

**INVESTIGATIONS
ON
ECO-FRIENDLY
NATURAL INSECTICIDES**

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1999

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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
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School of Environmental Studies, Cochin University of
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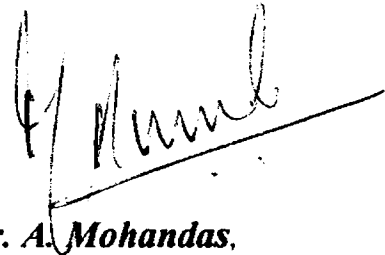
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CERTIFICATE

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 tiglium* 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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1

GENERAL INTRODUCTION

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 *tu/si* 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* disease 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 (*Oslon 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 doses; 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.

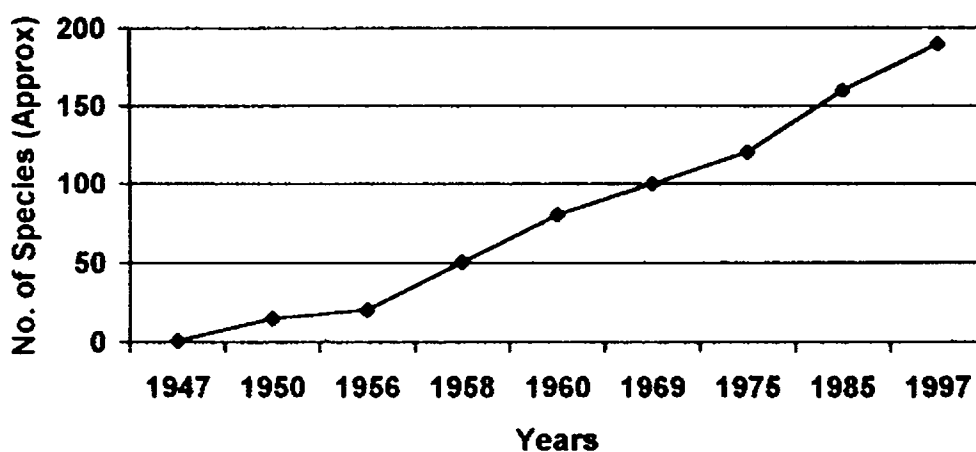


Figure 1.1

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 adequate 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 inspiring 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 (*Chakraborty, 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)

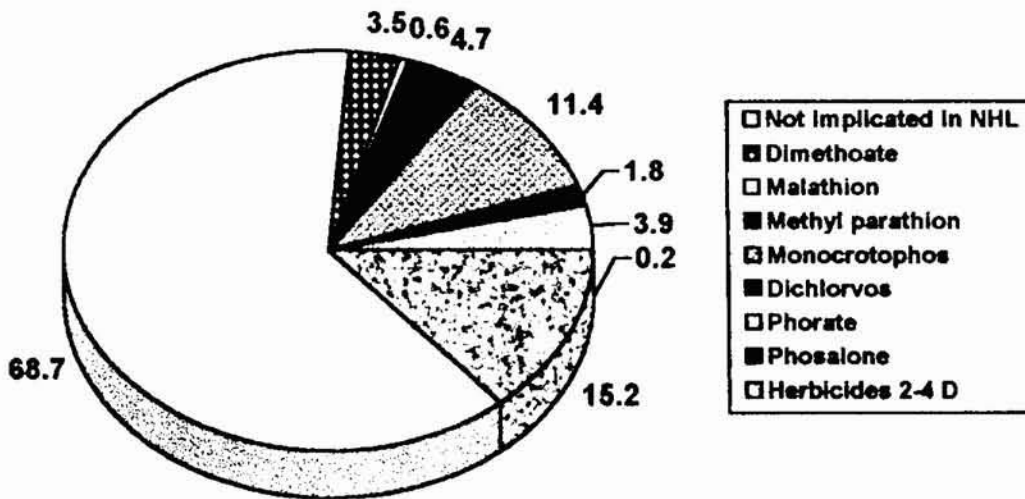


Fig.1.2 NHL Implicated pesticides and their % consumption in Indian Agriculture (1993-94)

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.

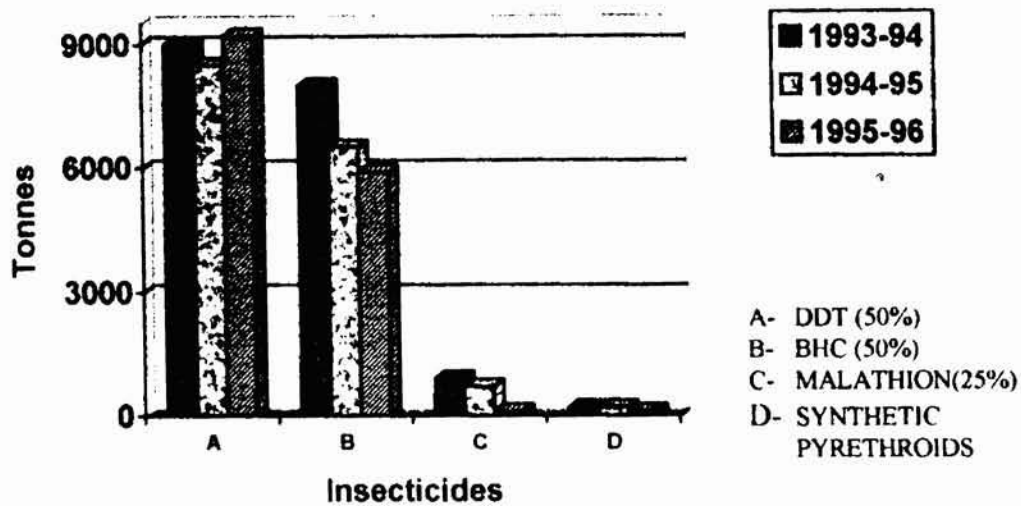


Figure 1.3 Use of Insecticides under National Malaria Eradication Programme (NMEP)

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 trophic 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 unstablisation 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 secretion of saliva and tears, diarrhoea, discomfort in the chest due to constriction of the bronchioles and retardation 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 phytochemicals 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 persistence on the crops under field conditions (Elliot et al., 1978., Collingwood et al., 1979). At present four synthetic pyrethroids viz. Permethrin, Fenvalerate, 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 (Elliott 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 pinnata*), 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 azadirachtin, a triterpenoid, 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 odoratissimus*, *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*.

