* acts as a very effective ovicidal agent against *C. chinensis*.

Figure IX.1. Ovicidal activity of petroleum ether extract of *C. infortunatum* at different concentrations on *C. chinensis*

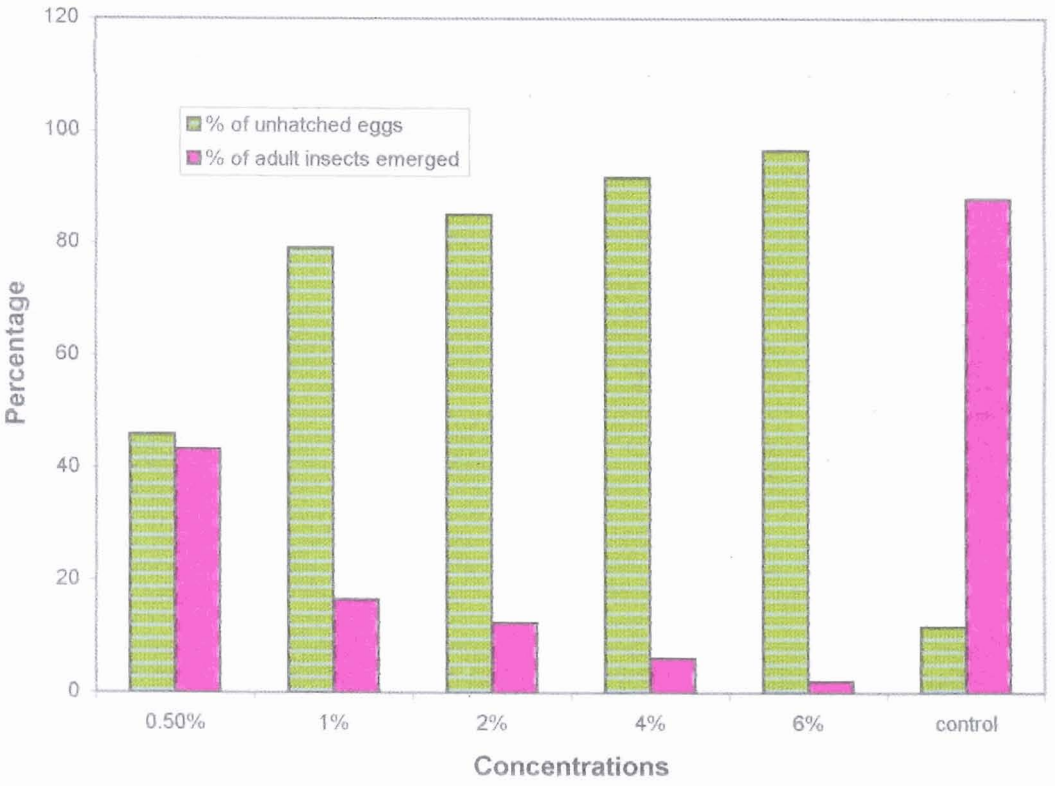
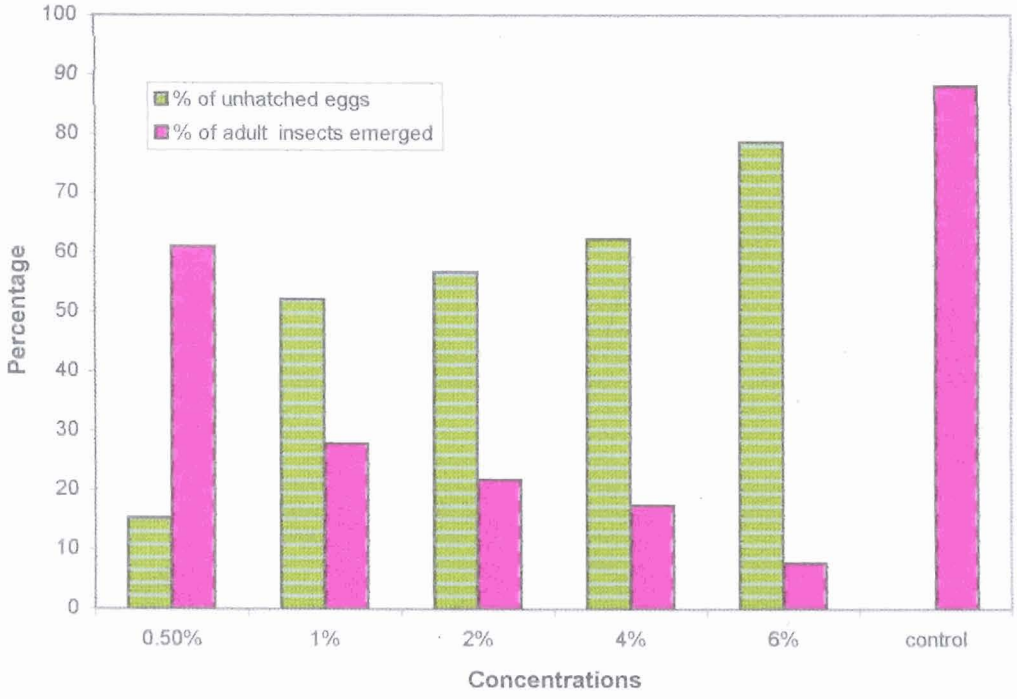


Figure IX.2. Ovicidal activity of methanol extract of *C. infortunatum* at different concentrations on *C. chinensis*



4. Discussion

One of the most conspicuous features of the *Callosobruchus* egg is the presence of a short funnel like structure at the posterior pole. This funnel leading to a space beneath the embryo, the micropyle, may be the site of gaseous exchange (Credland, 1992). The ovicidal and larvicidal effect of oils and plant extracts results from the asphyxiation caused by occluding the funnel and cutting off proper air supply to the developing insects. It has been suggested that the egg mortality is caused by a general property of the oil coating rather than a specific chemical action. Don Pedro (1989 a), on the other hand, has suggested that diffusion of vegetable oils over the egg or first instar larvae, caused the reduction in rate of gaseous exchange due to the barrier effect and /or direct toxicity by penetrated oil fractions. Another suggestion is that oil infiltration under the operculum may block respiration or disrupt water balance of eggs and developing embryos (Messina and Renwick, 1983).

In the present study, the ovicidal activities of extracts of *C. infortunatum* in two different solvents (methanol and petroleum ether) at different concentrations were compared. Data obtained proved that both extracts caused egg mortality to a great extent. The ovicidal activity of extracts increased with increase in dose level. In grains treated with 0.5% extract, 15.3% (Table IX.2) and 46% (Table IX.1) eggs were unhatched in the methanol and petroleum ether extracts respectively. Percentage of unhatched egg increased with concentration, i.e., at 1% concentration of the extracts, the egg mortality was 52.2% and 79.2% respectively. This further increased to 56.8% and 85.1% with 2% extracts and

62.4 and 91.8% with 4% extracts of methanol and petroleum ether respectively. Maximum percentage of unhatched eggs was obtained when 6% concentration of both the extracts were used (78.6% in methanol and 96.5% in petroleum ether) (Figure IX.1 & 2).

From the results, it was revealed that mortality of the egg was dose dependent. Dose dependent ovicidal activity of six plants on *Corcyra cephalonica* was reported by Dwivedi and Kumar (1999) and *Ipomoea palmata* against *C. chinensis* by Dwivedi and Kumari (2000 a). Similar observations were made by Dwivedi and Bajaj (2001) when different plant extracts viz. *Cassia fistula, Acacia nilotica, Lantana camara* and *Tagetus indica* were used as test chemicals (ovicides) against *Trogoderma granarium.*

Repellent and ovicidal properties of different plant oils have been reported by Varma and Pandey (1978) and Babu *et al.* (1989). Egg mortality has been attributed to toxic components present in plants (Su *et al.,* 1972) and also to physical properties, which cause changes in surface tension and oxygen tension within the egg (Singh and Kataria, 1986). According to Amonker (1973) and Fagoonee and Lauge (1981), reason for increase in egg mortality due to plant extracts is the interference of the extract with the normal embryonic development of the eggs by suppressing hormonal and biochemical processes. If oils or some of their constituents do penetrate eggs, they may act by hardening the egg shells, and interfere with the water balance of the embryo thus coagulating the egg proteins thus preventing hatching.. It has been shown that first instar larvae of

bruchids cannot enter the seed unless the egg is firmly attached to the seed surface (Fagoonee and Lauge, 1981).

Results from studies of Dwivedi and Kumar (1999) strongly supported the above findings. High ovicidal activity of *Vinca rosea* and *Withania somnifera* may be due to the presence of 'vincane' and 'withanin' respectively. These chemicals cause disturbance in embryonic development of *Corcyra cephalonica.* Pandey *et al.* (1981) reported that toxic constituents of the vegetable oils enter the eggs through the micropyle and kill the yolk.

Ovicidal activity of various vegetable oils and other plant extracts against *Callosobruchus* species were reported by many workers. Pereira (1983) reported the ovicidal activity of neem oil and other vegetable oils against the cowpea weevil *C. maculatus*. These results revealed that all oils exhibited significant ovicidal activity on cowpea weevil. On cowpeas, only neem oil significantly reduced the adult emergence. Su *et al.* (1972) reported the reduced emergence of *C. maculatus* on black-eyed peas treated with peel oils of lemon, grape fruit, lime and tangerine. Results of Khaire *et al.* (1992) in respect of ovicidal activity of neem, karanj, castor and palm oil against *C. chinensis* are in agreement with the present results. Ali *et al.* (1983) have reported the ovicidal activity of some plant oils (neem, mahua, palm, rape, mustard, sesame and coconut) against *C. chinensis*. Among these, mahua, rape and mustard oils were least toxic, sesame and coconut were at par. Samples treated with sweet flag afforded best protection to grain due to its ovicidal nature (Chander and Ahmed, 1985). Similarly, Khan and Borle (1985) observed that sweet flag at 0.1 to 0.5% was effective in arresting the development of *C. chinensis*.

From the data presented in the Table IX.1 and 2, it is also revealed that all the larvae hatched out from the eggs did not develop to adult stage. A smaller per cent of mortality during the larval/pupal stages occurred. A dose dependent effect was seen in the larval mortality. The effect was remarkable in the case of methanol extract treated samples (Table IX.2). In the case of petroleum ether extract treated samples, similar type of effect is seen, but the difference between hatched eggs and adult emerged are not remarkable (Table IX.1). From these results, it seems apparent that the extract coated on the seed surface would inhibit the development of the larvae of *C. chinensis*. The relatively higher rate of adult mortality observed in the case of methanol extract treated samples may be due to the persistence of the material or higher degree of penetration of the material into the tunnel. Similar results were reported by Don-Pedro (1989 b). The oil coat on seeds would inhibit the penetration of *C. maculatus* larvae. These oils probably interfered with attachment of eggs by weakening the cement with which *C. maculatus* female attach their eggs to seeds. Larson and Fisher (1938) also reported similar data. It has been shown that first instar larvae of bruchids cannot enter the seeds unless the egg is firmly attached to the seed surface. At lower doses even though the larvae hatched, they could not survive for more than two days (Suryakala *et al.*, 1995). The results obtained in the present study thus are in conformity with these results. It was further revealed that, irrespective of solvents, *Clerodendrum* extract acts as an effective ovicide against *C. chinensis*. Its effect is dose dependent. It is also found to inhibit the post-embryonic development of the insects.

Chapter X

SEPARATION AND IDENTIFICATION OF TOXIC PRINCIPLES OF *CLERODENDRUM INFORTUNATUM*

1. Introduction

The subject of phytochemistry or plant chemistry, has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the elucidation of chemical structures, their biosynthesis and metabolism, natural distribution and biological functions of these substances. More than 4,00,000 chemicals are estimated to be produced by plants. Of these, about 10,000 are secondary metabolites, which are produced as by-products of major biochemical pathways, whose major role is to provide defence to the plant especially against insect pests because the overall pressure of insect on plant is much more than any other herbivore (Swain, 1977). Some of the secondary metabolites are allelochemicals that influence plant-insect relationship. These chemicals elicit food-searching behaviour in insects. Such chemicals are examples of synomones, since the polyphagous insects benefit as receivers and the host plants benefit as emitters by attracting natural enemies of the former. Chemically, all these secondary metabolites include alkaloids, terpenoids, phenolics, glycosides, polyacetylene etc. Many of the toxic components among these chemicals have been successfully exploited by humanity for the control of arthropod pests (Singh, 2000).

Among the toxic principles, alkaloids, saponins and essential oils are important. Their main function was thought to be defence against herbivorous attack by acting as repellents, inhibitors or toxins. In addition to this, they deter feeding, disrupt development, disrupt digestion and many are neurotoxic to herbivorous pests. They can be stored inside or outside cells, some are stored for relatively long period of time while others undergo rapid turn over within plants. Expression levels of these substances can be modified by ambient environmental conditions.

The neem plant is unique in that it contains a fairly good number of pesticidal constituents including triterpenoid, vermicillin, salannin, neemol, nimidin, vepacide, ninabiole, nimbidin, nirabinic acid, nimicidin and meliantriol. Most of these are found in leaves, fruits, seeds and bark. All these compounds have been found to control many pest species including insects, mites and nematodes. Neem cake enriched with fatty acids like myristic, palmitic, linolic, capric, caproic, stearic and linolenic acids have been applied as insecticides. A number of congeners of azadirachtin have also been isolated from the neem seed kernel (Rembold, 1989; Govindachari and Gopalakrishnan, 1998). Among the triterpenoids, C-ring modified limonoids such as salanin were shown to be effective antifeedants as azadirachtin against *Epilachna verivestis* and *Pieris brasssica* (Schwinger *et al.*, 1984; Luo *et al.*, 1995).

Intensive studies in this field had led to the isolation of various toxic components from some other plants also. Custard apple (*A. squamosa*) is an important source of botanical pesticides. So far, 20 acetogenins were isolated

from *A. squamosa*, which include squamosin, neo-annonin and squamostatin (Zafra-Polo *et al.*, 1988). Among these, squamosin is important and is characterized as dihydroxy- bis- tetrahydrofuran fatty acid containing 30 carbon atoms (Kawazu *et al.*, 1989). They are potent cytotoxic agents, insecticides, fungicides, repellents and anti-tumour agents (Ruprecht *et al.*, 1990) and also a potent mitochondrial inhibitor (Xing-wang and Hong, 1999). Kumar *et al.* (1989) had succeeded in isolating three new isoflavone from the stems of *Milletia recemosa*. All these compounds showed promising insecticidal activity against fourth instar larvae of *Spodoptera litura*.

A terpenoid lactone isolated from costus root, *Saussurea lappa,* drastically reduced fecundity, egg hatching and adult emergence in the pulse beetle, *C. maculatus* (Singh, 1998). Insecticidal activity of chromatographic fractions of Himalayan cedar wood oil against the pulse beetle, *C. analis* and the housefly, *Musca domestica* were reported by Singh and Agarwal (1988). Their study revealed that almost all fractions inhibited insecticidal activity against both the species. Su *et al.* (1982) have isolated, purified and identified two insect repellent compounds ar-turmeron and turmeron from *Curcuma longa.* These compounds showed strong repellency against *T. castaneum*.

The plants of the genus *Clerodendrum* are also well known for their pesticidal properties. Several flavonoids and diterpenoids (Vendantham and Subramanian 1977) have already been reported from a few plants of this genus. Akihisa *et al.* (1988) have studied the CNMR spectroscopy of the 24-ethylcholesta-5,22 E-dien-3-2-ol isolated from the aerial parts of *Clerodendrum*

fragrance and *C. infortunatum*. A new neo-clerodane type diterpenoid was isolated from the aerial part of *C. inerme* by Raha *et al.* (1991). A steroidal glycoside, 3-0-2-D-galactopyranosyl-24-2-ethyl cholesta-5-22, 25-triene was isolated from *C. inerme* by Rahman *et al.* (1997).

Since in the present study it was revealed that petroleum ether extract of *C. infortunatum* exhibited maximum toxic effect, this extract was used for further analysis to find out the chemical nature of the active toxic principles by chromatography. Different fractions separated by chromatography were tested against *C. chinensis* to find out the extent of toxicity. Attempts were also made to isolate and purify sufficient quantity of some of the active fractions for further structural studies.

2. Methods

A. Separation and identification of toxic principles from *C. infortunatum* by TLC

As described in the general methodology, five chromatograms were run with the crude extract of *C. infortunatum*. Different bands separated on TLC were marked, scrapped and the gel of the corresponding bands from the 5 plates were pooled. The combined silica gel was eluted with 20 ml of a mixture of acetone and petroleum ether (2:1 ratio) and the eluted extracts of the different fractions were dried separately. These fractions were used for further bioassay.

(a) Evaluation of Toxic effect of TLC-fractions against *C. chinensis.*

For evaluating the toxicity of each fraction of *C. infortunatum* extract on *C. chinensis,* topical application method was used (see Materials and Methods). Experiment was replicated ten times for each fraction and five insects were subjected to treatment in each set. A control set in which the insects were treated with the eluting solvent alone was included in each set of bioassay. Experiment was conducted at 28 ± 2°C. The number of insects knocked down after 1 h, 3 h and 24 h and death after 24 h were recorded.

(b) Identification of fractions separated by TLC

Fractions separated by TLC were identified using various visualizing reagents. These reagents could give an idea of the group in which they belonged. The chromatograms were sprayed with the visualizing reagents as described by Stahl (1969) and Harbone (1973). Various methods and visualizing reagents used to detect some of the secondary metabolites were:

Tests for terpenes

Terpenes are lipid soluble compounds that are extracted from plant tissues with light petroleum ether or chloroform. Specific reagents to detect terpenoids, triterpenoids, steroids, glycosides and carotenoids were used.

Vanillin- sulfuric acid mixture **(specific test for terpenes):** A solution of vanillin (1%) was prepared in sulfuric acid and was sprayed on the chromatogram. Heated the TLC plates at 120°C for 10-20 min until the spots attained maximum colour intensity.

***Liebermann- Burchard reagent* (detect the presence of triterpenoid):** This reagent was prepared by mixing 1.0 ml conc. H_2SO_4, 20 ml acetic anhydride and 50 ml chloroform. After spraying this reagent on the TLC plates, they were heated at 85- 95°C for 15 min.

***Anisaldehyde- H_2SO_4* (test for terpenes and steroids):** Conc. H_2SO_4 (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 plates were heated at 100-105°C until the spot attained maximum colour intensity.

***Perchloric acid* (to detect the presence of steroids):** Sprayed the chromatogram with 2% aqueous perchloric acid and heated at 150° C for 10 min.

***Copper sulphate solution* (to detect the presence of glycosides):** Sprayed the TLC plates with 10% $CuSO_4$ solution and heated the plates at 120° C for 20 min.

Tests for phenols

***Ferric chloride-Hydrochloric acid* (to detect the presence of phenol):** Ferric chloride solution (5%) prepared in 0.5 N HCl was sprayed on the TLC plates.

***Aluminium chloride solution* (detect flavonoids):** Sprayed 1% aluminium chloride in ethanol on the TLC plates and viewed the plates under long wave UV lamp.

B. Column chromatographic separation and evaluation of toxicity of fractions of *C. infortunatum* against *C. chinensis*

(a) Separation of chemical components

By column chromatographic method, different fractions were isolated from the crude petroleum extract of *C. infortunatum* as described in Materials and Methods (Chapter III). Each fraction collected was spotted on an activated TLC plate and run in the solvent system ethyl acetate: petroleum ether (1: 4 v/v). Spots were visualized by using the reagents mentioned earlier in this chapter and noted the R_f value of each spot. Fractions whose samples had same R_f values (determined by TLC) were pooled and dried as described in the Materials and Methods (Chapter III). Eleven such fractions were collected (from the column) and volume reduced to 3.0 ml and each fraction was used for toxicity studies.

(b) Evaluation of toxicity of fractions isolated by column chromatography

From the fractions, 5 µl each were taken with the help of a Hamilton syringe (10 µl capacity) and applied on the dorsum of the test insect, *C. chinensis* (5 insect / set) (topical application) as described in the Materials and Method. In a control set, acetone was applied on the insect instead of the extract. The experiment was replicated five times. Knock down after 1 h, 3 h and 24 h and death after 24 h were noted. Since these fractions were highly concentrated, almost all insects were found dead within 30 min after treatment. Hence all the fractions were diluted 5 times with acetone and repeated the bioassay.

(c) Isolation and purification of 2nd fraction

Since the 2nd (combined) column chromatographic fraction was found to be the most effective from the bioassay studies, sufficient quantity of this fraction was purified and collected by preparative TLC (as mentioned in Materials and Methods).

Ten samples of (500 µl each) of fraction 2 were spotted as bands on TLC plates (preparative) and were developed in the solvent system ethyl acetate: petroleum ether (1: 4). After developing the plates, one narrow track in each plate was sprayed with visualizing reagent (vanillin- H_2SO_4). A single band was detected and its R_f value was noted. The silica gel from the corresponding areas from the remaining plates was scrapped out and pooled. It was then eluted in ethyl acetate by constant stirring in a water bath until the ash color was completely washed out from the gel. The mixture was centrifuged at 1000 rpm for about 10 min. The supernatant was concentrated up to 2 ml by warming in a water bath. The purity of the compound was tested again by using TLC.

(d) Visible Spectrophotometric analysis

Purified 2nd fraction was subjected to Spectrophotometric analysis. For this, UV-visible Spectrophotometer, SHIMADZU- UV 1601 was used. Spectrum was taken in the wavelength of 300-700 nm using petroleum ether as the reference blank. Since there seemed to have more than one peak, further separation was found necessary using a new solvent system.

(e) Fractionation of 2nd component using a new solvent system

In the above- mentioned context, further experiments were conducted to find out the actual chemical principles present in this fraction. This fraction was further purified by TLC using another solvent system (acetone and petroleum ether, 1: 4). Developed plates were dried and sprayed with visualizing reagents as mentioned earlier (Vanillin-H_2SO_4).

(f) Test for biological activity of new compound separated from 2nd fraction

Sufficient quantity of new compound was isolated from the 2nd fraction by preparative TLC method, using the solvent system mentioned earlier. For this, compound was applied on 10 TLC plates (as bands) and developed in the solvent system (acetone and petroleum ether 1: 4). A narrow margin of each plate was sprayed with visualizing agent (Vanillin- H_2SO_4) and heated at 110°C for 5 min (Stahl, 1969). A pinkish new band appeared at the R_f of 0.607. The colourless area corresponding to this band and original ash coloured spot were scrapped out and pooled. The compounds present in the gel were eluted in acetone. The eluted solutions were concentrated by keeping in water bath and the volumes were reduced to 2 ml. From this sample, 5µl volume applied on the dorsum of insects (topical application). Knockdown and death rate were observed after 1 h, 3 h and 24 h of the treatment.

(g) Gas chromatographic-Mass spectrometric analysis (GC-MS) analysis

To find out the actual chemical components present in 2nd fraction and new compound separated from the 2nd fraction, GC-MS analysis was carried out (as described in Materials and Methods).

(h) Statistical analysis.

Data obtained from the present investigation were subjected to One way ANOVA and its level of significance of differences in fractions were calculated by DMRT at 5% level.

3. Results

A. Separation and identification of toxic principles in the petroleum ether extract of *C. infortunatum* by TLC

Thin layer chromatography of petroleum ether extract of *C. infortunatum* using the solvent system, ethyl acetate: petroleum ether (1: 4 ratio) showed six coloured bands. Fractions were numbered from top to bottom of TLC plate (Plate X.1). Characteristic colour and R_f values of the bands are presented in Table X.1.

Evaluation of toxicity of fractions isolated by TLC against *C. chinensis*

Data on bioassay of TLC fractions of *C. infortunatum* against *C. chinensis* are presented in the Table X. 2. The table summarizes the knock down effect of each fraction after 1 h, 3 h and 24 h and mortality effect after 24 h of treatment. Persual of the Table X. 2 reveals that the fractions have poor insecticidal activity and a moderate knock down effect.

Table X. 1. Different bands separated from *C. infortunatum* by thin layer chromatography

Bands	Colour	R_f value
1	Orange	0.893
2	Dark ash	0.571
3	Light ash	0.457
4	Dark yellow	0.3
5	Light yellow	0.179
6	Brown	0.036

Table X. 2. Knock down effect of TLC fractions of *C. infortunatum* on *C. chinensis*

TLC fractions	R_f	Knocked down (%) after			% mortality (after 24 h)
		1 h	3 h	24 h	
1	0.893	40	16^{ab}	8^{a}	8
2	0.571	60	47^{c}	28^{b}	20
3	0.457	52	28^{abc}	4^{a}	8
4	0.3	52	40^{bc}	12^{a}	16
5	0.179	56	32^{abc}	16^{b}	8
6	0.036	44	8^{a}	0^{a}	0
Control	-	10	4^{a}	0^{a}	0
P value		0.4375 **NS**	0.0185	0.0081	

Data presented in the Table are mean percentage of KD of insects of five replicates. Within the vertical column, mean values having the same superscripts are not significantly different at 5% level of DMRT, NS - non significant.

Table X. 3. Table showing the ANOVA of KD effect of TLC fractions of *C. infortunatum* on *C. chinensis*

Duration	Source	D.F.	Sum of Squares	Mean Squares	F ratio	P value
1 h	Between group	6	2880.00	480.00	1.0120	0.4375 NS
	Within group	28	13280.00	474.285		
	Total	34	16160.00			
3 h	Between group	6	7890.00	502.857	3.1046	0.0185 *
	Within group	28	11860.00	136.786		
	Total	34	19750.00			
24 h	Between group	6	3017.143	1315.00	3.6762	0.0081 **
	Within group	28	3830.00	423.571		
	Total	34	6847.143			

* Significant at P< 0.01 level; ** Highly significant at P< 0.01 level; NS = non-significant

Considering the knock down (KD) effect, it is found that the intensity of KD activity decreased with time. After 1 h of application, more than 50% insects were knocked down with most of the fractions tested, except those from 1st and 6th bands. Among the six fractions tested, highest KD effect (60%) was shown by fraction made from 2nd band (R_f 0.571). Fifth fraction (R_f 0.179) showed about 56% KD. Third and 4th fractions showed 52% KD and other two (1st and 6th) showed comparatively low level of KD (40% and 44% respectively). After 1 h the order of KD effect were 2> 5 > 3 and 4> 6 > 1 (Table X. 2).

After 3 h, significant decrease in KD activity was observed (Table X. 2). During this period also, 2nd fraction showed maximum effect (47%) and least active was 6th fraction (8%). All others had activities in between these two. The order of fractions based on their activity is 2 > 4 > 5 >3 >1>6. Percentage of KD is 47> 40> 32> 28> 16> 8 respectively.

After 24 h, there was a considerable decrease in the KD effect (Table X. 2). In the case of 6th fraction all insects revived from the KD effect. A maximum of 28% insects remained in the KD condition for 2nd fraction treated sample. Fifth and 4th fraction treated samples showed 16% and 12% KD respectively and 1st and 3rdshowed 8% and 4% KD effect respectively.

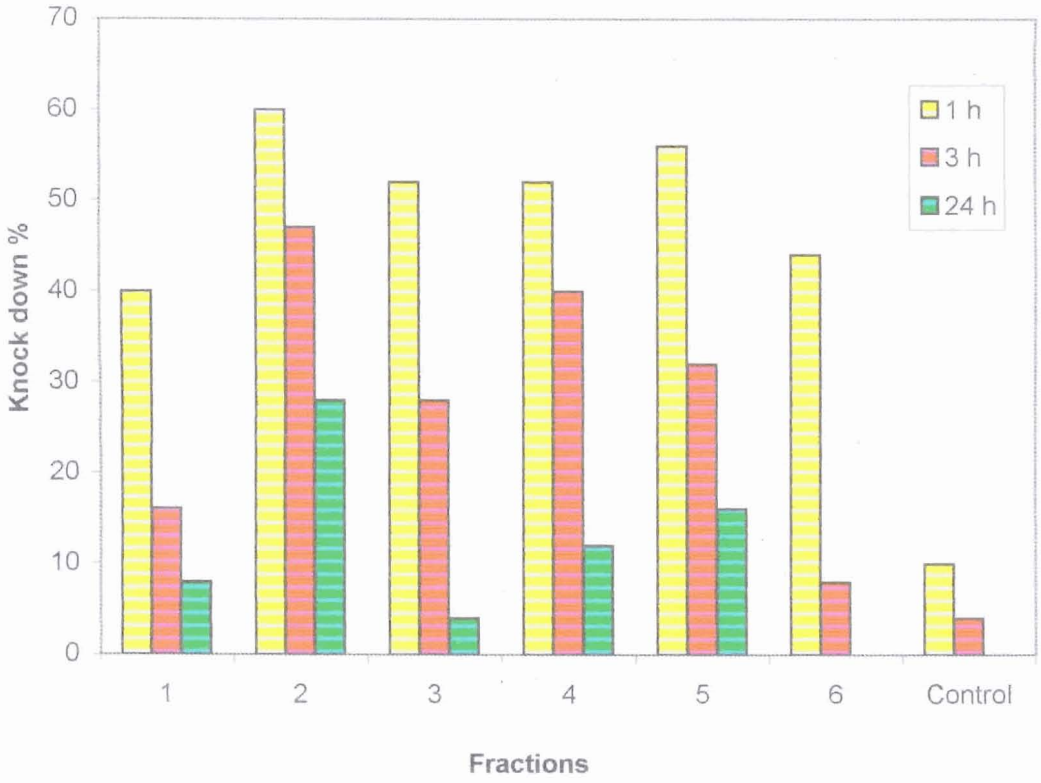
The percentage of KD effect at different exposure period (1, 3 and 24 h) were analysed statistically by one way ANOVA and its differences were compared by DMRT at 5% level. It was found that after 1 h, differences in KD effect between fractions were non-significant at 5% level (Table X.3). Whereas, after 3 h and 24 h, these differences were significant at 5% DMRT ($P< 0.02$ and

0.01 respectively) (Table X.3). From the data, it is revealed that after 3 h, effect of 2nd fraction significantly differed from the effect of other five bands. However, overlapping of activity was observed between different fractions. Based on the percentage of KD effect, 5th and 3rd fractions belong to 3 subsets (subset a, b and c). Whereas the 4th fraction belongs to the subset 'b' and 'c' and 2nd fraction belongs to subset 'c'. The 6th and control belong to the same subset (subset 'a') and 1st fraction in subset 'a' and 'b' (Table X. 2). After 24 h, based on the percentage of KD effect, fractions were categorized into 2 subsets. Thus, 2nd and 5th belongs to same subset, 'b' and all others (1st, 3rd, 4th, 6th and control) come under the same subset 'a' (Table X. 2).

While comparing the percentage of mortality after 24 h, a poor toxic effect was observed for the TLC fractions, compared to the crude extract. Among the 6 fractions tested, 2nd fraction showed comparatively higher toxicity (20%) and 4th fraction exhibited 16% mortality against *C. chinensis*. Other three fractions (5th, 3rd and 1st) showed similar extent of toxic effect (8%) whereas, none of the insects were found dead in samples treated with 6th fraction and in the control.

It was also found that in the control, there was some initial KD effect (about 10% and 4%) after 1 h, 3 h respectively, while after 24 h, almost all insects were found to recover from this effect.

Figure X.1. Knock down effect of TLC fractions of *C. infortunatum* on *C. chinensis*



Identification of chemical components

By using various visualizing reagents (see section X. 2.) presence of some of the components were detected. Their characteristic colour after spraying the reagents and the R_f values calculated are as follows:

Terpenes

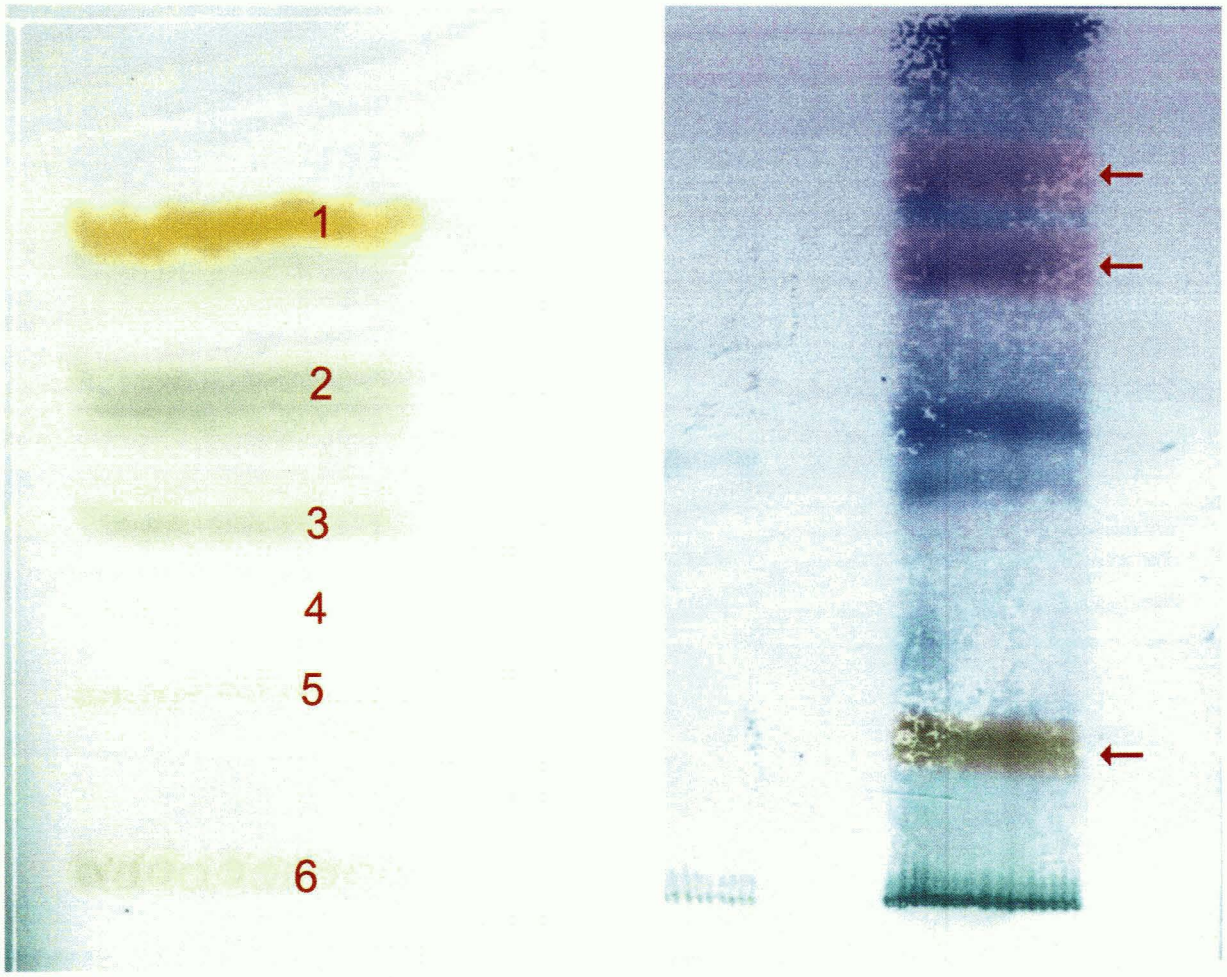
Spraying with vanillin–sulfuric acid mixture, resulted in the appearance of several coloured spots at different R_f values. Purple coloured spot appeared at the R_f 0.657, brown colour of R_f 0.179 and dark brown of R_f 0.086 (arrow mark shown in the plate X.1) were the major ones. All these colours strongly indicated the presence of terpenoid compounds. Spraying the chromatogram with Liebermann-Burchard reagent (see method), a bluish green colour appeared at the R_f 0.57, indicated the presence of triterpenoid. By spraying with vanillin- sulfuric acid, a dark blue colour appeared at the R_f 0.83 indicated the presence of carotenoid (Plate X.1.).

When sprayed with anisaldehyde-sulfuric acid mixture, the pinkish violet colour appeared at R_f 0.16 that turned brown when kept at room temperature. This is characteristic of terpenes including steroids. Presence of steroid was confirmed by spraying the TLC plates with 2% aqueous perchloric acid solution. The brown spot appeared at the R_f 0.16 confirmed the identity of the spot as steroids.

Phenols

Spraying the TLC plates with ferric chloride-HCl mixture, a blue green spot appeared at the R_f 0.57 indicated the presence of phenol. Presence of flavonoid

PLATE X -1



TLC fractions of *Clerodendrum infortunatum* extract

a- Before spraying the visualizing reagent

b- After spraying the visualizing reagent

was also detected by spraying 1.0% $AlCl_3$ solution (see method section 2. b) on TLC plate. An yellow fluorescent spot appeared at the R_f 0.23 which disappeared soon, strongly suggested the presence of flavonoids.

B. Column chromatographic separation and evaluation of toxicity of fractions of *C. infortunatum* against *C. chinensis*

(a) Separation of chemical components.

By column chromatographic method, eleven fractions were isolated from the crude petroleum ether extract of *C. infortunatum* (Table X. 4). Different fractions were eluted in different volumes of the solvent. First fraction (R_f 0.848 on TLC) was eluted in 20 ml of the solvent. 2^{nd} fraction (R_f 0.586) was eluted in 50 ml of the solvent. 3^{rd} fraction (R_f 0.510) was eluted in 35 ml of solvent. 4^{th} fraction was eluted in 20 ml solvent and its R_f value was 0.455. 5^{th} and 6^{th} fractions were eluted in 30 ml and 40 ml solvent respectively. Their R_f values were 0.385 and 0.310 respectively. 7^{th} and 8^{th} fractions eluted in 50 ml solvent each had R_f values of 0.241 and 0.212 respectively. Similarly, fractions 9^{th}, 10^{th} and 11^{th} were eluted in 50 ml each of the solvent and the R_f values were 0.179, 0.086 and 0.036 respectively. Out of 500 ml solvent used for elution, a total of 445 ml solvent contained different fractions.

(b) Evaluation of toxicity of fractions

In the present study, 5µl of different fractions (drawn from column chromatographic fractions reduced to 3.0 ml by evaporation) were applied on to the dorsum of each insect and found out the KD effect during 1 h, 3 h and 24 h

Table X. 4. Fractions isolated from *C. infortunatum* extract by column chromatography

Fractions	Colour	R_f value
1	Orange	0.848
2	Dark ash	0.586
3	Colourless	0.510
4	Light gray	0.455
5	Dark yellow	0.385
6	Colourless	0.310
7	Light yellow	0.241
8	Dark yellow	0.212
9	Colourless	0.179
10	Light brown	0.086
11	Brown	0.036

Column chromatography was carried out as described in Materials and Methods section. The stationary phase was silica gel 60-120 and the mobile phase was petroleum ether – ethyl acetate (4:1 v/v). R_f values were determined by running samples of the eluents on TLC.

Table X. 5. Knock down effect of column chromatographic fractions of *C. infortunatum* on *C. chinensis*

Fractions	R_f	Knock down(%) after			% of death after 24 h
		1 h	3 h	24 h	
1	0.848	72^{c}	48^{b}	18^{ab}	16
2	0.586	76^{c}	85^{c}	63^{d}	48
3	0.510	56^{bc}	46^{b}	24^{abc}	24
4	0.455	64^{bc}	44^{b}	17^{ab}	20
5	0.385	64^{bc}	44^{b}	16^{ab}	12
6	0.310	64^{bc}	52^{b}	22^{abc}	16
7	0.241	52^{bc}	32^{ab}	10^{ab}	8
8	0.212	60^{bc}	56^{bc}	32^{bc}	12
9	0.179	68^{c}	64^{bc}	49^{cd}	32
10	0.086	56^{c}	36^{b}	21^{ab}	12
11	0.036	36^{ab}	32^{ab}	16^{ab}	12
control		20^{a}	4^{a}	0^{a}	0
P value		0. 0022 **P< 0.01**	0. 0007 **P< 0.001**	0. 0006 **P< 0.001**	

Data presented in the Table are mean percentage of KD of insects of five replicates. Within the vertical column means having same superscripts are not significantly different at 5% level of DMRT

and mortality effect after 24 h. Results obtained from this study revealed that the KD effect is at its maximum with in the period of 1 h and it gradually decreased with time. After 24 h treatment, a very good percentage of insects were found dead in sample treated with some of the fractions.

Perusal of the Table X. 5, shows that during 1 h after treatment, almost all fraction except 11^{th} and control exhibited more than 50% KD effect. Among these, 2^{nd} fraction (R_f 0.586) exhibited maximum KD effect (76%). Ninth fraction (R_f 0.179) exhibited next better KD effect (68%). Fractions 4, 5 and 6 showed more or less the same KD effect (64%). Lowest level of KD effect (36%) was shown by 11^{th} fraction which was, however, better than that shown by the control (20%). All other fractions have their KD effect in between 64% and 36%.

After 3 h, a slight decrease in KD effect was shown by all fractions except fraction 2. In the case of 2^{nd} fraction, more insects (85%) were knocked down. In this exposure period also, 9^{th} fraction exhibited next better KD effect (64%). Lowest KD was exhibited by 7^{th} and 11^{th} fractions (32% each).

A fairly high percentage of insects were found to revive from the KD effect after 24 h. In the case of control, 100% insects revived. Only about 10% insects treated with 7^{th} fraction remained KD. Here also, maximum KD effect was shown by 2^{nd} and 9^{th} fractions. Their activities were 63% and 49% respectively.

When the percentage of KD at different exposure periods were subjected to one way ANOVA and difference in effects were compared by DMRT at 5% level, it was found that differences in effect between fractions were highly significant

($P<0.01$) after 1 h treatment and very highly significant ($P<0.001$) after 3 h and 24 h, of treatment (Table X. 6).

Based on the percentage of KD effect, these fractions were grouped into three subsets (a, b, and c). After 1 h, fractions 1, 2 and 9 belonged to the same subset (c). Effects of fraction 3, 4, 5, 6, 7, 8 and 10 exhibited an overlapping effect between 'b' and 'c'. Control belonged to the subset 'a' and fraction 11^{th} overlapped in to the two subsets 'a' and 'b'.

After 3 h also, fractions were categorized in to three subsets. In this, activity of 2^{nd} fraction belongs to the subset 'c' and activity of the fraction 9^{th} have overlapping status with 'b' and 'c' subsets. The subset 'b' contain following fractions, 1, 3, 4, 5, 6, 7, 8, 9, 10 and 11. Among this 7^{th} and 11^{th} fractions were overlapping with subset 'a' and 'b'; fractions 8^{th} and 9^{th} have overlapping effect with subset 'b' and 'c' (Table X. 5).

Very highly significant difference in effect ($P< 0.001$) was exhibited by the different fractions after 24 h treatment. After 24 h, fractions were categorized into four subsets (a, b, c, and d). Among these, 2^{nd} fraction belonged to the subset 'd' (KD is 63%) and 9^{th} overlap the two subsets, c and d. All other fractions were found to have overlapping effect between subsets a, b, and c (Table X. 5).

When the mortality percentage after 24 h were considered, it was found that in samples treated with the 2^{nd} and 9^{th} fractions, more insects died (48 and 32% respectively). Only 8% insects were killed in samples treated with the 7^{th} fraction. Insects treated with 3^{rd} fraction recorded only 24% mortality, while the

Figure X. 2. Knock down effect of column chromatographic fractions of *C. infortunatum* on *C. chinensis*

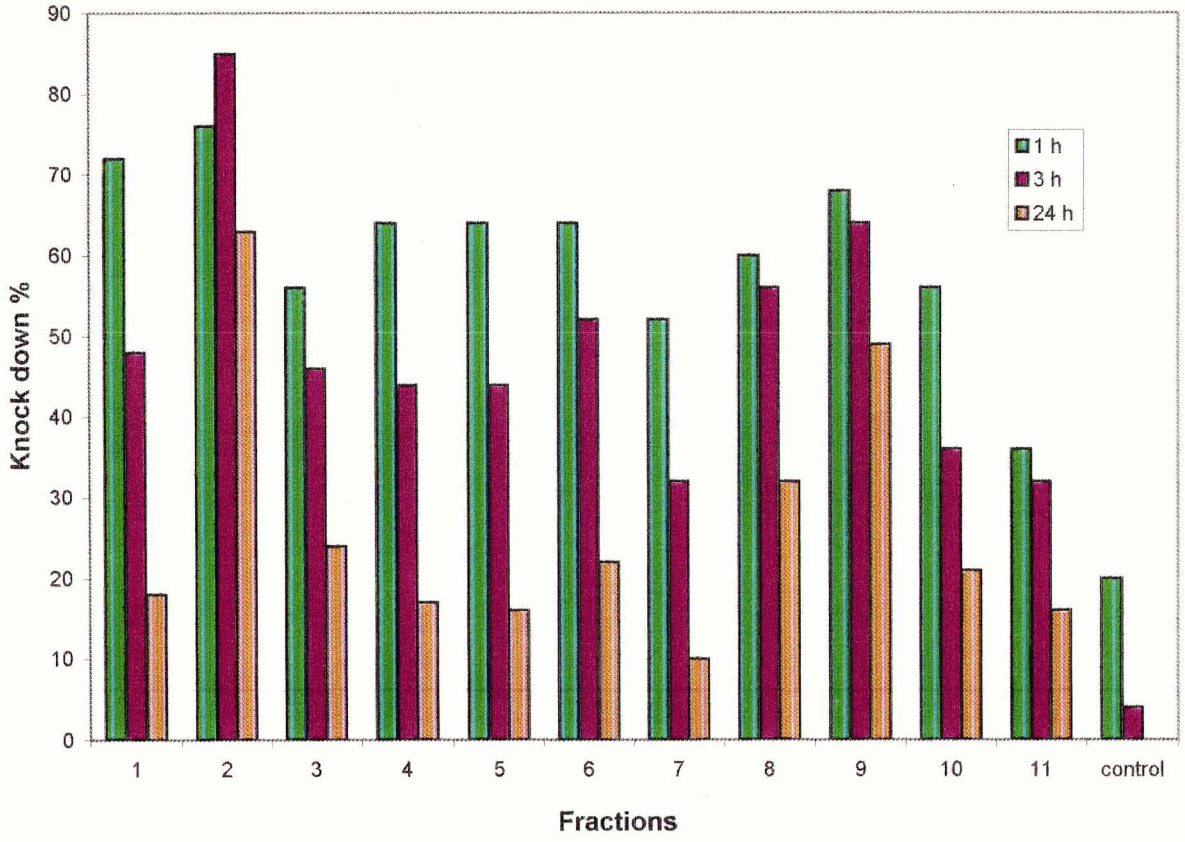


Table X. 6. ANOVA Table showing the significance of KD effect of chromatographic fractions of *C. infortunatum* on *C. chinensis*

Duration	Source	D.F.	Sum of squares	Mean squares	F ratio	P value
1 h	Between group	11	13493.33	1226.66	3.2566	0.0022
	Within group	48	18080.00	376.66		**
	Total	59	31573.33			
3 h	Between group	11	21347.17	1940.65	3.7244	0.0007
	Within group	48	25011.11	521.06		***
	Total	59	46358.28			
24 h	Between group	11	16040.00	1458.18	3.7834	0.0006
	Within group	48	18500.00	385.41		***
	Total	59	34540.00			

** Highly significant at P< 0.01 level; *** very highly significant at P< 0.001 level.

Table X.7. Toxic effect of 2nd fraction (P) and new fraction (Q) isolated from P on *C. chinensis*

Samples	Knock down (%)after			Mortality after 24 h
	1 h	3 h	24 h	
P (R_f. 0.586)	70	46.7	26.67	16.67
Q (R_f. 0.607)	83.33	46.7	23.3	10
Control	3.3	3.3	-	-

Data presented in the table are mean percentage of five replicates; 'P'-2nd fraction isolated by column chromatography; 'Q'- new fraction separated from 'P' using a new solvent system for TLC.

mortality was 16% in insects treated with 1st and 6th fractions. In the case of insects treated with fractions 5, 8, 10 and 11, mortality was 12% in each case. In the present experiment, no insects were killed in the control sample.

(c) Purification of second fraction:

Second fraction obtained by column chromatographic method was further purified by TLC technique using the solvent system ethyl acetate and petroleum ether (1: 4 ratio) until a single spot (whose R_f value correspond with the active fraction- 2nd of TLC of crude extract) was obtained (Plate X. 2. a). Spraying with the visualizing reagent (see section A. (b) of this chapter.) indicated the presence of phenols and terpenoids. When sprayed with ferric chloride and HCl mixture, the dark grey colour changed into blue green spot. This colour indicated the presence of phenol. While spraying with Liebermann-Burchard reagent, dark grey colour changed into bluish colour. This indicated the presence of terpenoids and steroids.

(d) Visible Spectrophotometric analysis:

Purified 2nd fraction was subjected to visible spectrophotometric analysis. Ten definite peaks were obtained. The detectable peaks appeared at the wavelengths, 307, 350, 360, 364, 408, 505, 534,561, 661 and 668 nm. The characteristic peaks at 400 nm and those between 500 and 600 nm were indicative of the presence of chlorophyll-like substances in the second fraction. But considering its strong KD and lethal activity, further chemical analysis were carried out.

(e) Further purification of second fraction:

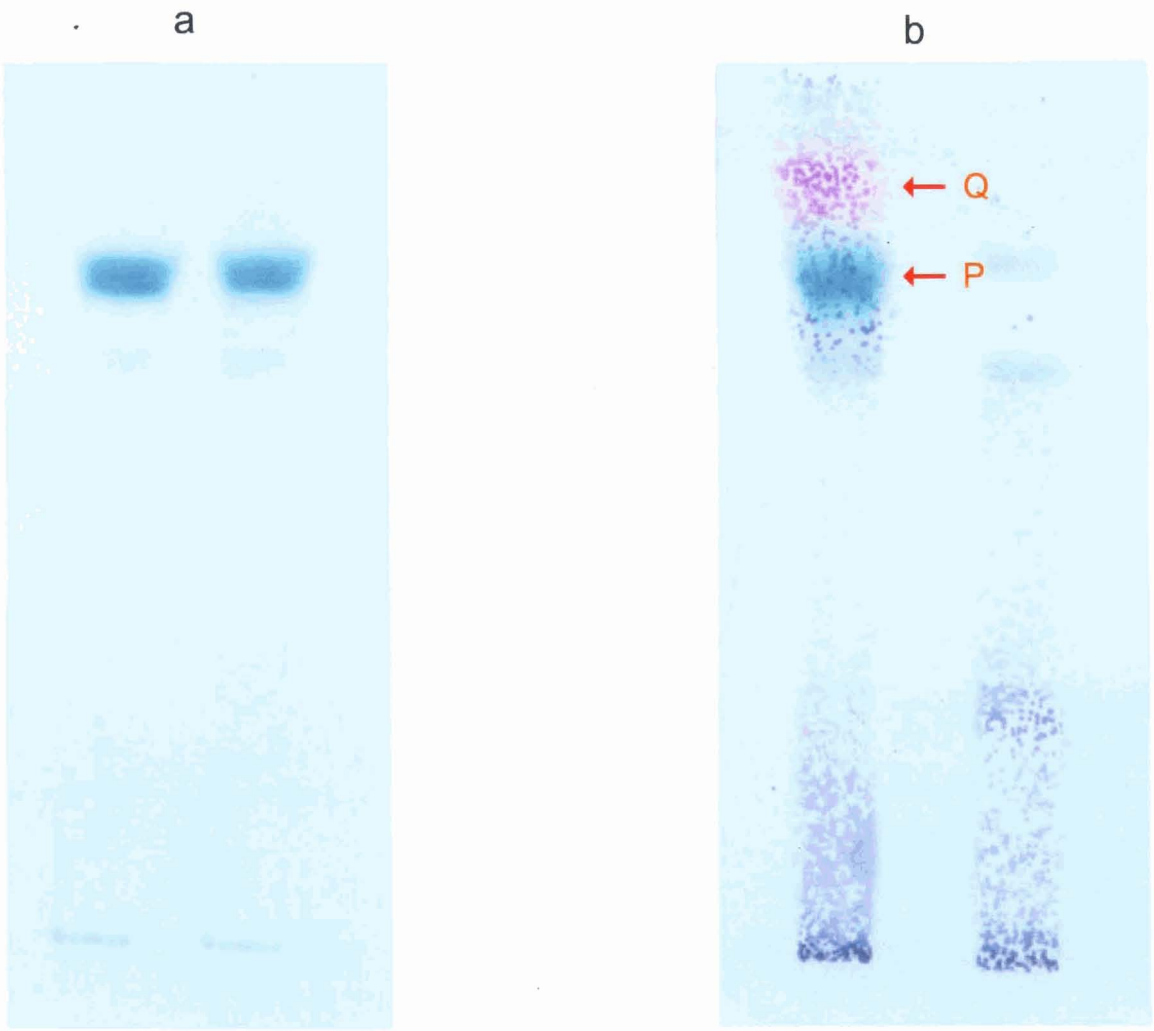
Second fraction purified in the above method (see Method B. (c) of this chapter), was further purified by TLC using another developing solvent system (acetone and petroleum ether, 1: 4 v/v). After developing the chromatogram and spraying with visualizing reagent, (vanillin-H_2SO_4), a pinkish spot appeared (R_f 0.607) just above the gray band (Plate X. 2. b). This region was colourless in the unsprayed plates. These results indicated the presence of terpenes or steroids. By this purification, a new component was separated out from the second column chromatographic fraction. For convenience original second fraction is designated as 'P' and the new compound as 'Q' (Plate X. 2. b).

(f) Bioassay using the components P and Q:

By preparative TLC, a sufficient quantity of new component (Q) was isolated from 2nd fraction (P) as mentioned in the method section B.(e). Applied 5µl of each sample on the dorsum of insect by topical application method and data obtained are presented in the Table X. 7.

Data presented in the Table X. 7 indicate the toxic effect of P and Q separated using the solvent system acetone: petroleum ether (1:4). From the table, it is revealed that both samples show relatively high level of toxicity against the pulse beetle. In both fractions, KD effect was the highest within 1 h after treatment. Among the samples, the new compound (Q) showed slightly higher KD activity than 2nd fraction (P) (83.33% and 70 % respectively). However, after 3 h, both fractions exhibited same KD effect (46.7%). But after 24 h, fraction P

PLATE X -2



TLC Separation of 2nd CC fraction using acetone : PE (1:4)

a- Before spraying the visualizing reagent

b- After spraying the visualizing reagent

P- Original fraction; Q- new fraction separated from the 2nd fraction

showed higher KD effect (26.67%) than the new fraction (Q). In this case, fraction 'Q' exhibited about 23.3% KD effect.

After 24 h, both samples showed very low level of lethal effect against this insect. About 16.67% mortality was exhibited by the 2nd fraction (P) and it was only 10% in the case of Q. A very low level of KD effect (3.3%) was exhibited in the control after 1 h and 3 h duration and this was overcome afterwards. Unlike the fractions tested, there was no mortality in the control sample.

C. GC-MS analysis

Purified 2nd fraction (P) and the new fraction (Q) isolated from the 2nd fraction, were analysed by GC-MS (Plate X. 3. 4). From both fractions, an array of chemical components were separated. The peaks obtained for these components were matched with peaks of known components of computer Library of Data base and matching quality was noted. Peaks with matching quality of above 90% were considered to be identical with the components already known. List of fractions with matching quality of 90% and above to the known compound, their area percentage and retention time are given in the Tables X. 8 and X. 9. Results obtained from the GC-MS analysis revealed that these two purified fractions (P and Q) themselves contained more than 20 distinct compounds.

Most prominent constituent in component 'P' is Di (2-ethyl hexyl) phthalate. Area percentage of this is 74.27. It exhibited about 91% matching quality. 1,2-Benzene di-carboxylic acid stands second. Its area is 9.62% and

matching quality 95%. In addition to this, phenol (0.14%), azulene (0.39%), neophytadiene (0.52%), pindolol N- butyl boronate (0.66%), ?-octyl-?-1-phenylethyl diphenyl amine (0.12%); retinoic acid (0.12%), 2`4`7- trimethoxy isoflavone (0.17%) are the other components. All these components exhibited more than 90% matching quality. Alkanes such as 2- hexadecane-1-ol (0.52%), methyl 2-N- pentyl 1-cycloheptene (0.5%), bis-(octyl phenyl) amine (1.49%) are also detected.

Other compounds having more than 90% matching quality were identical with styrene, cinnamene and styrol. Retention time of these compounds was 4.26 min. Another component present in this fraction, exhibited about 96% matching quality to salicylic aldehyde (RT. 7.96min). Phytol was detected at the retention time 32.65 min and octyl diphenylamine (93%) at the retention time 36.42 min. Butyl-2-ethyl hexyl phthalate was another prominent component present in this fraction. Its area was 2.92%. However, it exhibited only 83% matching quality (Table X. 8).

In sample 'Q' (isolated from 2nd fraction), fatty acid and aliphatic compounds are the major chemical components. Among the fatty acids, myristic acid, palmitic acid and stearic acids are the prominent ones. The area of myristic acid is 0.61%, palmitic acid is 1.73% and stearic acid is 0.84%. Aliphatic compounds present in this sample include alkanes and their derivatives. They are tetradecanoic acid (Area 0.61%), heptadecanoic acid (Area 0.61%), hexa decanoic acid (area 1.73%), octadecanoic acid (area 0.84%), pentadecanoic acid, decanoic acid etc. and their esters such as heptadecane (0.58%), docosane (0.58%),

Table X. 8. GC-MS Analysis of sample 'P' (purified 2nd fraction)

Sl. No.	RT	Area %	Library	Matching quality
1	4.269	ND	Styrene	95
2	4.269	ND	Styrol	95
3	4.269	ND	Cinnamene	94
4	6.59	0.14	Phenol	94
5	7.96	ND	Salicylic acid	96
6	11.73	0.39	Azulene	91
7	27.49	0.52	Neophytadiene	96
8	27.49	0.52	2- Hexadecane 1-ol	91
9	29.92	9.62	1.2- Benzenedicarboxylic acid	95
10	32.65	ND	Phytol	76
11	35.21	1.72	Butyl-2-ethylhexyl phthalate	83
12	35.34	1.72	Methyl 2-N-pentyl-1-cycloheptene	97
13	36.41	ND	Octyl- diphenyl amine	93
14	37.95	ND	1- Phenanthrene carboxylic acid	90
15	39.26	ND	1- Phenyl ethyl diphenylamine	93
16	40.02	74.27	Di- (2- ethyl hexyl) phthalate	91
17	43.02	0.12	?- Octyl?-1-phenyl ethyl diphenyl amine	99
18	43.04	0.12	Retinoic acid, methyl ester	95
18	44.32	0.16	Pindolol N- Butylboronate	91
20	45.19	1.49	Bis- (octyl phenyl) amine	94
21	46.42	0.17	2' 4' 7- Trimethoxy isoflavone	92
22	48.62	ND	1-Aza-1(methoxy iminomethyl) 5-carbamoyl)-cyclonona (6-7 b) indol	96

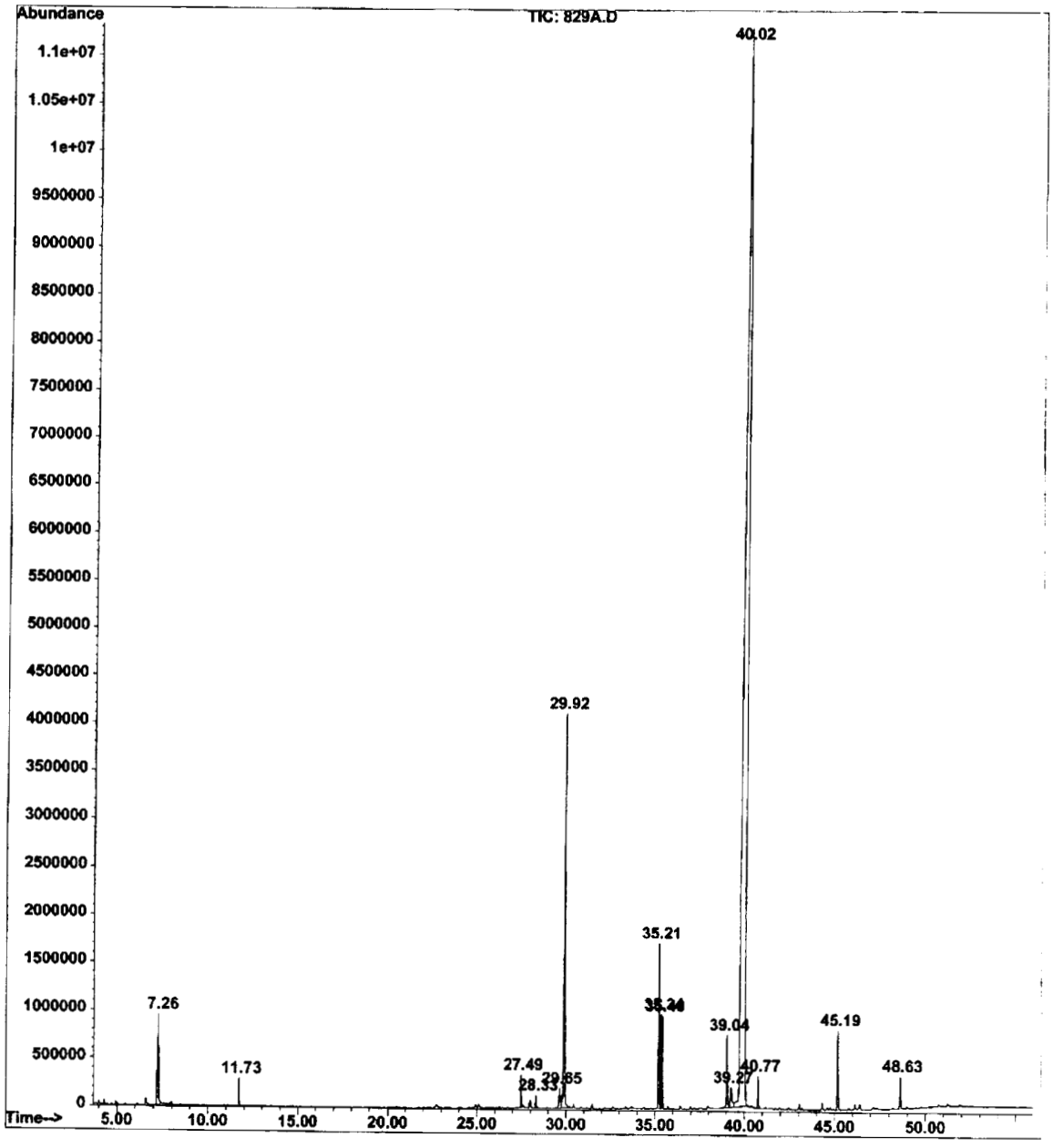
In the Table RT is the retention time; ND = not detected

Table X. 9. GC-MS analysis of sample 'Q'
(compound separated from 2nd fraction)

Sl. No.	RT	Area %	Library	Matching quality
1	21.68	ND	Lauric acid	99
2	25.98	0.61	Tetradecanoic acid	98
3	25.98	0.61	Myristic acid	94
4	29.97	1.73	Hexadecanoic acid	99
5	29.97	1.73	Palmitic acid	99
6	32.12	ND	+ Delta 4`-dehydroxyjurabione	38
7	33.57	0.84	Octadecanoic acid & stearic acid	99
8	37.49	0.58	Heptadecane	90
9	39.05	1.49	Pentacosane	96
10	39.05	1.49	Eicosane	95
11	39.78	74.27	Di- (2-ethylhexyl)phthalate	91
12	39.78	23.94	1,2-Benzene dicarboxylic acid	91
13	40.56	3.07	Hexacosane	97
14	40.56	3.07	Eicosane	96
15	42.01	4.73	Heptacosane	98
16	43.42	5.30	Octacosane	97
17	47.23	ND	Cholesterol	97
18	47.84	0.96	Androst-4-en-19 al-3, 17-diox - ol	53
19	48.59	2.74	Octadecane	96
20	48.59	2.74	Triacontane	93
21	49.0		Cholesta-5, 24. dien-3,20 diol	37
22	49.05	33.23	Stigmasta-7, 16, 25- trien-3-ol (3,beta, 5-alph stigmasterol)	92
23	49.05	33.23	23-Ethyl cholesta-5, 23, 28 trienol	90

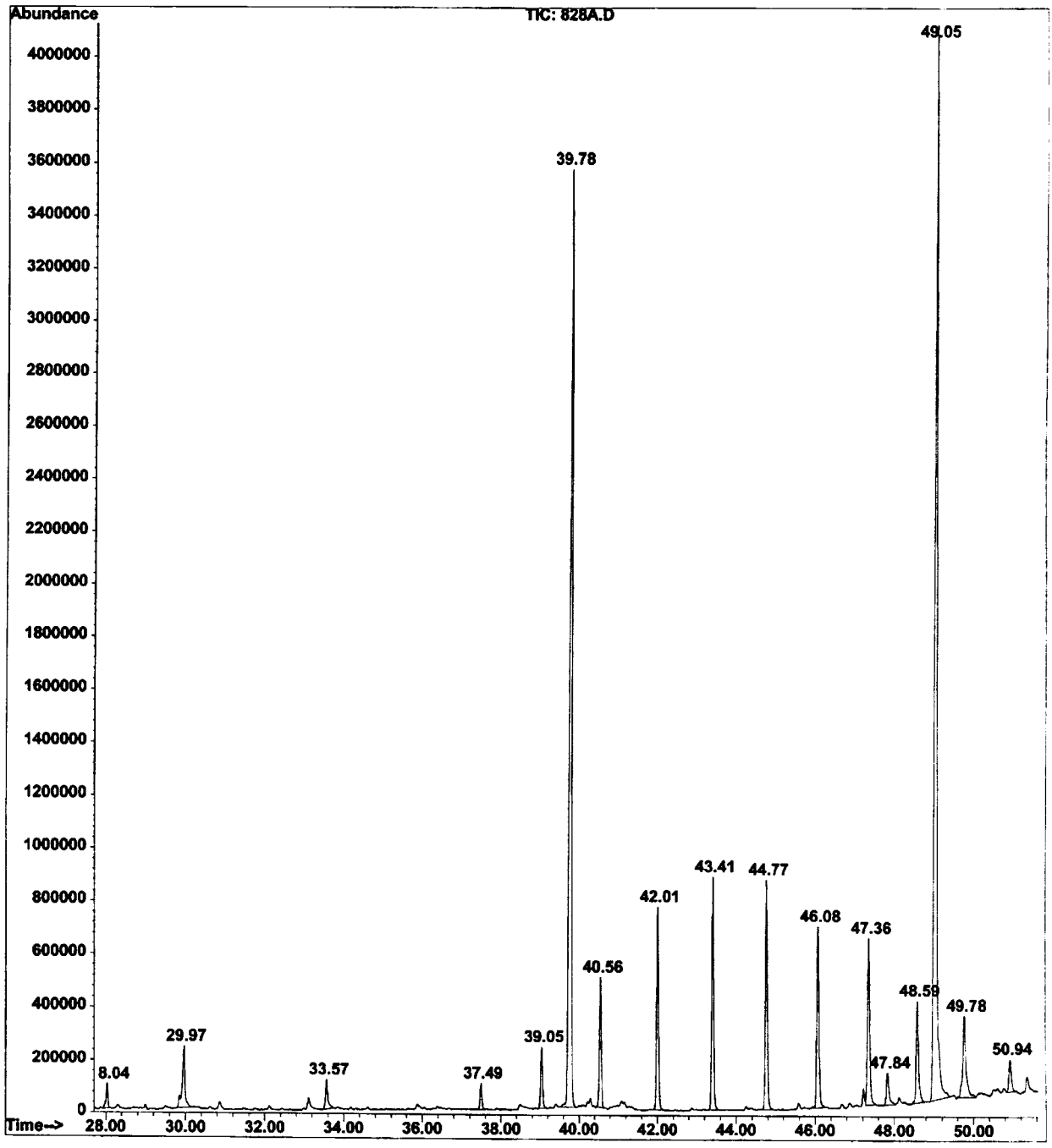
In the Table RT is the retention time; ND = not detected

PLATE X-3



GC - MS analysis of sample 'P'

PLATE X-4



GC - MS analysis of sample 'Q'

tetracosane (0.58%), pentacosane (1.49%), eicosane (1.49%), hexacosane (3.07%), octacosane (5.30%) and heptacosane (4.73%) (Table X. 9). Lauric acid, hexadecanoic acid, palmitic acid, octadecanoic acid and stearic acid exhibited about 99% matching quality.

Aromatic compounds include 1,2-benzene dicarboxylic acid (23.9%), which occur in two peaks of retention time 28.04 and 39.78 min. In sample Q, some steroidal components were also detected. Among the steroids, the prominent one was stigmasta-7,16, 25- trien-3-ol (stigmasterol). Area of this component peak is 33.23%. It showed about 92% matching quality. In addition to this, 23-ethyl cholesta-5, 23, 28, trien-3-ol (ethyl cholesterol) (33.23%) is also present. Presence of cholesterol at retention time 47.23 min was also detected. It exhibited about 97% matching quality. A terpene, (+) delta 4`-dehydrojurabione was detected at retention time 32.12 min. But its matching quality was only 38%. Similarly, cholesta-5, 24, dien- 3, 20, diol, was detected at retention time 49.0 min whose matching quality was only (37%) (Table X.9).

By the GC-MS analysis, it was found that in addition to the fatty acids, their esters, steroids and phenolic compounds, a large number of other aliphatic and aromatic compounds are also found in exceptionally high amounts (Tables X. 8 and 9).

4. Discussion

Using TLC and column chromatography, a number of fractions were separated from the crude petroleum ether extract of *C. infortunatum*. Crude

extract was separated into six distinct coloured bands by TLC. The chemical identity of the separated compounds were confirmed by spraying the plates with specific visualizing reagents. After analysis of the bioactivity of the bands, the most active fraction (2^{nd} band R_f 0. 571) was re-run on TLC using another solvent system, which resulted in resolving the band into two. These two bands were again found to be mixture of several chemical compounds when analysed using GC-MS. Therefore, it becomes clear that fractionation by TLC and identification by specific colour reagents cannot give the actual composition of plant extracts.

Fractions separated by column chromatography and TLC were subjected to bioassay against the stored grain pest, *C. chinensis* and results obtained are presented in the Tables X. 2 and X.6. Perusal of the data of both the experiments proved that KD effect reached the maximum with in one hour duration and gradually it decreased. This may be due to the effect of some highly volatile compounds present in these extracts. A mean of 76% insects were knocked down when treated with 2^{nd} fraction collected by column chromatography (CC), corresponding band from TLC (2^{nd} band), exhibited 60% KD after 1 h. The KD effect gradually decreased with time. Even after 24 h, the maximum KD effect was given by 2^{nd} fraction of CC (63%) and corresponding TLC fraction, the 2^{nd} band (28%). This similarity was observed in the percentage of mortality also. In the case of TLC fractions, 2^{nd} band exhibited 20% insect mortality, and with the 2^{nd} fraction of CC, about 48% mortality was obtained.

From the results presented in the Tables X. 2 and X. 6, it is revealed that 2^{nd} fraction of column chromatography and corresponding TLC fraction (2^{nd}

fraction) were comparatively more active than other fractions. When statistical analysis by One Way ANOVA and differences in KD effect among fractions at different time interval were compared, it was shown that there was more or less significant difference ($P<0.01$) at 5% level of DMRT. In the case of TLC extracts, KD effect of fractions after 1 h exposure was found to be statistically non- significant. However, after 3 h and 24 h, difference in KD effects among fractions were highly significant ($P<0.01$). On the other hand, in the case of fractions obtained from CC, difference in KD effect among fractions, at all the tested durations were statistically highly significant ($P< 0.01$) at1 h, 3 h duration and very highly significant ($P<0.001$) at 24 h duration.

Although the second fraction of CC and 2nd fraction of TLC were showing the most prominent toxic effect against insects, other fractions also exhibited activity to a greater or lesser extent. This was suggestive of the toxic effect of this plant being due to the synergistic effect of all these components. The identity of the different components were thus determined by spraying with specific reagents. TLC plates were sprayed with these visualizing reagents (vanillin-sulphuric acid). Most of the bands showed positive indication for terpenoids or steroids. Purple coloured spots appeared at the R_f 0.729, 0.657, 0.179 and brown colour at 0.086. All these spots indicated the presence of terpenoids including sterols. Various previous reports indicated the occurrence of terpenoids and sterols in this plant species. Akihisa *et al.* (1988) isolated a clerosterol (24- ethylcholesta-5, 22 E-dien-3 2-ol) from the aerial parts of *C. fragrans* and *C. infortunatum.* Leaves and flowers of *C. infortunatum* contain clerodin, hentriacontane, fumaric acid, ethyl and methyl esters of caffic acid, 2-sitosterol and its glucoside and flavone

glycoside (Akihisa *et al.*, 1988). In the present study, GC-MS analysis of the sample 'Q' (compound isolated from 2nd fraction, 'P') showed that major component of this fraction was stigmasterol (stigmasta-7, 16, 25, trien-3-ol). Its peak area was 33.23%. In addition to this, 23-ethyl cholesta-5, 23,28,trien-3-ol (area 33.23%) and trace of cholesterol were also detected.

Biological assay, using the fractions isolated by TLC as well as column chromatography showed that a very good percentage of insects were knocked down immediately after the application of these fractions. This may be due to the neurotoxic effect of some of the components like terpenoids or steroids present in these fractions. Plant terpenes (mono-terpenoid, sesquiterpenoid, diterpenoid and triterpenoids) are neurotoxic, feeding deterrent and toxic to insects and steroids acts as development inhibitors (Panda and Khush, 1995). Results obtained from the present study were in agreement with these findings. Wide ranges of monoterpenes have insecticidal effects and depressive effects on the reproductive development of several insect species (Weaver *et al.*, 1991). According to Jacke and Rimpler (1983), the diterpenes, clerodendrin A and B are the widely distributed phytosterol present in plants, which seem to be of great toxicological importance. According to Ryan and Byrne (1988) terpenoid toxicity to insect is due to the inhibition of acetylcholine esterase or is linked to oxido-reduction reactivity.

Other major components detected in the sample 'Q' are alkenes and their derivatives. Octadecane, triacontane, tetradecane, heptacosane, eicosane, hexacosane, pentacosane and their acids such as Octadecanoic acid, hexadecanoic

acid, tetradecanoic acid etc. are the major derivatives. Presence of alkenes such as decanol, decanal and geraniol in essential oil of *C. infortunatum* were reported by Jirovetz *et al.*(1999). These alkenes, aldehydes and ketone derivatives in plants act as strong barriers to insect attack (Panda and Khush, 1995). Peterson *et al.* (1989) reported the use of mixture of a (z)-11- hexadecanal and (z)-13-octadecanal in 4: 5 ratio to control the striped rice borer *Chilo suppressalis* in the field.

Another prominent component of this sample is long chain fatty acids and their esters. GC-MS analysis enabled the detection of the presence of some of these long chain fatty acids. They were myristic acid, stearic acid, palmitic acid, lauric acid etc. Plamitic acid was the main component among these (peak area 1.73%). Akihisa *et al.* (1988) also reported the presence of long chain fatty acids such as palmitic, oleic and linoleic present in the seed extract of the plant, *C. infortunatum*. These components can take part in the complex effect of a laxative or for a support of dermal application in the case of skin diseases because of the skin penetration effects of these compounds (Jirovetz *et al.*, 1999).

In the samples 'P'and 'Q, the most dominant constituents were phthalic acid (1,2, Benzene dicarboxylic acid) and phthalate (Di-2ethyl hexyl phthalate). Peak areas of these components are 23.94% and 74.27% respectively. Peterson *et al.* (1989) suggested that these phthalate might come from the silica used in the column and thin layer chromatography since they are commonly used as binders. It has generally been observed that during GC-MS, phthalic acid and phthalates appear as major peaks which are suggested to be column bleeding and are usually discounted.

GC-MS analysis may help to explain the biological activity of plant materials (extracts), based on the chemical structure of this constituent. Toxic fractions 'P' and 'Q' isolated from the petroleum ether extract of *C. infortunatum* contain mainly long chain hydrocarbons and fatty acid derivatives. In addition to this, alkanes, sterols and phenolic compounds are also detected. All these components act together (synergistic) resulting in promising toxic effects against the tested insect. From this study, it is also revealed that plant secondary metabolites act together and result in better toxic effect than the individual components. That explains why, crude extract exhibited much higher level of KD, insecticidal and antifeedant effect than isolated fractions. These results are in agreement with the results reported elsewhere. Peterson *et al.* (1989) reported that crude plant extract exhibited higher level of insecticidal activity than its fractions. Gbewonyo and Candy (1992) also supported this. According to their report, root extract of *Piper guineense*, lost about half of its insecticidal potency during passage down the GLC column. This may be due to the loss of some insecticidal components per se or to the loss of synergists. Hence they found out that crude petroleum ether extract was more potent than its fraction against *Musca domestica.* Present experiment was also in agreement with these findings. Insecticidal property of the fractions isolated by TLC or column chromatography were comparatively less effective than crude petroleum ether extract of *C. infortunatum.* Hence, detailed toxicological studies on crude extracts and their various fractions are required before they could be recommended for use in stored product protection.

Chapter XI

GENERAL DISCUSSION

Protection of crop, stored food grains and public health continues to place heavy reliance upon the use of pesticides. The history of synthetic insecticide development has been instructive to use them in terms of benefits derived as well as the hazards, which accompany the indiscriminate use of these poisons. Serious scientific and public health concerns on the toxic effects of pesticides have led to eco-compatibility requirement of these products. Alternative forms of crop protection have elicited interest for decades. Plants appear to produce a wide variety of secondary metabolites as defensive weapons. Such natural defence chemicals are far superior to the synthetic pesticides. The isolation of these protective chemicals from the plants and their determination of chemical structures would provide a valuable pointer to the development of new pesticides and novel methods of pest control.

For centuries man has been striving hard to find safe means for storing grain ensuring full protection from various pests. By trial and error, he has progressed far but none of the methods adopted has been fool-proof. India produces around 12.65 million tones of different pulses per year but a good percentage of the same is lost during post harvest handling and storage. Among the pests of stored pulses, the pulse beetle, *C. chinensis* is the major pest causing severe damage and economic loss. Various plant products have been tried recently against this pest with a good degree of success.

During the present investigation, the extracts made from eight indigenous plants in petroleum ether were tested for their toxic effects on the pulse beetle, *C. chinensis*. Results obtained indicated that all the eight plants contained several toxic components with varying toxic effect on the insect. Among the eight plants screened, *C. infortunatum* exhibited the highest and *A. vasica* exhibited the lowest toxic effect. Earlier, various workers have reported the insecticidal effect of *C. infortunatum* on some agricultural pests (Johnson *et al.*, 1979; Rajamma, 1982; Sheela, 1997). Even though, *A. vasica* exhibited poor insecticidal effect in this study, Tripathi and Singh (1994) have found that this plant exhibited the highest level of toxicity against *Spilosoma obliqua.* Another observation of our experiment is that the mortality has increased with increase in time. With all the plants tested, comparatively higher mortality of insects was obtained within 6 days after treatment. Irrespective of plants, there occurred a gradual increase of mortality rate from 2^{nd} day to 6^{th} day. A similar observation was made by Lawrence *et al.* (1993) in *T. castaneum.* The treatment of different plant extracts against *T. castaneum* revealed a gradual increase of mortality from 24 h to 72 h.

Plants are the richest source of organic chemicals on earth. Some of their components are highly volatile while others are low or nonvolatile. Many volatile compounds have been found to act as insect deterrents. In the present study, five plants were selected to determine their repellent effect against *C. chinensis* at different exposure time durations (30 min, 1 h, 3 h, 24 h). From this experiment it was revealed that repellent effect of different plant extracts at different time durations were significantly different. All the tested plants exhibited highest

repellent effect against this insect within 30 min, after which a considerable decline was observed. From these experiments it was clear that some of the aromatic plants possess repellent activity against insects and this repellent effect persists for a few hours and then slowly decreases with time. Repellent effect observed immediately after the treatment could be due to some highly volatile or unstable components in the extracts. This causes some behavioural changes in insects and they tend to move away from the treated sample. With time, such compounds get evaporated off or the insect get accustomed or acclimatized to the condition, which may be the reason for low level of repellency after 3 h and 24 h exposure. Similar results were reported by Malik *et al.* (1984) and Urs and Srilatha (1990). In the present study, however, the repellent activity of the extract of *C. infortunatum* was found to persist even after 24 h. This indicated the presence of some possibly less volatile, toxic components in the extract of *C. infortunatum.*

When different concentrations (0.5, 2, 4 and 6%) of *C. infortunatum* were used against *C. chinensis* to test their repellent effect, it was observed that highest repellency occurred within 30 min of exposure, irrespective of the concentrations and it gradually decreased with time. These observations were in conformity with the findings of Jilani and Su (1983) and Ahmed and Eapen (1986). An interesting observation in this experiment was that, when the exposure period was extended up to 72 h and more, repellent effect of these extracts were lost and a considerable number of insects remained in the treated area. This resulted in negative values for the percentage repellency and was designated as attractancy (Talukder and

Howse, 1995). This attractancy was prominent in lower concentrations whereas this never occurred with higher concentrations, suggesting the presence of low volatile toxic principles in the extracts of higher concentrations. The attractancy was also found to be dose and time dependent. A considerable percentage of insects were found dead in this repellency experiment, especially in higher concentration (6% and 4%). This mortality rate increased with time. Similar observations were made by Jilani *et al.*(1988), Tripathi *et al.* (2000) and Sahayraj and Paulraj (2000). From our findings, it was revealed that presence of highly volatile chemical components in *C. infortunatum* extract caused immediate repellent effect and the loss of these components might have led to the decline of repellent effect. In other words, substances causing persistent repellency was absent in the extract. Whereas, substances that are more toxic to the insects were in great abundance, which resulted in the mortality of the insects. Sahayaraj and Paulraj (2001) reported that there is no relationship between toxicity and repellency. Present study is also in agreement with these findings. Even though, *C. infortunatum* does not act as a persistent repellent against *C. chinensis*, it acts as a very effective insecticide for a long period of time. Some of the low volatile essential oils such as terpenes, steroids and glycosides are responsible for this.

Another finding of this investigation is that, solvent used for extraction, play a significant role in the extraction of principal insecticidal constituents from the plant materials concerned. In the present experiment, petroleum ether extract of *C. infortunatum* was found to cause higher mortality than methanol extract. In the case of highest concentration (6%) of petroleum ether extract, 100% insects

were killed within 6 days (Chapter VII). While in the case of methanol extract, only 80 % insects were found dead within this period. This observation was in conformity with the findings of Mukherjee and Govind (1959); Pandey *et al.* (1976); Usharani and Jamil (1989) and Sheela (1997). According to their report, among the various extracts, petroleum ether extract was the most effective followed by ether and alcoholic extracts. However, with all the concentrations tested, mortality response was found to be dose and time-dependent irrespective of solvent used for extraction. El-Nahal *et al.* (1989) proposed that the period of exposure could be the most important factor to determine the efficacy of extracts rather than dosages. The LC_{50} value in the case of both the extracts were inversely proportional to the duration of exposure. As the time of exposure extended, a lower concentration of the extracts were required to kill 50% insects. Similar observations were made by Sharma and Srivasthava (1998). According to their findings, LC_{50} values for the extracts of *Artemisia annua*, *Carica papaya*, *Cuscuta reflexa* and *Lantana camara* were 0.29, 0.67, 0.15 and 1.19 after 24 h exposure and it decreased to 0.16, 0.66, 0.01 and 1.05 respectively after 48 h exposure.

Three plants (*C. infortunatum, C. fistula* and *C. citratus*) were screened for their action as oviposition deterrents with extracts prepared in petroleum ether. It was found that *C. infortunatum* clearly stood out as an effective oviposition deterrent (Chapter VI). This experiment was repeated with various concentrations of petroleum ether and methanol extracts of *C. infortunatum* to study the effect of the extracts as oviposition and feeding deterrents. Comparing the methanol and

petroleum ether extracts, it was seen that petroleum ether extract exhibited high reproductive control than methanol extract. However, here also a concentration dependent effect was observed. According to Pandey and Khan (1998b), the decline in oviposition at higher doses was due to the interruption of vitellogenesis and damage to the egg chambers in the ovaries of *C. chinensis*. Dhar *et al.* (1996) reported that oviposition was possibly regulated by the volatile compounds absorbed through the cuticle. Number of adult insects emerged from the grains were inversely proportional to the concentration of the extracts. Only very few insects emerged from grains treated with the highest concentration (6%) of both the extracts. Khaire *et al.* (1992) and Prakash and Rao (1989) also reported similar results from their experiments on pulse beetles. In addition, percentage of weight loss of grain was also found to be related to the concentration and type of solvent used for extraction. Present findings are thus, in agreement with the earlier observations of Ali *et al.* (1981), Kumari *et al.* (1990), Chiranjeevi (1990) and Khaire *et al.* (1992). More grains were destructed in methanol extract treated sample and weight loss was at its maximum. Even though there is a significant difference between the activities of the two extracts, there was a common pattern in the increase of the reproductive control and grain protecting activity with an increase in concentrations of the extract.

Effect of plant extract on egg mortality has also been carried out during the present investigation. Egg mortality has been attributed to the toxic components (Su *et al.*, 1972) and also to physical properties, which cause changes in surface tension and oxygen tension within the egg (Singh *et al.*, 1978). According to

Amonker (1973) and Fagoone and Lauge (1981), reason for increase in egg mortality due to plant extract is its interference with the normal embryonic development of the eggs by suppressing hormonal and biochemical processes. In addition to this, the egg mortality is also caused by anoxia resulting from the diffusion of these extracts into the egg, which may block respiration, causing death (Don-Pedro, 1989a). It has also been observed that the seeds coated with oil would inhibit the penetration of *C. maculatus* larvae. These oils probably interfered with the attachment of the eggs by weakening the cement with which *C. maculatus* females attach their eggs to seeds (Don-Pedro, 1989a)

In the present investigation, when the ovicidal effect of the different concentrations of two extracts of *C. infortunatum* were compared, it was revealed that irrespective of solvent used for extraction, *C. infortunatum* acted as a very efficient ovicidal agent against *C. chinensis.* However, a dose dependent effect was observed among the two extracts. In this experiment, even though some of the eggs hatched after treatment with both the extracts, not all of them developed into adults. A majority of them were killed at post-embryonic development. Here also a dose dependent effect was seen. From these results, it was revealed that some of the extract coated on seed surface, reaching the interior through the funnel, would inhibit the development of the larvae of *C. chinensis.* Similar observation was made by Don-Pedro (1989). Abivardi (1977) also reported the growth inhibitory effect of plant extracts on earlier stages of larvae and mortality of embryos at different stages of development. Pandey *et al.* (1981) reported that toxic constituents of vegetable oils enter the eggs through micropyle and kill the

yolk. Thus it was recommended that complete coverage of extracts/ oils over the surface of grains was necessary to prevent the egg laying and further development of the pests.

Identification of the active principles, which act as effective antifeedant, repellent, ovicide, oviposition deterrent etc. is important. Having identified this, the isolation of the ingredients from the raw products is another major task. In the present experiment, few fractions of *C. infortunatum* were separated by TLC and column chromatographic methods. From the petroleum ether extracts, 6 fractions were separated by TLC and 11 fractions were separated by column chromatography. Each fraction isolated by these techniques were subjected to bioassay against *C. chinensis*. From these experiments it was revealed that the fractions had poor insecticidal activity and moderate KD effect. The KD effect was at maximum with in 1 h of treatment, which gradually decreased. From the bioassay, it was found that almost all fractions exhibited KD activity to a greater or lesser extent. But comparing the toxic action of crude extract, it was found that crude plant extract was always more effective. From these results, it was concluded that highest toxicity of this plant is due to the synergestic action of all these fractions. By chemical analysis of some of these fractions using specific visualizing reagents, presence of terpenoids, steroids and glycosides were detected at various R_f values. Presence of these components were confirmed by GC-MS analysis of two selected (active) fractions. When these fractions were analysed by GC-MS method, an array of chemical components were separated. Among these, fatty acids, alkanes, aliphatic compounds and steroids are prominent.

From the present investigation, it has been concluded that the locally available plant *C. infortunatum* acts as a promising protectant against the attack of the pulse beetle, *C. chinensis*. It acts as an effective oviposition deterrent, antifeedant, ovicidal agent and toxicant against this insect. But comparing the toxic action of crude extract with different fraction separated from this, it was revealed that crude plant extract was more effective. High toxicity of this plant is thus believed to be due to the synergistic action of all these fractions.

Results from the investigation, thus reveals that a thorough knowledge of the various components of different plants, the natural world of chemical ecology is definitely going to provide new principles of insect pest control in the most natural way. This study therefore, opens a new line of work for the management of stored grain pests with indigenous plant materials in a very safe way, avoiding operational and residual hazards that are usually involved in the use of synthetic insecticides. Plants contain a hidden treasure of ingredients, which can effectively be employed in the pest management of crops. Some of these plants may be incorporated as a desirable and easily accepted components of IPM programme on stored grains.

For those who are interested in insecticide research, these natural agents (botanicals) offer a continual source of inspiration and challenge. The isolation of active principles from these plants and the determination of their chemical structures would provide a valuable pointer to the development of new pesticides and novel methods of pest control. These active principles can be tested against other pests of agriculture as well as insect vectors besides stored grain pests.

These active molecules obtained as insecticidal principles under insect bio-directed isolation may also be used as a basic design of a new class of compounds, naturally occurring insecticides, for future industrial application. With an ever-increasing public interest and awareness in the environment, in both developed and developing countries, positive public perception of natural pesticides is an added incentive for their development and use.

Summary

SUMMARY

In the present investigation eight indigenous plants (*Clerodendrum infortunatum, Cassia fistula, Cymbopogon citratus, Hyptis suaveolens, Adathoda vasica, Achyranthus aspera, Glyricidia sepium* and *Uvaria narum)* were screened for their insecticidal effect on the pulse beetle, *Callosobruchus chinensis*. Extracts were prepared in petroleum ether. All the tested plants exhibited toxicity to a greater or lesser extent. However, *C. infortunatum* was found to be the most effective. Repellent effect of five plants selected from this group (*C. infortunatum, C. fistula, C. citratus, A. vasica, and H. suaveolens*) were also studied. The insects were exposed to the test materials for different time intervals ranging from 30 min to 24 h. Among these five plants, *C. infortunatum* exhibited higher repellency against these insects and *C. citratus* came next to this. Based on the results obtained from this study, *C. infortunatum* was subjected to further detailed study on repellency against the insect *C. chinensis*. From the results obtained, it was revealed that repellency is dose and time dependent. Repellency increases with concentration and decreases with time. This suggested the presence of certain highly volatile components in this extract, which causes immediate repellent effect. When the exposure period was extended up to 72 h, repellency was very much reduced especially in lower concentrations. However, a remarkable degree of mortality was observed at this duration. This indicated the presence of certain non-volatile, highly toxic components in the extract. From these results it was found out that besides its repellent effect, this plant extract act as a very good toxicant against the pulse beetle, *C. chinensis*.

Toxic effects of methanol and petroleum ether extracts of *C. infortunatum* was also carried out in the present study. From this, a dose and time dependent effect was observed. However, petroleum ether extract showed promising toxic effect compared to methanol extract.

Comparison of the oviposition deterrent effect of three plants (*C. infortunatum, C. fistula* and *C. citratus*) revealed that *C. infortunatum* exhibited the highest oviposition deterrent effect and hence, extracts of this plant in two solvents (methanol and petroleum ether) were used for further study for oviposition deterrence. From this study, it was found out that petroleum ether extract exhibited high reproduction control than methanol extract. Although there appeared to be a significant difference between the two extracts, there was a dose-dependent reduction in the mean number of eggs laid on grains treated with both the extracts. A dose dependent effect was also observed on the number of insects emerged from the grains with both the extracts. In addition to this, percentage of weight loss of grain also was found to be related with the concentration and type of solvent used for extraction.

When the ovicidal activities of the extracts of *C. infortunatum* prepared in two different solvents (methanol and petroleum ether) at different concentrations were compared, petroleum ether extract was found to exhibit promising results than methanol extract. However, both the extracts caused more than 50% egg mortality even with 1% concentration. It was also revealed that mortality of the egg was dose-dependent in both the cases. Maximum percentage of unhatched eggs were obtained when 6% concentration of both the extracts were used.

In the present investigation, attempts were also made to isolate the chemical components from the extract of *C. infortunatum* and to evaluate the toxicity of each fraction on *C. chinensis*. From the bioassay, it was found that almost all fractions exhibited knockdown activity to a greater or lesser extent. But when compared to the toxic action of the crude extract, it was found out that the crude plant extract was more effective. From these results, it was presumed that better activity of the crude extract than the individual fractions, was due to the overall synergestic action of all these fractions. By applying various visualizing reagents, presence of some of the active components like terpenes, steroids etc. were detected. Based on the results of bioassay of activity of these fractions, the 2^{nd} band of TLC and corresponding 2^{nd} fraction of column chromatography were found to be the most effective components. GC-MS analysis of purified samples of these fractions showed the presence of an array of chemical components. Among these, steroids, alkanes and fatty acid derivatives were prominent.

From this investigation, it becomes evident that the country weed, *C. infortunatum* contains some toxic principles, which showed good insecticidal, oviposition deterrent and ovicidal effect on the pulse beetle *C. chinensis*. Even though the pest incidence occurred immediately after the harvesting or at granaries, as a control measure, this material either in a crude form or a commercial product prepared from this plant extract, could be recommended as an eco-friendly contact poison resulting in oviposition and feeding deterrency, egg mortality etc. and ultimately providing protection against the invasion of this insect.

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