INVESTIGATIONS ON ECO-FRIENDLY NATURAL INSECTICIDES

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dedicated to
the self immolated
farmers of
Warangal

DECLARATION

I here by declare that the thesis entitled

'Investigations on Eco-friendly Natural Insecticides'

is a bonafide record of the original research work done by me under the guidance of Dr. A. Mohandas, Director, School of Environmental Studies, Cochin University of Science And Technology, and that no part of the thesis has previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of this university or of any other university or society.

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CERTIFICATE

Certified that the thesis entitled ***'Investigations on Eco-friendly Natural Insecticides'*** *is a bonafide record of the original research work done independently by* ***Mr. K.M. Abdul Khader****, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowships, or associateship.*

Dr. A. Mohandas,
Supervising Guide

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ABSTRACT

Instead of developing easily degradable, and low-priced insecticides, we are going after highly sophisticated chemicals. Here, an attempt is being made to develop safer formulations of insecticides of botanical origin.

Different parts of the plants were chosen based on their use in countryside and villages. The dried plant materials were extracted with petroleum ether, and were applied on *Tribolium castaneum*. The results were statistically analysed.

The active principles from *Croton tigilium* and *Leea sambucina*, the most potential plants, were isolated using Column Chromatography, TLC, and Hydrolysis.

The isolated principles were analysed spectroscopically (UV-Vis., IR, NMR, and MS) to identify their chemical nature. The active principles from *Leea* and *Croton* were identified as a cholisterate derivative and a phorbol derivative respectively.

In order to ascertain the environmental combatibility of the principles, degradation by soil bacteria was studied.

The isolated principles were made into three type of formulations using stabilizers. The formulations were applied on *Snake gourd semilooper*, Pulse beetle, and mosquito larvae. Also the biocidal activity of the formulations was studied.

Both *Leea* derivative and *Croton* derivative could be formulated effectively and were effective against a variety of pests. They are eco-friendly, as there is no artificial chemicals involved.

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GENERAL INTRODUCTION

1

GENERAL INTRODUCTION

Our ancestors had enough indigenous system of knowledge on medicinal plants. But western system of knowledge in agriculture and medicine was defined as the only scientific system. Thus, instead of strengthening research on safe and sustainable plant- based pesticides such as Neem and Pongamia, we fund extensively on the development and promotion of hazardous and non-sustainable chemical pesticides such as DDT and Sevin. The use of DDT causes millions of deaths each year, and has increased the occurrence of pest 12000-fold *(Siva, 1996).*

The growing concern about protecting the environment, recognizing the importance of ecological balance and evidence of changes in the surroundings by extensive and indiscriminate use of pesticides with persistent residues has emphasized the need to review our existing strategies for insect pest control. Among the several avenues explored for integrated insect pest control programs, the use of plant insecticides, being safe, easily bio-degradable, and practically innocuous to non-target species, has drawn attention all over the world. *(Ahmed and Chander, 1983).*

At present only two botanical products, Rotenone from *Derris* species and Pyrethrins from *Chrysanthimum* species, are commercially used as insecticides, although a third, Azadirachtin from the Neem tree *(Azadirachta Indica)*, is under commercial development in several countries *(Isman et al., 1992)*.

What the present generation of farmers fed by voluminous literature on pesticides do not know is the use of certain varieties of plants to fight pests. For example, ***turmeric*** mixed with cow's urine is a good pesticide, and *tulsi* extract is a good insect repellent *(Ramiah, 1998)*.

1.1 A Global Scenario

The use of chemical pesticides has created a lot of irreparable damages on the global ecology. Developed countries banned many organochlorine pesticides such as DDT, BHC etc. during the early seventies. Still, the process of regulations on pesticide uses has been continuing with their detrimental effects on the environment being unraveled.

Experts in the National Cancer Institute, USA, believe that the rising incidents of Non-Hodgkin's Lymphomas, a form of cancer, are due to increased use of organophosphate pesticides and phenoxy herbicides, and the cumulative effects of these pollutants on the human system.(*Agarwal, 1997*).

A study by the US Environmental Protection Agency has shown that ***Saku*** desease is caused by the non-target effect of pesticides like Ethyl Thromitone, Fenthion and Fenitrothion *(Dementi, 1994)*.

DDT and PCBs have been proven to be carcinogenic and the incidence of breast cancer in the USA and other major industrialized countries has been correlated with these chemicals *(Epstein, 1993).*

During 1990-91 a study in Spain showed that residues of five organochlorines in milk (in ng/ g fat) averaged 25.0, 26.7, 33.9, 18.7, 13.9 for α-HCH, β-HCH, lindane, heptachlor epoxide and p,p' DDE, respectively. Mean residues in meat from cattle, pigs, lambs and chicken varied from 0.6 to 54.1 ng/g fat *(Herandez et al., 1994).*

About 32 species of raptors in Australia were reported to have shown a significant reduction in egg shell thickness, of which nine birds species showed significant reduction after DDT was introduced in agriculture *(Oslen et al., 1993).*

Many chemical pesticides are found to be detrimental to soil micro organisms. Lindane at a concentration of 3.5 to 15 Kg/ha can decrease nitrifying bacteria *(Martinez-Toledo et al., 1993).*

Toxaphene, Hexachloro cyclohexane, Trichlorophenol, Strobane, Perthane, TCDD, Dieldrin, DDT, 1,2, dichloro ethane, Heptachlor, Picloram etc. have been proven to be lymphatic cancer causing pesticides (*Agarwal, 1997*).

Several studies carried out in Canada, Sweden and the USA have shown a strong correlation between the risk of NHL (Non- Hodgkin's Lymphomas) and use of pesticides (*Zahm, 1992*).

The very wide use of modern synthetic insecticides, beneficial in many ways, has had some undesirable consequences. One of these is the emergence of resistant strains, due to the widespread and persistent destruction of normally susceptible insects while the abnormally tolerant ones managed to survive. Emergence of resistant strains, therefore, is a selection of pre-existing genetic types, analogous to the selection of new types and varieties by natural selection. The idea that resistance can be developed in individual insects by exposure to sub-lethal dozes; is a misconception (*Busvine, 1980*).

Prior to the second world war, resistant strains were rare curiosities because, until the introduction of modern synthetic insecticides, the attack on pests by toxic chemicals was very rarely on scale sufficient to exercise appreciable selective effect on the natural population. Beginning with the report of DDT-resistant flies in 1947, however, the phenomenon has appeared in pest after pest all over the world until well over three hundred species are now involved. About a third of these are pests of public health importance and growth in their number is shown in Figure 1.1.

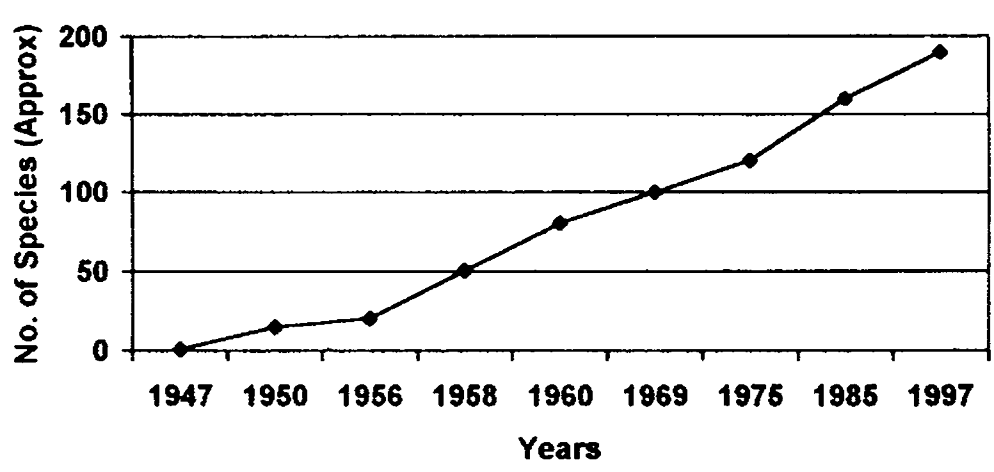


Figure . 1. 1

Development of resistance to synthetic insecticides over the last fifty years

Resistance is most likely to develop in species which are regularly and extensively exposed to the selective effects of insecticides; hence, it occurs in most of the important vector groups.

The numbers of cases of resistance which have been recorded, though significant, do not give an adequated picture of the seriousness of the problem. This depends on: (i) importance of the species concerned (e.g., if it is a disease vector), (ii) whether the degree and extent of the resistance actually prevent effective control, (iii) whether there are other non-chemical means of control, and (iv) **whether there are alternative insecticides suitable for maintaining control**. The first three criteria demand appropriate information and sound judgement from experts in the field. The fourth point involves an understanding of the **toxicology** and genetics of resistance. Since resistance develops in response to powerful selection, excessive use of insecticides should be avoided. Alternative methods of control with improved hygiene, and **low residue compounds** may be resorted to.

With the progress of pest control science, the older crude inorganic poisons were being replaced by synthetic organic compounds, specifically toxic to arthropods rather than to man or higher animals. During 1960's, however, immense quantities of organic chlorine insecticides were used under the impression that they were harmless. Indeed, they caused little harm to man or his domestic animals, and a great deal of good in saving crops and preventing disease. But all the time they were building up persistent residues in the environment and also in the bodies of vertebrates. Furthermore, excessive use (mainly in agriculture) was responsible for considerable intermittent death of wild life. These incidents, dramatized in Rachel Carson's *Silent Spring*, created a strong reaction in the press, the public, and the Government.

In short, global ecology is facing severe threat from the use of pesticides. So the search for safer pesticides of plant origin is an awe inspring field of research.

1.2 India's Malady of Using Chemical Pesticides

After the much celebrated Green Revolution, India, like other third world countries, have switched over to chemical-centered farming techniques. Though our buffer stock beefened up above the food security level, the ecological cost incurred has been greatly underestimated. We were left with diseased soil, pest-infested crops, and water logged - deserts *(Siva, 1991)*. With the introduction of high yield varieties of seeds replacing our traditional varieties, the farmers were forced to use chemical fertilizers and pesticides. As our farmers were not aware of the safer use of these chemicals, like one in developed countries, they were used indiscriminately. Last year's cotton pest attack and the resultant mass suicide at Warangal district of Andhra Pradesh is an example of the green revolution ghost still pursuing us. Though Warrangal district alone has 13000 pesticide shops (with a turn over of 200 crores, selling 93 products) none of them could save the crops *(Chakraborthy, 1998)* because the pests had turned resistant to pesticides even to methomyl, a highly poisonous formulation.

If India's consumption of pesticides in the mid eighties was 99,000 tons, the figure has shot up to 1,20,000 tons in the later eighties *(Muraleedharan, 1994)*. Pan Pesticide Action Network has labeled twelve pesticides in the market at present as 'Dirty Dozen'.

Of these, six (DDT, BHC, Aldrin, Heptachlore, Methyl Parathione and Toxephene) are in rampant sale in India.

Since the use of pesticides, particularly phenoxy herbicides (2,4-D, 2,4,5-T and MCPA) and organophosphate pesticides, has increased over the last forty years, The National Cancer Institute, US, argues that they could have played a significant role in contributing to the rising incidents of NHL (Fig. 1.2)

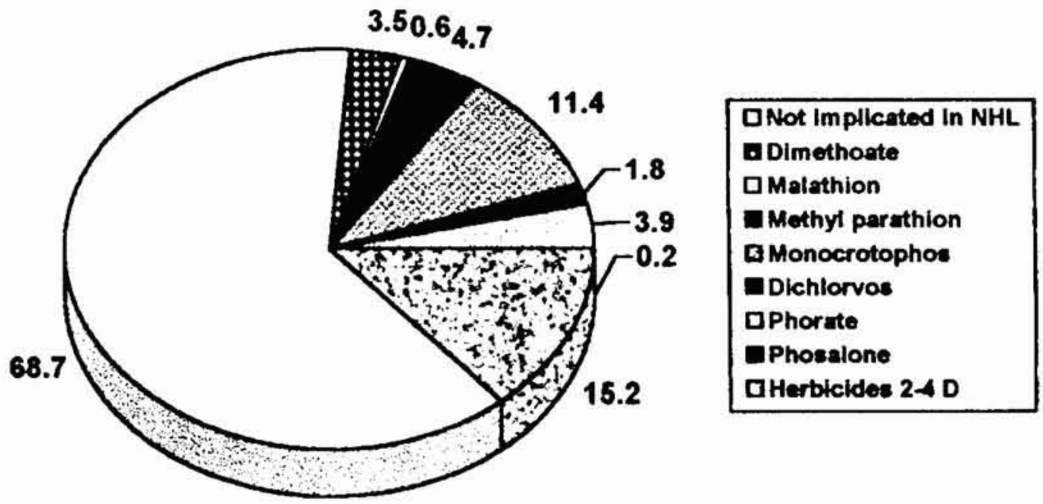


Fig. 1.2 *NHL Implicated pesticides and their % consumption in Indian Agriculture (1993-94)*

Indian planners doggedly persist in using DDT, BHC, Malathion etc., in high amount for the National Malaria Eradication Programme (Fig.1.3). The less eco-damaging pyrethroids are used negligibly.

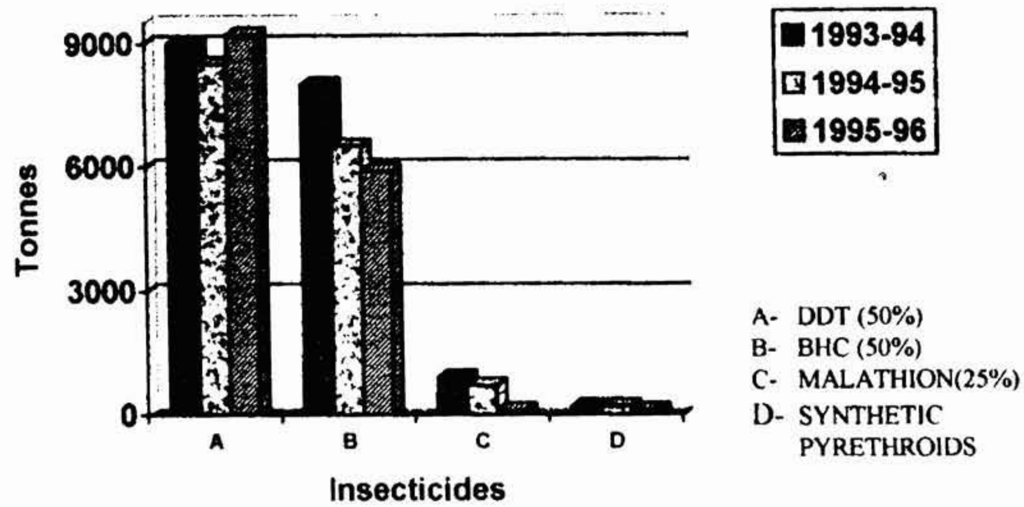


Figure 1.3 Use of Insecticides under National Malaria Eradication Programme (NMEP)

The findings of an unpublished study by ICMR quoted by *Agarwal (1997)* are the following:

- Detectable residues of alpha, beta and gamma isomers of HCH were found in 87,85 and 85 per cent of the samples, respectively. The percentages of samples exceeding the scientifically determined tolerance limits were 21,42 and 28 in the case of alpha, beta-and gamma-HCH, respectively.

- The worst contamination was in the states of Andhra Pradesh ,Uttar Pradesh and Bihar. Dietary intake of beta-HCH was about twice the acceptable daily intake amongst populations with high income in urban areas of Andhra Pradesh.

- DDT residues were detected in about 82 per cent of the samples. About 37 per cent contained DDT residues above the tolerance limit of 0.05 milligram per kilogram.The maximum level of DDT residues was found to be 44 times above the tolerance limit-2.2mg/Kg.

- Maharashtra had 74%samples with DDT residues above the tolerance limit, Gujarat 70%, Andhra Pradesh 57%, Himachal Pradesh 56% and Punjab 51%.
- Industrial milk- Infant formula, for instance, also had pesticide residues. Out of 186 samples of 20 commercial brands of infant formula, 70% showed DDT residues and 94% revealed the presence of HCH-isomers. The dietary intake of β-HCH by an infant fed on infant formula was 90% of the ADI.

1.3 Conventional Insecticides

Conventionally, the insecticides are of three types viz., organo phosphates, carbamates, and organochlorines.

Most of these compounds are broad-spectrum insecticides. Organo-phosphorous compounds and carbamates are easily degradable in the environment and have low residence time. But they are highly toxic and most often pose threat to non-target organisms. These compounds produce delayed neurotoxic action in animals *(Johnson, 1969)*.

Organo chlorines are highly persistent in the environment and they biomagnify in mammals including man. If one takes a concentration factor of 10 at each tropic level, then a theoretical concentration of several hundred fold is possible *(Qurashi, 1971)*.

DDT is known to act preferentially on sensory nerves and appears to cause unstabilisation of the polarisation of the nerve axons so that nerve impulses tend to be reduplicated As a result, both insects and mammals show the effects of overstimulation causing continual tremors and muscular inco-ordination.

Less is known about the mode of action of γ HCH and cyclodiene series; the site of action of which appears to be the central nervous system. Mammals poisoned by these compounds tend to show hyper-excitability and later suffer severe clonic and tonic convulsions, even in non-fatal cases.

Considerably more is known on the mode of action of organo phosphorous and carbamates group, which act as anti-cholinesterase poisons, both in mammals and insects. Accordingly, the more characteristic signs and symptoms of intoxication are those due to excessive acetyl choline. In mammals this can be recognised by the excessive stimulation of the parasympathetic system. There is contraction of the pupils, profuse secreation of saliva and tears, diarrhoea, discomfort in the chest due to constriction of the brachioles and ratardation of the heart.

1.4 Search for Eco-friendly Insecticides

Insecticides and pesticides with lesser non-target effect and residue problems are what the modern agriculturists are looking for. The pathway for the development of such insecticides can be understood only from naturally occurring compounds having insecticidal effect.

In response to the strong selective pressures of herbivores, plants have evolved a variety of chemical and structural defences *(Harbone, 1986)*. A multitude of phyotochemicals evolved since chemical defences by photoautotrophs produce varied toxic responses in herbivores. The toxic phytochemicals that primarily constitute the secondary metabolites, synthesized perhaps as a result of aberrant bio synthetic pathways, affect the nerve axons and synapses, muscles, respiration and behaviour of insects *(Klocke, 1989)*. In fact, some of these phytochemicals have been used to develop commercial insecticides and could serve us models for new insect control agent *(Balandrin et al., 1985)*. However, studies on the chemical defences evolved by tropical species have been little understood *(Tyler, 1986)*.

The synthetic pyrethroids were reported as a group of pesticides with high insecticidal activity, low mammalian toxicity (*Hascoet and Cavelier, 1978*), and with moderate persistance on the crops under field conditions *(Elliot et al., 1978., Collingwood et al., 1979)*. At present four synthetic pyrethroids viz. Permethrin, Farvalerate, Azpermethrin, and Deltamethrin are available.

Pyrethroids as a group is one of the oldest of insecticides known *(Schmeltz, 1971)*. Later, by modifying the Rethronal moiety and Replacement of Alcohol moiety, synthetic pyrethroids were made and thereby their insecticidal activity could be enhanced. For example, when the trans-(E)- methyl group was replaced by a but-1-enyl side chain, compounds of greatest insecticidal activity were obtained *(Elleott et al., 1973)*. Similarly, modified pheromones and azadiractin- derived compounds are being widely tested for the effective control of insecticides. This shows that phytochemicals or their modified derivatives are promising safer insecticides of next millenium.

In India, Persian Lilac *(Melia azedarach)*, garlic*(Allium sativum)*,Onion(*Allium cepa)*, Pongam (*Pongamia pinneta)*, aloe (*Aloe barbedensis)*, thulasi (*Ocimum* species), tobacco (*Nicotina tobacum*) etc. have been used extensively since ancient times. But many of them are still used in the crude form. Little work has been done to isolate active principles and formulate safer insecticides. But countries such as US are doing extensive research in this field (*Muraleedharan, 1994*). The recent patenting of azedirachtin, a triterpinoid, isolated from neem by a US company is an evidence. In India, Osmania University Chemistry Department, and Bhabha Atomic Reserch Centre(BARC), Trombay have prepared two commercial formulations from neem and garlic under the label Allitin and Neemguard, respectively.

1.5 Literature Review

At present there are a number of literature available on screening of phytochemicals for insecticidal, antimicrobial, antifungal, and larvicidal activities. But there are very few literature available on isolation, identification, and formulation of active principles.

Sharma et al. (1998) have studied the larvicidal effect of *Gluricia spium* against mosquito larvae of *Anopheles stephansi*, *Aedes aegypti* and *Culex quinquefasciatus*. The crude ethanol extract shows genetic variability with respect to their tolerance. The authors have found out that it is likely that the toxicity, atleast in some cases, could be due to more than one toxic principle. *Ouda et al. (1998)* have studied the insecticidal and ovicidal effects of the four seed extract of *Atriplex canescens* against *Culex quinquefasciatus*. The exposure of early fourth instar mosquito larvae was found to be very effective. *Govindachari et al. (1998)* have isolated and identified the antifungal compounds from the seed oil of *Azidurachta indica* using preparative HPLC. *Osawa et al. (1994)* have isolated antibacterial diterpenes, trichrabdals A,B,C and H from the leaves of *Rabdosia tricocarpa*, and the relationship between their conformations, analysed by spectroscopic and computational methods. Their antibacterial activity was discussed. *Tripathi and Singh (1994)* have screened 37 plants against *Spilosoma obliqua* Walker for the antifeedant activity, where *Ailanthum excelsa* gave 90.81% feeding deterency. *Singh et al.(1994)* have tested the effectiveness of vegetable oils on the fecundity and development of *Callosobruchus chinensis*. Castor, mustard, soybean and taramira were found to be effective. *Saradamma et al.(1994)* have evaluated the toxicity of twenty plant extracts against three important stored pests *Spodoptera litura, Dysdercus cingulatus and Aphis craccivora. Theratic neraiifolia, Pandanus odoratissiumus, Clerodendron infortunatum, Azadiracta indica, and Eupatorium odaratum* have shown promising results. *Regnault-Roger and Hamraoui(1993)* have done extensive work to test the efficiency of thirteen plant species against a stored pest *Acanthoscelides oblectus*. Plants from the family *Labiatae* provide the best direct and indirect insecticidal effect and among them, *Origanum serpyllum* is the most effective. *Chitra et al. (1993)* have tried some field evaluation of three plant extracts and one commercial formulation each of garlic and neem in comparison with the two chemical formulations, *Endosulfan and Monocrotophos*. *Argenone mexicana* was found to be superior among plant products and has close efficacy with *Monocrotophos and Endosulfan. Xei and Isman (1992)* have reported the antifeedant and growth regulatory effect of Tall Oil and its derivatives against cutworm *Peridroma sancia*. Their result shows that an environmentally sound, low cost natural pest control agent may be developed based on tall oil. *Mishra et al. (1992)* have studied the efficacy of nine plant species against the wheat seed infesting pest *Sitophilus oryzae*.

Custard apple and neem powder were found to be effective in this study. *Nieber et al. (1992)* have tested the toxicity of twelve plant extracts against three storage beetles of *Coleoptera* family and have found that *Ricinus communis* and *Solanum nigrium* were most effective. *Shukla et al. (1992)* have examined, in laboratory, the effectiveness of nine edible oils for the protection of stored grains against the insects *Silophilus oryzae, Rhizopertha domnica* and *Tribolium castaneum*. They found ground nut oil to be effective. *George and Patel (1992)* have observed that minta leaf to be highly effective against *Callosobrachus analis* in protecting greem gram. *Schmidt et al.(1991)* and *Risha et al. (1990)* have established that the vapours of *Acorus calamus* oil can considerably bring down the progency of *Coleoptera; Tribolium castanium, T. confusum, Sitophilus oryzae, Callosobruchus chinesis,* and *Trogederma granarium* in stored products. *Makanjoula (1989)* has evaluated in laboratory as well as in field the neem extract for the control of stored pests such as *Collasobrucus maculatus, Sitophilus oryzae* and *Cylas pucticollis* and found that the extract gave good protection to cowpea. *Caasi-Lit and Marallo-Ryesus (1989)* have studied the effect of *Aristolochia* extracts on the Asiatic cornborer, *Ostrinia funnacalis*. The two *Aristolochia* species tested revealed to be growth inhibitory, repellent, and antifeedant. *Delobel and Malonga (1989)* have tested the insecticidal properties of six plant materials against *Caryleon serratus*. Of these, *Tephrosia vogelii* and *Chenopodium ambrosiodes* were found to be effective. *Okello-Ekochu and Wilkins (1994)* have isolated a few principles from maize leaf extracts active against African army worm. *Vokou et al. (1993)* have studied the effect of essential oils isolated from *Lonandula angustifolia, Mentha pulegium, Mentha spicata, Rosmarium officinalis* and *Salvia fruticosa* and found that they could suppress potato sprout growth.

Miles et al. (1989) have isolated and spectroscopically identified heritorin, a new pesticide, from the mangrove plant *Heritiera littoralis*. *Gunzinger et al. (1986)* have isolated two toxic saponins having molluscicidal activity from *Cassonia spicara*. *Mary et al. (1986)* have learned the distributions of Avenacins in oat roots and their anti-fungal activity towards 'take-all' fungus. *Cojocaru et al. (1986)* have isolated 5-(12-Hepta decenyl) - Risorcinol from the peel of mango fruit. This compound possess antifungal activity. *Anna-Karin et al. (1986)* have isolated pinene, 2-heptanol, 2-nonanol, 2-heptanone and 2-nonanone and small amounts of linalool from four species of Oprys *(Orchidaceae)* and found that those compounds could be used as insect mimic attractants. *Arnason et al. (1986)* have investigated the phototoxic effect of naturally occuring polyacetylenes and thiophenes of the *Asteraceae sp.* against mosquito larvae and proved that Methylation at 2- position increased the activity. *Klocke et al.(1986)* have isolated Ellagitagitannin geranin and its hydrolysis products from semi-arid land plants. These products are excellent insect growth inhibitors. *Jondico (1986)* has isolated N-Isobutyl-2E, 4E, 8E, 10 z- dodeca- 2,4,8,10- tetranamide from *Spilanthus mauritiana* and it is a potential mosquito larvicide. *Chander and Ahmed (1981)* have evaluated the efficacy of embelin, isolated from *Embelia ribes*, agianst the stored insect pests of wheat. It was found that embeline acted as a stomach poison to *Triholim castaneum* while it showed reduction in productivity of *Rhyzopertha demnica*. *Al-Shamma et al.(1981)* have isolated and identified harmine, a microbial agent, from *Peganum harmal*. *Ahamed et al. (1983)* have shown the insecticidal potential and biological activity of Indian indigenous plants against *Musca domestica*. *Mitscher et al. (1980)* have isolated a few antimicrobial isoflavanoids and related substances from *Glycyrphyza glabra*. *Torres et al.(1979)* have isolated coumarins from *Gymnophyton isatidicarpum*. The antimicrobial property of coumarines have already been established

1.6 Relevance of Present Work

The literature reviewed above shows that screening, isolation and identification of phytochemicals as pesticides are quite relevant and may open a small pathway to the search for sustainable and eco-friendly bio-pesticides.

In general, it can be stated without fear of contradiction, that scheduled application of insecticides has caused problems such as residues in soil, water, air, food, and fodder, adverse effect on non-target organisms, development of resistance in pests to insecticides, induction of resurgence of target and non-target pests. For these reasons, importance of phytochemicals and their use as candidates for inclusion in the arsenal of weapons for pest management has increased during recent years.

Many species of plants are known to possess a variety of chemical substances which act as anti feedants, repellents, juvenile hormone mimics, insectistats and insecticides.

Though India's ancient knowledge of phytochemicals as insecticide is laudable, there are not much work done in developing them into formulations suited to Integrated Pest Management of modern times. Only during the eighties we could make some breakthroughs. So the major objectives of this study are:

- Screening of indigenous plants for their efficacy as pesticide against a resistant pest *Tribolium castaneum*.

- Isolation of most effective Principles using chemical and physical methods such as hydrolysis, and chromatography,
- Identification of Active Principles through spectroscopic, chemical, and physical methods,
- Developement of possible commercial methods of isolation and formulation of the active principles, and
- Application of the commercial formulation to test their potential against vegetable pest, mosquito larvae, grain beetle, and micro organisms

This thesis entitled **'Investigations on Eco-friendly Natural Insecticides'** comprises seven chapters including this general introduction.

The second chapter deals with screening of ten indigenous plant species against *Triboilum castaneum* in order to study their potential as pesticide.

The third chapter encompasses the isolation techniques used for separating the Active Principles from the two most potential plant extracts

The fourth chapter attempts to identify the derivatives with the help of spectroscopic, chemical, and physical methods

The fifth chapter contains the degradation study of the principles with soil micro organisms.

The sixth chapter carries the commercial methods for the isolation and formulation of the active principles, and the last and the

seventh chapter summarises the entire work with concluding remarks. This chapter is followed by Bibliography

2

SCREENING OF PLANT EXTRACTS FOR EFFICACY EVALUATION

2

SCREENING OF PLANT EXTRACTS FOR EFFICACY EVALUATION

2.1 INTRODUCTION

Central to any scheme for evaluating natural or synthetic compounds as agents for insect control must be the development of appropriate assays. Such assays must be rapid, must be applicable to small quantities of both purified compounds and crude extracts, must be applicable to different insect species and they must provide information concerning toxicity of a compound or extract.

Here, the standard toxicity test for the evaluation of new candidate material as toxicants used by *Mc Donald et al. (1970)* has been followed. The insect pest used for the biossay is *Tribolium castaneum*, a cosmopolitian pest *(Jagdish et al., 1992)*.

2.2 Materials and Methods

2.2.1 The Plant Species

There are several approaches possible in the search for new products with commercial potential. One obvious way is collecting the information about traditional plant-based insecticides in the country side. For this study ten such plant varieties were selected and their botanical, agronomical and economic details as per *Gamble* are mentioned in Section 2.2.1.a to 2.2.1.j.

2.2.1.a Croton tiglium

Mal: *Nervalam*

Family: *Euphorbiaceae*

This as an evergreen tree with smooth, ash coloured bark, the young shoots of which are covered with stellate hairs. The leaves are yellowish-green and the flowers are small. The fruit is a capsule. The seeds and oil have irritant, rubefacient and cathastic properties.

Seeds are mucilaginous and purgative, useful in fever, and constipation. Oil from seeds is used as an anthelmintic. Seeds form a drastic purgative. The oil is used in incipient apoplexy, visceral obstruction and in dropsy. Seeds mixed with honey and water are applied to obstruct bubces. Oil is applied in rheumatism. Sublimated seed in minute quantity is given in stomach-ache, asthma, and other diseases. Warmed oil is applied in rheumatism. Leaves ground and taken in water cause purging. Crushed seed mixed with honey can be applied to boils. Ground bark causes vomition and perspiration. Dried seeds and leaves ground are applied to snake bite.

The Plant is naturalized and cultivated in Bengal, Assam and South India, also in gardens throughout the country.

2.2.1.b Vitex leucoxylon

Mal: *Attanochi*

Family: *Verbanaceae*

Seen in low level forests and on the banks of streams.

A deciduous tree with spreading head and white rather large flowers with hairs. Bark grey, smooth, wood light greyish brown, moderately hard. The leaves are widely used as a mosquito repellent.

2.2.1.c Euphorbium anticorum

Mal *Kallipala*

Family : *Euphorbeaceae*

Seen in low hills and rocky ground in most dry districts, commonly cultivated as a hedge plant.

An erect tree with terete or three to six angled fleshy winged branches containing a white sap.

2.2.1.d Blumea balsamifera

Mal: *Kozhichedi*

Family: *Compositae*

Blumea balsamifera, a shrub-like plant of the family compositae is a native of India. It occurs wild from the Himalaya Mountains to Singapore, in Burma, South China, Formosa, Malayan Archipelago and the Philippine Islands

The stalks and leaves of the plant contain from 0.1 to 0.4 % of an essential oil known as blumea camphor and the leaf extract is used in the treatment of excitement and insomnia

2.2.1.e Ervatamea dichotima

Mal: *Kuruttupala*

Family: *Apocynaceae*

A small tree with stout woody resinous branches, oblonge or abovate coriaceous leaves with about 20 main nerves at right angles to the midrib and meeting in loops impressed above, flowers large white with yellow follicles 4-5 inch long 1-2 inch broad, organic yellow.

2.2.1.f Lea sambucina

Mal: *Njezhu*

Family: *Leeaceae*

This small shrub grows commonly in the tropics. The leaves are pinnately compound The stem is usually sympodial. The main axis ends in tendril and is pushed aside while axillary branch grows more vigorously and takes the position of the main axis. The stem is cylindrical The Plant has been widely used for louse control.

2.2.1.g Crataeva religosa, Linn

Mal: *Neermathalam*

Family : *Capparidaceae*

A small or medium sized tree with broad terminal corymbs of white flowers which turn yellow soon after opening, filaments purple, berry 1-2 inch thick, globose or ovate.

Found in patches in almost all districts of Kerala. Often planted, along river banks.

Wood yellowish white, ever-grained but not durable.

The plant is used for the treatment of rheumatic ailment, kidney stone etc.

2.2.1.h Sphaeranthus indicus

Mal *Adakkamaniyan*

Family. *Compositae*

It is a common plant seen in all districts and in wet places, especially rice fields

An aromatic herb with glandular hairy stem and branches, and pink or purple flowers

The plant is used for the control of stored pests.

2.2.1.i *Ficus asperrima, Linn*

Mal · *Theragam*

Family. *Moraceae*

Seen in all hilly tracks up to 4500 ft and down to sea-level on the west coast. A small medium sized tree without aerial roots. Bark pale, smooth, wood whitish, soft but useless. Leaves used as sand paper in wood carving.

The plant extract is often used as an anti infectant

2.2.1.j *Premna latifolia, Roxly*

Mal *Kozhiappa*

Family *Verbanaecae*

Seen in North Circass and Carnatic to Tirunelvelli near coast in dry forest areas.

A small-tree reaching 25 ft. in height. The leaves usually turn blue or black, after drying and the flowers greenish-white

The plant is widely used for louse control in chickens.

2.2.2 Preparation of Plant Material

Different parts of plants were taken for the extraction. The plants and parts taken are listed in Table 2.1

The plant materials were dried in an air oven kept at 40°C and powdered using a motor-driven pulverizer. The dried materials were then kept in a refrigerator until extraction.

2.2.3 Extraction

The powdered materials were extracted with petroleum ether (40°C - 60°C) by using Soxhlet apparatus.

Non polar compounds are more penetrating to the cuticle of insects than polar compounds. So, petroleum ether, a non-polar solvent, was selected for the extraction as it would preferably dissolve non-polar constituents.

The extract was then concentrated by distillation under reduced pressure technique. The solvent was finally evaporated in an evaporating dish at 40°C and the residue was used to prepare solutions of different concentration for bioassay study.

2.2.4 The Insect Species

Tribolium castanium, the insect species chosen for the present study, is a cosmopolitan pest. It belongs to the family *Coleoptera* and is a very common test animal for efficacy study of insecticides.

The insect is brown in colour with 3 – 4.5mm in length. Antenna with a distinct, moderately compact three segmented club. Eyes separated ventrally by much less than two diameters of an eye. No ridge was present above the eye.

Tribolium is a common and serious pest of cereal products, especially flour. They do not appear to be able to attack sound grain, but often add to the damage of primary grain pests. *T. castanium* is the most commonly intercepted insect on many kinds of imported food, being especially numerous on oil seed, oil cake and rice bran *(Baker, 1988)*.

The eggs are laid singly, 2-10 per day, depending on the temperature, over a period of many months and reaching a total of about 450. Oviposition ceases below 15°C. The eggs are sticky when laid and become coated with food particles or other debris. The larvae grow from a length of about 1mm to 5mm, moulting 5-11 times (usually about 7 or 8). The pupae lie in the food, gradually darkening before emergence as adult. The adult feeds on the same substance as the larvae. Under very warm conditions, the adults readily fly. Males have been recorded to live for about 600 days, and females about 450. At 15°C, the larvae starve to death around 50 days, and the adults in half the time.

2.2.5 Rearing of Insect

The wheat flour was disinfected by chilling at 0°C for 48 hrs. Adults, 200-300 in number, were then added to a small quantity of the disinfested wheat flour in a glass jar of 400ml and left to lay eggs overnight before they were removed. The eggs were then allowed to hatch in an incubator at 28 ± 2 °C temperature and R.H.70± 5% *(Shukla et al., 1992)*. The two-week old adults were used for the study of bioeffectiveness.

2.2.6 Treatment

The insects were immobilized in a refrigerator at 0°C for about 2-3 minutes. An amount of 0.5 µl of suitably diluted extract was individually dropped on the dorsal surface of the thorax with a microapplicator. The insects were released to the flour after 30 minutes. There were three replicates each with 20 beetles (whose sex were not determined). The control was treated with the solvent alone.

2.2.7 Recording of data

The treated insects were examined for mortality 24 hrs after treatment. Moribund insects were recorded as dead. Percentage mortality for each replication was calculated individually

2.2.8 Statistical Analysis and Interpretation of the data

The average percent mortality of three replication was corrected by *Abbott's formula (1925)*. This took into account natural mortality as might be obtained in the case of control. The regression equation, LD_{50} values, and $\chi^2_{(3)}$ for each extracts were calculated by Probit analysis *(Finney, 1971)*. Lower the LD_{50}, higher was the toxicity of the extract

2.3 Results and Discussion

The results are presented in Table 2.1. *Croton tiglium, Vitex leucoxylon, Euphorbium anticorum, Blumea balsamifera, Ervatamea dichotima*, and *Leea sambucina* have considerable activity while *Crataeva religosa, Sphaeranthus indicus, Ficus asperrima*, and *Premna latifolia* do not have activity at ≤ 1% w/v concentration. The more active six were analysed for probit. Regression equation, LD_{50} and $\chi^2_{(3)}$ were found out Rest of the results were not analysed satistically because this study was intended to characterise and formulate only two potential principles.

Table 2.1
Insecticidal Potential of the Petroleum Ether Extracts of Plant Materials

Plant	Parts taken	Conc (%w/v)	% Mortality	Regression equation	LD_{50}	$\chi^2_{(3)}$
Croton tiglium	Seed	2.5 2.0 1.5 1.0 0.5	75 65 50 30 10	y = 2.78x - 5.76	0.75	0.235
Vitex leucoxylon	Aerial	2.5 2.0 1.9 1.0 0.5	55 45 35 30 15	y = 1.49x - 1.48	2.24	0.185
Euphorbium anticorum	Aerial	2.5 2.0 1.5 1.0 0.5	35 30 20 10 5	y = 2.04x - 4.36	3.89	0.180
Blumea balsamifera	Whole plant	2.5 2.0 1.5 1.0 0.5	40 20 10 5 0	y = 3.57x - 10.99	3.02	0.385
Ervatamea dichotima	Aerial	2.5 2.0 1.5 1.0 0.5	80 75 60 45 20	y = 4x - 11.12	1.07	0.086
Lea sambucina	Aerial	0.18 0.16 0.14 0.12 0.10	75 65 55 45 40	y = 0.23x - 4.29	0.126	0.159
Crataeva religose	Aerial	No Mortality at ≤ 1% w/v				
Sphaeranthus indicus	Whole plant	- do-				
Ficus asperrima	Aerial	- do-				
Premna latifolia	Aerial	-do-				

From the results it is obvious that *Croton tiglium* and *Leea sambucina* are the most potential plants with LD_{50} values of 0.75 and 0.126, respectively and $\chi^2_{(3)}$ value very small in all the cases (<3). This $\chi^2_{(3)}$ value is clearly sufficient to be attributed to random fluctuations about the relation specified in regression equation. *(Finney, 1971)*.

Further study was conducted on *Croton tiglium* and *Leea sambucina* to isolate and identify the active principles

3

ISOLATION OF ACTIVE PRINCIPLES FROM *CROTON TIGLIUM* AND *LEEA SAMBUCINA*

3

ISOLATION OF ACTIVE PRINCIPLES FROM CROTON TIGLIUM AND LEEA SAMBUCINA

3.1. Introduction

In the examination of natural products and compounds derived from them, perhaps the most important experimental stage is the isolation and purification of the material in its unaltered natural state The frequently occurring difficult problems that must be overcome, are fractionation, degradation, chemical modification etc

Techniques dependent on differential migration like chromatography are now much more widely used than crystallisation, distillation, sublimation and fractional precipitation.

The striking success of chromatographic methods in practically all fields of chemistry is so well known

In the isolation of the active principles from *Leea samucina,* Adsorption Column Chromatography and Thin Layer Chromatography have been made use of while for isolating the active principle from *Croton tiglium* ; alkali hydrolysis, solvent extraction and column chromatography have been combined.

3.2 Materials and methods

3.2.1 Isolation of Active Principle from *Leea sambucina*

The petroleum ether extract from Soxhlet was subjected to TLC technique in order to identify the number of fractions and to fix the suitable eluent for column chromatography

3.2.1.a Thin Layer Chromatography

TLC has, within the last few years, leaped into prominence as one of the simplest, most useful and most widely applicable forms of chromatography yet devised. Because of sharpness of separation, high sensitivity, speed of separation and ease of recovery of the separated compounds, TLC has found wide acceptance *(Dean, 1969)*.

TLC represents one of the several means for resolving mixtures of trace organic substances. TLC is generally applicable except for volatile or reactive substances. The only requirements are suitable mobile phases and sorbents with which competing equilibrium systems can be established for solutes

The low cost of TLC has always been emphasized. Sample preparation and cleanup are special problems relating to the compound of interest. In order to separate a mixture of compounds, the mobile phase should be selected according to the principles of chromatography. The mobility of the compound of interest should be related to solvent strength and adjusted between an R_f of 0.3 to 0.7. Sample application must be accurate, uniform and reproducing (*Touchstone and Rogers, 1980*)

Silica gel (TLC grade, Qualigens) was made to a slurry in dichloromethane. Glass slides of 5 x 1.5cms size were dipped in it so as to get a uniform coating. The slides were allowed to dry in air. A solution of 0.1% (w/v) in petroleum ether was prepared and spotted on each slide with a capillary tube.

The slides were then kept in closed jars containing in CCl_4, Benzene, Chloroform, Dichloromethane, Ether and Ethyl acetate, respectively. The solvents were allowed to dry naturally.

For visualization of various fractions an iodine chamber was used. The spots were visible as follows.

CCl_4 - 4, Benzene - 4, Ether -2
Chloroform-4, Dichlromethane - 3
Ethyl acetate - 3, Methanol- 2

The experiment was repeated with mixed solvents and it was found that Benzene, Ethyl acetate and Methanol in the ratio of 3:2:1 was very effective. TLC was repeated with normal plates, spreader, chamber etc. Five spots were obtained with R_f values 0.12, 0.19, 0.78, 0.84, 0.98 respectively.

3.2.1.b Column Chromatography

Adsorption chromatography, the oldest of the various chromatographic techniques, is based on the retention of solute by surface adsorption. Although overshadowed to some extent in recent years by other chromatographic techniques, it remains one of the simplest and most effective ways of separating mixtures of rather non-polar substances and constituents of low volatility *(Dean, 1969)*.

It is a very common method of isolating natural products. *Javett (1967)* has reported that when petroleum ether extract of dried leaves was placed on top of a MgO column, several coloured zones were formed.

Petroleum extract of the aerial part of *Leea sambucina* was concentrated in a vacuum still and then evaporated to a dry mass. About 2 gm of this residue was dissolved in benzene-ethyl acetate-methanol (3:2:1) mixture. A chromatographic column of 600mm x 3.5mm size was filled with silica gel (60-120 mesh, Qualigens) in 3:2:1 ratio of Ben-eth.acetate- MeOH mixture. The above prepared solution of the plant material was then poured on top of the column and was eluted with the solvent mixture. Fractions were collected in 25ml intervals till all the material had percolated. TLC of each fraction was done on glass slides with silica gel and some solvent mixture. Fractions that gave the same R_f values were combined together. Five fractions were obtained. All the fractions were subjected to bioassay study on *Tribolium castaneum* by preparing 0.05% solution in petroleum ether. (Similarly as done in screening)

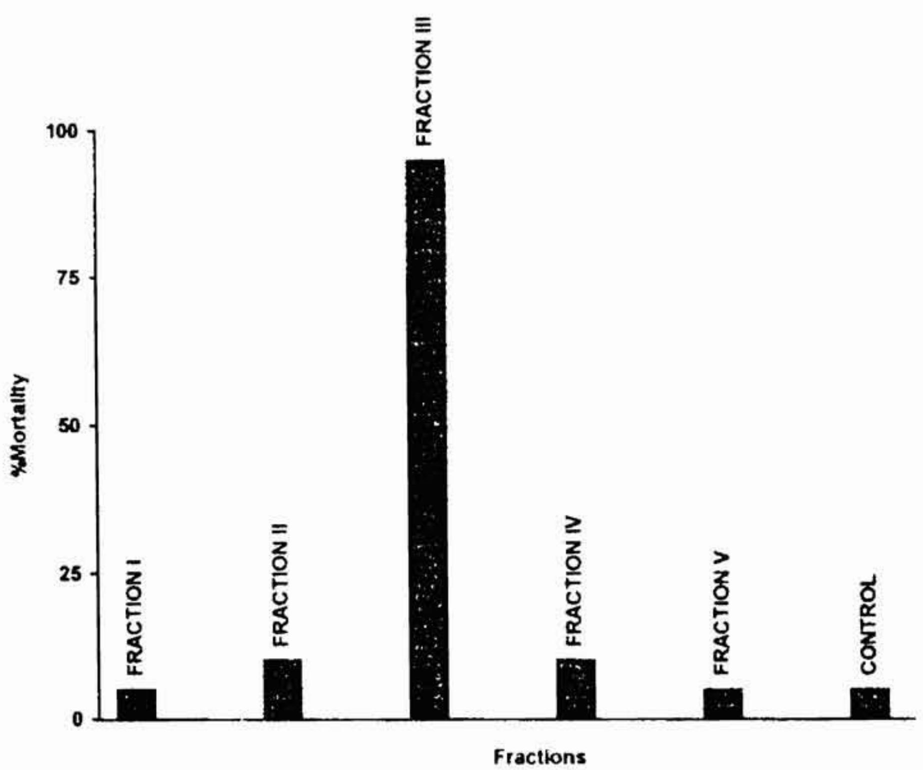


Fig. 3.1
Percentage Mortality of Different Fractions (0.05% w/v in Petroleum Ether) of Leea sambucina against Tribolium castanium

There were three replicates each with 20 beetles and a control. The result of the bioassay study is diagrammatically represented in Fig.3.1. Average % mortality has been used in the diagram.

Fraction III was found to be the active principle with 95% mortality. This fraction was further purified by repeated column chromatography and purity was confirmed with TLC (Silica gel, Benzene, R_f 0.07, single spot). Although GC-MS analysis (with Fisons 8000 series, Silica gel column, Helium carrier gas) was done, no sharp peak could be obtained. This may be due to the non- ionizable heavy molecular structure of the compound.

3.2.2 Isolation of Active Principles from *Croton tiglium*

The croton oil contains a toxic resin, besides vesciculant and purgative principles, dissolved in fixed fatty oil. The fixed oil is glyceryl esters of higher fatty acids and is hydrolyzable, whereas the solutes are non-hydrolyzable. This property was made use of in separating the major basic principle from the oil.

About 10g of the oil was mixed with 5g of Sodium hydroxide dissolved in 20ml distilled water and 20ml of 95% ethanol. The mixture was then heated on a water bath for 45 min. Forty ml of 1:1 ethanol - water mixture was prepared and added in small portions to the reaction over a 45 min. period with stirring.

The hot mixture was poured to about 150ml of 35% (w/v) NaCl solution in cold, stirred thoroughly for several minutes, and cooled in an ice bath. The precipitated soap was removed by vacuum filtration and the filtrate was collected, neutralized with hydrochloric acid, and was extracted with petroleum ether for four times. The extracted portion was dried with anhydrous sodium sulphate and filtered. The filtrate was concentrated in a vacuum still at 40-50°C.

The concentrate was subjected to TLC on glass slides with benzene, dichloromethane, acetone, methanol etc. as mobile solvents. Methanol was found to be the most effective eluent. It spots with R_f values; 0.53,0.67, and 0.96, respectively.

The isolation of the active fraction was done using column (600 mm x 35 mm) chromatography with silica gel and methanol as stationary and moving phases, respectively. Fractions were collected 25 ml each. Fractions with the same R_f values were combined together to get three fractions.

All the three fractions were diluted to 0.1% w/v in petroleum ether and were applied on *Triboleum castaneum* (as in the case of screening) for bioassay analysis. The result is interpreted in Fig. 3.2. There were three replicates with 20 beetles each and a control. Average percentage mortality has been used in the graph.

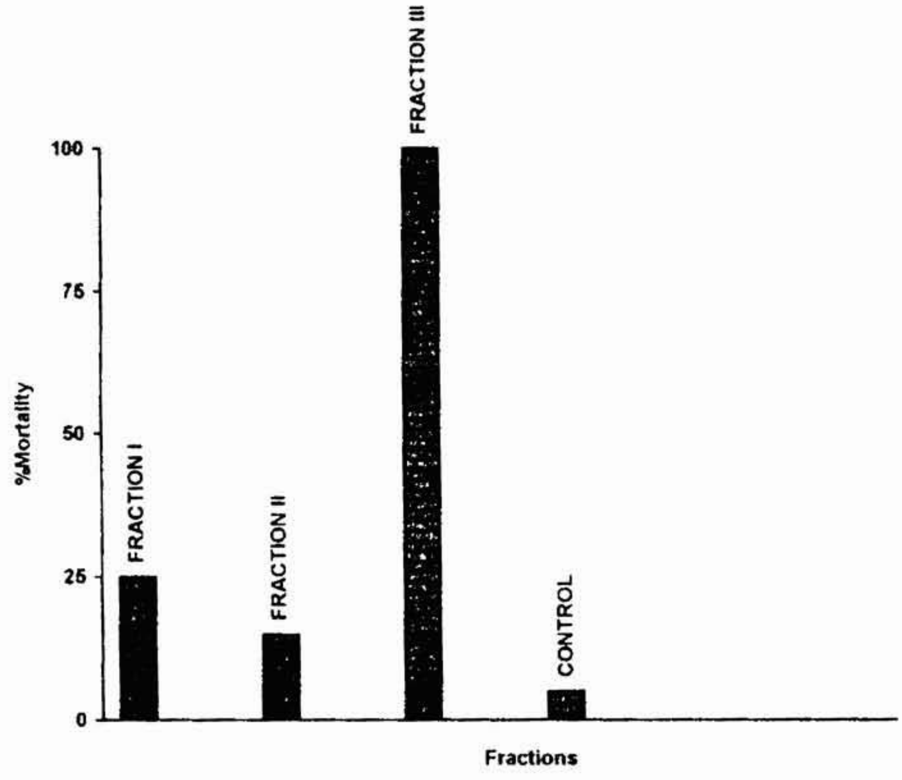


Fig. 3.2
Percentage Mortality of Different Fractions (0.1% w/v in Petroleum Ether) of Croton tiglium against Tribolium castaneum

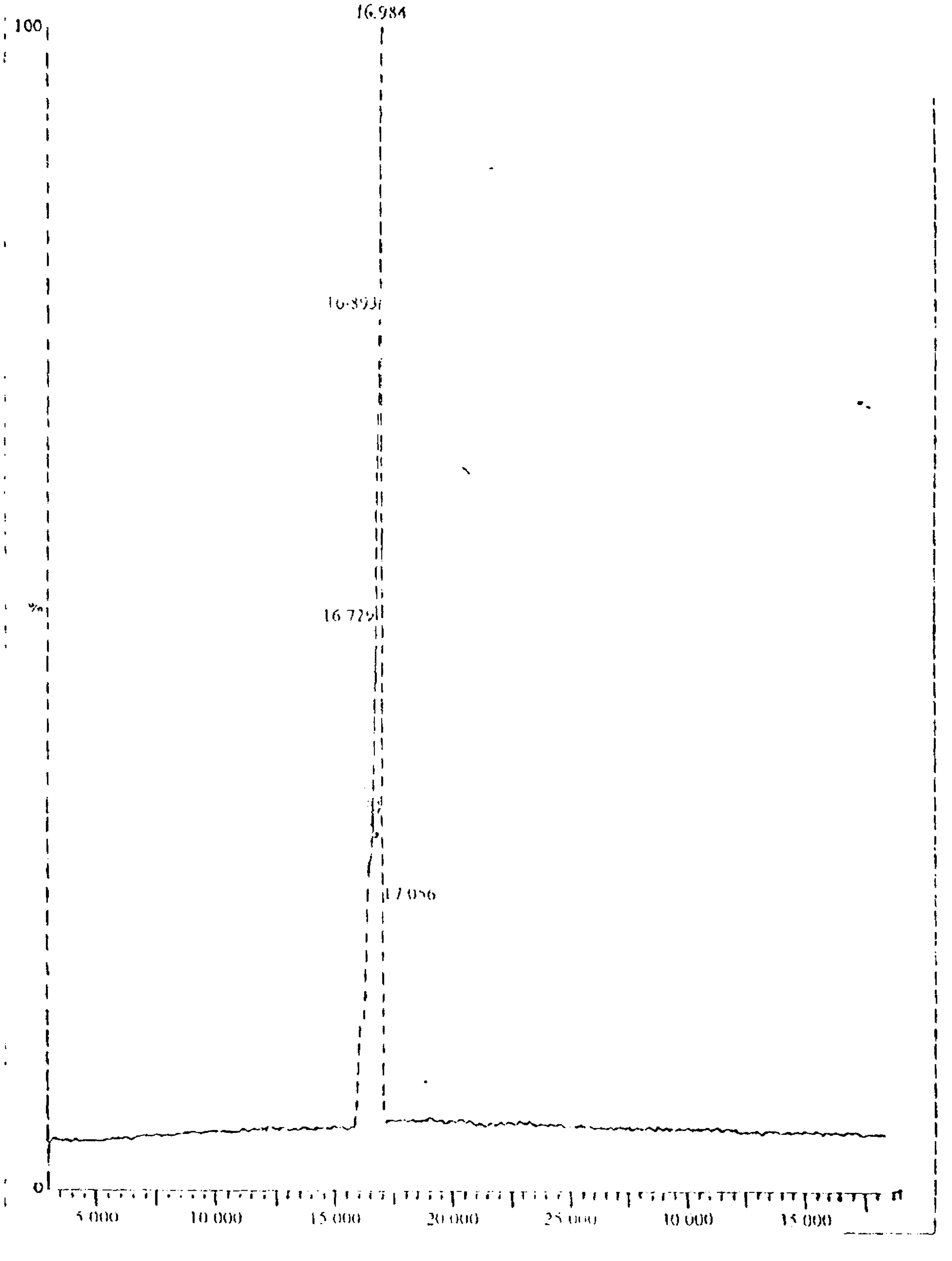


Fig. 3.3 Chromatogram of the Croton derivative

Fraction III with 100% mortality was further purified by repeated column chromatography and was analysed in GCMS. (Fisons 8000 series, Silica gel scan EI+, TIC, 8.97 e5) The chromatogram is shown in Fig 3.3. There is only one sharp peak at R_t=16.98 minutes

3. 3 Results and Discussion

For the isolation of the active principle from *Leea*, a solvent mixture of benzene, ethyl acetate, and methanol in the ratio 3:2:1 was ideal. The active principle could be isolated in pure form with repeated column chromatography using the same solvent mixture. Fraction III with R_f vaule 0.78 in TLC was the active principle and it gave 95% mortality when applied on *Tribolium castaneum*, while rest of fractions gave only less than 10% mortality.

The isolation of active principle from *Croton* could be achieved by saponification followed by petroleum ether extraction. The TLC experiment showed that methanol was the ideal solvent. Repeated column chromatography with methanol as solvent helped the purification of the active principle. Third fraction in the column gave 100% mortality when applied on *Tribolium castaneum* while fraction I and fraction II gave 24.9% and 13.2% mortality respectively.

GCMS analysis confirms the purity of the isolated principle since there is only one peak

4

IDENTIFICATION OF ACTIVE PRINCIPLES FROM *CROTON* AND *LEEA*

4

IDENTIFICATION OF ACTIVE PRINCIPLES FROM CROTON AND LEEA

4.1. Introduction

Until recently, structural determinations were based almost completely on purely chemical evidence Nowadays, physical methods are considered as necessary tools for elucidating structures (*Finar, 1994*).

Ultraviolet, infrared, and nuclear magnetic resonance spectroscopy are among the most important techniques the organic chemist now uses routinely to gain information about a particular substance (*Dyer, 1991*). Almost all parts of the electromagnetic spectrum, particularly energy absorption from three or four regions (ultraviolet-visible, infra red, micro wave, and radio frequency are made use of) are used for studying matter in Organic Chemistry (*Kemp, 1987*).

Here, purely physical methods have been used in the identification of the active principles from *Leea* and *Croton*.

4.2 Materials and Methods

4.2.1 UV-Vis Spectroscopy

The absorption of UV/Vis radiation by a molecule leads to transitions among the electronic energy levels of the molecule All organic compounds absorb UV light, albeit in some instances of very short wave length But for practical reasons, the absorptions above 200nm are considered Though other spectroscopic techniques like IR spectroscopy and NMR spectroscopy superceded, the strength of UV-Vis spectroscopy lies in its ability to measure the extend of multiple bonds, the non-bonding electrons on oxygen, nitrogen, and sulphur

The technique can, in general, differentiate conjugated dienes from non-conjugated dienes, conjugated dienes from conjugated trienes, α β-unsaturated ketones from their β γ-analogues etc Since the degree of conjugation may suffer in strained molecules, electronic spectroscopy may be used to study such strain by correlating the change in spectrum with angular distortion The position of absorption may also be influenced in a systematic way by sustituents, and a particularly successful application has been the correlation of substituent shifts in conjugated dienes and carbonyl compounds.

The electronic transitions involved in the UV-Vis spectroscopy are $\sigma \rightarrow \sigma^*$, $\pi \rightarrow \pi^*$, $n \rightarrow \sigma^*$ and $n \rightarrow \pi^*$. Of these $n \rightarrow \sigma^*$ and $\pi \rightarrow \pi^*$ are allowed transitions (need high energy) while the other two are forbidden transitions (need low energy) in terms of symmetry.

4.2.2 IR Spectroscopy

When IR light is passed through a sample of an organic compound, some of the frequencies are absorbed while other frequencies are transmitted through the sample. If the percent absorbance is plotted against frequency, the result is an infra red spectrum.

At ordinary temperatures, organic molecules are in a constant state of vibration, each band having its characteristic stretching and bending frequency, and being capable of absorbing light of that frequency (within the IR range). The vibrations of two atoms joined together by a spring: using this analogy, several features of IR spectra can be rationalised, i.e., to stretch a spring requires more energy than to bend it; thus the stretching energy of a bond is greater than the bending energy, and stretching absorptions of a bond appear at higher frequencies in the IR spectrum than the bending absorptions of the same bond.

The vibrational frequency of a bond is expected to increase when the bond strength increases and also when the reduced mass of the system decreases. Thus, C=C and C=O str will have higher frequencies than C—C and C—O str, C—H and O—H str absorptions at higher frequencies than C—C and C—O str.

There are many vibrational modes in organic molecules viz., scissoring, rocking, twisting, wagging etc. These vibrations are often referred as fundamental vibrations. Other frequencies can be generated by modulation of the fundamentals. They appear as overtone bands at integer multiples of fundamental vibrations.

IR spectrum is a valuable 'finger print' data of organic compounds.

4.2.3 NMR Spectroscopy

Nuclear Magnetic Resonance is concerned with the magnetic properties of certain atomic nuclei, notably the nucleus of hydrogen atom- the proton- and that of C^{13} isotope.

Studying a molecule by NMR spectroscopy enables one to record differences in the magnetic properties of the various magnetic nuclei present and to deduce in large measure what the positions of these nuclei are within the molecule. One can deduce how many different kinds of environment are there in the molecule, and also which atoms are present in neighbouring groups. Usually one can also measure how many atoms are present in each of these environments.

Proton NMR (HNMR)

The nucleus of the hydrogen atom behaves as a tiny spinning bar magent, and does so because it possesses both electric charge and mechanical spin. Any spinning charged body will generate a magnetic field, and the nucleus of hydrogen is no exception.

When an external magnetic field is applied, this tiny magnet will align itself with the field. Because of quantum restrictions, the alignment will be either with the field (lower energy state) or opposed to the field (higher energy state). Besides this alignment, the precessional frequency of the proton also varies proportionally.

If a proton is precessing in the aligned orientation, it can absorb energy and pass into the opposed orientation. Subsequently, it can lose this extra energy and relax back into the aligned position. If the precessing nuclei are irradiated with a beam of radio frequency energy of the correct frequency, the low energy nuclei may absorb this energy and move to a higher energy state The precessing proton will only absorb energy from the radiofrequency source if the precessing frequency is the same as the frequency of the radio frequency beam When this occurs the nucleus and the radio frequency beam are said to be in resonance, hence the term Nuclear Magnetic Resonance.

The simplest NMR experiment consists in exposing the protons in an organic molecule to a powerful external magnetic field; the protons will precess, although they may not all precess at the same frequency. The absorption of the radio frequency is recorded to get an NMR spectrum.

The precessional frequency of all protons in the same external applied field is not, however, the same and the precise value for any one proton depends on a number of factors. Depending on the chemical environment, each proton shows marked shift in the frequency. This shift is termed as chemical shift (δ).

To measure the precessional frequency of a group of nuclei in absolute frequency units is extremely difficult and rarely required More commonly, the differences in frequency are measured with respect to some reference group of nuclei. For proton NMR, the universally accepted reference is tetra methyl silane (TMS) because it gives an intense sharp signal even at low concentrations. The common solvent used is $CDCl_3$. The δ value is measured in parts per million (ppm).

4.2.4 Mass Spectroscopy

Organic chemists use mass spectroscopy in two principle ways: (a) to measure relative molecular masses (molecular weights) with very high accuracy; from these one can deduce exact molecular formulae, and (b) to detect within a molecule the places at which it prefers to fragment, from this can be deduced the presence of recognisable groupings within the molecule.

In the mass spectrometer organic molecules are bombarded with electrons and converted to highly energetic positively charged ions (molecular ions or parent ions); the loss of an electron from a molecule leads to a radical cation, represented as $M \rightarrow M^{\dot{+}}$ The molecular ions, the fragment ions, and the fragment radical ions are separated by deflection in a variable magnetic field according to their mass and charge, and generate a current (the ion current) at the collector in proportion to their relative abundance. A mass spectrum is a plot of relative abundance against the ratio mass/ charge (m/z value).

Most organic molecules form molecular ions ($M^{\dot{+}}$) when the energy of electron beam reaches 10-15 eV ($\approx 10^3$ kJ mol^{-1}). While this minimum ionization potential is of great theoretical importance, fragmentation of the molecular ion only reaches substantial proportions at higher bombardment energies, and 70eV ($\approx 6\times10^3$ kJ mol^{-1}) is used for most organic work. When the molecular ions have been generated in the ionization chamber, they are expelled electrostatically by means of a low positive potential on repeller plate in the chamber. Once out, they are accelerated down the ion tube by the much higher potential between the accelerating plates. The ion beam is focused by a series of slits. The

68214

ions are separated in a magnetic analyzer based on the m/z values *(Bentley and Kirky, 1972)*

4.3 Identification of Active Principles by Spectroscopy.

The active principles isolated were subjected to UV-Vis., IR, NMR and GC-MS spectral analyses.

UV spectra were recorded using a Shimadzu UV 160 Spectrophotometer with a 0.1 mg/l solution in methanol

The infrared spectrum was measured using a Shimadzu IR Spectrophotometer. The sample was prepared by palletizing in KBr crystals

The NMR spectrum was read in a Perkin- Elmer HNMR Spectrophotometer using TMS as standard

GC-MS analysis was done using Fisons 8000 series GC-MS Silica gel as column and He as carrier gas

4.3.1 *Croton tiglium*

The UV-Vis, IR, NMR and GC-MS spectra are given in Fig 4.1, 4.2, 4.3 (a&b), and 4.4

The data have been summerised as follows

UV λ^{MeOH}_{max} nm(log ε) 215(4.05)

G 8214

IR ν^{KBr}_{max} cm^{-1} 3441, 3017, 2933, 2862, 1731, 1708, 1647, 1566, 1476, 1424, 1282, 941

HNMR : 0.8-2.4(multiplet), 4.15(quartet), 5.3(sextet)

m/z : 308(m), 279, 264, 262, 223, 220, 178, 164, 150, 149, 135, 123, 121, 109, 96, 95, 81, 79, 69, 67, 57, 55, 54, 41

4.3.1.1 Estimation of Nitrogen

Nitrogen was estimated by Kjeldahl's method. Approximately 1 g of the sample was mixed with 25ml of 25% Sulphuric acid and about 5 g of digestion mixture ($CuSO_4$ / K_2SO_4). It was diluted to 250 ml , boiled to reduce the volume to 1/4th, cooled and 20 ml of 40% NaOH added. The Kjeldahl's flask was connected to a water condenser and boiled. The vapours were absorbed in 50ml 1N Sulphuric acid. Nitrogen was estimated by back titrating the acid using Methyl red as indicator.

4.3.2 *Leea sambucina*

UV: λ^{MeOH}_{max} nm(log ε) 220 (4.61)

IR ν^{KBr}_{max} cm^{-1} 3452, 2929, 2860, 1731, 1708, 1679, 1641, 1511, 1473, 1385, 1269, 1049, 938

HNMR: 0.7 – 2.4 (multiplet)

m/z : No characteristic fragments obtained but a spreaded peak.

The UV, IR, NMR Spectra have been given in fig 4.5, 4.6 and 4.7.

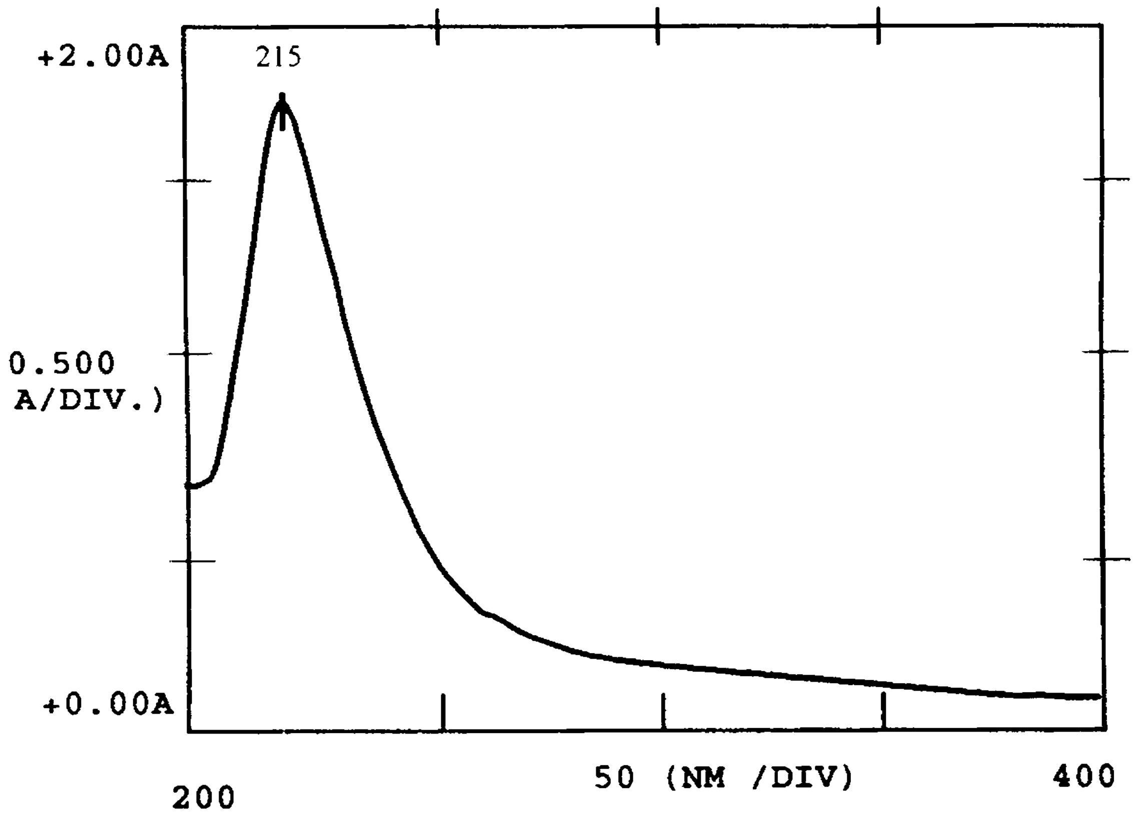


Fig. 4.1 UV Spectrum of the Croton Derivative

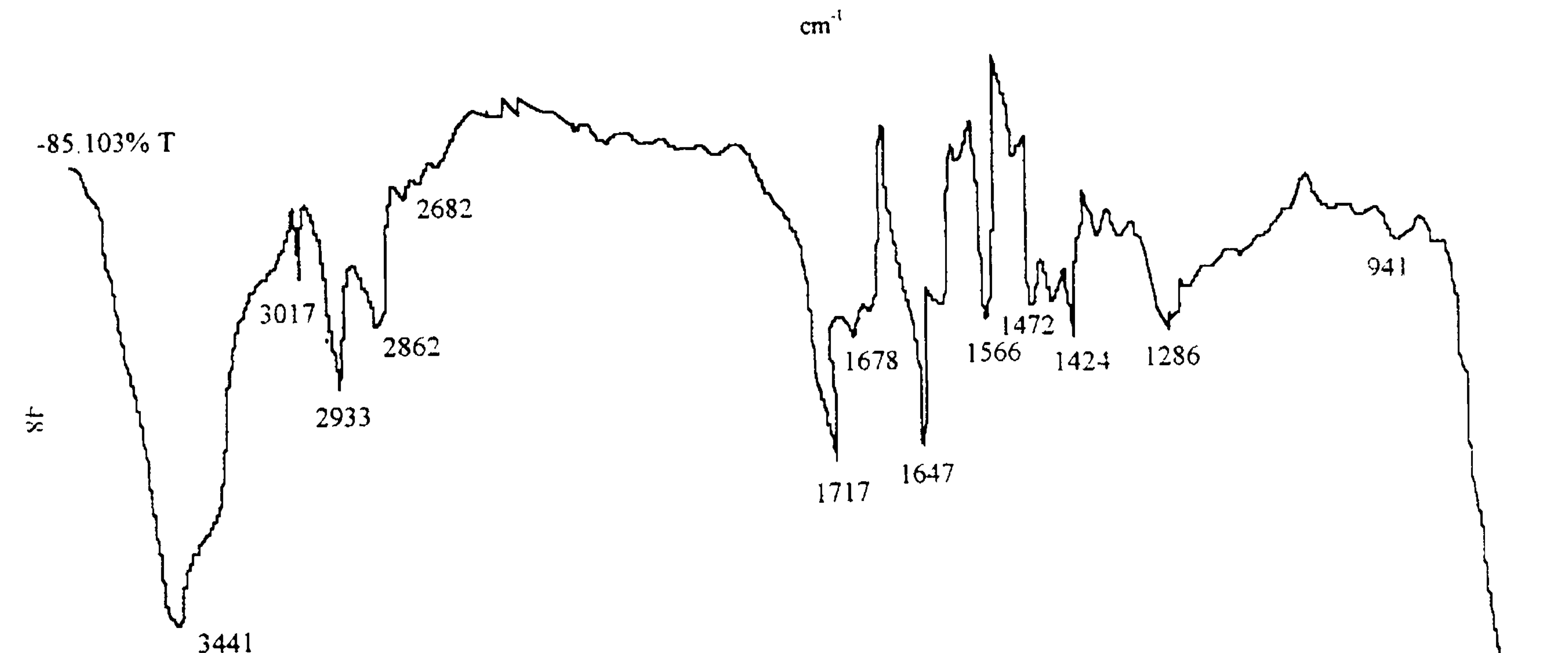


Fig. 4.2 ***IR spectrum of the Croton derivative***

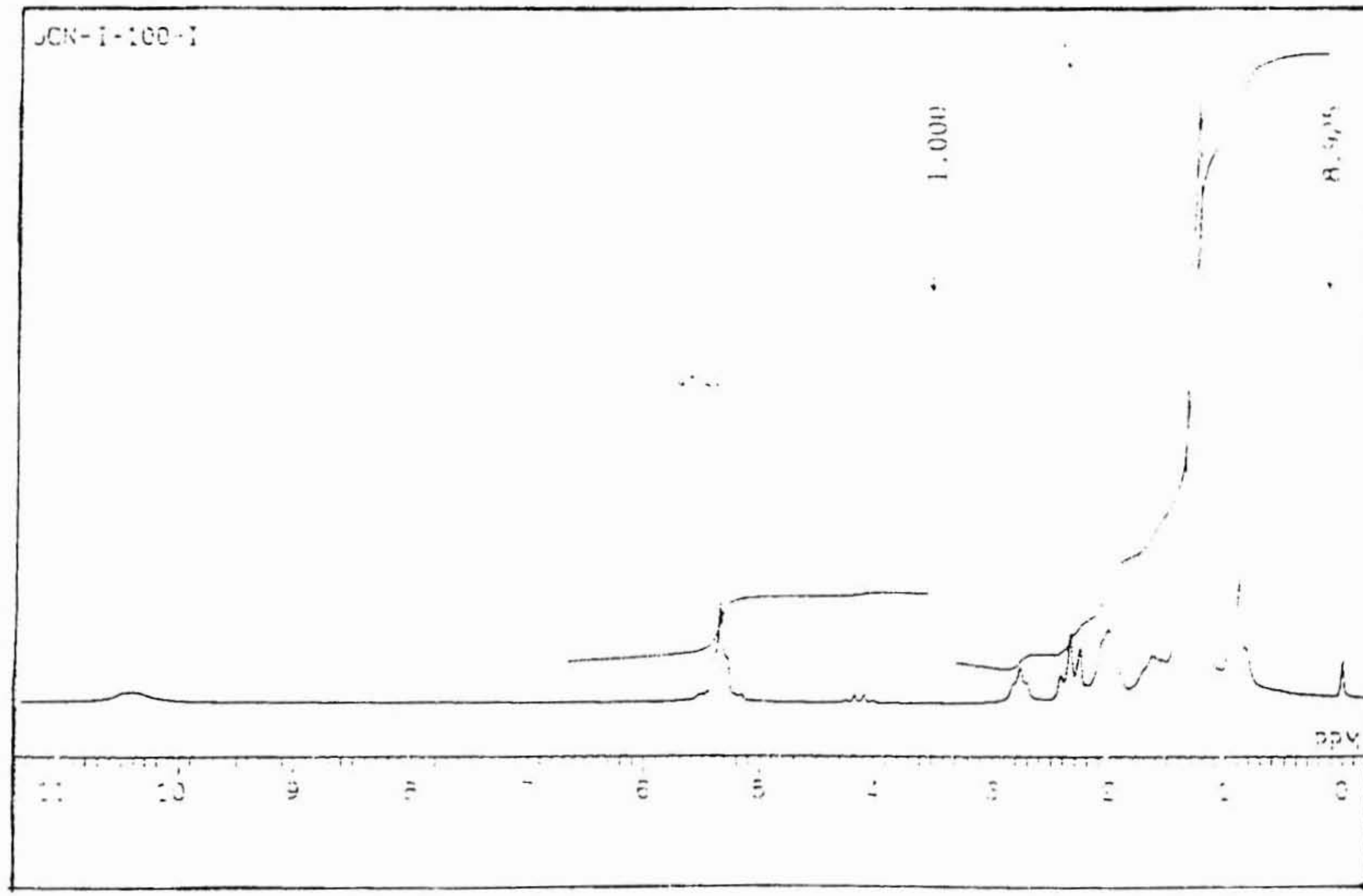


Fig. 4.3a NMR spectrum of the Croton derivative

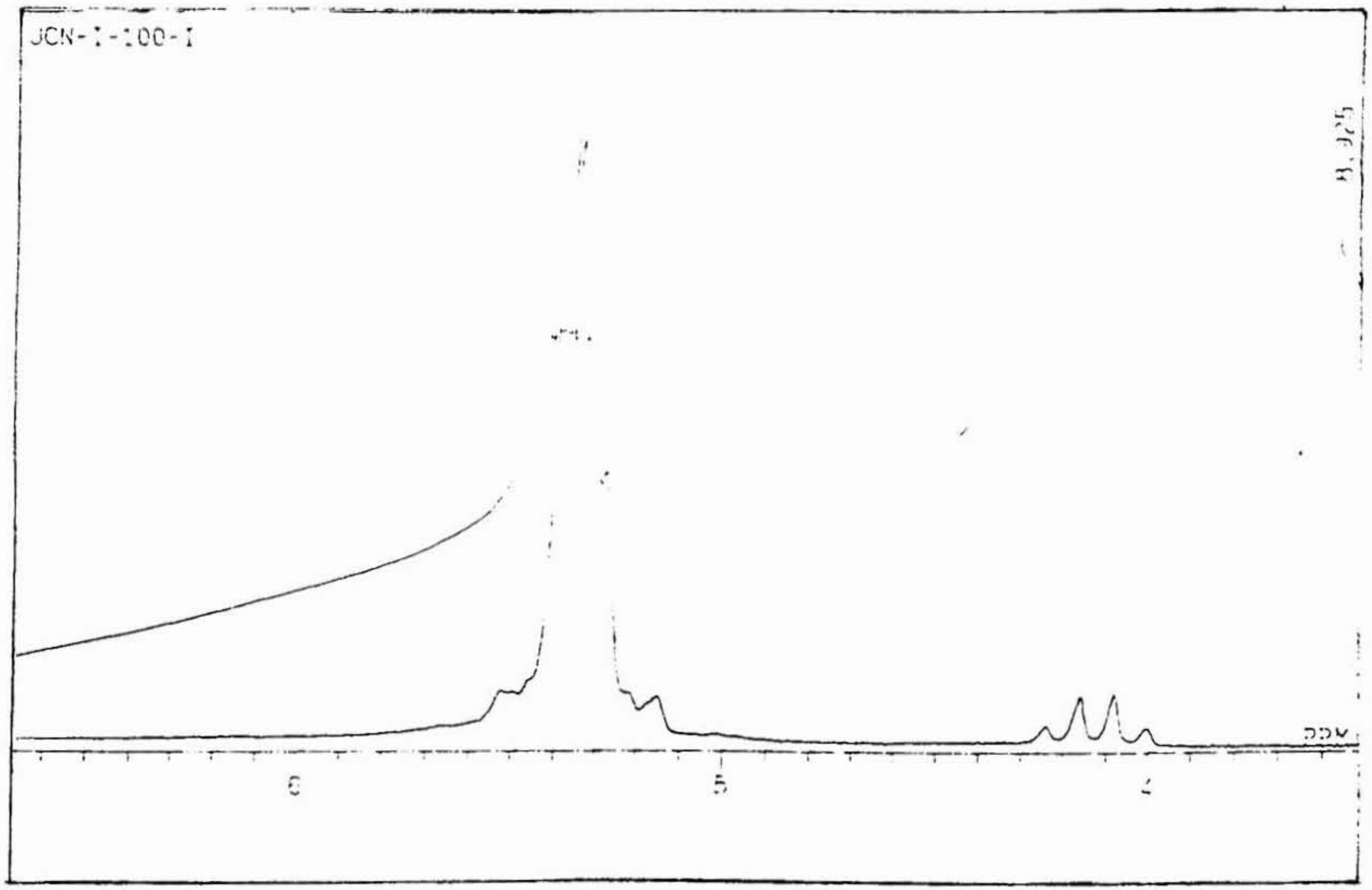


Fig. 4.3b NMR spectrum of the Croton derivative

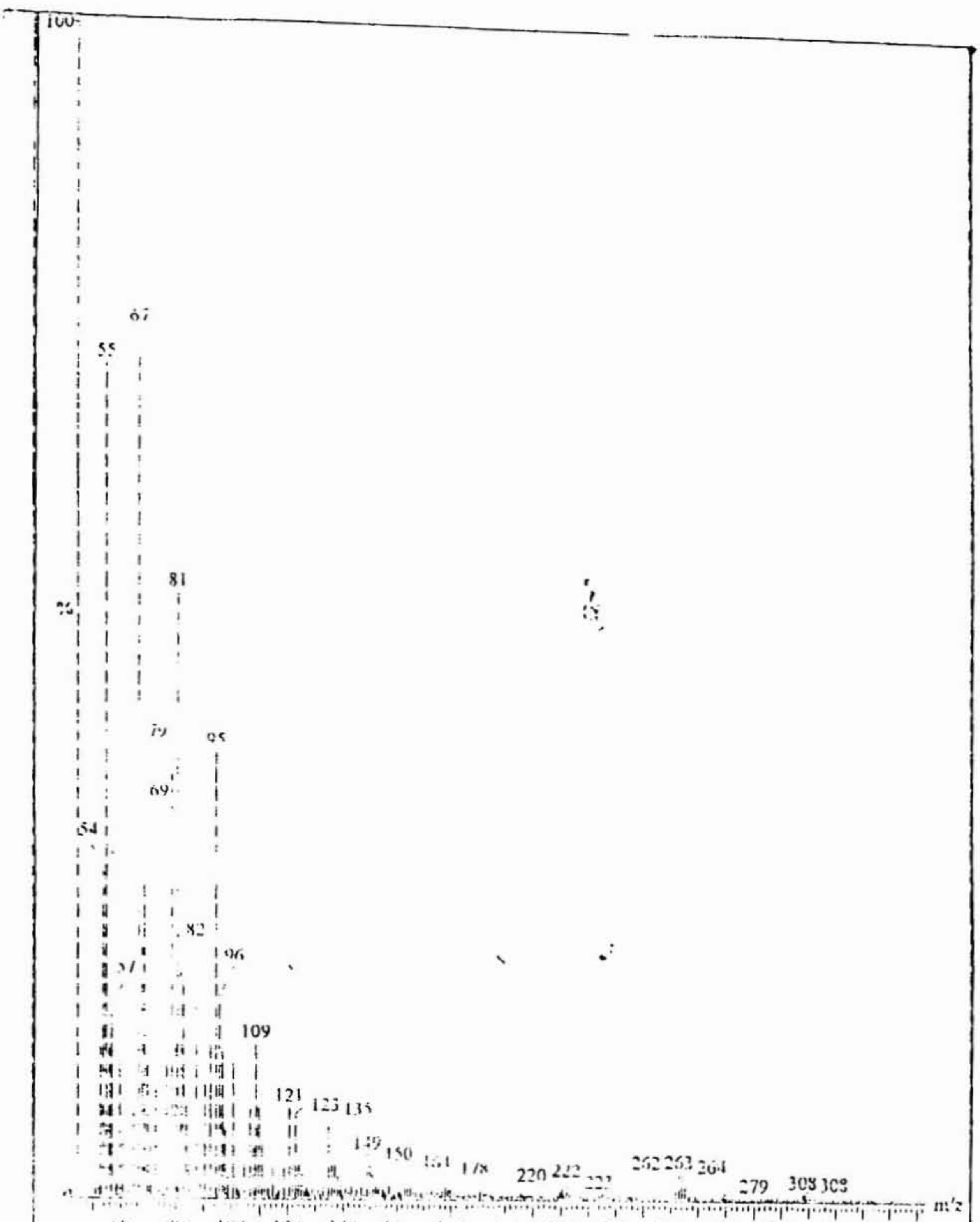


Fig. 4.4 Mass spectrum of the Croton derivative

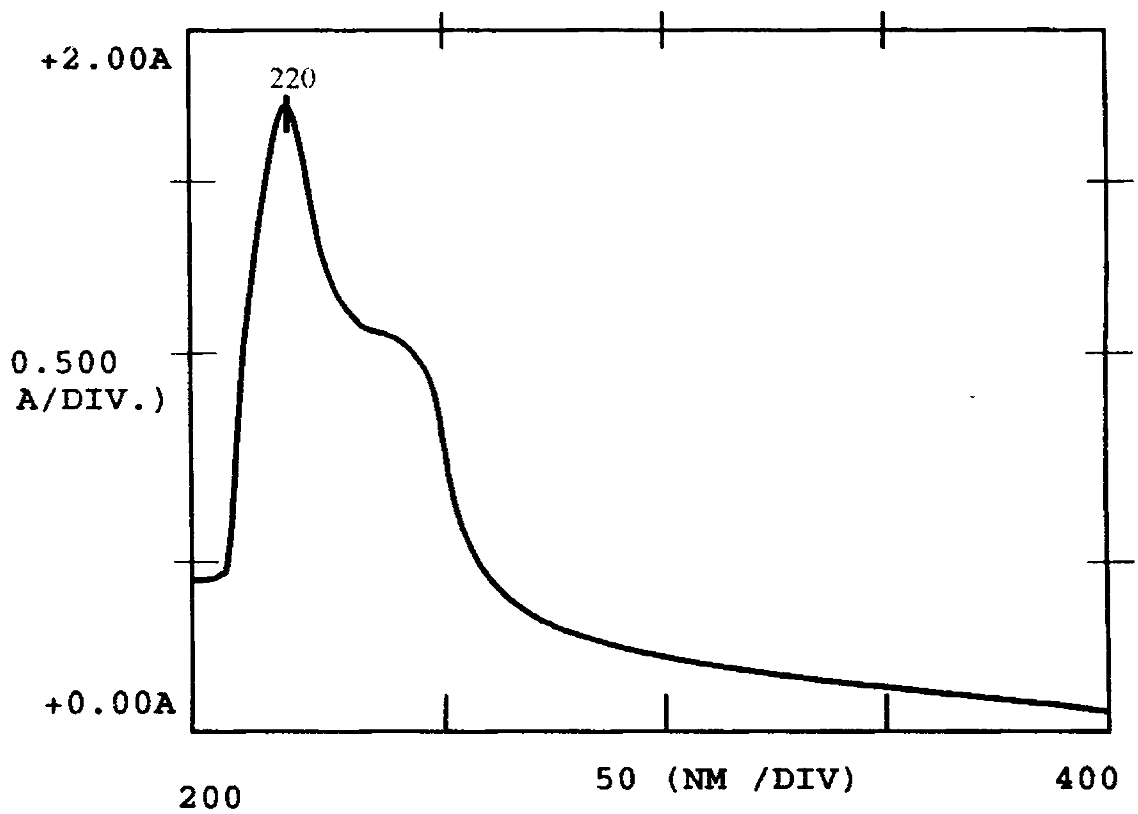


Fig. 4.5 UV Spectrum of the Leea Derivative

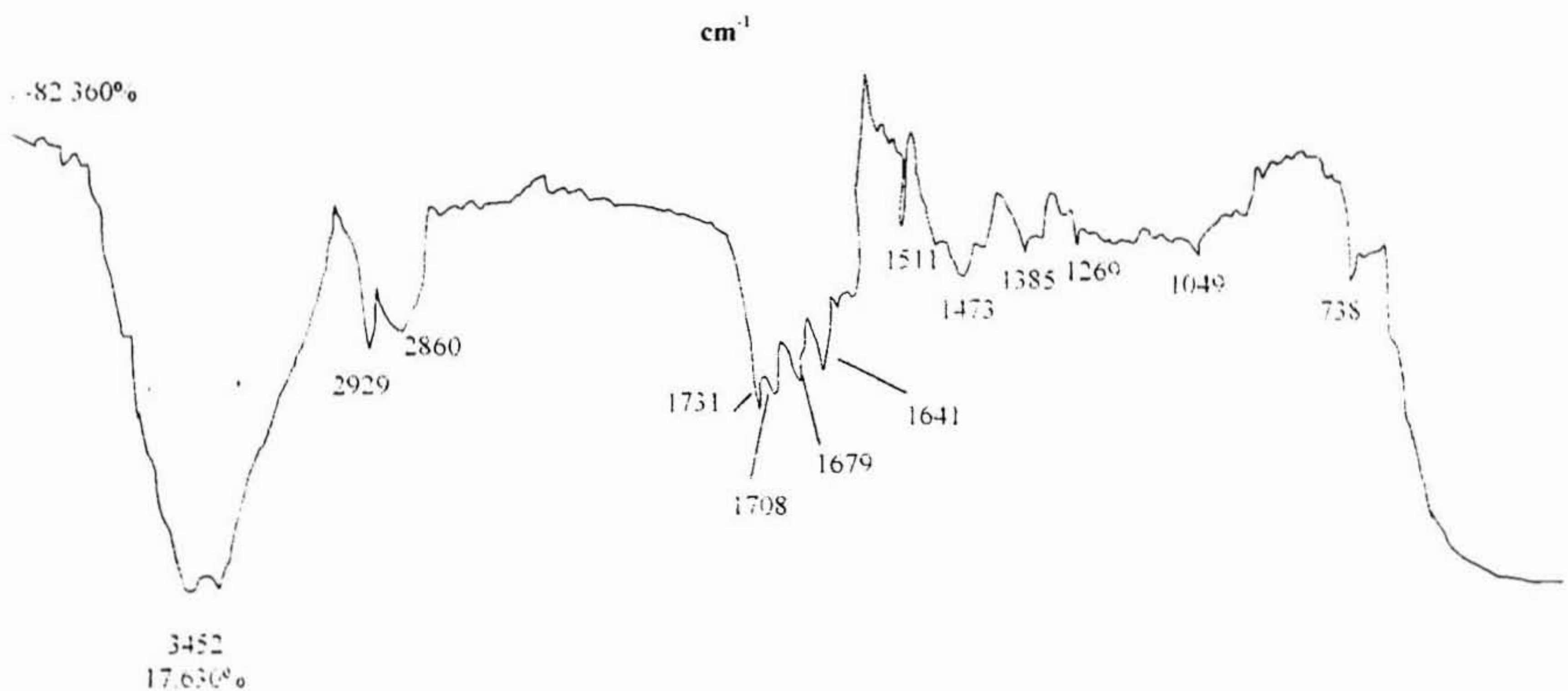


Fig. 4.6 IR spectrum of the Leea derivative

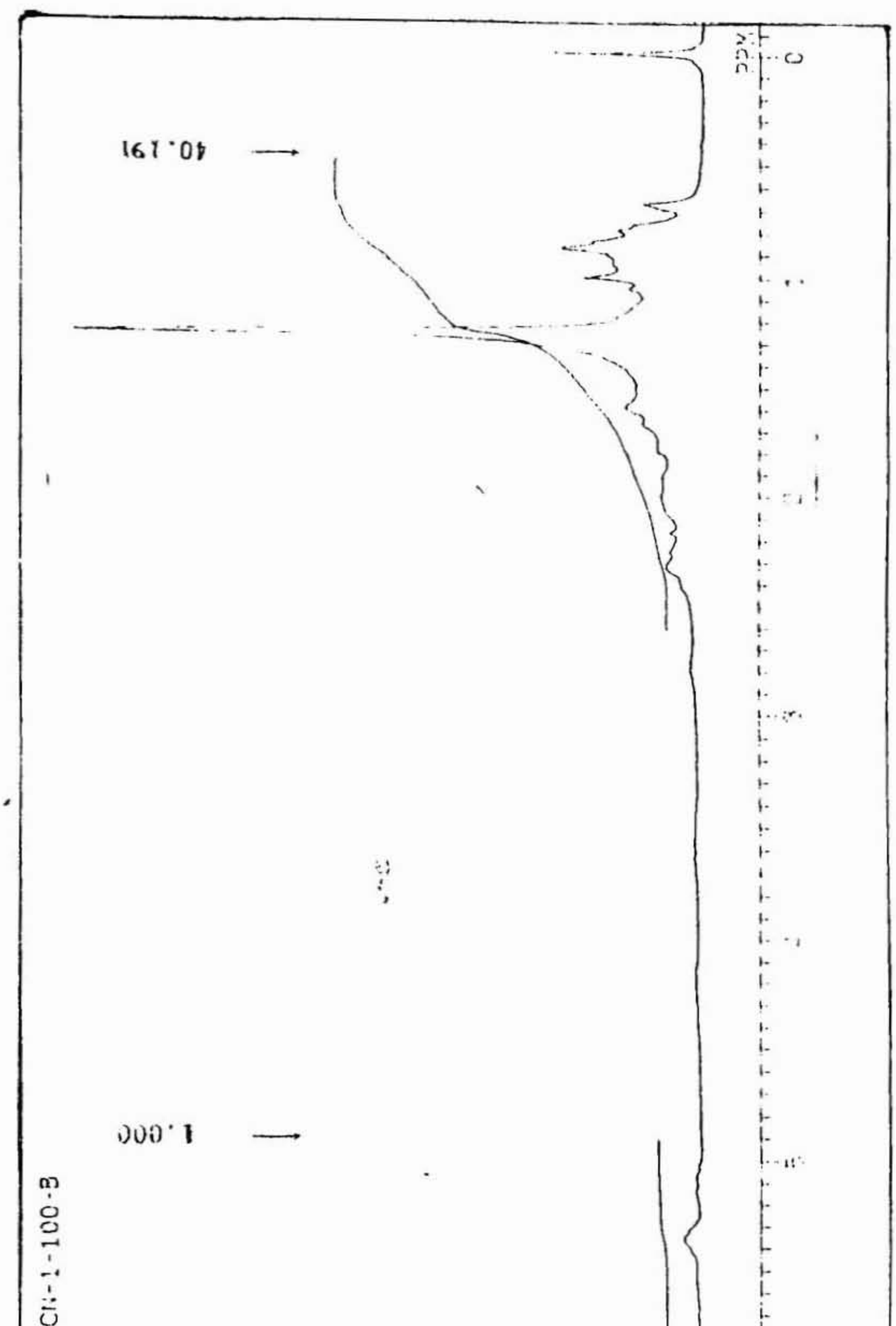


Fig. 4.7 NMR spectrum of the Leea derivative

4.3.2.1 Rosenhein- Callow reaction

A little of the pure compound was added to a reagent by dissolving 25gm mercuric acetate in 100cc of HNO_3 . A bluish green colour followed by a transient pink colour was observed *(Merk Index. 1949)*

Nitrogen was estimated by Kjeldahl's method

Since no mass spectrum was obtained, the molecular weight was determined by Rast's method.

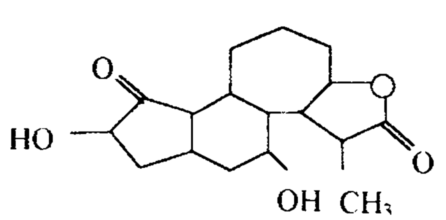
4.4 Result and Discussion

4.4.1 *Croton tiglium*

The molecular weight of the compound is 308 (from Mass Spectrum). There was no nitrogen obtained by Kjeldahl's method The compound is not an alkaloid

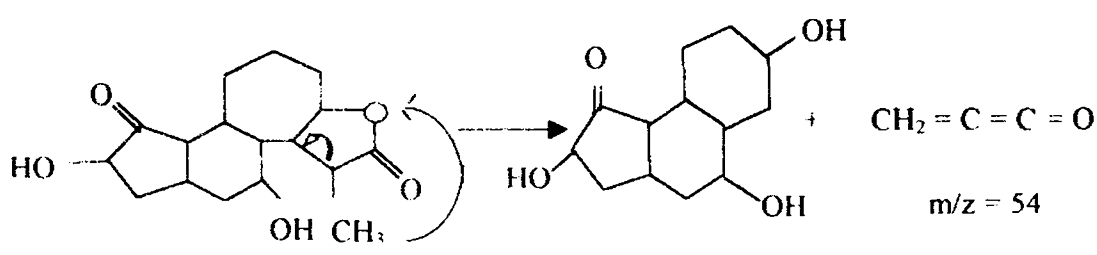
The UV data shows that the compound is a fused cycloalkane with two exodouble bond λ_{max}^{MeOH} 215nm (205 + 2 x 5)

The IR data, 3441(alcohol), 1731 (ketone), 3017, 2933 (cycloalkanes), Mass Spectral data m/z :308(m) 279(m-29), 264(m-44)(m-CO_2 ester), 69, 55,41(cycloalkane) and the HNMR data quartet 4.15 (-C-CO-), multiplet 5.38 (-OH), multiplet 0.8 - 2.38 (cycloalkanes) show that the compound might be a phorbol with following molecular structure



Boiling Point 75 ° C

Further, the compound undergoes Mc Lefferty rearrangement as follows to give fragmented m/z 54.



The presence of m/z 54, therefore, confirms the structure assigned to the compound.

4.4.2 Leea sambucina

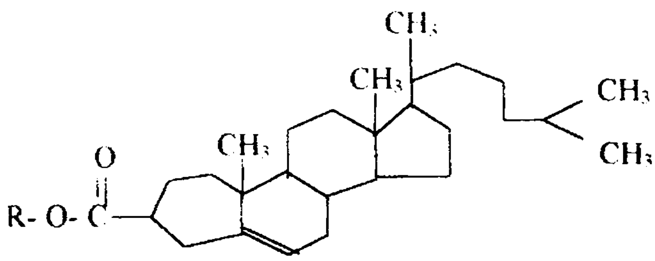
The compound does not give nitrogen by Kjeldahl's method, it is not an alkaloid.

The compound has a λ_{max} at 200nm which is close to a theoretical value of 221 (215 base value + 5 ; 1-exocyclic double bond)

IR Spectrum values 1734, 1708 (ester C=O), 2929, 2860 (EH stretching), 1385, 1269, 1049(C-O stretching of esters), 1473 (cyclo pentane), 738 (mono substitution) 1641 (C=Cstr)

HNMR signals are spread between 0.7 - 2.4 and is similar to cholisterate (*Adrich*) of a fatty acid

Rosenhein - Callow test confirms that the compound is a sterol. From the spectral data the compound was identified as a cholisterate, which is a sterol compound. The molecular weight (by Rast's method) was found to be 485 The molecule can be assigned the following structure



The compound has a melting point 92 °C.

5

DEGRADATION STUDY OF THE ACTIVE PRINCIPLES

5

DEGRADATION STUDY OF THE ACTIVE PRINCIPLES

5.1 Introduction

Human beings stand at the top of every food chain in which they are involved, and accumulation of persistent pesticides, therefore, occur in them. Synthetic pesticides such as BHC, DDT, Endosulfan, Parathion etc. are highly persistent in the environment. They not only contaminate water and soil but also enter the living organisms through food. They biomagnify in living beings including man, and cause millions of death every year.

Pesticide properties differ widely, both among and within chemical classes. Accordingly, their potential as environment contaminants can vary. Among the important properties related to environmental pollution are (i) the tendency to vaporise, (ii) the tendency to dissolve in water and other solvents, and (iii) the resistance to various degradation processes *(Stoker and Seger, 1977)*.

The third is of particular importance in determining the effects of a given pesticide on the environment. This property is related to persistence, which is defined as the time required for a pesticide to loose at least 95 percentage of its activity under normal environmental conditions and rates of application *(Moriber, 1974)*. Activity loss is complete when the pesticide has been totally decomposed or otherwise inactivated by chemical or biological processes. Non-persistent pesticides remain in the environment for 1 -12 weeks; moderately persistent ones remain 1 – 18 months; and persistent pesticides endure 2 years or longer. Obviously, if the degradation is rapid, there is little potential for accumulation or movement of the toxic species within the environment *(Stoker and Seager, 1977)*.

Environmental combatibility of a pesticide is therefore a priori for the wide application of a derivative.

5.2 Materials and Methods

Degradation is a process of fractionation by micro organisms which are capable of making use of the molecule for their food. It is a slow process in which the component is structurally altered. So, if the absorbance of the component in a suitable solvent is measured prior to degradation and after the degradation there will be reduction in the absorbance at the λ_{max}.

For pesticide degradation in soils, factors such soils type, amount of organic matter present, and extent of cultivation have all been shown to have significance *(Kenneth and Lesley, 1992)*.

5.2.1 Degradation

The degradation study was done with soil bacteria. The purified principles (0.1 g for phrobol derivative and 0.02 g for cholisterate derivative) were mixed aseptically with 100ml soil extract. The soil used for this study was surficial (0-15cm), taken from Eloor industrial area. Soil samples were combined, mixed thoroughly, then sieved to remove debris and large particles The prepared soil was then stored at 4°C prior to use to minimize effects of microbial activity Properties of the soil analysed *(Jackson, 1973)* were

P - 0.026% K - 0.130% OC- 2.7%

Twenty ml each of this were transferred aseptically to each of the five test tubes and a control was also kept All the tubes plugged with cotton were incubated at 30° C. After 48 hrs, one of the tubes was taken and the mixture extracted with petroleum ether. The ether was evaporated and extracted with 10ml methanol (*Kenneth and Lesley,1992)*. The methanol extract was filtered and the asborbance was measured at 215nm for phorbol and at 220nm for cholesterate. The experiment was repeated at 48 hour interval. The result is interpreted in Fig 5.1 and 5.2. The absorbance values were corrected to the control value by using the control extract as blank.

5.2.2 Shelf Life

The phorbol derivative isolated from *Croton* plant was active to the same extent after being kept for two months in room temperature whereas the cholisteràte derivative from *Leea* had 18% reduction in activity against *Tribolium castanium*.

5.3 Result and Discussion

Residence time generally refers to the length of time necessary for the compound to be degraded or changed in any way so that it can no longer be considered a potential or a real problem. It may also be classified as the length of time a compound or element will remain in the environment before it is removed by one process or another *(Mullins, 1977)*.

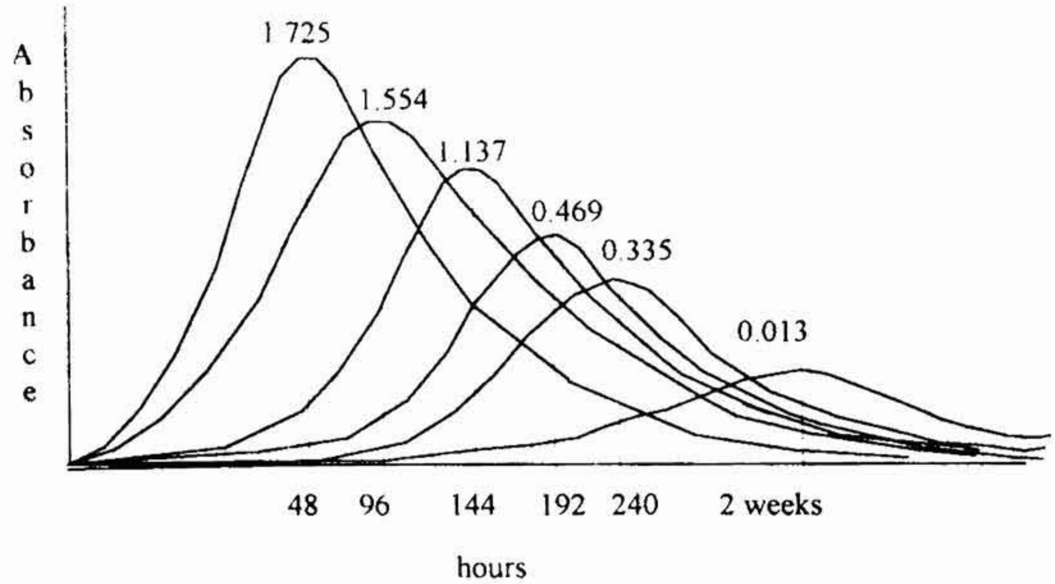


Fig. 5.1
Degradation of Cholisterate derivative by Soil Microorganisms

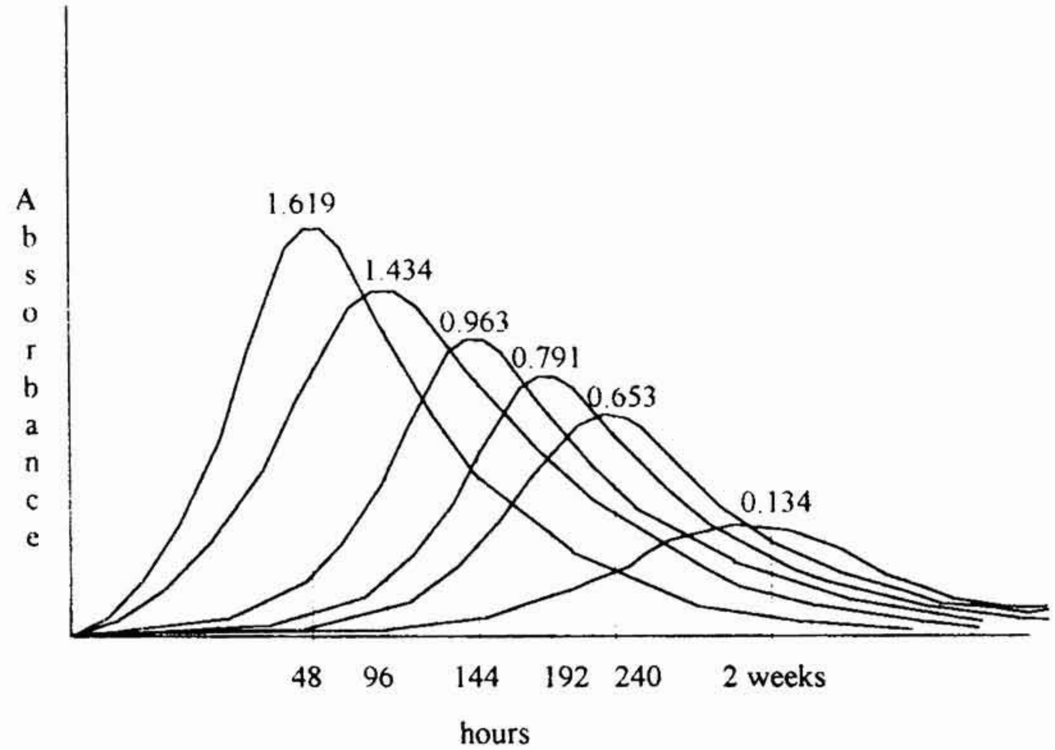


Fig. 5.2
Degradation of Phorbol derivative by Soil Microorganisms

The principles isolated from both *Croton* and *Leea* are fast degradable by soil micro organisms. These compounds can be considered as non-persistent.

The shelf life for phorbol derivative > 2 months while that for cholesterate derivative is < 2 months. This can be improved by suitable formulations.

Both the compounds can be considered environmentally combatible as they degrade at a rate not to pose any residual effect on mammals *(Mc Euwen, 1979)*.

6

COMMERCIAL FORMULATION OF THE ISOLATED PRINCIPLES

6

COMMERCIAL FORMULATION OF THE ISOLATED PRINCIPLES

6.1 Introduction

In spite of the wide recognition that many plants possess insecticidal properties, only a handful of pest control products directly obtained from plants i.e., botanical insecticides, are in use in developed countries. The demonstrated efficacy of the botanical neem (based on seed kernel extracts of *Acedirachta indica*) and its recent approval for use in the US has stimulated research and development of other botanical insecticides. However, the commercialisation of new botanical insecticides can be hindered by a number of issues The principal barriers to commercialiastion of new botanicals are . (i) scarcity of the natural resource, (ii) standardisation and quality control and, (iii) registration (*Isman , 1997*).

In the next decade, biologicals will either begin to play a meaningful role in specialty crops or become a curious relegated to organic farming. (*Gaugler, 1997*)

So, the formulation of bio-pesticides is equally important as isolation and identification of bio active principles.

6.2. Materials and Methods

6.2.1 Formulation of phorbol derivative from *Croton tiglium*

Formulation 1

Croton oil is a glyceryl ester in which a toxic resin, a vesiculant, and toxic proteins are found in dissolved form. Since the glyceryl esters can be saponified, a simple technique used in soap manufacture is made use of to isolate and formulate the active principles.

The seed is pulverised and extracted with petroleum ether (60-80°C) at 60-70°C sequentially for two times. The cake is removed and dried. The ether extract is evaporated at reduced pressure to get the pure oil. The oil is saponified with 15% NaOH solution. The saponification mixture is then poured into 30% NaCl solution for salting out the soap. Soap is removed, filtered and cleaned. The filtrate is neutralised with HCl and concentrated by vacuum evaporation. The NaCl crystallised out is removed by centrifugation. The centrifugate containing the active principles can be used as insecticides. A flow diagram of the production stages are given in Figure 6.1.

The Cake has a P_2O_5 content of 0.98gm/kg and a K_2O content of 12.04gm/ kg and is a useful manure *(Furman, 1969)*.

Formulation 2

The croton oil was saponified as above and the active part was extracted with petroleum ether. Ether was evaporated and one gram of active part was mixed with 25ml of 1% teepol (*Saxena and Kidwai, 1997*).

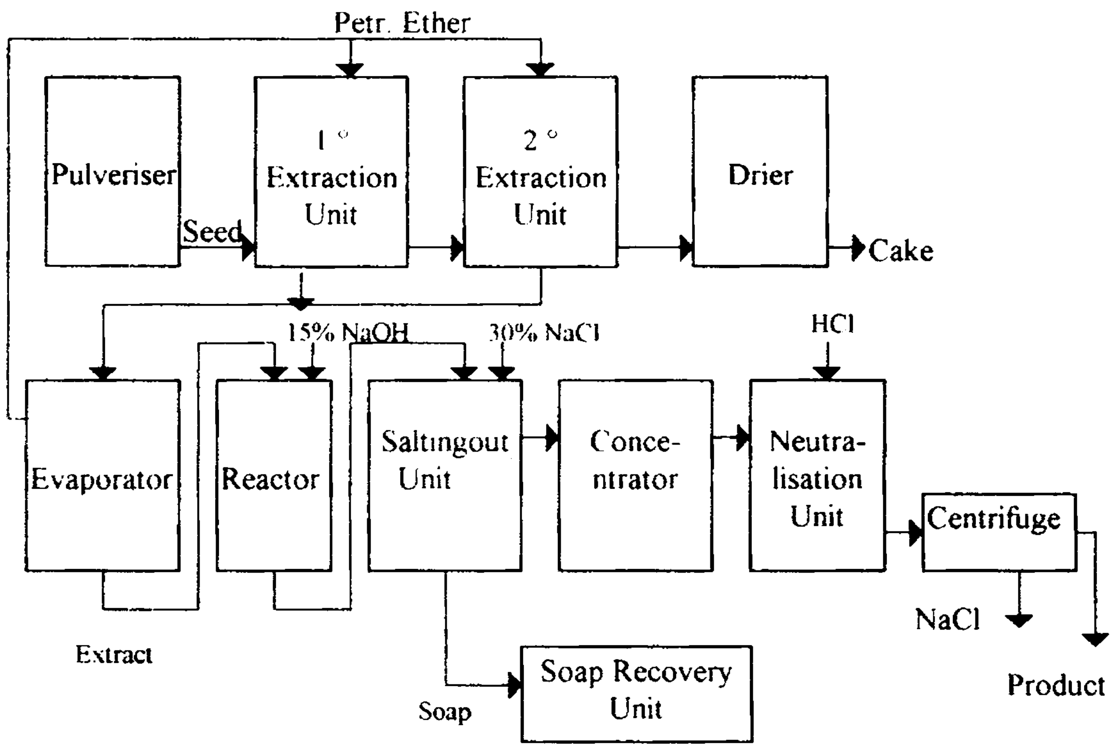


Figure 6.1 Flow diagram for the production of Phorbol formulation from Croton tiglium

6.2.2 Formulation of Cholesterate Derivative from *Leea sambucina*

A sample of 0.1g cholesterate derivative isolated by column chromatography was mixed with 25ml of 5% water extract of the dried fruit of soap berry and 0.1% solution of teepol in water (Formulation 3). The soap berry extract, being easily degradable and reported to be insecticidally active and a surfactant was used for formulation. The extraction was made as follows.

Berry (dried)
↓
Methanol (Soxhlet Extraction)
↓
Fractionation (Water + 1 butanol)
↓
Evaporation to dryness ⟶ Dry Residue

Alternatively, the large scale extraction of cholisteryl derivative from *Leea* was attempted. Fresh plant material was dried and extracted with hot methanol(MeOH). The methanol extract, after the removal of the solvent under reduced pressure, was partitioned between n-butanol (n-BuOH) and water

A flow chart for the extraction and formulation is shown in Figure 6.2

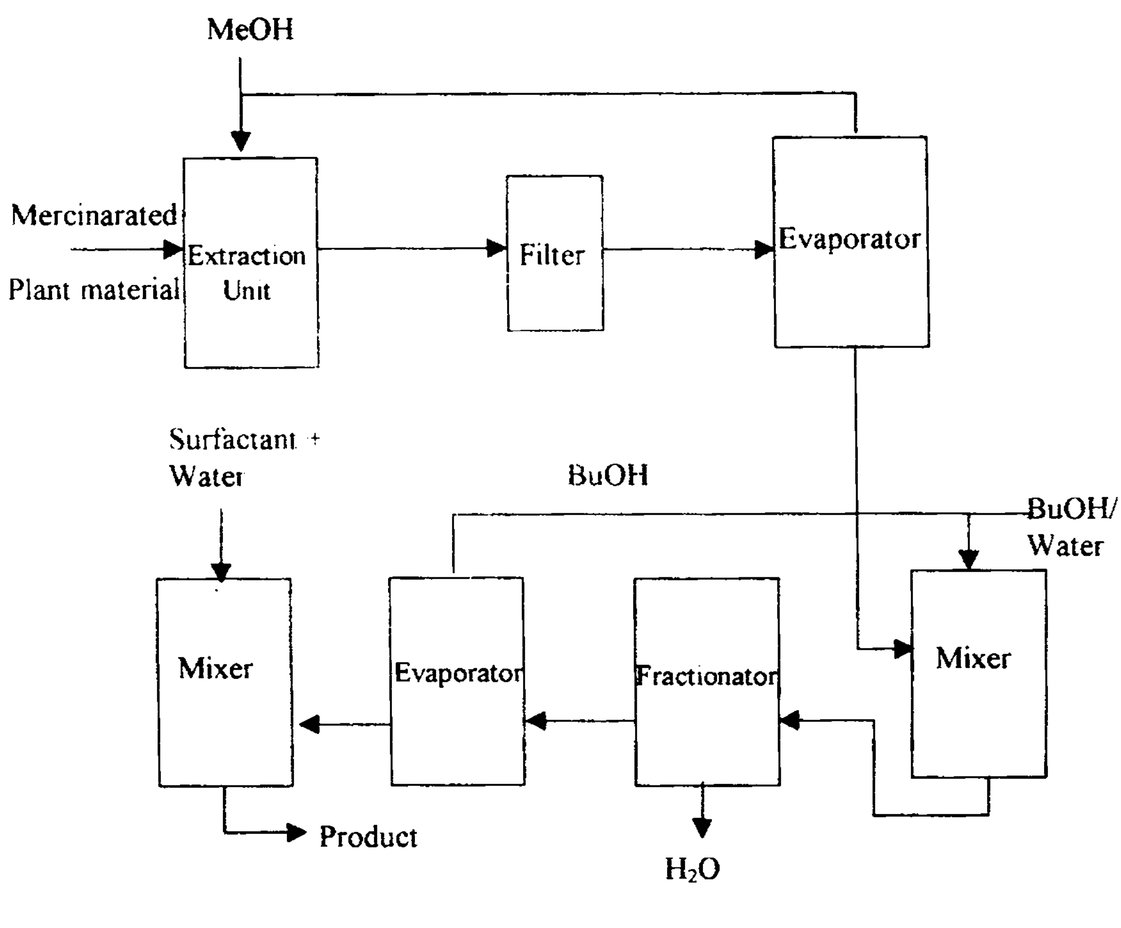


Figure 6.2 Flow diagram for production of Cholisteryl formulation from Leea sambucina

6.2.3. Wide Spectrum Application of Formulations

Formulation 1 and 2 on Snake gourd Semilooper (*Phlusia peponis F*)

Vegetables have an important role in human diet. Insect pests are one of the major constraints in vegetable production all over the world, especially under tropical conditions. These crops are subject to attack by a series of pests with varying feeding habits from sowing to harvest. This necessitates application of insecticides at different occasions to control the insect pests of these crops. As the products are also consumed without any processing, the insecticides applied on these crops must be essentially of low mammalian toxicity and of less waiting period, but at the same time, highly effective against the pests.

Gourds are an important groups of vegetables under the broad classification of cucurbits and these are widely cultivated in India. Of these snake gourd *(Trichosanthes anguina L)* is an important crop with high nutritive value *(Arkroyd et al., 1962)*. This crop suffers severe damage and crop loss is due to insect pests such as melon fly, spotted beetle, pumpkin beetle, pumpkin caterpillar, *snake gourd semilooper*, jassids, aphids and others.

Insecticides belonging to various groups viz., chlorinated hydrocarbons, organo phosphates and carbamates have been evaluated against these pests and some are reported to give effective but partial control of the pests *(Das et al., 1968, Nagappan et al., 1971, Nole, 1975)*. Many of these insecticides possess disadvantages like long and undue persistence in the environment, high toxicity to higher animals and other non-target organisms, pest resurgence, pest resistance to insecticides, residue hazards and environmental pollution.

The snake gourd semilooper belonging to the family *Noctiridae* is a serious pest of snake gourd all over India The larva is a greenish semilooper with black warts on its body. It grows to a length of about 3 8cm and is humped on its anal segment. It hides within a leaf foil and feeds on the leaf blades. The damage caused is often serious and often when young plants are attacked. They are totally denuded of their leaves. Pupation takes place in a leaf fold within a thin cocoon. The caterpillar is parasited by *Apentilis taragamae* Vier, *A. plusiae* Vier, and *Mesochorus plusiaephilus* Vier.

The semilooper grubs were collected from field of snake guard. The healthy tender leaf of the snake guard was sprayed with 1% , 5%, 10% solution of the formulations. The leaves were allowed to dry under a ceiling fan and were put in glass jars. Five grubs were transferred carefully to the jars A control sprayed with water alone was also kept. All the jars were closed with muslin cloth. Observations were made after two days. Percentage damage was calculated by measuring the area of the leaves. The result is interpreted in Table 6.1, Figures 6.3 and 6.4.

Formulation 2 on mosquito larvae

Economically, mosquitoes are of utmost significance owing to their functioning as the intermediary hosts of malaria, yellow fever, filariasis, dengue and numerous viruses. Increased knowledge of these insects and diseases transmitted by them has rendered vast areas of tropical countries no longer a menace.

The medically important mosquitoes belong to the tribes *Culicini* and *Anophilini*. The medical importance of the latter as vectors of malaria is well known.

Formulation 2 was diluted suitably to 250ml to get 50ppm, 100ppm, 150ppm, 200ppm. The solution was kept in 500ml beakers. Twenty five larvae of *Culex quinquefasciatus* collected from field were transferred to the beaker. A control with water and teepol mixture was also kept. Mortality was counted after 24 hrs. *(Thangam and Kathresan, 1993).* The mortality value has been corrected to the control using *Abbott's* formula. The result is interpreted in Table 6.2 and Figure 6.5.

Formulation 3 on *Callosobruchus chinensis L.*

Callosobruchus chinensis belonging to the family *Bruchidae* is a common pest attacking pulses. It breeds in stored peas or beans. Loss of seed depends on whether the germ or only the cotyledons are badly damaged. If the former, the seed will not germinate. If the latter, the seed may grow but will be handicapped by lack of nutrient.

The eggs are laid among the dried beans. The first stage larvae are active with well developed legs. They bore into the beans and eat them from inside. The second and third stage larvae grow to fat and sessile grubs. They pupate in small oval cells excavated immediately under the seed coat so that the adults can escape easily *(Patil et al., 1994).*

Green gram was disinfested by chilling at 0°C for 48 hours. Fifty grams of the grain was sprayed with 1% (w/v), 3% and 5% solution of the formulation in a petri dish. The grain was then transferred to glass jars and one day old 20 adult beetles were transferred carefully to the jars. The jars were closed with muslin cloths *((Chander and Ahmed, 1982).* A control was also kept using suitable concentrations of soap berry extract and teepol. Observations were made after two weeks. Percentage damage was calculated by counting damaged grains. The result is interpreted in Table 6.3 and Figure 6.6.

Formulation 2 and 3 as Biocide

Minimum Inhibitory Concentration (MIC) is the concentration of a biocide which inhibits microbial growth. This necessarily means that in a system, if biocide concentration is present at MIC level, the micro organisms, at least, will not be able to grow and multiply (*Aquapharm Manual*.)

Nutrient broth was prepared as per the standard methods. The formulations were diluted so as to get 50, 100, 150, 200, and 250 ppm for formulation 2, and 50, 75, 100, 125, and 150 ppm for formulation 3 in the medium. Methylene bis thiocyanate, a commonly used biocide in cooling water systems, was used to get a comparison of the activity. One ml of cooling water (collected from cooling tower of Fertilizers And Chemicals Travancore Ltd., Udyogamandal) was inoculated to the test tube containing the medium and the formulations. All experiments were done aseptically. The tubes were incubated at 38° C for 48 hours. Tubes with turbidity were reported as positive, and negative, if otherwise. The results are given in Table 6.4. In Figure 6.7 the concentration of formulation Vs. turbidity in NTU (Nephelometric Turbidity Unit) has been plotted.

6.3 Results and Discussion

Table 6.1 Activity of formulation 1 & 2 against Snake guard Semilooper	
Formulation 1	% Leaf damage
Control	71.8
1%	32.1
5%	5.6
10%	0.0
Formulation 2	% Leaf damage
1%	27.2
5%	2.7
10%	0.0

Table 6.2 Activity against mosquito larvae Formulation 2	
Concentration	% Mortality
50ppm	28
100ppm	43
150ppm	76
200ppm	100

Table 6.3 Activity against Callosobruchus chinensis L Formulation 3	
Concentration	% Damage
Control	76.8
1%	26.7
3%	11.1
5%	0.01

Table 6.4
Biocidal activity

Biocide	Concentration ppm					MIC ppm
Methylene bis Thiocyanate	10	20	30	40	50	
	+	+				30
Formulation 2	50	100	150	200	250	
	+	+	+		-	200
Formulation 3	50	75	100	125	150	
	+	+				100

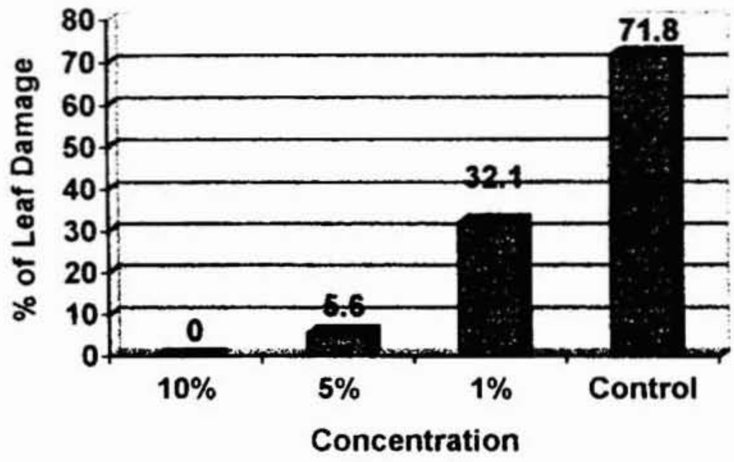


Fig. 6.3 *Activity of formulation 1 against Snake gourd Semilooper*

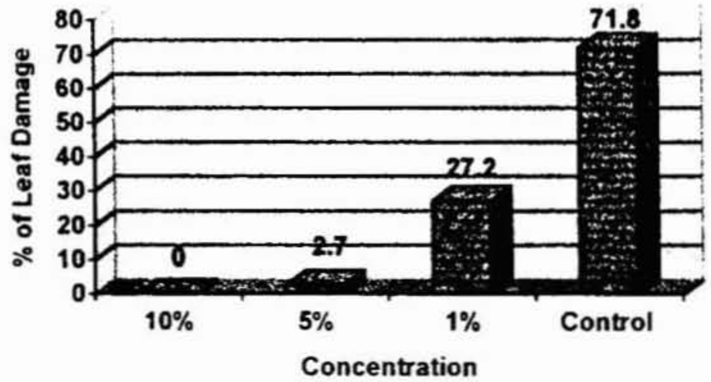


Fig. 6.4 *Activity of formulation 2 against Snake gourd semilooper*

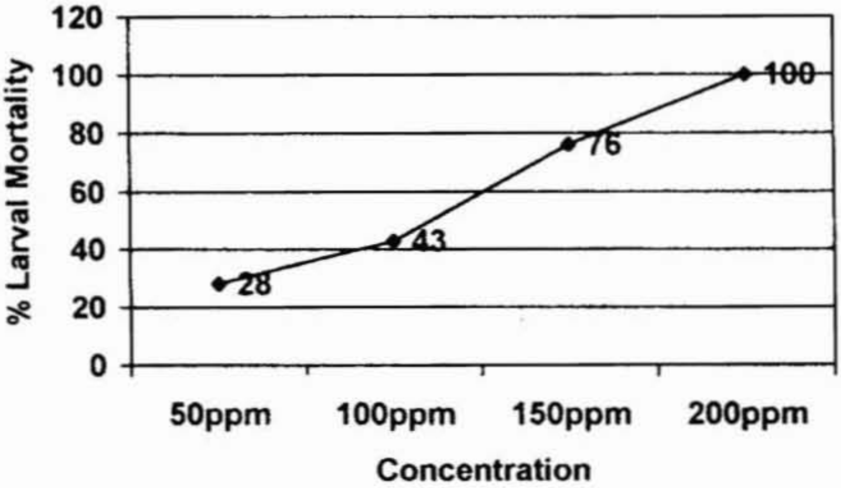


Fig. 6.5 *Larval Mortality (%) of Culex quinquefasciatus with different doses of formulation 1*

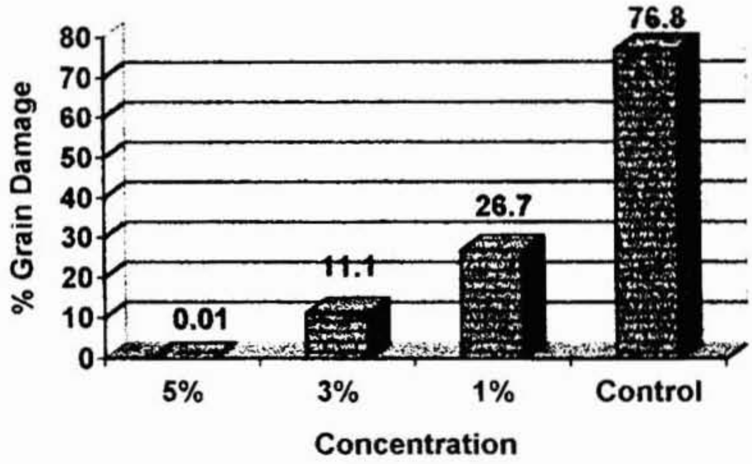


Fig. 6.6 *Activity of formulation 3 against Callosobruchus chinensis*

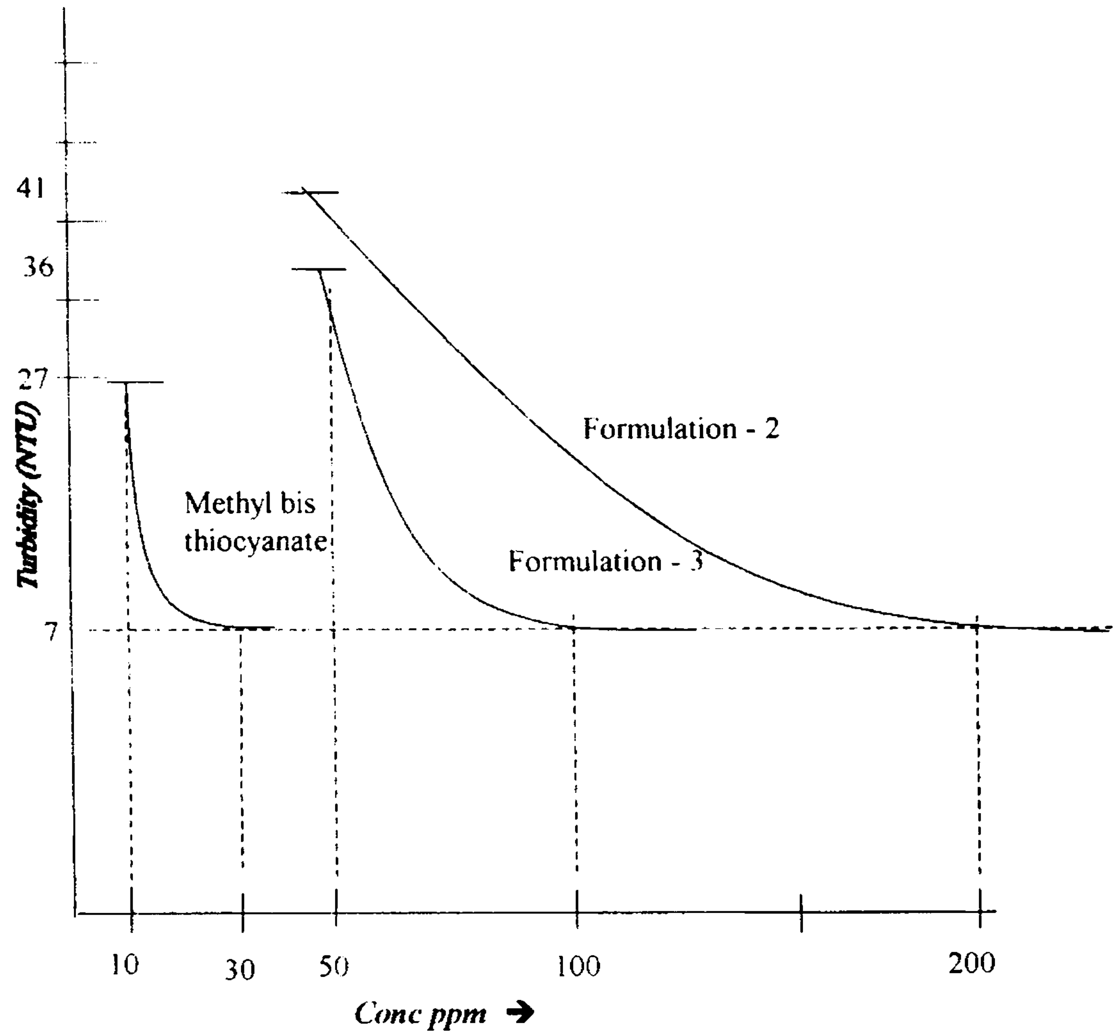


Fig.6.7 Activity of formulation 2 & 3 as biocide

The result shows that formulation 1 and 2 are active against Snake gourd semilooper A 5% concentration of the formulations gives protection of 94.4% and 97.3% With 10% concentration the leaf protection is cent percent.

Formulation 2 gives excellent larvicidal activity against *Culex quinquefasciatus* larvae, with 200 ppm solution the larval mortality is 100%.

Formulation 3 gives negligible (0.01%) loss to green gram grains at 5% concentration.

Formulations 2 and 3 give excellent anti- bacterial effect with cooling water which mainly contains SRB, nitrifying and various soil bacteria.

All the formulations can be widely applied against cut worm, mosquito larvae, pulse beetle and as a biocide. The method of formulation 1 can be used for the large scale production of insecticide from Croton seed. The cake is a valuable byproduct. The method of formulation 3 can be used for the large scale production of insecticide from *Leea*.

Since there is no environmentally detrimental molecules involved, all the formulations are eco-friendly. However, the phorbol derivative may have some mammalian toxicity as croton oil is reported to be a strong irritant to human beings.

Formulation 1 and 2 may be useful for the control of vegetable pests and mosquito larvae while formulation 3, being a sterol, may be an excellent grain protectant.

7

SUMMARY AND CONCLUSION

7
SUMMARY AND CONCLUSION

7.1 Findings of the Study

Out of ten plants screened for efficacy as eco-friendly insecticide, *Leea sambucina* and *Croton tiglium* were the most effective. Four plants viz., *Crataeva religosa, Sphaeranthus indicus, Ficus asperrima, Premna latifolia* were the least active, while the remaining four viz., *Vitex leucoxylon, Euphorbium anticorum, Blumea balsamifera, Ervatimea dichotima* had medium activity.

The active principles from *Leea* could be isolated in almost pure state with repeated column chromatography The active principle from *Croton* could be isolated by hydrolysis, solvent extraction, and column chromatography.

The active principles could be identified with UV-Vis, IR, NMR and GC-MS techniques The active principle isolated from *Leea* is a cholisteryl derivative and that from *Croton* is a phorbol. However, advanced techniques like C^{13} NMR, FTIR and FABMS may have to be used to confirm the structural identity and stereo chemistry of the active principles.

Both the cholisteryl derivative and phorbol could be formulated effectively with eco-friendly emulsifiers. Methodologies for the bulk production of insecticide formulation from *Croton* seed as well as from *Leea* have been developed.

The croton seed cake after extraction, can be used as manure since it contains 0.98g/kg P_2O_5 and 12.4 g/kg of K_2O.

Formulations (1) and (2) are effective against snake gourd semilooper. Formulation (2) is also very active as mosquito larvicide. Formulation(3) can be used for the protection of pulses. Formulations (2) and (3) are also effective as a biocide.

Both the derivatives are highly degradable by soil micro organisms. However, the mammalian toxicity of these compounds have to be tested before being put into use.

7.2 Impediments to Commercialisation

For the Indian farmer, the strategy for employing more toxic chemicals on pesticide and herbicide resistant varieties is suicidal, in a literal sense (*Siva, 1993*). However, the commercialisation of new botanical insecticides can be hindered by a number of issues. The principal barrier to commercialisation of new botanicals are (i) scarcity of the natural resource (ii) standardisation and quality control; and (iii) registration (*Isman, 1997*).

As far as *Croton tiglium* is concerned, since it is being cultivated commercially, the problem of scarcity may not be an issue. But the other two problems have to be sorted out. But for *Leea sambucina* tissue culture is the only alternative to get the bio mass in large quantity. Callus cultures of *Acedirachta indica* has already been reported to have improved the yield by hundred fold (*Morgan, 1996*).

Whereas the possibility remains that insects will not develop resistance as quickly to a botanical insecticide as to synthetic insecticide, the main reason for using a botanical insecticide is practical one. Specifically, it is too difficult or costly either to isolate the principal active ingredient or synthesise it. Pyrethroid is an exception to this. Natural pyrethrum remains in use and is cost effective in many market segments. But standardisation and quality control of such formulations are difficult. However, standardisation and quality control of one or two ingredients are the alternative method (*Isman, 1997*).

In the case of the derivatives from *Croton tiglium* and *Leea sambucina* the formulation can be standardised on the basis of phorbol and sterol content respectively.

Since the costs of studies in support of registration (non target effect study) is costly, a provisional registration for five years is the solution. This facility is now available in India. Within this relaxation period, the required tests have to be done for the registration with regulatory authorities.

7.3 The New Paradigm

The study of the pesticide crisis of the last couple of decades indicates that it was partly technical and partly a cultural phenomenon. Resistance, resurgence and secondary pest outbreaks were the major aspects of the techincal problems, and health and environmental hazards highlighted the cultural difficulties (*Perkins, 1985*).

The reorientation of entomological research towards a sustainable path began after *Rachel Carson (Carson, 1962)* launched a crusade against the widespread use of insecticides through her well known book - *Silent Spring*.

A paradigm shift is quintessential for pest and vector control programmes. The Integrated Pest Management (IPM) is an accepted strategy. A paradigm defined by *Kuhn* is a scientific change based upon the emergence, use, and eventual discard of ideas. As far as pest control strategy is concerned the old paradigm that chemical pesticides are the sole methodology for the protection of agriculture and human being has to be shifted to a new paradigm which is IPM In IPM, biological control, cultural control, and pheromone based control alongwith prudent use of chemicals is the methodology. If sustainable and eco-friendly chemicals of natural origin are used, the IPM programme gets a boost. In this context, the present study opens a new series of pesticides alongwith the presently wide acclained neem, pyrethroids, rhotinones etc..

A new biologically based paradigm for the botanical insecticide industry might emulate business sectors that have successfully overcome biological limitations. For example, the food industry has shown that low stability products are not a fatal flaw. Similarly the microbrewery industry has demonstrated that there is a strong market for fresh products sold without preservatives. The keys have been local small-batch custom production and quick turnover leading to high quality products. This concept might be adapted to botanical insecticides.

Phytopesticides are at a cross road. The early vision of biologicals becoming significant pest management tools for major raw crops faded in this decade. No foreseeable new technology, including genetic engineering is likely to change this reality. In the next decade, biologicals will either begin to play a meaningful role in speciality crops or become a curiosity relegated to organic farming. Industry, growers, extension and researchers must come together and think 'outside the mold' to realise the former *(Gaugler, 1997)*.

With the clock ticking towards next millenium, the modern man has become an ecological man, supported by the ecological paradigm. He is searching for technologies and practices which are lasting and capable of catering the generations yet to come (*Skolmowski, 1983*). The search for new botanical insecticides is anchored on this philosophy.

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