DETERMINING THE EFFICACY OF ORGANIC INSECTICIDES AND SYNERGISTS AGAINST A RANGE OF REPRESENTATIVE INSECT AND MITE PESTS USING A POTTER TOWER.

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GENERAL ABSTRACT

A variety of insecticidal formulations were examined for their efficacy against a range of insects under laboratory conditions using a Potter Tower technique.

The insecticides Yates Pyrethrum, Eco-oil, Defender, Orchex, Confidor, Agrimec, Encapsulated Pyrethrum, Encapsulated Pyrethrum and Neem, Azatin, NeemAzal, as well as a combination of the insecticides Azatin plus Eco-oil were tested at both the half as well as the full recommended field rate against a range of insect pests.

In addition, the ability to synergise natural pyrethrum by some or all of the natural compounds including sesame oil, the crystalline extract of a sesame oil crude extract, dillapiole, and the synthetic synergist PBO, in spray emulsions at a variety of rates and ratios was examined in tests against the pea aphid *Acyrthosiphon pisum,* passionvine hopper (*Scolypopa australis),* greenhouse thrip, and lightbrown apple moth.

Confidor and Yates Pyrethrum gave marked control (i.e. mortality > 80%) of the greatest number of test species including the aphid, thrip and mealybug and two of the moth species for Confidor and all of the moth, aphid and thrip species for Yates Pyrethrum.

It is suggested that the use of the synthetic synergist PBO could be replaced by the natural synergists including dillapiole, as well as the crystalline extract of a sesame oil crude extract at the highest ratio that was tested against the pea aphid, passionvine hopper, and lightbrown apple moth. Results with greenhouse thrip and tomato fruit worm were inconclusive.

A complete summary of the results is given in abstract form for each of the three parts of this dissertation.

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GENERAL INTRODUCTION

With the increasing emphasis by consumers and agricultural export markets, particularly in developed countries, towards sustainability, environmental agriculture, and organic production, there is a growing recognition that if New Zealand is to retain or expand access to these markets then it must respond to this demand.

Agricultural policy is being increasingly driven by these sociopolitical forces (Hall et al 1989) and in response to this pressure the adoption of organic practices and values in new farming systems are emerging with more sustainable methods and growing markets (McCrae et al 1989).

Although there is little consensus as to what sustainability is precisely, and a myriad of definitions have been proposed, in the wider sense it clearly involves biology, ecology, economics, and sociocultural and political considerations (Singh & Thornton 1992).

World Bank economist Herman Daly, (Daly 1991) suggested three conditions that would have to be met by society's material and energy throughputs in order to be physically sustainable. Firstly its rates of use of renewable resources do not exceed their rates of regeneration, secondly its rates of use of nonrenewable resources do not exceed the rate at which sustainable renewable resources are developed, thirdly its rates of pollution emission do not exceed the assimilative capacity of the environment.

MAF New Zealand (1994) defines sustainability in general terms as the ability of a system to endure, by maintaining the resource base, and the absence of unacceptable effects on people or the environment.

An alternative and defensible term of sustainability is sustainable development. The most widely adopted definition of sustainable development, which is the key concept of the Brundtland Report (1987), is defined as 'development that meets the needs of the present without compromising the ability of the future generations to meet their own needs'.

Included in its strategy for sustainable food security, the Brundtland Report states that 'Pest control must be based increasingly on the use of natural methods....The legislative, policy, and research capacity for advancing non-chemical and lee-chemical strategies must be established and sustained'. Further, included in its strategy for sustainable industrial development, the Brundtland Report states that 'The chemical producer and user industries...should bear the responsibility for ensuring that their products meet the highest standards of safety, have the fewest adverse side effects on health and the environment, and are handled with appropriate care by workers and users'.

Organic farming is therefore one of several systems, other than Integrated Pest Management, that can move New Zealand farming practices towards the goal of sustainability by the use of a range of primarily biological (as opposed to synthetic chemical inputs) strategies that produces a commodity free of conventional chemical use, while maintaining soil fertility and plant and animal health.

While some writers such as McCrae et al (1990) have pointed out that most organic farmers are only part of the way along a continuum between input dependent farming (conventional) and sustainable systems, others view sustainability differently. For instance Fisher (1989) from an economical viewpoint believes that a system using some

chemical inputs can be more sustainable than a strictly organic system as it is more able to endure than a system that does not use any chemical inputs.

Never the less, the use of organic pesticides is a valuable tool in the development of a sustainable agriculture, whether it is used by organic farming systems or more conventional systems.

Pyrethrum is the only chemical compound, other than soaps and oils and *Bacillus thuringiensis* which is accredited by the organic production standards in New Zealand and is the most dependable of these chemicals (Epenhuijsen et al 1992).
However, the most frequently used pyrethrum synergist at present, piperonyl butoxide (PBO) is synthetic (Wachs 1949) and non-organic.
As the ability of a synergist to be "environmentally friendly" or organic is now becoming more desirable with changing consumer attitudes (Hall et al 1989). There is a growing need for their synergists to be organic also, even though they may be less cost effective than the alternative synthetic synergists.

The widely accepted definition of a pesticide synergist was described by Matsamura (1985) as " compounds that greatly enhance the toxicity of an insecticide, although they are usually practically non-toxic by themselves".

Originally sought as a means to increase the insecticidal activity of pyrethrum in order to conserve it, today pyrethroid synergists are predominantly used to increase the cost

efficiency of pest control by pyrethroids (Brown 1971; Yamamoto 1973; Bond et al 1973), by reducing the concentration of toxicant necessary to achieve a desired mortality when it is combined with the synergist.

The history of pyrethroid synergists stretches back to their origins in the first World War.

In 1940, the synergistic action of sesame oil was first discovered by Eagleson, its active components each having the methylenedioxyphenyl (MDP) moiety.
Recognition of the importance of the MDP moiety for synergism led to the testing for synergistic potency for hundreds of MDP compounds that were synthesised, or isolated from natural sources and is still continuing to a somewhat lesser extent today.

PBO, the first truly effective commercially viable synergist was patented in 1949 and as is previously mentioned it is still a requirement in most pyrethrum insecticides. PBO is an MDP synergist.

Today it is accepted that synergists primarily increase the toxicity of insecticides by inhibiting their metabolism (Casida 1970; Georghiou 1983; Sun & Johnson 1972). In particular, the synergistic effect of MDP synergists has been related to the greater competitive ability of these synergists (in comparison to the chemical insecticide) to form metabolic complexes with cytochrome P450 (Perry & Buckner 1970), as well as their production of adducts which inhibit metabolism of other substrates (Werringloer & Estabrook 1979), and thus inhibiting the oxidative metabolism of the insecticide by the mixed function oxidase system (mfo) of microsomes. In other words, the synergists act

as alternative substrates of metabolism, sparing the chemical insecticide from detoxification. Although it is known that MDP synergists are metabolised by oxidation on various sites on their long aliphatic side chains, it is still unclear why the synergists are competitively superior to the insecticide in their ability to form P450 metabolic complexes. This may be due to either improved fit on the active site of the pyrethroid metabolising enzymes or to the availability of the additional sites on the synergists.

This study is divided into three separate parts and although they each have three different aims they are somewhat interrelated.

The aim of the first study was to undertake an assessment on a range of insect species of the efficacy of a range of pesticides, the majority of which are 'soft' pesticides i.e. are natural products which offer lower levels of impact on the environment than the alternative conventional synthetic chemicals. Some of these pesticides have not been released by pesticide companies in New Zealand. Three of these pesticides contain the toxicant natural pyrethrum which is examined more closely in the remaining two parts of this thesis.

Secondly, an assessment of the efficacy of the natural oils sesame and dill, and their extracts, as synergists for natural pyrethrum against the pea aphid *Acyrthosiphon pisum* were examined in comparison to the relative efficacy of the synthetic natural pyrethrum synergist piperonyl butoxide (PBO).

Thirdly, as both toxicity and the degree of synergism of synergists is specific to an insect species, the efficacy of the natural synergists from Part B were examined against a wider range of insect species.

The last two parts were undertaken with the ultimate aim of developing an effective natural pyrethrum product in New Zealand which contains an organic synergist.

PART A

Determining the efficacy of several 'soft'insecticides against a range of representative insect and mite pests using a Potter Tower.

ABSTRACT

The insecticides Yates Pyrethrum, Eco-oil, Defender, Orchex, Confidor, Agrimec, Encapsulated Pyrethrum, Encapsulated Pyrethrum and Neem, Azatin, NeemAzal, as well as a combination of the insecticides Azatin plus Eco-oil were tested at both the half as well as the full recommended field rate against lightbrown apple moth larvae, *Epiphyas postvittana*; diamondback moth larvae, *Plutella xylostella*; codling moth larvae, *Cydia pomonella*; pea aphid, *Acyrthosiphon pisum* (nymphs and adults); Greenhouse thrip adults, *Heliothrips haemorrhoidalis*; predator mite, *Phytoseiulus persimilis* (larvae and adults); and the mid-instar obscure mealybug, *Pseudococcus affinis* populations, under laboratory conditions.

Confidor and Yates Pyrethrum gave marked control (i.e. mortality > 80%) of the greatest number of test species (i.e. five) including the aphid, thrip and mealybug species and two of the moth species for Confidor and all of the moth, aphid and thrip species for Yates Pyrethrum. Encapsulated Pyrethrum, and Encapsulated Pyrethrum and Neem, both gave marked control of lightbrown apple moth, codling moth, aphid, and thrip species. Azatin and NeemAzal gave marked control of the diamondback moth and codling moth. Agrimec only gave marked control of the moth species while Defender only gave marked control of the pea aphid. Eco-oil, Orchex and Azatin plus Eco-oil did not markedly control any of the test insect species. Confidor was the only pesticide which markedly controlled the obscure mealybug.

The biocontrol agent *P. persimilis* was only controlled markedly by Azatin plus Eco-oil. This was unexpected.

CHAPTER ONE

INTRODUCTION

An increasing demand for organic produce, and the increasing occurrence of insect resistance to insecticides has resulted in a growing need for the production of organic control products both overseas as well as in New Zealand. 'Soft' insecticides are natural products that have been obtained from plants, fungi or bacteria. These 'new' products offer less of the damage and risk associated with synthetic chemicals while still maintaining an acceptable level of insect control.

The aim of this study was to undertake an assessment of the efficacy of ten insecticides, of this 'soft' nature on a range of economically important insect pests in New Zealand. Some of these insecticides have not been released by pesticide companies in New Zealand.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 INTRODUCTION:

This chapter briefly describes ten 'soft' insecticides that are either in a developmental stage or are already available for use in horticultural based systems.

2.2 YATES PYRETHRUM :

Yates Pyrethrum contains Pyrethrum flower (*Chrysanthemum cinerariaefolium*) extracts from which have been isolated six chemicals, namely Pyrethrin I , Pyrethrin II, Cinerin I, Cinerin II, Jasmolin I and Jasmolin II , as well as the synergist piperonyl butoxide (PBO). These six chemicals are often referred to collectively as the 'Pyrethrins'.

Yates Pyrethrum has been reported to control a broad range of insect pests (Yates product information leaflet) including aphids, whitefly, mealybug, caterpillars, earwigs, pear slug, and passion vine hopper.

Pyrethrum works by contact action and acts very quickly and effectively on the insect's nervous system. Although Pyrethrum may harm some natural enemies, due to its rapid degradation the potential effects on such insects as well as on the environment are lessened and there are no associated withholding periods with its use. The future of Pyrethrum looks promising despite the sometimes high costs associated with its production.

2.3 ECO-OIL:

Eco-oil is a vegetable oil based product that reportedly controls Two spotted mite and European red mite, aphid, thrip, scale, mealy bug and whitefly. Eco-oil controls these pests by contact action. At the time of printing of this report, Eco-oil was available for experimental use only.

2.4 DEFENDER:

Although the insecticidal properties of soaps have been known for a long time (Siegler & Popenoe 1925), it is only within the last two decades that soaps and surfactants have proven to be commercially effective in reducing insect and mite populations. However, field applications of insecticidal soaps are not always effective in controlling the pest without disruption to predators (Osborne & Petitt 1985).

Defender is an insecticidal soap whose active ingredients, namely potassium salts of fatty acids, occur naturally in foods such as corn and peanuts.

Yates Defender has been reported to rapidly control a range of soft-bodied insects, by contact action, including European Red Mite and Two Spotted Mite (Dentener & Peetz 1992), ideally for late season control in pip and stonefruit; and also controls aphids, whitefly (Epenhuijsen et al 1992), and thrips.

This insecticide has been found to be highly selective to target pests with little or no activity on beneficial insects such as predatory mites, bees, ladybirds and parasitic wasps. (Lo and Blank 1992) found that applications of Defender did not disrupt

predation by ladybirds *Orcus chalybeus* on *Ceroplastes destructor* on Citrus in laboratory trials.

2.5 ORCHEX 692:

Orchex 692 is a specially refined mineral oil with high paraffinic content and may be used as an insecticide, or as an adjuvant for crop oil formulations with the function of spreader, sticker, penetrant, and low-volatility carrier.

This insecticide has been reported to provide effective control notably of specified mites and scale (Product Information Bulletin,1989). For the control of other insects, specific pesticides may be added to the mixture by the formulator.

2.6 CONFIDOR 5%WDG:

Confidor is a systemically acting, low toxicity insecticide, its active ingredient, imidacloprid, belongs to the nitroguanadine group of active ingredients (Schroeder & Flattum 1984).

Confidor has a broad spectrum of control covering many of the most economically important pests including "sucking" insects such as aphids, thrips and mealybugs as well as soil pests, but is less effective against the larger lepidopteran larvae (Elbert et al 1990)

This insecticide acts rapidly via stomach and contact action and interferes with the transmission of nerve impulses (acting as an agonist on the nicotinic acetylcholine receptors). Imidacloprid, applied as a seed treatment, has been found to control *Myzus persicae* through repellency (Dewar and Read, 1990).

Imidacloprid is an active ingredient which is likely to have good potential for control of insect pests. Although this insecticide is very persistent in the environment, due to its new mode of action, and suitability for use in rotations, the build up of resistant pest populations is hindered.

Further, imidacloprid may be applied as a variety of different treatments on a wide variety of crops. Due to its systemic nature, effects on non-target organisms are minor and short-lived with damage occurring immediately after application. Damage can however occur in bee populations.

2.7 AGRIMEC:

2.7.1. INTRODUCTION

Agrimec contains the active ingredient abamectin (MK-936), the latter consists of ≥80% avermectin B1a and ≤ 20% avermectin B1b. The avermectin family of macrocyclic lactones, fermentation products of the soil microorganism *Streptomyces avermitilis* were first isolated in Japan in the 1970's. They are systemic insecticides.

2.7.2. SPECTRUM OF ACTIVITY

Abamectin is an extremely potent broad spectrum acaricide as well as insecticide of members of the orders Diptera, Hymenoptera, Homoptera, Coleoptera, and Lepidoptera. However, this high innate activity is shown only against specific insect pests.

Abamectin is active primarily as a stomach poison as indicated by its residual activity, however contact activity is also important for some insects such as aphids (Green &

Dybas 1984).

2.7.3. MODE OF ACTION

Abamectin has a unique mode of action in arthropods. It acts on the chloride ion channel where it functions as an agonist of the inhibitory neurotransmitter gamma - aminobutyric acid (GABA) to increase chloride ion entry into the post-synaptic region of GABA cells (Mellin et al 1983), thus immobilising and killing. In addition to its toxic and delayed activity, avermectin B1a has been shown to inhibit feeding (Pienkowski & Mehring 1983), and to inhibit pheromone production (Wright 1984).

2.7.4. COMMERCIAL PROSPECTS

2.7.4.1. EFFECTS ON NON-TARGET ORGANISMS AND HUMANS

Avermectins appear to be relatively innocuous to many natural enemies, including predator mites, and therefore have the potential to be compatible with IPM programs. Further, they have low mammalian toxicity.

2.7.4.2. DEVELOPMENT OF RESISTANT INSECTS

Due to its novel mechanism of action, abamectin has been shown to control pests which have become resistant to conventional acaricides and insecticides.

Nevertheless, some evidence of cross-resistance to abamectin was reported in a multi-insecticide-resistant population collected in Thailand 1982, prior to abamectin's introduction (Abro et al 1988). Further, Iqbal et al (1996) gave "what may be the first recorded evidence of marked resistance to abamectin in field populations of *Plutella xylostella* in Malaysia".

2.7.4.3. ENVIRONMENTAL EFFECTS

Agrimec has low environmental persistence and bioaccumulation (Lasota & Dybas 1991). However, its residual action (and control) in field use can be enhanced further by combination with mineral oil (Wright et al 1985a,b).

2.8 ENCAPSULATED PYRETHRUM:

Encapsulated Pyrethrum contains 1.4% of the active ingredient pyrethrin, as does Yates Pyrethrum. However, the latter also contains the synergist piperonyl butoxide in the ratio 1:4 parts pyrethrin:PBO.

Encapsulated Pyrethrum persists longer in its environment and degrades less readily than non-encapsulated pyrethrum in sunlight as a result of a protective oil coating around the pyrethrum molecules.

At the time of printing of this report, this product was available for experimental use only.

2.9 ENCAPSULATED PYRETHRUM AND NEEM (AZADIRACHTIN):

This pesticide contains 0.1% neem.

At the time of printing of this report, this product was available for experimental use only.

2.10. INSECT GROWTH REGULATORS:

Insect Growth Regulators, (IGR's), are a relatively new group of insecticides.

2.10.1 AZATIN (an IGR): (4.5% W.P.)

Azadirachtin, the active ingredient in Azatin; is derived from the neem tree *Azadirachta indica* and chinaberry *Melia azedarach*, and is therefore available as a raw material in a number of tropical and subtropical countries. This natural product is capable of controlling a huge diversity of insect species as its chemical structure is similar to the insect hormone ecdysone.

This product controls insect larvae when they ingest or come into contact with it, by interfering with the insect's ability to grow, develop, and moult. In most cases azadirachtin's effects on ecdysis are preceded by reduced growth. It therefore has Insect Growth Regulator Activity (IGR). Further, contact with azadirachtin based products has been shown to have antifeedant effects, disrupt the behavioural pattern of egg laying, exhibit varying degrees of toxicity to eggs and early insect stages, as well as increase the locomotory activity of many insects (Saxena, 1989). It is effective on most developmental instars and stages of various orders. Infected insects do not complete their moult and eventually die (AgriDyne Technologies Inc.).

The future of azadirachtin based products looks particularly good for smaller-scale and organic horticultural systems due to a lack of acute toxicity associated with its use, and its non-toxic nature and limited affects on non-target organisms, respectively. However, care must be taken in the choice of timing of applications, as they need to coincide with the most sensitive and active stages of the target pest (Koul et al; 1990). Azadirachtin's multifarious mode of action is an added advantage for controlling a number of insect pests.

Azatin contains 3% azadirachtin.

2.11 NEEMAZAL-T/S (an IGR):

The active ingredient of NeemAzal-T/S is azadirachtin, which has been described previously in section 2.10 Azatin. The azadirachtin component in NeemAzal-T/S is one third of the concentration in Azatin. NeemAzal-T/S is compatible with organic and IPM programs.

CHAPTER THREE

MATERIALS AND METHODS

3.1. SOURCE AND ESTABLISHMENT OF EXPERIMENTAL INSECTS

3.1.1. Lightbrown Apple Moth (*Epiphyas postvittana*)

3.1.2. Diamondback Moth (*Plutella xylostella*)

3.1.3. Codling Moth (*Cydia pomonella*)

E. postvittana, P. xylostella, and *C. pomonella* used in this experiment were supplied by the Insect Rearing Group, HortResearch , Auckland, as eggs. These were hatched at 22°C ± 1°C, with a 12:12 light:dark photoperiod.

3.1.4. Pea Aphid (*Acyrthosiphon pisum*)

The source of the pea aphids used in the experiment were from an established laboratory colony, at Massey University. These were maintained on potted broadbean plants in a controlled atmosphere room, at 23°C with a 16:8 light:dark photoperiod, and were supplied to the population as required.

3.1.5. Greenhouse Thrip (*Heliothrips haemorrhoidalis*)

The Greenhouse thrips used in the experiment were collected as needed from Rhododendron trees, which are found throughout the grounds of Massey University. Males of this species are unknown in New Zealand.

3.1.6. Predator Mite (*Phytoseiulus persimilis*)

Both the predator mites, and their food source the twospotted spider mite (*Tetranychus pyri*), were supplied by the Insect Rearing Group, HortResearch, Auckland , as a variety of life stages on dwarf bean leaves in sealed plastic containers. The predator mites were stored in their containers at 10°C.

3.1.7. Obscure Mealybug (*Pseudococcus affinis*)

The mealybugs used in the experiment were supplied by the Insect Rearing Group, HortResearch, Auckland, as a variety of life stages on potatoes.

3.2. LABORATORY EXPERIMENT

3.2.1. Insecticide Treatment

The following insecticides and rates (Table 1) were used to treat the insects. Half rate and full rate treatments represent applications at half the recommended New Zealand field rate, and the recommended New Zealand field rate respectively, for all pesticides other than for Encapsulated Pyrethrum and Encapsulated Pyrethrum and Neem. For these two pesticides, recommended manufacturer experimental rates were not tested as they appeared to be very high! It was recommended by the maufacturer that the concentrated form of these pesticides should be diluted with water at a 9:1 ratio.

One water-only control was applied immediately prior to application of a given insecticide treatment. Each insecticide treatment was replicated five times consecutively.

Table 1: The insecticides, active ingredients, and rates used for treating each test species (All insecticides were supplied by Yates New Zealand Ltd).

Product Name	Active Ingredient	Product Rate
		Full,Half field rate.
Yates Pyrethrum	14g pyrethrum/l + 56.5g piperonyl butoxide/l	500ml/100l 250ml/100l
Eco-oil	825g/l vegetable oil	500ml/100l 250ml/100l
Defender	250g/l C12-C18 fatty acids	2l/100l 1l/100l
Orchex 692		1l/100l 0.5l/100l
Confidor	50g/kg imidacloprid	100g/100l 50g/100l
Agrimec	18g/l abamectin	37.5mls/100l 18.8mls/100l
Encapsulated Pyrethrum RD-232-337-6	1.4% pyrethrum	500ml/100l 250ml/100l
Encapsulated Pyrethrum and Neem RD-233-338-6	1.4% pyrethrum 0.1% neem	500ml/100l 250ml/100l
Azatin and Eco-oil	30g azadirachtin/l 825g/l vegetable oil	80g/100l + 500ml/100l; 40g/100l +250ml/100l
Azatin (IGR*)	3% azadirachtin	80g/100l 40g/100l
NeemAzal-T/S (IGR*)	1% azadirachtin	500ml/100l 250ml/100l

* = Insect Growth Regulator

3.2.2. Bioassay Set Up for Each Test Insect Species

3.2.2.1. Lightbrown Apple Moth (*Epiphyas postvittana*)

Twenty three millimetre diameter apple (*Malus domestica*) leaf discs were punched out

and placed, upper surface down, on damp filter paper into 90 mm plastic Petri dishes. Twenty neonate lightbrown apple moth larvae were placed, using a fine paint brush, on each leaf disc's upper surface.

3.2.2.2. Diamondback Moth (*Plutella xylostella*)

Twenty three millimetre diameter cabbage leaf discs were punched out from a whole cabbage, and placed on damp filter paper into 90 mm plastic Petri dishes. Twenty, neonate larvae were placed, using a fine paint brush, on top of each disc.

3.2.2.3 Codling Moth (*Cydia pomonella*)

Twenty three millimetre diameter apple (*Malus domestica*) leaf discs were punched out and placed, upper surface down, on damp filter paper into 90 mm plastic Petri dishes. Twenty neonate codling moth larvae were placed, using a fine paint brush, on each leaf disc's upper surface.

3.2.2.4. Pea Aphid (*Acyrthosiphon pisum*)

Twenty three millimetre diameter broadbean leaf discs were punched out and placed, upper surface uppermost, on damp filter paper into 90 mm plastic Petri dishes. Twenty late instar nymphs of the pea aphid were placed, using a fine paint brush, on each leaf disc's upper surface.

3.2.2.5 Greenhouse Thrip (*Heliothrips haemorrhoidalis*)

Twenty three millimetre diameter *Rhododendron* spp leaf discs were punched out and

placed, upper surface down, on damp filter paper into 90 mm plastic Petri dishes. Twenty adults of the greenhouse thrips were placed, using a fine paint brush, on each leaf disc's upper surface.

3.2.2.6 Predator Mite (*P. persimilis*)

One dwarf bean leaf discs was placed, upper surface uppermost, on damp cotton wool into 90 mm plastic Petri dishes containing filter paper. Twenty adult female predator mites and twenty twospotted mites were placed, using a fine paint brush, on each leaf's upper surface.

A ring of sticky "Tanglefoot" was placed around the circumference of the base of each Petri dish to prevent the mites from escaping.

3.2.2.7 Obscure Mealybug (*Pseudococcus affinis*)

Twenty three millimetre diameter potato tuber discs were punched out and placed, upper surface uppermost, on damp filter paper into 90 mm plastic Petri dishes. Twenty mid-instar mealybugs were placed, using a fine paint brush, on each disc's upper surface.

3.2.2.8. IGR'S

Thirty test insects, in their juvenile stages were added to each Petri dish and their food supply (unsprayed) was added as required. For the pea aphid and predator mite bioassays, the size of the food supply was altered slightly. Whole leaves were placed into each Petri dish rather than leaf discs, as the leaves provided a longer lasting food

supply. For the predator mite bioassays, the bean leaves that were initially added before spray were infested with all life stages of the two-spotted mite *Tetranychus urticae* and with predator mite eggs.

3.2.3. Insecticide Application

The Potter precision tower (Burkhard Manufacturing Co Ltd, Rickmansworth, U.K.) (Plate 1) was used to apply each insecticide treatment to the leaf discs and leaves. This apparatus can spray insecticides to simulate field spraying deposits and reproduce these with acceptable precision (Potter, 1952).

The operating instructions described by Potter (1941, 1952) for the Potter tower were followed throughout the experiment. The air pressure to operate the tower was maintained at 9 psi. A standard volume of 2ml was sprayed onto each of the leaf discs (or leaves) and a settling period of 10 seconds was allowed after all the spray had been discharged from the Potter tower sprayer.

With codling moth, diamond back moth, lightbrown apple moth, and thrip bioassays, both sides of the leaf discs were sprayed to ensure that if the larvae were to move to the underside of the leaf, contact with the insecticide would be maintained. This was achieved by initially spraying the bottom of the leaf disc with 1ml of insecticide then applying the larvae to the top surface of the leaf disc (or leaf) and spraying the disc again.

The upperside only of the food discs were sprayed with 2ml of insecticide, after having added the test insects for mealybug, pea aphid, and predator mite bioassays.

To eliminate spray contamination, precautions were taken between treatments to decontaminate the sprayer, i.e., two flushings with acetone followed by deionised water.

3.2.4. Mortality Assessment

After treatment each Petri dish was held at 21.5 ± 1°C, and a 14:10 light:dark photoperiod for all the test insect species other than for the predator mites. The latter were held at 10°C, and a 24:0 light:dark photoperiod. Mortality was assessed at 24 and 48 hours for all of the pesticides apart from the Insect Growth Regulators. Assessment of the latter occurred after two and fourteen days for the lightbrown apple moth, codling moth, and diamondback moth bioassays; two and five days for the aphid bioassays; and two and nine days for the predator mite bioassays. For the latter bioassay, the number of predator mites which had reached adulthood was recorded along with their "vitality status" i.e. whether they were alive or dead.

The following table lists for each species, and life cycle stage, the criterion used to determine mortality.

Table 2: The mortality criterion for each species and the lifecycle stage tested.

Species	Stage Tested	Mortality Criterion
Lightbrown apple moth	1st instar larvae	failure to move one body length
Diamondback moth	1st instar larvae	failure to move one body length
Codling moth	1st instar larvae	failure to move one body length
Pea aphid	late instar nymph; early instar nymph	failure to move one body length
Greenhouse thrip	adult;	failure to move one body length
Predator mite	adult	failure to move one body length
	larvae	failure to develop into adults;failure of developed adults to move one body length
Obscure Mealybug	mid-instar larvae	failure to move one body length

3.2.5. Analysis of Laboratory Results

The test insect's mortality response to the insecticide treatments was analysed using Analysis of Variance (ANOVA) in SAS (see Appendix 1 for programme).

The corrected percentage mortality values (in response to natural mortality) were calculated using Abbott's formula (Abbott, 1925).

Plate 1: The Potter tower

CHAPTER FOUR

RESULTS

This chapter presents the results of the mortality of various economically important insect pests to ten 'soft' insecticides.

NOTE: The significance levels which are given in the results are defined as:-

*** = Control mortality and mean replicate mortality are signifcantly different at $p \leq 0.001$.

* = Control mortality and mean replicate mortality are significantly different at $p \leq 0.05$.

n.s.= Control mortality and mean replicate mortality are not significantly different.

4.1. YATES PYRETHRUM:

The calculated mortality values for Yates Pyrethrum, at rates of 250ml/100l and 500ml/100l are shown in Table 3.

Table 3: Insect mortality values for Yates Pyrethrum.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	250ml/100l	24	72.0	2.55	*
			48	92.9	3.20	*
		500ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Diamondback Moth	1st Instar Larva	250ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
		500ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Codling Moth	1st Instar Larva	250ml/100l	24	82.8	2.60	*
			48	85.7	2.88	*
		500ml/100l	24	97.0	2.00	*
			48	100.0	0.00	-
Pea aphid	Late Instar Nymph	250ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
		500ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Greenhouse thrip	Adult	250ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
		500ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Predator mite	Adult	250ml/100l	24	58.7	11.19	*
			48	65.3	9.90	*
		500ml/100l	24	76.8	7.18	*
			48	75.2	6.85	*
Mealybug	Mid-instar juvenile	250ml/100l	24	0.0	0.00	-
			48	0.0	0.00	-
		500ml/100l	24	2.2	1.37	-
			48	2.2	1.37	-

4.2. ECO-OIL:

The calculated mortality values for Eco-oil at rates of 250ml/100l and 500ml/100l are shown in Table 4.

Table 4: Insect mortality values for Eco-oil.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	250ml/100l	24	0.0	0.00	-
			48	-10.5	2.04	*
		500ml/100l	24	0.0	0.00	-
			48	4.7	3.43	n.s.
Diamondback Moth	1st Instar Larva	250ml/100l	24	12.4	3.89	*
			48	-3.8	22.6	n.s.
		500ml/100l	24	19.7	3.68	*
			48	24.3	6.56	*
Codling Moth	1st Instar Larva	250ml/100l	24	-1.45	2.32	n.s.
			48	6.5	7.30	n.s.
		500ml/100l	24	-0.5	3.24	n.s.
			48	4.9	3.56	n.s.
Pea aphid	Late Instar Nymph	250ml/100l	24	0	0	n.s.
			48	0	0	n.s.
		500ml/100l	24	32.4	12.72	***
			48	8.4	12.54	n.s.
Greenhouse thrip	Adult	250ml/100l	24	4.0	4.00	n.s.
			48	4.0	4.00	n.s.
		500ml/100l	24	44.5	9.51	*
			48	77.7	8.15	*
Predator mite	Adult	250ml/100l	24	2.7	1.82	n.s.
			48	-7.2	3.15	***
		500ml/100l	24	5.5	3.04	n.s.
			48	5.7	2.00	*
Mealybug	Mid-instar juvenile	250ml/100l	24	0.0	0.00	*
			48	5.0	2.24	n.s.
		500ml/100l	24	1.0	1.00	*
			48	15.0	5.24	*

4.3. DEFENDER:

The calculated mortality values for Defender at rates of 1l/100l and 2l/100l are shown in Table 5.

Table 5: Insect mortality values for Defender.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	1l/100l	24	11.0	5.79	n.s.
			48	17.0	3.74	*
		2l/100l	24	9.12	4.56	n.s.
			48	17.8	5.02	*
Diamondback Moth	1st Instar Larva	1l/100l	24	8.4	8.57	n.s.
			48	41.7	9.34	*
		2l/100l	24	39.5	8.51	*
			48	40.0	8.88	*
Codling Moth	1st Instar Larva	1l/100l	24	24.9	4.27	*
			48	-37.2	19.59	n.s.
		2l/100l	24	31.1	5.81	*
			48	45.9	13.50	*
Pea aphid	Late Instar Nymph	1l/100l	24	23.8	4.93	*
			48	1.9	6.19	n.s.
		2l/100l	24	90.1	2.13	*
			48	92.7	2.52	*
Greenhouse thrip	Adult	1l/100l	24	35.8	3.71	*
			48	50.2	4.87	*
		2l/100l	24	46.8	9.44	*
			48	59.6	8.80	*
Predator mite	Adult	1l/100l	24	-4.3	1.24	*
			48	5.1	3.71	n.s.
		2l/100l	24	-2.9	1.47	n.s.
			48	2.5	5.82	n.s.
Mealybug	Mid-instar juvenile	1l/100l	24	13.2	1.99	n.s.
			48	13.2	1.99	*
		2l/100l	24	14.0	6.60	*
			48	15.0	6.71	*

4.4. ORCHEX 692:

The calculated mortality values for Orchex 692 at rates of 0.5l/100l and 1l/100l are shown in Table 6.

Table 6: Insect mortality values for Orchex 692.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	0.5l/100l	24	0.0	0.00	-
			48	4.0	3.25	n.s.
		1l/100l	24	13.2	5.46	***
			48	41.6	3.71	*
Diamondback Moth	1st Instar Larva	0.5l/100l	24	-0.2	3.02	n.s.
			48	19.2	8.00	***
		1l/100l	24	55.2	8.09	*
			48	70.8	14.78	*
Codling Moth	1st Instar Larva	0.5l/100l	24	41.7	13.15	*
			48	45.4	12.89	*
		1l/100l	24	-66.7	6.09	*
			48	-44.8	26.06	n.s.
Pea aphid	Late Instar Nymph	0.5l/100l	24	31.9	8.95	*
			48	-188.9	83.74	***
		1l/100l	24	52.5	9.81	*
			48	47.8	16.47	*
Greenhouse thrip	Adult	0.5l/100l	24	2.1	6.47	n.s.
			48	-15.4	10.56	n.s.
		1l/100l	24	60.4	9.50	*
			48	76.4	7.02	*
Predator mite	Adult	0.5l/100l	24	0.5	2.11	n.s.
			48	2.0	5.50	n.s.
		1l/100l	24	5.1	2.49	n.s.
			48	3.7	2.65	n.s.
Mealybug	Mid-instar juvenile	0.5l/100l	24	1.0	1.00	n.s.
			48	-10.0	1.11	***
		1l/100l	24	9.4	4.69	*
			48	11.2	7.24	*

4.5. CONFIDOR:

The calculated mortality values for Confidor at rates of 50g/100l and 100g/100l are shown in Table 7.

Table 7: Insect mortality values for Confidor.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	50g/100l	24	2.2	3.95	***
			48	3.2	3.63	n.s.
		100g/100l	24	8.9	6.99	n.s.
			48	19.5	5.15	*
Diamondback Moth	1st Instar Larva	50g/100l	24	94.7	3.38	*
			48	94.7	3.38	*
		100g/100l	24	97.7	2.29	*
			48	100.0	0.00	-
Codling Moth	1st Instar Larva	50g/100l	24	80.3	9.57	*
			48	100.0	0.00	-
		100g/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Pea aphid	Late Instar Nymph	50g/100l	24	62.1	25.27	***
			48	81.1	18.92	*
		100g/100l	24	82.0	5.19	*
			48	85.7	4.56	*
Greenhouse thrip	Adult	50g/100l	24	100.0	0.00	-
			48	100.0	0.00	-
		100g/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Predator mite	Adult	50g/100l	24	-6.7	3.10	***
			48	-11.4	3.99	*
		100g/100l	24	15.4	4.24	*
			48	-9.6	16.82	n.s.
Mealybug	Mid-instar juvenile	50g/100l	24	100.0	0.00	-
			48	100.0	0.00	-
		100g/100l	24	100.0	0.00	-
			48	100.0	0.00	-

4.6. AGRIMEC:

The calculated mortality values for Agrimec at rates of 18.8mls/100l and 37.5mls/100l are shown in Table 8.

Table 8: Insect mortality values for Agrimec.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	18.8mls/100l	24	44.6	4.34	*
			48	69.1	3.04	*
		37.5mls/100l	24	92.6	4.85	*
			48	94.2	3.58	*
Diamondback Moth	1st Instar Larva	18.8mls/100l	24	100.0	0.00	-
			48	100.0	0.00	-
		37.5mls/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Codling Moth	1st Instar Larva	18.8mls/100l	24	82.4	4.82	*
			48	82.9	3.81	*
		37.5mls/100l	24	98.4	1.07	*
			48	97.8	1.57	*
Pea aphid	Late Instar Nymph	18.8mls/100l	24	1.0	1.00	n.s.
			48	36.7	13.34	***
		37.5mls/100l	24	7.3	2.97	***
			48	73.3	14.84	*
Greenhouse thrip	Adult	18.8mls/100l	24	3.0	1.22	***
			48	18.3	5.30	*
		37.5mls/100l	24	49.2	8.14	*
			48	59.1	9.82	*
Predator mite	Adult	18.8mls/100l	24	6.9	2.06	*
			48	4.0	2.52	n.s.
		37.5mls/100l	24	5.8	4.73	n.s.
			48	27.1	0.96	*
Mealybug	Mid-instar juvenile	18.8mls/100l	24	0.0	0.00	-
			48	0.0	0.00	-
		37.5mls/100l	24	5.4	1.92	n.s.
			48	5.2	1.93	n.s.

4.7. ENCAPSULATED PYRETHRUM:

The calculated mortality values for Encapsulated Pyrethrum at rates of 250ml/100l and 500ml/100l are shown in Table 9.

Table 9: Insect mortality values for Encapsulated Pyrethrum.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	250ml/100l	24	61.5	2.96	*
			48	64.6	2.96	*
		500ml/100l	24	99.2	0.81	*
			48	100.0	0.00	-
Diamondback Moth	1st Instar Larva	250ml/100l	24	65.8	5.82	*
			48	75.3	8.67	*
		500ml/100l	24	87.5	1.96	*
			48	97.0	3.00	*
Codling Moth	1st Instar Larva	250ml/100l	24	93.6	4.92	*
			48	98.0	2.04	*
		500ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Pea aphid	Late Instar Nymph	250ml/100l	24	14.6	5.81	***
			48	3.8	21.81	n.s.
		500ml/100l	24	77.6	5.66	*
			48	80.0	15.88	*
Greenhouse thrip	Adult	250ml/100l	24	99.0	1.00	*
			48	99.0	1.00	*
		500ml/100l	24	100.0	0.00	-
			48	100.0	0.00	-
Predator mite	Adult	250ml/100l	24	6.8	4.90	n.s.
			48	12.9	6.62	n.s.
		500ml/100l	24	0.0	0.00	-
			48	21.0	4.77	*
Mealybug	Mid-instar juvenile	250ml/100l	24	1.0	2.12	n.s.
			48	6.0	3.80	n.s.
		500ml/100l	24	6.7	3.04	***
			48	7.7	2.63	*

4.8. ENCAPSULATED PYRETHRUM and NEEM:

The calculated mortality values for Encapsulated Pyrethrum and Neem at rates of 250ml/100l and 500ml/100l are shown in Table 10.

Table 10: Insect mortality values for Encapsulated Pyrethrum and Neem.

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	250ml/100l	24	65.7	5.54	*
			48	78.4	3.91	*
		500ml/100l	24	88.9	0.00	-
			48	94.7	1.66	*
Diamondback Moth	1st Instar Larva	250ml/100l	24	54.6	6.18	*
			48	54.6	6.18	*
		500ml/100l	24	62.5	10.16	*
			48	72.9	7.77	*
Codling Moth	1st Instar Larva	250ml/100l	24	70.1	2.24	*
			48	75.2	4.61	*
		500ml/100l	24	89.3	4.45	*
			48	92.7	3.51	*
Pea aphid	Late Instar Nymph	250ml/100l	24	5.0	2.04	***
			48	10.0	7.96	***
		500ml/100l	24	80.0	5.24	*
			48	82.6	4.92	*
Greenhouse thrip	Adult	250ml/100l	24	99.0	1.00	*
			48	100.0	0.00	*
		500ml/100l	24	99.0	1.05	*
			48	99.0	1.05	*
Predator mite	Adult	250ml/100l	24	26.6	9.32	*
			48	29.9	17.34	n.s.
		500ml/100l	24	8.2	6.05	n.s.
			48	33.5	7.66	*
Mealybug	Mid-instar juvenile	250ml/100l	24	0.0	0.00	*
			48	0.0	0.00	*
		500ml/100l	24	6.7	2.13	*
			48	8.7	2.74	*

4.9. AZATIN and ECO-OIL:

The calculated mortality values for Azatin and Eco-oil applications at rates of 40g/100l and 250ml/100l , and 80g/100l and 500ml/100l respectively are shown in Table 11.

Table 11: Insect mortality values for Azatin plus Eco-oil .

Insect Species	Life Stage	Application Rate	Assessment Period (Hours After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	40g/100l and 250ml/100l	24	-60.2	27.09	***
			48	-121.1	36.72	*
		80g/100l and 500ml/100l	24	-10.6	15.67	n.s.
			48	18.6	27.44	n.s.
Diamondback Moth	1st Instar Larva	40g/100l and 250ml/100l	24	8.1	1.19	*
			48	17.5	5.74	*
		80g/100l and 500ml/100l	24	-1.9	1.21	n.s.
			48	18.4	6.23	*
Codling Moth	1st Instar Larva	40g/100l and 250ml/100l	24	24.0	8.88	***
			48	32.3	19.2	n.s.
		80g/100l and 500ml/100l	24	17.3	3.64	*
			48	20.4	5.04	*
Pea aphid	Late Instar Nymph	40g/100l and 250ml/100l	24	0.0	0.00	-
			48	0.0	0.00	-
		80g/100l and 500ml/100l	24	-4.0	1.02	*
			48	-4.5	6.61	n.s.
Greenhouse thrip	Adult	40g/100l and 250ml/100l	24	36.0	19.78	n.s.
			48	20.0	15.08	n.s.
		80g/100l and 500ml/100l	24	3.0	1.22	***
			48	4.0	1.87	***
Predator mite	Adult	40g/100l and 250ml/100l	24	34.4	11.18	*
			48	43.6	12.01	*
		80g/100l and 500ml/100l	24	65.8	9.00	*
			48	71.6	7.59	*
Mealybug	Mid-instar juvenile	40g/100l and 250ml/100l	24	0.0	0.00	*
			48	0.0	0.00	*
		80g/100l and 500ml/100l	24	1.7	1.29	n.s.
			48	7.4	2.10	*

4.10. AZATIN:

The calculated mortality values for Azatin at rates of 40g/100l and 80g/100l are shown in Table 12.

Table 12: Insect mortality values for Azatin.

Insect Species	Life Stage	Application Rate	Assessment Period (Days After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	40g/100l	2	2.0	2.02	n.s.
			14	1.3	2.78	n.s.
		80g/100l	2	3.6	1.60	***
			14	27.1	9.76	*
Diamondback Moth	1st Instar Larva	40g/100l	2	6.5	4.25	n.s.
			14	40.0	81.68	n.s.
		80g/100l	2	20.9	9.15	***
			14	95.5	4.55	*
Codling Moth	1st Instar Larva	40g/100l	2	39.5	6.11	*
			14	89.3	10.67	*
		80g/100l	2	10.0	9.61	n.s.
			14	92.9	3.91	*
Pea aphid	Early Instar Nymph	40g/100l	2	0.0	0.00	n.s.
			5	0.0	0.00	n.s.
		80g/100l	2	4.7	5.33	n.s.
			4	32.6	6.63	n.s.
Greenhouse thrip	Adult	40g/100l	2	0.0	0.00	-
			8	0.0	0.00	-
		80g/100l	2	0.0	0.00	-
			8	0.0	0.00	-
Predator mite	Neonate Larvae	40g/100l	2	0.0	0.00	-
			9	3.2	1.43	***
		80g/100l	2	0.0	0.00	-
			9	0.0	0.00	-

4.11. NEEMAZAL-T/S:

The calculated mortality values for NeemAzal-T/S at rates of 250ml/100l and 500ml/100l are shown in Table 13.

Table 13: Insect mortality values for NeemAzal-T/S.

Insect Species	Life Stage	Application Rate	Assessment Period (Days After Application)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Lightbrown Apple Moth	1st Instar Larva	250ml/100l	2	3.7	1.77	n.s.
			14	17.6	9.17	n.s.
		500ml/100l	2	6.3	1.98	*
			14	36.9	6.55	*
Diamondback Moth	1st Instar Larva	250ml/100l	2	38.4	6.96	*
			14	80.7	6.18	*
		500ml/100l	2	3.0	3.94	n.s.
			14	91.5	2.60	*
Codling Moth	1st Instar Larva	250ml/100l	2	19.1	1.97	*
			14	85.7	14.29	*
		500ml/100l	2	43.0	9.30	*
			14	68.6	22.91	*
Pea aphid	Late Instar Nymph	250ml/100l	2	0.0	0.00	-
			5	0.0	0.00	-
		500ml/100l	2	6.0	5.59	n.s.
			5	-36.6	41.01	n.s.
Greenhouse thrip	Adult	250ml/100l	2	0.0	0.00	-
			8	0.0	0.00	-
		500ml/100l	2	0.0	0.00	-
			8	0.0	0.00	-
Predator mite	Neonate larvae	250ml/100l	2	-12.4	1.78	*
			9	-9.27	3.04	*
		500ml/100l	2	-1.94	3.31	n.s.
			9	-1.94	3.31	n.s.

CHAPTER FIVE

DISCUSSION

The format of this chapter follows the same order as presented in the result section. It interprets the results and evaluates the methods used, and compares these to similar past studies including M. Grassam's report to Yates NZ Ltd, 1994.

Pesticide induced mortality varies with many factors such as species, sex, life-stage, and environmental temperature of the test insect. For instance, generally, mortality of pesticide treated insects decreases with increasing temperature. These factors need to be taken into consideration when making comparisons of mortality values obtained by various different research programmes. Comparisons with Melanie Grassam's report are often undertaken in this discussion. After treatment, she held pesticide treated insects at 20° C ± 1°C. For this report, pesticide treated insects were held at 21.5 °C ± 1°C for all test insects other than for the predator mite (as previously mentioned).

For the purpose of summarising insect mortality values, the efficacy of pesticides has been assigned five categories as defined below.

Table 14: Efficacy categories for insect mortality values.

Percentage mortality (corrected)	Efficacy category
0	no effect
10	minimal
50	slight
50-80	moderate
80	marked

It is important to note that as *P. persimilis* is a biocontrol agent which preys exclusively on phytophagous spider mites such as *Tetranychus* spp; that for *P. persimilis* the most desirable outcome of pesticide applications is for no mortality to occur.

5.1. YATES PYRETHRUM:

Pyrethrum application has been reported to control a broad range of insect pests including aphids, mealybugs, and caterpillars. Most of these findings are supported by previous research as well as by research undertaken in this report. Table 3 shows that at both rates of 250ml/100L and 500ml/100L, Yates Pyrethrum has a marked effect on first instar larvae of the lightbrown apple moth, diamondback moth, and codling moth; as well as on adult Greenhouse thrips and adult pea aphids. Similar efficacy results for Greenhouse thrips were found by Blank & Gill (1995) in contact toxicity trials. They found that Yates Pyrethrum at 3.5 g.a.i./100L and 7g.a.i./100L gave significantly marked mortality (100%) for three life stages. This suggests that for these insect pests , effective mortality can be achieved through a 50% reduction in active ingredient quantity. Further, these results also mimic the results given in Melanie Grassam's report which examined mortalities after Yates Pyrethrum applications for lightbrown apple moth, diamondback moth, pea aphid, and New Zealand Flower thrips at the same life-stages as tested in this report. This earlier report also showed marked mortality for all these species at both 250ml/100L and 500ml/100L applications with the exception of the former rate in which efficacy was only moderate for the lightbrown apple moth.

Mortalities for the obscure mealybugs were not as promising. Even with the addition

of the synergist piperonyl butoxide to the Yates formulation, no significant difference in mortality was achieved with either 250ml/100L or 500ml/100L applications despite the claim that Yates Pyrethrum controls mealybug. These results differ somewhat from Epenhuijsen et al's report (1992), in which 0.5% spray applications on another mealybug species, the long-tailed mealybug, had a slight effect on mortality of crawlers and nymphs.

Moderate control of the beneficial predator mite was achieved.

Yates Pyrethrum works rapidly on insects to cause mortality, as expected. Increases in mortality as assessed at 24 and 48 hours were very small for most of the species tested.

5.2. ECO-OIL:

Although Eco-oil reportedly controls aphids, thrips and mealybug amongst various other species, this report reveals that its associated efficacy is at best moderate for adult Greenhouse thrips at 500ml/100L applications with no significant effects at half this rate. Further, for obscure mealybug nymphs, after 48 hours only minimal and slightly greater than minimal percentage mortality was achieved (5.0 and 15.0 % at half and full rates as outlined in Table 4.).

Finally, for adult pea aphids at the application rate of 500ml/100L there was no significant effect on mortality after 48 hours.

For all the other species tested, results indicated no significant mortality at both field rates for lightbrown apple moth and codling moth first instar larvae, and the greatest mean mortality for diamondback moth, for which slight, and significant, mortality was

achieved at 500ml/100L.

Only minimal efficacy (5.7%) at the 500ml/100L rate was observed for the predator mite.

5.3. DEFENDER:

Yates Defender has been reported to control a range of soft bodied insects including aphids and thrips. Nevertheless, although results showed marked mortality within 24 hours of application for the pea aphid at the 2L/100L rate only, at best moderate mortality occurred for the adult Greenhouse thrips after 48 hours, but at both the 1L/100L and 2L/100L rates. Also, very little increase in mean mortality was achieved for the latter species amongst rates and/or times.

For the first instar larvae of the species including lightbrown apple moth, diamondback moth and codling moth, only slight efficacy of Defender was shown for both rates after 48 hours.

No significant mortality for predator mites was shown, as was expected.

Slight efficacy of Defender was shown for the mealybug, and there was almost no difference in mortality amongst either rates or times. These results agree with the research by Epenhuijsen et al (1992) for which slight efficacy (17%) for crawlers and nymphs was recorded for 2% Defender spray applications on the long-tailed mealybug.

Overall, the results indicate that Defender works rapidly as little change in mortality was achieved over time. Further, for the adult aphids, only, was a significant increase in mortality observed between 1L/100L and 2L/100L rates, suggesting that for all other species a reduction in 50% of active ingredient quantity would achieve no changes to their corresponding mortalities.

5.4. ORCHEX:

Orchex has been reported to provide effective control of specified insects and mites.

Moderate mortality of diamondback larvae, pea aphid and Greenhouse thrips was achieved at 1.0L/100L applications of Orchex 692, but was slight or not significant at applications of 0.5L/100L.

Slight mortality of 1st instar lightbrown apple moth and codling moth larvae was achieved for half rate applications. No significant mortality was shown for the mealybug. For all species except codling moth, significantly greater mortality occurred at 1L/100L rates of Orchex as opposed to the consequent mortality at half this rate.

No significant mortality was observed for the predator mite.

There did not appear to be much change in mortality over time for all of the test species.

5.5. CONFIDOR:

Confidor's active ingredient, imidacloprid, is highly effective against sucking insects such as aphids, thrips and mealybugs. Elbert et al (1991) found that imidacloprid was

extremely effective against *Myzus persicae* and *Aphis gossypii* aphids as well as against *Hercinothrips femoralis* thrips and *Pseudococcus comstocki* mealybugs; was very effective against biting insects such as certain species of the Lepidoptera and Coleoptera. Due to imidacloprid not having any acaricidal activity, no adverse effects have been shown to occur with predator mites upon Confidor application .

Marked efficacy of Confidor application at 100g/100L application rates was obtained for Greenhouse thrips, mealybug, codling moth, and diamondback moth (all 100% mortality), as well as for the pea aphid although mortality (86%) was slightly reduced for this species as was expected. Mortality to the lightbrown apple moth was slight.

There was no significant mortality to the predator mite at 100g/100L application rates.

Most of these results were expected, but there was some discrepancies in results with Melanie Grassam's report in which marked mortality occurred at full rate applications for lightbrown apple moth and only slight mortality occurred for diamondback moth bioassays. It seems unlikely that diamondback moth mortality would indeed be so low. Elbert et al (1991) found that approximately 250ppm of imidacloprid in a foliar application resulted in 95% mortality of *P. xylostella*.

50g/100L applications of Confidor resulted in marked mortality of mealybug, thrip, codling moth, diamondback moth, and pea aphid bioassays. This suggests that for these insect pests, effective mortality can be achieved through a reduction in Confidor's active ingredient quantity.

Confidor has been shown in this report to cause rapid mortality, with very little

increase in mortality between 24 and 48 hours for most species.

5.6. AGRIMEC:

Agrimec shows high innate pesticidal activity only against specific insect pests.
In this report, at both half and full rates, mortality was marked for both diamondback and codling moth bioassays resulting in 100% mortality for the former species. This suggests that for these insect pests, marked mortality could be achieved through a 50% reduction in active ingredient quantity. Reed et al (1985) suggested that "Avermectin B1, may in some manner, prevent neonate larvae from feeding. This may be due to non-functional mouthparts because of the physiological action of avermectin, rather than an antifeedant activity". Indeed at both the full and half rates it was observed that larvae of diamondback, codling and lightbrown apple moths has not burrowed into their food discs in the spray replicates as they has done in their controls.

Lightbrown apple moth mortality was also marked, but only at the full 37.5mls/100L rate, as it was at best moderate at the half rate. This was to be expected and compares favourably with research by Suckling et al (1985). They found that in Agrimec field spray trials of seven day old lightbrown apple moth larvae on potted apple trees, that marked mortality (91%) at field rates and moderate mortality (68%) at 36% field rate applications occurred.

Both pea aphid and thrip bioassays showed slight mortality at half rate applications, and at best, moderate mortality at the full rate.

The effect of Agrimec on the obscure mealybug was only evident at full rate applications, but was still minimal (5.4%).

It was expected that Agrimec effects would be fairly minimal on predator mites as Agrimec is compatible with IPM programmes, especially if releases of the active stages of this species occurs after treatment (Trumble & Morse 1993). However, Agrimec has been reported to depress numbers temporarily". Indeed, significant mortality occurred at only half the full rate treatment, and was slight as it resulted in 27% mortality.

As abamectin is slow acting, assessment should take account of the time required for abamectin (and other IGR'S) to work, usually several days. In this report, mortality was assessed after 24 and 48 hours. It is suggested that it would be of interest to assess mortality at 94 hours at least, in order to fully determine the efficacy of Agrimec.

5.7. ENCAPSULATED PYRETHRUM:

As previously discussed in section 2.1; pyrethrum application has been reported to control a broad range of insect pests including aphids, mealybug, and caterpillars. Table 9 shows that Encapsulated Pyrethrum had a marked effect on thrip and codling moth populations at both tested rates suggesting that for these insect pests, effective mortality can be achieved through a 50% reduction in active ingredient quantity. At the full rate only, marked mortality was shown for lightbrown apple moth,

diamondback moth and pea aphid bioassays. At half this rate, the first two populations showed moderate mortality upon application of this pesticide, while no significant mortality occurred for the pea aphid populations.

Only minimal mortality was achieved at full rate applications to the mealybug nymphs, (this was unexpected).

Slight efficacy at the full rate for the adult predator mite was achieved.

A comparison of Encapsulated Pyrethrum with Yates Pyrethrum is of interest as the latter has the same pyrethrin content as Encapsulated Pyrethrum plus the addition of the synergist PBO. Therefore the part that PBO plays in increasing mortality can be examined. There was no apparent difference in efficacy at both half and full rates for thrips and codling moth amongst Encapsulated Pyrethrum and Yates Pyrethrum applications as both resulted in marked mortality of these populations. In fact, at 250ml/100L, applications of Encapsulated Pyrethrum resulted in a slightly higher mortality for codling moth than for applications of Yates Pyrethrum. These results suggest that the addition of PBO to this pyrethrum insecticide does not result in a corresponding increase in the mortality of either adult thrips or codling moth.

As there was no significant mortality at both half and full rate applications for mealybugs which had been treated by either Yates Pyrethrum or Encapsulated Pyrethrum, it can be concluded that this population is highly resistant to pyrethrum.
For all other species, a Yates Pyrethrum application resulted in higher mortalities than

a corresponding Encapsulated Pyrethrum application at the same rate. Marked mortality was shown for half rate Yates Pyrethrum applications for lightbrown apple moth, aphid, and diamondback moth populations (93.0, 100.0, and 100.0 % respectively). However, such a level of efficacy could only be achieved by full rate applications of Encapsulated Pyrethrum for lightbrown apple moth, aphid, and diamondback moth populations, resulting in 100.0, 80.0, and 97.0 % mortalities respectively. It therefore appears that for these species, only half the amount of active ingredient (i.e. pyrethrin) is required to result in the same degree of efficacy that is obtained for Encapsulated Pyrethrum so long as PBO is added in the ratio 1:4 parts pyrethrin:PBO.

Full rate application of Encapsulated Pyrethrum resulted in only slight mortality for predator mites while half rate applications of Yates Pyrethrum resulted in close to marked mortality (77%).

5.8. ENCAPSULATED PYRETHRUM and NEEM:

This formulation contains the same concentration of pyrethrins as Encapsulated Pyrethrum outlined in 5.7. i.e. 1.4%, as well as the addition of 0.1% neem.

As previously mentioned, Neem has Insect Growth Regulator Activity and azadirachtin treated insects are not directly killed by the application of this compound, but succumb to the physiological and behavioural stresses and starvation that results from its use (Saxena ,1989). Therefore as a result of insect mortality being recorded at 24 hours and 48 hours, it is to be expected that no increase in mortality for a given rate will be achieved for Encapsulated Pyrethrum and Neem applications in comparison

with Encapsulated Pyrethrum treatments. This was shown to be the case for all of the insect pest species tested.

The only increases in efficacy occurred for the beneficial adult predator mites at half rate applications of Encapsulated Pyrethrum and Neem as at this rate, slight mortality was shown for this pesticide. No significant mortality was observed for the corresponding Encapsulated Pyrethrum application.

It is recommended that if the effects of the addition of neem based products on mortality is to be examined that mortality should be assessed after a greater period of time than 48 hours.

5.9. AZATIN plus ECO-OIL:

Table 15 below examines the effects on mortality of the addition of Azatin to Eco-oil as opposed to their individual effects. Only those bioassays that have shown higher mortality in the Azatin pus Eco-oil trials have been included. For the remaining species, there was no significant difference in mortality.

Table 15: Insect mortality criterion for given species and rates for Eco-oil; Azatin; and Azatin plus Eco-oil after 48 hours.

SPECIES	RATE	ECO-OIL	AZATIN	ECO-OIL PLUS AZATIN	LEVEL OF SIGNIFICANCE
Diamondback moth	Half	No effect	No significant effect	Slight (17.5%)	*
Codling moth	Full	No significant effect	No significant effect	Slight (20.4%)	*
Mite	Half	No significant effect	No significant effect	Slight (43.6%)	*
	Full	Minimal (5.7%)	No significant effect	Moderate-marked (71.6%)	*

It was expected that the Azatin/Eco-oil combined pesticide might show an increased effect after only 48 hours for both diamondback moth and codling moth (i.e. an immediate, rather than delayed effect on mortality) as for these species only, Azatin application had resulted in some degree of mortality within this time period.

The results obtained for the predator mite combined pesticide bioassays were far from expected. Individually both Eco-oil and Azatin at either rates showed either no significant or minimal effects. However a huge increase in mortality was observed when these two pesticides were combined. This suggests some sort of interaction between these two pesticides is occurring, which at the full rate results in close to marked mortality for this species. This warrants further investigation.

5.10. AZATIN:

Azatin's active ingredient, azadirachtin, is reported to have a broad spectrum of

insect control. However, marked mortality at field rates was only shown by codling moth and diamondback moth larvae.

Mortality was shown to be marked for codling moth at both full rate and half rate applications. This compares favourably with results obtained by Burballa and Sarasua (1995) in laboratory studies examining the effect of Azatin incorporated in codling moth larval diet, on feeding. They observed an altered feeding behaviour of codling moth larvae, causing larvae not to penetrate their diet medium, i.e. a repellent or antifeedant effect. Also, larvae which fed on neem extracts were smaller and had a longer development time than for untreated larvae. For this report, such observations were made for codling moth, diamondback moth and lightbrown apple moth populations. The results for codling moth suggested that effective mortality could be achieved through a 50% reduction in active ingredient quantity.

Diamondback moth bioassays also showed marked (or close to marked) mortality, but only at the full 80g/100L rate. The mortality level achieved for diamond back moth is similar to that obtained by Streets (1976), where crude extracts containing 2.5 and 5.0 percent azadirachtin revealed high mortality of 5th instar diamondback moth larvae during the first week after spraying of cabbage leaf discs serving as food. Only one individual in the 2.5% treatment reached the pupal stage, in which it died. Ruscoe (1972) in spray trials with 3rd instar larvae at 10ppm/L rates on cabbage leaf discs observed 100% mortality of diamondback moth, most during the next moult. Interestingly, slight mortality (20.9%) was observed within 48 hours of application for full rate application for the diamondback bioassays and for the half rate codling

bioassay only, suggesting that azadirachtin causes a significant, immediate toxic effect (as opposed to delayed IGR activity) for these species. The pea aphid mortality results are much higher than for Melanie Grassam's report in which field rate applications of Azatin achieved no significant mortality.

Light brown apple moth mortality is also dissimilar to that shown in Melanie Grassam's report where 100% mortality had occurred after 14 days.

Nevertheless, no significant mortality was shown for the thrip, or pea aphid species tested in this or Melanie Grassam's report, and therefore in this case results compare favourably. These results were expected. Ascher at al (1992) found that adult western flower thrips (*Frankliniella occidentalis*), when introduced for 24 hours to seedlings that had been sprayed on the previous day and removed, showed no mortality. However, at low concentrations, Azatin prevented development of thrips from 1st to 2nd nymphal instar.

Neem is claimed to have low toxicity to beneficial insects and natural enemies and to be compatible with predator mites. Mansour et al (1987) found no significant mortality of adult predator mites were caused by one hour old residue tests of neem extracts containing 0.05% neem. However, although both full and half rate applications of Azatin showed no significant consequent reduction in ability of larvae to reach the adult stage, and no significant mortality of larvae, it was observed that the number of predator mite eggs that hatched, which has been sprayed with their leaf discs were lower in the spray replicates than in the controls. Further, the number of

eggs laid by predator mites which had developed into adults had significantly decreased. Very little information exists on the influences of neem ingredients on insect eggs. From the data available at present, it may be concluded that applications of high concentrations result in slight effects if any. As yet, no published research has been carried out to determine the effect of neem on predator mite eggs. The observation that the number of eggs laid per adult had decreased may be expected. "In many female insects ovulation has also become rare with treatment with azadirachtin, with the oocytes and ovaries shrinking and the vitellarium and oviducts being absorbed" (Schmutterer 1990; Saxena 1989).

5.11. NEEMAZAL:

NeemAzal also contains the active ingredient azadirachtin, but at the rates tested in this report, the concentration of azadirachtin in NeemAzal is 1.25 times the concentration of azadirachtin in Azatin.

An increase in mortality in comparison to the results obtained for Azatin was observed for diamondback moth bioassays at the half rate from no significant mortality to marked mortality after 14 days. This appears to be the result of the addition of azadirachtin at a higher concentration. Further, at half rate applications of NeemAzal, slight (38.4%) mortality was achieved within 48 hours of pesticide application for the diamondback moth. This compares favourably with results obtained for Azatin, where slight mortality was observed within 48 hours at full rate applications.

The decrease in codling moth mortality for full rate NeemAzal in comparison to full rate Azatin was unexpected. However, this may be accounted for by the large standard error obtained for mean percentage mortality in the NeemAzal bioassay.

No changes in mortality (between Azatin and NeemAzal applications) were shown for lightbrown apple moth, pea aphid, or greenhouse thrip pest species.

No changes were also found for the predator mite.

5.12. EVALUATION OF THE METHODOLOGY USED IN THE POTTER TOWER INSECT BIOASSAY:

Although laboratory bioassays are an essential and invaluable tool in understanding the action of pesticides, they have their limitations. Indeed, mortalities achieved in laboratory pesticide bioassays only give an indication of mortalities that will be achieved with these pesticides in the field.

When testing pesticides it is preferable that replication of bioassays occur. The purpose of replication is to randomise effects related to uncontrollable laboratory procedures and conditions so that experimental error can be estimated. Experimental error includes effects caused by unexplained correlations among the subjects in the same treatment, as well as unexplained variation that occurs each time an experiment is carried out. A true estimate of experimental error can only be obtained by testing more than one treatment group with the same dose in the same experiment and repeating the experiment at least twice more at different times but under the same test

conditions as possible. Unfortunately, due to the large number of tests involved in this report, it was not feasible to test either control or spray replicate mortalities at different times.

Another reason for replication is to detect errors in formulation (i.e. in the preparation of the treatments that are tested). This, as well as incomplete cleaning of the Potter Tower between different spray treatments may have resulted in the observed high variation in control mortality between treatments. This variation was particularly evident for the diamondback moth and pea aphid bioassays. However, there was almost no variation observed in control mortalities for the obscure mealybug. Interestingly, replicate mortalities for the obscure mealybug for different pesticides was either low or not significant while replicate mortalities for the pea aphid and diamondback moth were often marked. Therefore it seems reasonable to conclude that incomplete cleaning of the Potter Tower between spray treatments may have played a major part in control mortality. Indeed, the design of the Potter Tower is such that cleaning of the Potter Tower was very difficult.

Nevertheless, it is important to remember that replicate mortality has been corrected with Abbot's formula to allow for control mortality.

Finally, when control treatments are tested, they must include everything except the pesticide. However, as the control treatments in this report were comprised of water only, it is important to recognise that although these series of tests are spraying different pesticides, that they are actually testing the action of each pesticide's active ingredient.

CHAPTER SIX

CONCLUSION

From the experiments undertaken the following conclusions were drawn.

1). Yates Pyrethrum gave marked control of lightbrown apple moth, diamondback moth, and codling moth larvae; as well as of adult greenhouse thrip and pea aphids at half the recommended field rate. No mortality of mid-instar mealybug or adult predator mites was achieved.

2). Eco-oil did not give marked control of any of the species tested. At best, moderate efficacy was shown, but only for the adult greenhouse thrip. Minimal mortality was observed for the adult predator mite.

3). Defender gave marked control of the late-instar of the pea aphid, but gave poor control of the remaining test insect species. No significant mortality was shown for the adult predator mite.

4). Orchex did not give marked control of any of the species tested. At best, moderate efficacy was shown but only for the larvae of the diamondback moth, late-instars of the pea aphid and the adult greenhouse thrip. No significant mortality was observed for the adult predator mite.

5). Confidor gave marked control of the adult greenhouse thrip, mid-instar mealybug, late-instar pea aphid and first instar larvae of the codling moth and diamondback moth. No significant mortality of the adult predator mite was observed.

6). Agrimec gave marked control of the first instar diamondback, codling, and lightbrown apple moth. Slight mortality of the adult predator mite was observed.

7). Encapsulated Pyrethrum gave marked control of the adult thrip, larvae of the codling moth and lightbrown apple moth, and the late-instar pea aphid. Slight mortality of the adult predator mite was observed.

8). Encapsulated Pyrethrum and Neem gave the same level of control for all the species tested as afforded by Encapsulated Pyrethrum, other than for the adult predator mites where slight mortality was observed at half the recommended field rate.

9). Azatin plus Eco-oil gave the same level of control for all the species tested as afforded by Eco-oil alone, other than for notably the adult predator mite, as well as the larvae of the diamondback moth and the codling moth. A huge comparative increase in the level of control of the adult predator mite was observed and warrants further investigation.

10). Azatin gave marked control of the larvae of the codling moth and diamondback moth. No significant reduction in the ability of larvae of the predator mite to reach the adult stage was observed. Further, no significant mortality was shown for the developed adult predator mite, although a reduction in the number of eggs laid was observed.

11). NeemAzal gave the same comparative level of mortality to Azatin.

PART B

Determining the efficacy of dill and sesame oil synergists in enhancing the toxicity of pyrethrum against the pea aphid using a Potter Tower.

CHAPTER ONE

INTRODUCTION

The most frequently used synergist at present, piperonyl butoxide (PBO) is synthetic (Wachs 1949) . As the ability of a synergist to be "environmentally friendly" or organic is now becoming more desirable with changing consumer attitudes (Hall et al 1989), there is a growing need for their synergists to be organic also, even though they may be less cost effective than the alternative synergists.

The aim of this study was to undertake an assessment of the efficacy of sesame oil and a crystalline extract of its crude extract, as well as of the dillapiole fraction of dill oil, as synergists for natural pyrethrum against the pea aphid *Acyrthosiphon pisum.* Further, the efficacy of the synthetic pyrethrum synergist piperonyl butoxide (PBO) was also examined, in order to compare relative synergistic abilities of the widely used synthetic synergist PBO with the experimental natural synergists tested in this report.

ABSTRACT

Sesame oil, the crystalline extract of a sesame oil crude extract, dillapiole, and the synthetic synergist PBO, were examined for their ability to synergise natural pyrethrum in spray emulsions at a variety of rates and ratios against the pea aphid *Acyrthosiphon pisum.* Testing was carried out under laboratory conditions using a Potter tower technique.

Sesame oil showed slight synergistic abilities, the crystalline extract of a sesame oil crude extract showed moderate-marked synergistic abilities, PBO showed moderate synergistic abilities at the 1:1 ratio of natural pyrethrum:PBO, dillapiole showed marked synergistic abilities for natural pyrethrum.

It is suggested that the use of the synthetic synergist PBO could be replaced by the alternative natural synergists including dillapiole, as well as the crystalline extract of a sesame oil crude extract at the highest ratio that was tested.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. INTRODUCTION:

This chapter describes the synergists that are tested in this report and also reviews relevant associated past literature for all the synergists tested except for PBO.

A search of biological abstracts from 1987 to September 1997 and current contents (CAB winSPIRS) from mid 1985 to the present for "pyrethrum" and "sesame"resulted in no matches.

2.2. SESAME OIL:

The seeds of cultivated *Sesamum indicum* L. (also known as *S. orientale* L.) yield sesame oil, otherwise known as till (India), gingelly (India), simsim (Africa), benne (Africa), or ajonjoli (Latin America) as do a number of wild *Sesamum* species (Swingle 1945), (Pearman et al 1951). Sesame oil is an edible source of nutritious protein food for human consumption, and is one of the oldest oilseeds known to man..

Sesame oil was the first pyrethroid synergist to be discovered, and was thus patented, in 1940 by Eagleson as a result of the screening of 42 animal and vegetable oils as constituents of livestock and household sprays (Eagleson 1942), in which only sesame oil enhanced the toxicity of pyrethrum. Since then, sesame oil has been found to markedly increase the effectiveness of natural and synthetic pyrethrum on a range of insect pests.

Eagleson first recorded that the addition of 1 to 5% of sesame oil to a kerosene solution of pyrethrum increased its effectiveness against houseflies *Musca domestica* (Eagleson 1942) in an insecticidal spray. This occurred apparently as a result of the production of a greater initial effect per unit amount of toxicant, and prolongation of the duration of that effect, making recovery impossible for most of the affected insects. That the increase in toxicity was due to a synergistic effect, and not to the addition of the insecticide pyrethrum, was shown by the failure of sesame oil alone to kill flies. Dichlorodifluoromethane solutions of pyrethrum and sesame oil in aerosols have also been found to be toxic to the adult cheese skipper *Piophila casei* (Billings et al 1942); to adult *Culex*, *Anopheles*, and *Aedes* mosquitoes, to several species of flies (*Haematobia*, *Stromoxys*, and *Musca*), and to roaches and bed bugs (Sullivan et al 1942).

According to Eagleson " data from other experiments show that 5% of the total volume is about the optimum quantity of sesame oil for addition to a fly spray. Those compounded with 10% of sesame oil have not proved any more effective". The addition of 5% of sesame oil to pyrethrum saved about 50% of pyrethrum.

Following Eagleson's discovery, Haller et al (1942) carried out a chemical study of sesame oil. They separated the active ingredient sesamin in a crystalline form from the first two fractions of a high vacuum distillation of sesame oil. Although sesame oil was synergistic to pyrethrins against houseflies, sesamin was found to be a greater synergist, as the addition of the combined first and second fraction, at the same concentration, resulted in approximately one and a half times the mortality of the pyrethrins plus sesame oil insecticide. Although this superiority was found to be the

case for work by (Parkin and Green 1944), they found that the addition of only 0.05% w/v sesamin to pyrethrins resulted in the same mortality as the addition of 5% w/v sesame oil.

The non-crystalline fraction of sesame oil in the work of Haller et al (1942) (above) also showed considerable synergistic activity. Later it was revealed by other researchers that sesame oil in fact appeared to contain more than one synergist (Parkin and Green 1944). Examination of chromatographic fractions of sesame oil, (Beroza 1954; Erdtman et al 1955; Haslam et al 1955), yielded two pyrethrum synergists, sesamin and sesamolin (Simanton 1949).

As previously mentioned in the introduction, both sesamin and sesamolin are MDP synergists. The structures of both synergists are very similar (see Appendix 2), sesamolin differing from sesamin by having a methylenedioxyphenyl group attached to the central nucleus through an oxygen atom (Haslam & Haworth 1955; Beroza 1, 1955; Erdtman & Pelchowicz 1955), that is it has a methylenedioxyphenoxy group in place of one of the methylenedioxyphenyl groups of sesamin. Never the less, despite the slight structural dissimilarities, marked differences in synergistic abilities have been observed between sesamin and sesamolin. Gersdorff et al (1954) found that sesamolin, which had not been known to be synergistic, was found to be about five times as effective as sesamin against the housefly, an equiproportional mixture of sesamolin and natural pyrethrins increasing the insecticidal value of the latter 31 times. Thus sesamolin "is a far more effective synergist for natural pyrethrins than the best commercial synergist". The difference in synergistic abilities between sesamin and sesamolin resulted in a series of tests of the synergistic, synthetic

methylenedioxyphenoxy derivatives, one of these being PBO, and is beyond the scope of this project.

Neither sesamin nor sesamolin have been found in any other vegetable oil although a number of plant materials have been shown to contain related compounds (Haller 1947), as they have the 3, 4-methylenedioxyphenyl grouping in their structures. These include amongst others asarinin, found in various oriental plants and in the bark of American prickly ash; pinoresinol, a constituent of the exudate of spruce and related species; and eudesamin, a constituent of kino gum from eucalyptus.

2.2.1. The synergistic content of cultivated sesame oil:

The amounts of each synergist in sesame oil in a given crop is effected by many factors including strain, location grown, ageing, and frost damage (Beroza & Kinman 1955).

Parkin and Green (1944) estimated that the normal sesamin content of oil is approximately 1%, while Budowski 2 (1950) reported that four crude oils obtained from different varieties of sesame seed contained 0.3-0.5% sesamolin. On the basis of these figures and the knowledge that sesamolin is about five times as effective as sesamin, Budowski concluded that "most of the synergistic action of sesame oil with pyrethrins is due to sesamolin rather than sesamin".

The quantitative determination of these synergists can be carried out by chromatographic analysis (Beroza , 1954).

The method of commercial preparation of sesame oil also affects its synergistic content.

Commercial sesame oil contains a much lower synergistic component than non-refined sesame oil as a result of these processes.

Some of these note worthy processes include treatment with fuller's earth, during which processes a proportion of the sesamin originally present in non-refined oil is lost (Pearman et al 1951); further, bleached and hydrogenated sesame oils contain reduced concentrations of sesamolin compared to those contained in crude oil due to the splitting of sesamolin during these processes (Budowski et al 1950). It is preferable to have sesame oil extracted from the seed by solvent extraction in order to wholly retain the sesamin content of the oil.

2.3. DILL OIL

Anethum sowa L. (dill) or *A. graveolens* . a common herb of the Apiaceae family, has been used as a condiment in cooking for centuries. Both the seed as well as the leaves are used for culinary purposes worldwide.

The insecticidal and synergistic properties of dill seed extract were first reported by (Hartzell 1944), and later by (Lichtenstein et al 1974).

The subsequent isolation and identification of the main components from the insect-active extract of dill from various plant parts has been carried out by many researchers.

Two major compounds were revealed by (Su & Horvat 1988), in an investigation of the main components in insect-active dill seed extract. These were a carvone (d-carvone, 2-methyl-5-(1-methyl-phenyl)-2-cyclohexen-1-one); and an isomer of

apiole, dillapiole (4,6-dimethoxy-6-(2-propenyl)-1,3-benzodioxole) (Karrer 1958) (see below).

The major insecticidal principle obtained from dill greens (ie all aerial plant parts plus seeds) was characterised as d-carvone by (Lichtenstein et al 1974). This supported previous work by (Gladstone 1872) who reported that dill oil, prepared from seeds (a component of dill greens for the work of (Lichtenstein et al 1974) of dill plants, contained up to 60% of d-carvone. The presence of dillapiol in dill seeds was first reported by (Ciamician & Silber 1896), and although it was non-detectable in dill greens by (Lichtenstein et al 1974), the latter authors found that the major insecticidal and synergistic components in dill roots were apiol and dillapiol.

Dill is also a rich source of monoterpenoids, to which antifeedant and repellent effects have been attributed (Hough & Goldstein 1990).

2.3.1. DILL APIOLE

Dill apiole is found in dill oil and a number of other plant species.

Gulati and Parmar (1969) first reported, and patented dillapiole, one of the fractions of the petroleum ether extracts of dill seeds, to be a synergist for pyrethrins. This was later to be substantiated by (Singh et al 1976). They found that fraction 1V of a petroleum ether extract of *Anethum sowa* seeds, i.e. the dillapiole fraction, showed the highest factor of pyrethrin synergism (2.48), to the red flour beetle (*Tribolium castaneum*); while carvones and hydrocarbons richly found in fraction 1-1V failed to show any synergistic activity for this test insect. Lichtenstein et al (1974) reported similar biological activity in dill root extracts to houseflies (*Musca domestica*), and yellowfever mosquito larvae (*Aedes aegypti*).

Certain derivatives of dillapiole have been found to impart a greater synergistic activity than dillapiole. The dihydro derivative of dillapiole, dihydrodillapiole, was found to have superior synergistic activity to dillapiole at most levels in emulsion formulations against *Tribolium castaneum* (Parmar 1969); and with pyrethrins in dust formulations against *Tribolium castaneum* by about 9 to 11% (Handa & Dewan 1975). Further, the synergistic activity of dillapiole and dihydrodillapiole was enhanced through synthetic introductions at the vacant position in the aromatic ring and was comparable to synergism by PBO (Saxena & Sharma 1972).

Finally, mixed synergist studies using PBO combined with dillapiole and/or dihydrodillapiole in pyrethrin formulations, have shown knock-down rate results of houseflies *Musca domestica* to be similar to those for piperonyl butoxide (Parmar

1974) alone. This suggested that the use of piperonyl butoxide could be economised by using these test synergists with it. This enhanced synergism may have been the due to greater efficiency in blocking the sites of loss of the toxicant pyrethrum. In particular, combinations of dill apiole and dihydrodillapiole in pyrethrin formulations which did not contain PBO also showed results similar to PBO alone suggesting that the use of PBO could be dispensed with by using these test synergists.

2.4. PBO

PBO is a synergist for the pyrethrins and related synergists and is used with pyrethrins in ratios of 5:1 to 20:1, usually 8:1 by weight, either in solution, as aerosols, emulsions or dusts.

Yates Pyrethrum contains PBO in the ratio 1:4 pyrethrum:PBO.

CHAPTER THREE

MATERIALS AND METHODS

3.1. SOURCE AND ESTABLISHMENT OF TEST INSECTS

The source of the pea aphids (*Acyrthosiphon pisum*) used in these experiments were from an established laboratory colony, at Massey University. These were maintained on potted broadbean plants in a controlled atmosphere room, at 23°C with a 16:8 light:dark photoperiod, and were supplied to the population as required.

3.2. LABORATORY EXPERIMENT

3.2.1. Insecticide Treatment

Pyrethrum Extract (Pyr)
Crude extract of pyrethrum flowers was obtained from Yates N.Z. Ltd. and was reported to contain 50% pyrethrum.

Sesame Oil (SO)
Commercially available sesame oil (Healtheries cold pressed) was obtained and used. This oil is obtained from cultivated sesame seed by successive expressions in a hydraulic press, carried out in the cold. Healtheries cold pressed sesame oil is a high-grade oil, light in colour and agreeable in taste and odour.

Crystalline extract of crude sesame oil (SX)
A mixture of sesamin and sesamolin 2:1 was isolated in a yield of 0.3% from Healtheries cold pressed sesame oil by extraction, chromatography and recrystallisation (see Appendix 2 for extraction details). This was carried out by the

Gracefield Research Centre, Lower Hutt, New Zealand.

Dillapiole (DA)

Dillapiole was isolated in 6.5% yield from a commercially available dill oil (Auroma Therapy) by high vacuum distillation. This work was carried out by the Gracefield Research Centre, Lower Hutt, New Zealand (see Appendix 2).

Piperonyl Butoxide (PBO)

Commercially available PBO (Incite) was obtained and used.

Emulsifiable Concentrate

Emulsifiable concentrate formulation was carried out by Graysons Laboratories and Consulting, Auckland, New Zealand .

The pyrethrum only emulsifiable concentrate formulation consisted of:-

Pyrethrum crude extract (50% active)	0.0275 g (0.01375g of active pyrethrum)
Terric 200	0.109 g
Isopropyl alcohol	0.05 g
PEG 200	to 1ml

Crude pyrethrum extract (50%) was formulated with the different synergists in the proportions as indicated in Table 1. The emulsifiable concentrates were then diluted with water to give the range of tested concentrations of pyrethrum (50%) in the final spray given in Table 1. The concentrations for a given spray application were chosen from early ranging tests, not reported here, to cover a mortality range roughly within 5-95% of the test insects, and the mortality at these chosen concentrations were spaced roughly, as far as possible, at even intervals between 5 and 95%.

As only a limited amount of the crystalline extract of the sesame oil crude extract and dillapiole was available; the tests with these pyrethrin synergists unfortunately could not be as extensive as was desirable.

One control was applied immediately prior to testing of a given spray application treatment. This "control" consisted of all the ingredients found in the highest pyrethrum concentration spray application tested for a given synergist, minus pyrethrum. This was included in order to find out whether the emulsifying agents had any positive direct effects on mortality.

The range of concentrations of pyrethrum from lowest to highest for a given synergist were tested consecutively for a given spray application. Each spray application range was replicated five times for a given synergist and was prepared as fresh formulations for each replicate.

Table 1: The spray composition, ratios of pyrethrum:synergist, and application rates tested.

Spray composition	Ratio (Active Pyrethrum:synergist)	Application rate (mls/L)
Pyr (50%)	—	1.0; 3.0; 6.0; 9.0; 12.0; 15.0; 18.0; 21.0; 23.0
Pyr + (SO)	1:1	3.0; 6.0; 9.0; 12.0; 15.0; 18.0
	1:5	0.3; 1.0; 3.0; 6.0; 9.0; 12.0; 15.0; 18.0
	1:8	0.3; 1.0; 6.0; 9.0; 14.0; 15.0
Pyr + (SX)	1:1	2.1; 2.7; 3.3; 3.9; 4.5; 5.1
	1:5	0.3; 0.9; 1.5; 2.1; 2.4; 2.7
Pyr + (DA)	1:5	0.9; 1.2; 1.5; 1.8; 2.1; 2.4; 2.7
	1:10	0.6; 0.9; 1.2; 1.5; 1.8; 2.1
	1:15	0.3; 0.6 ; 0.9; 1.2; 1.5; 2.1
Pyr + (PBO)	1:1	1.5; 2.0; 2.5; 3.5; 4.5; 5.5
Controls		
– + SO	1:1	3.0; 18.0
– +SO	1:5	0.3; 18.0
– +SO	1:8	0.3; 15.0
– +SX	1:1	2.1; 5.1
– +SX	1:5	0.3; 2.7
– +DA	1:5	0.9; 2.7
– +DA	1:10	0.6; 2.1
– +DA	1:15	0.3; 2.1
– +PBO	1:1	1.5; 5.5

3.2.2. Bioassay Set Up

Whole broadbean leaves were placed, upper surface down, on damp filter paper in 90mm plastic Petri dishes. Forty mid-late instar nymphs of the pea aphid were placed, using a fine paint brush, on each leaf's lower surface.

3.2.3. Insecticide Application

The Potter precision tower was used to apply each insecticide treatment to the leaves. The operating instructions described by Potter (1941, 1952) for the Potter tower were followed throughout the experiment. The air pressure to operate the tower was maintained at 15 p.s.i. A standard volume of 2ml was sprayed onto each of the leaves and a settling period of 10 seconds was allowed after all the spray had been discharged from the Potter spray tower.

To eliminate spray contamination, precautions were taken between treatments to decontaminate the sprayer, i.e. two flushings with acetone followed by deionised water followed by hosing down of the spray column with tap water.

In order to eliminate any possible direct effects on mortality of circadian periodicity in response to the spray applications, all the insecticide applications were carried out within a defined two hour period.

3.2.4. Mortality Assessment

After treatment each Petri dish was held at 22.0° ± 1° C, and a 24:0 light:dark photoperiod. Mortality was assessed at 24 hours. Nymphs which failed to move one

body length after light prodding with a fine paint brush were recorded as "dead".

3.2.5. Analysis of Laboratory Results

The data were corrected (in response to control mortality) using Abbott's formula (Abbott, 1925) and the mortality responses to the various pyrethrum plus synergist treatments were subjected to probit linear; logistic dose; and complimentary log-log link analysis in SAS (see Appendix 3 for programme).

The expected LC95 (and LC50) values were used to calculate the degree of synergism for a given spray application. The degree of synergism, expressed as a number, was calculated by dividing the expected mean pyrethrum concentration in a pyrethrum only formulation by the expected mean pyrethrum concentration in a pyrethrum/synergist spray application at the LC95 and LC50 level.

CHAPTER FOUR

RESULTS

This chapter presents the calculated observed results of the mortality of the pea aphid to spray applications of natural pyrethrum containing one of the synergistic additives sesame oil, the crystalline extract of the sesame oil crude extract, dillapiole, or PBO. In addition, the estimated LC50's and LC90's and their corresponding fiducial limits are presented for each spray application.

Finally, two graphs are presented. Figure 1 plots the observed results of the mortality of the pea aphid (n=40) to the spray applications containing PBO, DA, and SX and gives 95% confidence intervals around each mean. Figure 2 plots the observed results of the mortality of the pea aphid to the spray applications containing PBO, sesame oil; and pyrethrum alone and gives 95% confidence intervals around each mean.

As the controls containing synergists did not generally cause a considerably greater mortality than the pyrethrum only controls at the concentrations tested, a possible contribution by the emulsifiers or synergists to the expected toxicity of the spray applications was deemed negligible. At the concentrations tested, the synergists were therefore shown to be true synergists of pyrethrum .

Upon subjection of the data to probit linear, logistic dose and complimentary log-log analysis, goodness-of- fit chi-square tests revealed that the logistic dose model was the best fit for the data and this model was therefore solely used in data analyses.

Although variability in the data was greater than expected,(a phenomenon known as

"overdispersion"), adjustments for this variability (nominal chi-square values were divided by the heterogeneity factor and the adjusted chi-square compared to the unadjusted chi-square) indicated that the logistic dose model fitted the data reasonably well. Justification for this procedure is given by P. McCullagh & J. A. Nelder. 1989. Although there is some overlapping in the effects of the synergists, due to the high variation in observed mortality, trends are indicated and differences in synergistic abilities are demonstrated.

It is important to note that the biological data presented in this section are only applicable to the insect species used i.e. the pea aphid, and cannot be applied to other insects however closely allied. Further, the effect of different bioassay techniques on the apparent activity of pyrethrum synergists may vary.

4.1. PYRETHRUM:

The calculated observed mortality values for pyrethrum spray applications containing no synergist ("pyrethrum only") at the given rates and ratios are shown in Table 2.

Table 2: Observed mortality values for pyrethrum only spray applications.

Application Rate (ml/L)	Active Pyrethrum conc. (%)	Mean control mortality	Mean no. "dead" (corrected) (4d.p.)	Standard error (4 d.p.)
1.0	0.1375	—	1.2104	0.5823
3.0	0.4125	—	0.4328	0.2652
6.0	0.825	—	11.0176	4.9648
9.0	1.2375	—	16.7248	4.2274
12.0	1.65	—	22.1520	2.7217
15.0	2.0626	—	31.4568	3.5500
18.0	2.43	—	31.2000	3.0826
21.0	2.8875	—	34.1776	0.7029
23.0	3.1625	2.4	37.0472	1.0848

4.2. SESAME OIL:

The calculated observed mortality values for pyrethrum plus sesame oil spray applications at the given rates and ratios are shown in Table 3.

Table 3: Observed mortality values for pyrethrum plus sesame oil spray applications.

Ratio Pyr:SO	Application Rate (ml/L)	Active Pyrethrum conc. (%)	Mean control mortality	Mean no. "dead" (corrected) 4d.p.)	Standard error (4 d.p.)
1:1	3.0	0.4125	—	2.1808	1.0410
	6.0	0.825	—	16.9000	3.7445
	9.0	1.2375	—	31.8928	3.0315
	12.0	1.65	—	33.3776	2.7541
	15.0	2.0625	—	37.5072	0.6874
	18.0	2.43	2.2	38.7688	0.5937
1:5	.3	0.04125	—	3.5408	1.9102
	1.0	0.1375	—	4.4896	0.9748
	3.0	0.4125	—	3.2104	1.7847
	6.0	0.825	—	8.6728	2.9526
	9.0	1.2375	—	24.2288	3.5414
	1.2	0.165	—	31.2928	3.9370
	1.4	0.1925	—	34.1664	2.6546
	1.6	0.22	—	36.4352	1.8406
	1.8	0.2475	9.0	38.6504	0.8794
1:8	.3	0.04125	—	1.9352	0.8983
	1.0	0.1375	—	3.8976	0.7968
	6.0	0.825	—	14.0768	4.2473
	9.0	1.2375	—	28.7568	4.1510
	14.0	1.925	—	31.4280	0.8378
	15.0	0.20625	4.4	38.9480	0.3327

4.3. CRYSTALLINE EXTRACT OF SESAME OIL CRUDE EXTRACT:

The calculated observed mortality values for pyrethrum plus the crystalline extract of sesame oil crude extract applications at the given rates and ratios are shown in Table 4.

TABLE 4: Observed mortality values for pyrethrum plus the crystalline extract of sesame oil crude extract spray applications.

Ratio Pyr:SX extract	Application Rate (ml/L)	Active Pyrethrum conc. (%)	Mean control mortality	Mean no."dead" (corrected) (4d.p.)	Standard error (4d.p.)
1:1	2.1	0.28875	—	1.7232	0.8650
	2.7	0.37125	—	5.7904	1.9970
	3.3	0.45375	—	16.6744	1.2890
	3.9	0.53625	—	22.2784	2.4664
	4.5	0.61875	—	28.812	4.5869
	5.1	0.70125	6.6	34.6168	2.0922
1:5	.9	0.1527 (4 d.p.)	—	9.8400	1.8567
	1.5	0.20625	—	30.768	2.1490
	2.1	0.28875	—	36.5392	0.8802
	2.4	0.33	—	37.852	0.7562
	2.7	0.37125	2.6	38.4864	1.0023

4.4. DILLAPIOLE:

The calculated observed mortality values for pyrethrum plus dillapiole spray applications at the given rates and ratios are shown in Table 5.

TABLE 5: Observed mortality values for pyrethrum plus dillapiole spray applications.

Ratio Pyr:DA	Application Rate (ml/L)	Active Pyrethrum conc. (%)	Mean control mortality	Mean no. "dead" (corrected) (4d.p.)	Standard error (4 d.p.)
1:5	1.2	0.165	—	6.5816	1.2175
	1.5	0.20625	—	16.8808	2.2876
	1.8	0.2475	—	25.0288	5.2132
	2.1	0.28875	—	36.0528	1.4771
	2.4	0.33	—	36.8888	1.3351
	2.7	0.37125	1.8	40.0000	0.0000
1:10	.6	0.0825	—	2.0256	0.8597
	.9	0.1527 (4 d.p.)	—	15.0152	3.2473
	1.2	0.165	—	30.8096	2.7429
	1.5	0.20625	—	34.3328	2.1270
	1.8	0.2475	—	38.5744	0.7658
	2.1	0.28875	0.8	40.0000	0.0000
1:15	.3	0.04125	—	1.5344	0.6482
	.6	0.0825	—	4.7408	2.5129
	.9	0.1527 (4 d.p.)	—	24.9704	2.0004
	1.2	0.165	—	29.8544	4.3083
	1.5	0.20625	—	33.8360	4.3069
	2.1	0.28875	2.8	39.7648	0.2352

4.5. PBO:

The calculated observed mortality values for pyrethrum plus PBO spray applications at the given rates and ratios are shown in Table 6.

TABLE 6: Observed mortality values for pyrethrum plus PBO spray applications.

Ratio Pyr:PBO	Application Rate (ml/L)	Active Pyrethrum conc. (%)	Mean control mortality	Mean no. "dead" (corrected) (4 d.p.)	Standard error (4d.p.)
1:1	1.5	0.20625	—	2.9712	1.5279
	2.0	0.275	—	10.3296	3.8080
	2.5	0.34375	—	22.1320	5.1031
	3.5	0.48125	—	35.0176	1.8504
	4.5	0.61875	—	34.0640	3.6788
	5.5	0.75625	3.4	38.1952	0.9332

4.6. COMBINED RESULTS OF ALL THE SPRAY COMPOSITIONS AND THEIR ASSOCIATED RATES AND RATIOS TESTED.

The estimated mortality values for pyrethrum spray applications containing each of the spray applications at the given rates and ratios are presented in Table 7.

TABLE 7: Estimated L.C.50's and L.C.95's and their associated fiducial limits and degrees of synergism for each spray application.

Test synergist	Pyr:Syn. ratio	Estimated L.C. 50 (ml/L)	Degree of synergism (L.C. 50)	95% fiducial limits (lower and upper)	Estimated L.C. 95 (ml/L)	Degree of synergism (L.C. 95)	95% fiducial limits (lower and upper)
Pyr:–	—	11.4131	—	L. 9.4755 U.13.3316	23.8108	—	L. 20.4745 U. 29.7784
Pyr:SO	1:1	7.2593	1.57	L. 4.8790 U. 9.1639	14.4501	1.65	L. 11.7839 U. 21.7284
	1:5	8.5289	1.34	L. 7.6362 U. 9.4139	17.3046	1.38	L. 15.7473 U. 19.4515
	1:8	7.1866	1.59	L. 4.3767 U. 9.9805	16.0892	1.48	L. 12.4555 U. 26.3581
Pyr:SX	1:1	3.7731	3.02	L. 3.5399 U. 4.0164	5.7406	4.14	L. 5.2618 U. 6.5414
	1:5	1.2746	8.95	L. 0.9786 U. 1.5339	2.2423	10.69	L. 1.9015 U. 2.9816
Pyr:DA	1:5	1.72129	6.63	L. 1.26583 U.1.90758	2.30004	10.35	L. 2.08257 U. 3.07850
	1:10	1.0351	11.03	L. 0.9032 U. 1.1549	1.6252	14.65	L. 1.4449 U. 1.9822
	1:15	1.5205	7.51	L. 1.1529 U. 1.8308	2.6772	8.89	L. 2.2598 U. 3.6744
Pyr:PBO	1:1	2.66049	4.29	L.1.90078 U. 3.42513	4.80428	4.96	L. 3.85192 U. 8.44037

The observed mean mortality values for pyrethrum spray applications containing PBO, DA, SX at the given rates and ratios are presented in Figure 1. 95% confidence intervals are given for each mean.

The observed mean mortality values for spray applications containing PBO, sesame oil, and the pyrethrum control (pyr alone) and the given rates and ratios are presented in Figure 2. 95% confidence intervals are given for each mean.

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1. SESAME OIL

Sesame oil has been found to increase the effectiveness of natural pyrethrum for a range of insect pests .

This increase in effectiveness was supported by the results in Table 7 in which the mortality values for the pyrethrum plus sesame oil applications to the pea aphid at ratios 1:1; 1:5; and 1:8 were not found to be significantly different.

At best, the estimated mean concentration of pyrethrum required to achieve 95% mortality for the pyrethrum plus sesame oil applications was approximately 1.65 times less (pyr:ses oil 1:1) than that necessary to achieve 95% mortality for the pyrethrum only applications. Therefore, the addition of sesame oil to pyrethrum ratio "saved" a maximum of approximately 40% of pyrethrum at 95% mortality.

No other studies have been conducted prior to this research that have examined the efficacy of sesame oil in enhancing the toxicity of natural pyrethrum against aphids, making comparisons in their synergistic abilities amongst relevant literature impossible.

Further, direct comparisons of the efficacy of sesame oil with other studies is made difficult by the fact that as the synergistic content of sesame oil varies, as discussed in Chapter 2, different oils will cause markedly different degrees of activation of pyrethrum. Further, it is important to note that synergism is specific with response to the test insect.

As no increase in the efficacy of sesame oil was found above 0.1375% addition of sesame oil, the results suggest that the "optimum quantity" of sesame oil for addition to an aphicide occurs somewhere below 0.1375%.

This "optimum quantity" is defined as the lowest quantity of sesame oil for addition to an aphicide at which any extra addition of sesame oil will not result in any increase in the spray application's effectiveness.

As this "optimum quantity" of sesame oil content in a natural pyrethrum aphicide remains to be determined, further work on these lines might prove useful in developing more economical formulations.

5.2. CRYSTALLINE EXTRACT OF SESAME OIL CRUDE EXTRACT

The superior synergistic effect of the recrystallised fractions of sesame oil crude extract (SX) in comparison to sesame oil has been attributed directly to the greater sesamin and sesamolin contents of the recrystallised fractions.

This marked synergistic ability was evident in this study, as was shown in Table 7, and was significantly greater for the pyrethrum plus SX application at the 1:5 ratio than the 1:1 ratio.

The estimated mean concentration of pyrethrum required to achieve 95% mortality for the pyrethrum plus SX applications at 1:1 and 1:5 ratios respectively were 4.14 and 10.69 times less than that necessary to achieve 95% mortality for the pyrethrum only applications.

Therefore the addition of SX (which contains sesamin:sesamolin in a ratio of 2:1) to pyrethrum at ratios of 1:1 and 1:5 saved about 76% and 91% of pyrethrum respectively at 95% mortality. Further, the addition of very small quantities of SX, in comparison to the quantities of sesame oil added, resulted in a much greater increase in the toxicity of natural pyrethrum for the pyrethrum plus SX spray application.

No other studies have been conducted prior to this research which have examined the efficacy of the sesame oil synergists in enhancing the toxicity of pyrethrum against aphids, making direct comparisons between their synergistic abilities amongst the relevant literature impossible.

Further, comparisons in the synergistic abilities of SX amongst the relevant literature between insect species is made difficult for two reasons.

Firstly, previous relevant literature has commonly tested the efficacy of sesamin and sesamolin as pyrethrum synergists separately, rather than as a crystalline extract of crude sesame oil extract which contains both sesamin and sesamolin as was used in this report. Secondly, when the efficacy of both sesamin and sesamolin as pyrethrum synergists has been tested as crystalline extracts of crude sesame oil extracts of sesame oil, their relative concentrations have been unknown. This makes comparisons difficult as the synergistic content of sesame oil varies.

5.3. DILLAPIOLE

Dillapiole was found to markedly increase the effectiveness of natural pyrethrum against the pea aphid as shown in Table 7.

The estimated factors of synergism for the pyrethrum plus dillapiole applications at the ratios 1:5; 1:10 and 1:15 were 10.35; 14.65 and 8.89 respectively. Therefore the addition of dillapiole to pyrethrum at these respective ratios saved about 90%, 93.17%, and 89% of pyrethrum at 95% mortality.

The mortality achieved by the use of dillapiole at the ratio 1:10 pyrethrum plus dillapiole was found to be significantly greater than the mortality achieved by the use of dillapiole at the ratios of 1:5 and 1:15 pyrethrum plus dillapiole.

Although these results supported work by Handa & Dewan (1975), in which dust formulations of pyrethrins with dillapiole gave maximum synergistic effect against *Tribolium castaneum* at a 1:10 ratio of pyrethrins:synergist, the factors of synergism at the LC50 for pyrethrum plus dillapiole 1:5, 1:10, 1:15 respectively (1.28, 2.47, and 2.04) were much lower than those found in this report. A comparatively lower factor of synergism was also found to be the case for work by Singh et al (1976), in which a pyrethrum plus dillapiole application at the ratio 1:5 gave a factor of synergism of 2.48 (LC50) against *Tribolium castaneum*. Perhaps this increased factor of synergism for the pea aphid may be explained on the basis that synergism is specific with respect to test insect.

As mentioned previously in the introduction, it has been suggested in mixed synergist studies (Parmar 1974) that the use of PBO could be economised or dispensed with by using PBO combined with dillapiole and/or dihydrodillapiole in pyrethrin formulations; or using combinations of dillapiole and dihydrodillapiole in pyrethrin formulations which do not contain PBO respectively . With the aim of developing an effective pyrethrum insecticide which includes the natural pyrethrum synergist dill, as opposed to one that contains the synthetic synergist PBO, more of such mixed synergist studies need to be conducted on a range of insects in order to find out which mixes are best.

5.4. PBO

PBO was found to increase the effectiveness of natural pyrethrum against the pea aphid as shown in Table 7.

The estimated mean concentration of pyrethrum required to achieve 95% mortality at the ratio of 1:1 pyrethrum:PBO was 4.96 times less than that necessary to achieve 95% mortality for the pyrethrum only applications.

Therefore, the addition of PBO at this ratio saved about 80% of pyrethrum at the 95% mortality level.

5.5. COMPARATIVE SYNERGISTIC ABILITIES OF THE TESTED SYNERGISTS.

It is evident that sesame oil, the crystalline extract of crude sesame oil extract, dillapiole and PBO were all true synergists of pyrethrum at all the rates tested.

Sesame oil had the comparatively inferior synergistic activity of all the synergists as apparent LC50 and LC95 values obtained with sesame oil combinations showed only slight synergistic properties, and were not significantly different amongst the three ratios tested.

The pyrethrum plus crystalline extract of crude sesame oil extract at a pyrethrum:SX ratio of 1:1 and the pyrethrum plus PBO at a pyrethrum:PBO ratio of 1:1 both had comparatively moderate synergistic properties and their apparent LC95 values were not significantly different.

The remaining pyrethrum plus synergist combinations showed marked synergistic activity.

Of these synergists, the combinations including pyrethrum plus dillapiole at the ratio 1:10, and pyrethrum plus the crystalline extract of crude sesame oil extract at a pyrethrum:SX ratio of 1:5 had the greatest synergistic activity and were not significantly different at 95% confidence. Pyr:DA 1:10 achieved the greatest factor of synergism (14.65).

The remaining synergist combinations (Pyr:DA 1:15; and Pyr:DA 1:5) were not found to be significantly different at LC95.

5.6. SYNERGISTIC ABILITIES OF THE NATURAL SYNERGISTS IN COMPARISON TO THE SYNTHETIC SYNERGIST PBO.

All of the tested synergists other than sesame oil and their associated spray applications were shown to have synergistic abilities similar to or superior to PBO at the ratio 1:1 pyrethrum:PBO.

Both the pyrethrum plus crystalline extract of crude sesame oil extract spray application at the ratio 1:1 and the pyrethrum plus PBO spray application at the ratio 1:1 showed moderate synergistic abilities and were not found to be significantly different.

Although the remaining synergists (pyr:DA 1:5; 1:10; 1:15 and pyr:SX 1:5) showed superior synergistic ability to PBO at the 1:1 ratio of pyrethrum:PBO, as PBO is often used in ratios of 1:5 or more in commercial applications it is probable that at these higher rates that the synergistic ability of PBO may have been superior to those shown by the "remaining" synergists and their associated application rates. Further work on testing these higher rates would prove useful in determining the comparative synergistic abilities of commercial rates of PBO with the natural synergists.
Nevertheless, as these remaining synergists showed marked synergistic ability, it is suggested that the use of PBO could be dispensed with by using any one of these synergists and associated ratios in a pyrethrum insecticide for the pea aphid.

However, it is important to note that whether crude oil, or specific crude oil extracts from either dill or sesame oil could be regarded as a more desirable synergist than piperonyl butoxide depends upon a number of factors other than synergistic ability including, importantly, the relative prices of the natural synergists in comparison to PBO. This in turn is determined by such things as extraction costs, availability of oils, and their synergistic component. Further, preferential demand for organic as opposed to non-organic pesticides is also important.

5.7. CONCLUSION

From the experiments undertaken the following conclusions were drawn:

1). Sesame oil, a crystalline extract of a crude sesame oil extract, dill apiole and the synthetic PBO were found to be true synergists of natural pyrethrum against the pea aphid for all of their tested application ratios.

2). Sesame oil showed slight synergistic abilities against the pea aphid for natural pyrethrum.

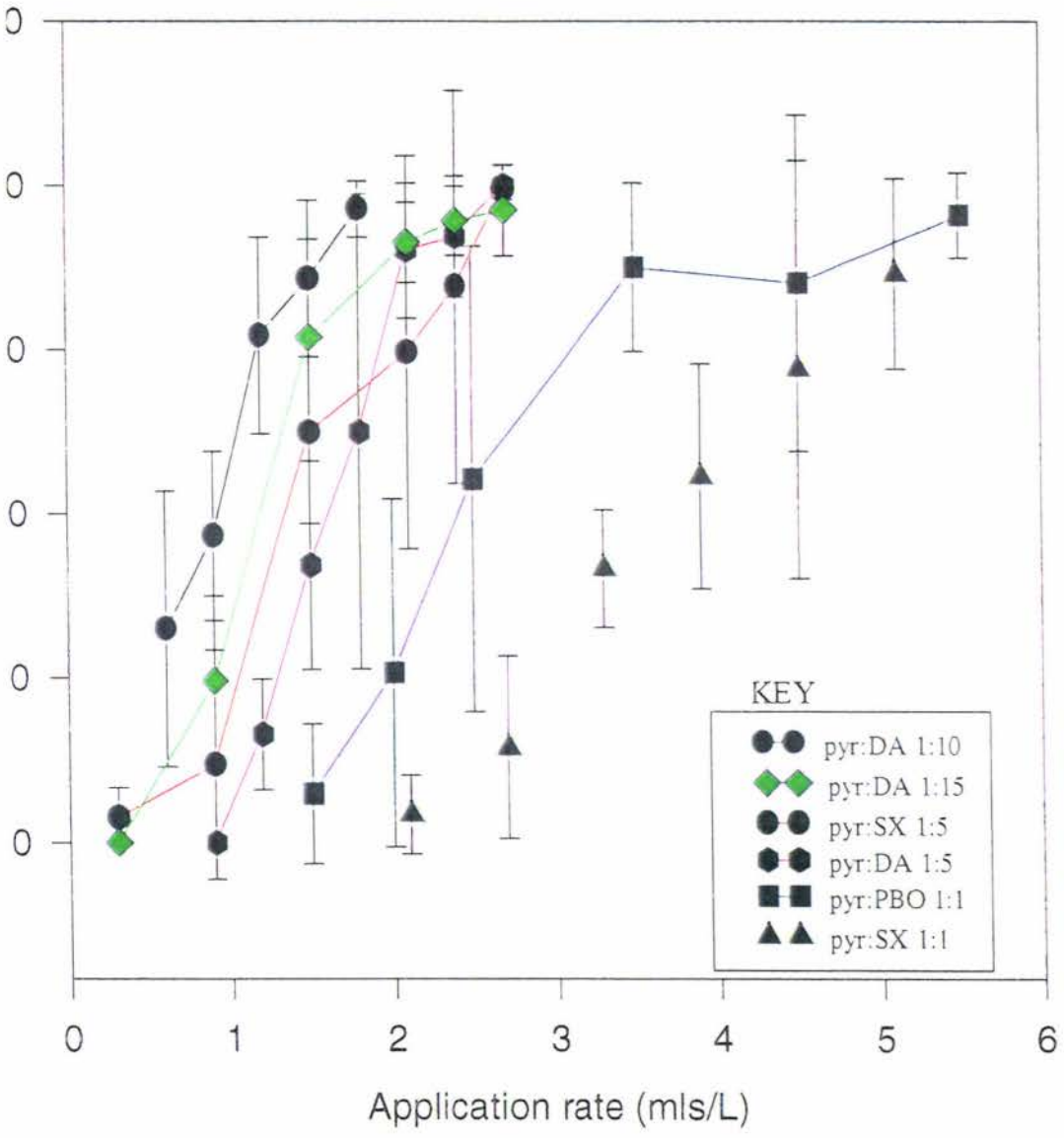
3). Both PBO and the crystalline extract of a crude sesame oil extract showed moderate synergistic abilities for natural pyrethrum at the ratio of natural pyrethrum:synergist 1:1.

4). The crystalline extract of a crude sesame oil extract at the ratio 1:5 pyr:extract, and all the ratios of pyrethrum:dillapiole tested showed marked synergistic abilities against the pea aphid for natural pyrethrum.

5). The use of PBO could be dispensed with by the use of dill apiole at all the tested application ratios, and by the crystalline extract of a crude sesame oil extract at the ratio of pyrethrum:synergist 1:5 against the pea aphid.

FIGURE 1.

tality for spray applications containing dillapiole, sesame oil extract, and PBO.



PART C

Determining the efficacy of dill and sesame oil as synergists for natural pyrethrum against a range of insect pests using a Potter Tower.

ABSTRACT

The crystalline extract of a crude sesame oil extract, dillapiole, and the synthetic synergist PBO were examined for their ability to synergise natural pyrethrum in spray emulsions at a variety of rates and ratios against the passionvine hopper (*Scolypopa australis*), greenhouse thrip (*Heliothrips haemorrhoidalis*), lightbrown apple moth (*Epipyhas postvittana*), and tomato fruit worm (*Helicoverpa armigera*). Testing was carried out under laboratory conditions using a Potter tower technique.

Based on the results with the passionvine hopper and LBAM, all of the natural synergists other than the crystalline extract of a crude sesame oil extract at the lowest ratio that was tested could replace PBO. Results with the greenhouse thrip and tomato fruit worm were inconclusive, and further research is needed in order to determine the precise natural synergist spray formulations which would allow conclusions regarding their efficacy against these insects.

CHAPTER ONE

INTRODUCTION

From an examination of the experimental findings gained in Part B against the pea aphid, it was suggested that the use of the natural synergists dillapiole and a crystalline extract of crude sesame oil could replace PBO in natural pyrethrum spray applications.

Both toxicity and the degree of synergism of a synergist is specific to each insect species. The aim of this section was to further examine the efficacy of these natural synergists on a wider range of insect pests.

CHAPTER TWO

MATERIALS AND METHODS

2.1. SOURCE AND ESTABLISHMENT OF TEST INSECTS

2.1.1. Passionvine hopper (*Scolypopa australis*)

The passionvine hoppers used in the experiment were collected as needed from a grape vine in a Palmerston North home garden and were contained in a fine mesh cage with a piece of grape vine until they were used in spray trials later that day.

2.1.2. Greenhouse thrip (*Heliothrips haemorrhoidalis*)

The adult greenhouse thrips used in the experiment were collected as needed from Rhododendron trees, which are found throughout the grounds of Massey University.

2.1.3. Lightbrown apple moth (*Epiphyas postvittana*)

2.1.4. Tomato fruit worm (*Helicoverpa armigera)*

E. postvittana and *H. armigera* used in this experiment were supplied by the Insect Rearing Group, HortResearch, Auckland as eggs. These were hatched at room temperature.

2.2. LABORATORY EXPERIMENT

2.2.1. Insecticide Treatment

A full description of the insecticides and their corresponding formulations is given in Part B.

The following spray applications were used to treat the insects tested (Table 1).

Although the pyrethrum plus sesame oil spray applications , as well as the pyrethrum plus dillapiole spray application at the ratio of 1:15 were tested in Part B, they were not examined in this section as the former spray applications were shown to be synergistically inferior to PBO at the ratio 1:1 pyrethrum:PBO and the latter spray application was shown to be synergistically inferior to pyr:DA 1:10.

The chosen application rates tested in the LBAM, greenhouse thrip and tobacco fruit worm bioassays were those that resulted in approximately 50% mortality for each of the spray applications in the pea aphid bioassays from Part B, other than for PBO spray applications. The latter were tested at 5mls/L, as it is the recommended full field rate for Yates Pyrethrum. Further, a lower ratio of pyrethrum:PBO was tested in Part B so that the LD50 at the higher ratio was not known.

As mortality values in preliminary tests for SX 1:5 with the passion vine hopper were

extremely low at the pea aphid LD50, (with a mean of approximately 4%) in comparison to the mortality values gained in the other insect bioassays(approximately 18-88%), the insecticide rate tested for this insect was 5ml/L for each insecticide treatment.

One control was applied immediately prior to testing of a given spray application treatment for the LBAM, greenhouse thrip and tomato fruit worm bioassays.
This "control" consisted of all the ingredients found in the pyrethrum concentration spray application tested for a given synergist, minus pyrethrum. This was included in order to find out whether the emulsifying agents had any positive direct effects on mortality
Two controls were applied prior to spraying for the passion vine hopper bioassays.
Each insecticide treatment was replicated five times consecutively for the LBAM, greenhouse thrip and tomato fruit worm bioassays and ten times consecutively for the passion vine hopper bioassay .

Table 1: The spray compositions, ratios of pyrethrum:synergist, and application rates tested

Test insect	Spray composition	Ratio (Active Pyr:synergist)	Application rate (mls/L)
Passion vine hopper	Pyr + (DA)	1:5	5.00
		1:10	5.00
	Pyr + PBO	1:4	5.00
	Pyr + (SX)	1:1	5.00
		1:5	5.00
Greenhouse thrip	Pyr + (DA)	1:5	1.70
		1:10	1.05
	Pyr + PBO	1:4	*
	Pyr + (SX)	1:1	3.80
		1:5	1.30
LBAM	Pyr + (DA)	1:5	1.70
		1:10	1.05
	Pyr + PBO	1:4	*
	Pyr + (SX)	1:1	3.80
		1:5	1.30
Tomato fruit worm	Pyr + (DA)	1:5	1.70
		1:10	1.05
	Pyr + PBO	1:4	5.00
	Pyr + (SX)	1:1	3.80
		1:5	1.30

* LBAM and greenhouse thrip were not tested in Pyr + PBO spray applications as they were tested previously in Part A (at application rates of 2.5ml/L and 5.0ml/L) under similar experimental conditions.

2.2.2. Bioassay Set Up for Each Test Insect Species

2.2.2.1. Passionvine hopper (*Scolypopa australis*)

5 cm length grape vine shoots were placed onto damp filter paper in 90mm plastic Petri dishes.

All of the adult passion vine hoppers for a given spray application were anaesthetised prior to their placement into Petri dishes to make handling easier. In order to anaesthetise the insects, their holding cage was placed into a coolstore which was set at 7° C ± 0.5° C until they were sufficiently immobile for placement into Petri dishes .

Ten of these anaesthetised adult passionvine hoppers were then placed, using a pair of entomological forceps, into each Petri dish. These Petri dishes were then removed from the coolstore and held at room temperature.

2.2.2.2. Greenhouse thrip (*Heliothrips haemorrhoidalis*)

Twenty three millimetre diameter *Rhododendron* spp leaf discs were punched out and placed, upper surface uppermost, onto damp filter paper in 90mm plastic Petri dishes.

Twenty adult greenhouse thrips were placed, using a fine paint brush, on each leaf disc's upper surface.

2.3.2.3. Lightbrown Apple Moth (*Epiphyas postvittana*)

Twenty three millimetre diameter apple (*Malus domestica*) leaf discs were punched out and placed, upper surface uppermost , onto damp filter paper in 90mm plastic Petri dishes. Twenty neonate lightbrown apple moth larvae were placed, using a fine paint brush, onto each leaf disc's upper surface.

2.3.2.4. Tomato fruit worm (*Helicoverpa armigera*)

Twenty three millimetre diameter tobacco (*Nicotiana tabacum*) leaf discs were punched out and placed, upper surface uppermost , onto damp filter paper in 90mm plastic Petri dishes. Twenty neonate tomato fruit worm larvae were placed, using a fine paint brush, onto each leaf disc's upper surface.

2.2.3. Insecticide Application

The Potter precision tower (Burkhard Manufacturing Co Ltd, Rickmansworth, U.K.) was used to apply each insecticide treatment to the leaf discs and leaves.

The air pressure to operate the tower was maintained at 12 psi. A standard volume of 2ml was sprayed onto each of the leaf discs (or leaves) and a settling period of 10 seconds was allowed after all the spray had been discharged from the Potter tower sprayer.

With lightbrown apple moth, tomato fruit worm and thrip bioassays, both sides of the leaf discs were sprayed to ensure that if the larvae were to move to the underside of the leaf, contact with the insecticide would be maintained. This was achieved by initially spraying the top of the leaf disc with 1ml of insecticide then applying the larvae to the bottom surface of the leaf disc (or leaf) and spraying the disc again. Spraying was carried out at room temperature.

For the passionvine hopper bioassays, the Petri dishes containing passionvine hoppers were sprayed only once and with 2ml of insecticide. Spraying was carried out in the cool store in order that the passionvine hoppers were kept anaesthetised throughout the procedure to make their handling easier.

To eliminate spray contamination, precautions were taken between treatments to decontaminate the sprayer, i.e., two flushings with acetone followed by deionised water and flushing with hose water were carried out.

2.2.4. Mortality Assessment

After treatment each Petri dish was held at 25°C ± 2°C and a 14:14 light:dark photoperiod for all the experimental insect species. Mortality was assessed at 24 hours.

The criterion used to determine mortality were identical for all the bioassays. Those insects which failed to move one body length were labelled as "dead", those which were able to move one body length were recorded as "alive".

2.2.5. Analysis of Laboratory Results

The experimental insect's mortality response to the insecticide treatments was analysed using Analysis of Variance (ANOVA) in SAS.

The corrected percentage mortality values (in response to natural mortality) were calculated using Abbott's formula (Abbott, 1925).

CHAPTER THREE

RESULTS

This chapter presents the results of the mortality of various economically important plant pests to five pyrethrum plus synergist spray applications.

NOTE: the significance levels which are given in the results are defined as :-

*** = Control mortality and mean replicate mortality are significantly different at p≤ 0.001

* = Control mortality and mean replicate mortality are significantly different at p≤ 0.05

3.1. Pyr:DA 1:5

The calculated mortality values for pyr:DA 1:5 at the rates of 5.0ml/l (passionvine hopper bioassay) and 1.70ml/L (remaining bioassays) are shown in Table 2.

Table 2: Insect mortality values for pyr:DA 1:5

Insect Species	Life Stage	Application Rate	Control mortality (%)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Passionvine hopper	Adult	5.00ml/l	10.0; 0.0	96.1	2.36	***
Greenhouse thrip	Adult	1.70ml/l	0.0	100.0	–	–
Lightbrown apple moth	1st Instar Larva	1.70ml/l	0.0	78.0	4.06	***
Tomato fruit worm	1st Instar Larva	1.70ml/l	20.0	28.0	13.56	*

*** = $p \leq 0.05$

* = $p \leq 0.001$

3.2. Pyr:DA 1:10

The calculated mortality values for Pyr:DA 1:10 at the rates of 5.0ml/l (passionvine hopper bioassay) and 1.05ml/L (remaining bioassays) are shown in Table 3.

Table 3:Insect mortality values for Pyr:DA 1:10.

Insect Species	Life Stage	Application Rate	Control mortality (%)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Passionvine hopper	Adult	5.00ml/l	0.0; 0.0	98.0	1.89	***
Greenhouse thrip	Adult	1.05ml/l	0.0	71.0	5.10	***
Lightbrown apple moth	1st Instar Larva	1.05ml/l	0.0	16.0	3.32	***
Tomato fruit worm	1st Instar Larva	1.05ml/l	0.0	33.0	6.82	***

*** = p≤ 0.001
* = p≤ 0.05

3.3. Pyr:PBO 1:4

The calculated mortality values for Pyr:PBO 1:4 at the rates of 5.0ml/l (all bioassays) are shown in Table 4.

Table 4: Insect mortality values for Pyr:PBO 1:4.

Insect Species	Life Stage	Application Rate	Control mortality (%)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Passionvine hopper	Adult	5.0ml/l	0.0; 20.0	96.7	2.40	***
Greenhouse thrip	Adult	5.0ml/l	**0.0	**100.0	–	–
Lightbrown apple moth	1st Instar Larva	5.0ml/l	** 0.0	**100.0	–	–
Tomato fruit worm	1st Instar Larva	5.0ml/l	0.0	100.0	–	–

*** = p≤ 0.001
* = p ≤ 0.05

** = results from Part A.

3.4. Pyr:SX 1:1

The calculated mortality values for Pyr:SX 1:1 at 5.0ml/l (passionvine hopper bioassay) and 3.8ml/L (remaining bioassays) are shown in Table 5.

Table 5: Insect mortality values for Pyr:SX 1:1.

Insect Species	Life Stage	Application Rate	Control mortality (%)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Passionvine hopper	Adult	5.00ml/l	0.0; 0.0	72.0	3.52	***
Greenhouse thrip	Adult	3.80ml/l	0.0	100.0	–	–
Lightbrown apple moth	1st Instar Larva	3.80ml/l	5.0	52.6	8.15	***
Tomato fruit worm	1st Instar Larva	3.80ml/l	20.0	34.3	13.25	*

*** = $p \leq 0.001$
* = $p \leq 0.05$

3.5. Pyr:SX 1:5

The calculated mortality values for Pyr:SX 1:5 at 5.0ml/l (passionvine hopper bioassay) and 1.3ml/L (remaining bioassays) are shown in Table 6.

Table 6: Insect mortality values for Pyr:SX 1:5.

Insect Species	Life Stage	Application Rate	Control mortality (%)	Mean % Mortality (Corrected)	S.E. mean	Signific.
Passionvine hopper	Adult	1.30ml/l * preliminary test	0.0; 0.0	4.0	2.31	*
	Adult	5.00ml/L	0.0; 0.0	88.0	8.89	***
Greenhouse thrip	Adult	1.30ml/l	0.0	73.0	9.30	***
Lightbrown apple moth	1st Instar Larva	1.30ml/l	0.0	41.0	10.54	*
Tomato fruit worm	1st Instar Nymph	1.30 ml/l	0.0	18.0	3.00	***

*** = $p \leq 0.001$
* = $p \leq 0.05$

CHAPTER FOUR

DISCUSSION AND CONCLUSIONS

The format of this chapter follows the same order as presented in the results section. It interprets the results and evaluates the methods used, and incorporates relevant results from Part A and Part B.

4.1. Pyr:DA 1:5

At approximately 1.70ml/L, Pyr: DA 1:5 application was found to result in 50% (moderate) mortality for the pea aphid in Part B.

At this application rate, the mortalities shown for the experimental insects were marked for the greenhouse thrip, moderate-marked for the LBAM, and slight for the tomato fruit worm.

At the 5ml/L application rate, marked mortality was achieved for the passionvine hopper.

4.2. Pyr:DA 1:10

At approximately 1.05ml/L (compared to 1.70ml/L for pyr:DA 1:5), pyr:DA 1:10 application was found to result in 50% mortality for the pea aphid in Part B.

At this application rate, the mortalities shown for the experimental insects were moderate for the greenhouse thrip, and slight for the LBAM, and tomato fruit worm.

At the 5ml/L application rate, marked mortality was achieved for the passionvine hopper. A reduction in the application rate from 1.70ml/L for pyr:DA 1:5 to 1.05ml/L for pyr:DA 1:10 has therefore resulted in a significant decrease in mortality for the LBAM and grrenhouse thrip. This decrease was particularly marked for LBAM.

As a decrease in mortality was not observed for the tomato fruit worm or the pea aphid (Part B), it is likely that the major factor involved in the decrease in mortality is not a decrease in application rate.

Rather, the decrease in mortalties for LBAM and greenhouse thrip alone may be explained by the fact that differing slopes of the mortality curve exist for each insect species as the degree of synergism is specific with response to the test insect .

Never the less, the dramatic drop in mortality for LBAM from the lower ratio to the higher ratio cannot be explained by the specific response of the experimental insect to the synergist. Keeping in mind that the control mortalities were nil at both ratios for this test insect and that the experimental conditions were kept constant, one possible reason for this dramatic drop in mortality may be explained by experimental error, most likely the pyr:DA 1:10 spray solution was not made up correctly.

4.3. Pyr:PBO 1:4

At approximately 2.66ml/L, pyr:PBO 1:1 application was found to result in 50%

mortality for the pea aphid in Part B.

At the higher application rate of 5ml/L, which is the full field application rate for Yates Pyrethrum, the mortalities shown were marked for all of the experimental insects.

4.4. Pyr:SX 1:1

At approximately 3.80ml/L, Pyr:SX 1:1 application was found to result in 50% mortality for the pea aphid in Part B.

At this application rate, the mortalities shown for the experimental insects were marked for the greenhouse thrip, moderate for LBAM, and slight for the tomato fruit worm.

At the 5ml/L application rate, moderate mortality was achieved for the passionvine hopper.

4.5. Pyr:SX 1:5

At approximately 1.30ml/L, Pyr:SX 1:5 application was found to result in 50% mortality for the pea aphid in Part B.

At this application rate, the mortalities shown for the experimental insects were moderate for the greenhouse thrip, and slight for the LBAM and tomato fruit worm.

Therefore, a reduction in the application rate from 3.8ml/L for pyr:SX 1:1 to 1.30ml/L for pyr:SX 1:5 resulted in decreased mortality for all of the experimental insects.

At the 5ml/L application rate, marked mortality was achieved for the passionvine hopper, where as at the 1.30ml/L application rate, minimal mortality was achieved for this insect species (4% mean mortality).

4.6. Natural synergists vs. PBO.

Generally, the overall results indicate that for any given pyrethrum plus synergist spray application that the mortality of the experimental insects in order from highest to lowest mortality is greenhouse thrip, LBAM, pea aphid, tomato fruit worm, passionvine hopper. This suggests that toxicity to pyrethrum is specific to the insect species (as was discovered in Part A).

In order to examine the specificity of the degree of synergism of a given spray application for each experimental insect, further research needs to be carried out of a nature similar to the research in Part B in which the degree of synergism was calculated for the pea aphid. Comparisons of the degree of synergism for each experimental insect species would indicate the specificity amongst these insects .

As different spray application rates were tested for each spray formulation, it is difficult to compare the relative synergistic abilities of the natural synergists with PBO for each experimental insect.

However, application rates for all of the different spray applications were kept constant at

5ml/L for the passionvine hopper. At this rate, marked mortality was observed for pyr:DA 1:5, pyr:DA 1:10, pyr:SX 1:5, as well as for pyr:PBO 1:4 spray applications. Moderate mortality only was shown for pyr:SX 1:1.

Therefore it is suggested that the use of the synergist PBO in natural pyrethrum spray formulations against the passionvine hopper could be dispensed with by the use of all of the natural synergist spray formulations tested in this report other than pyr:SX 1:1.

This same suggestion was also made in Part B for natural synergist spray formulations against the pea aphid.

Further, comparisons of insect mortality values for LBAM at the half rate Yates Pyrethrum from Part A (2.5ml/L gave a mean mortality of 72%) with the results for pyrethrum plus sesame oil extract at the ratio 1:1 from this Part (3.8ml/L gave 52.6% mean mortality) reveal that the latter formulation is less effective than Yates Pyrethrum against the LBAM. This suggests that the use of PBO cannot be dispensed with by the use of pyrethrum:sesame extract 1:1 for the LBAM.

As a result of differing spray application rates, conclusions relating to the relative synergistic abilities of the natural synergists in comparison to PBO could not be made for the greenhouse thrip or tomato fruit worm. Further work needs to be carried out in order to make these conclusions.

4.7. CONCLUSION

From the experiments undertaken the following conclusions were drawn:

1) Although their associated degrees of synergism were not calculated, the results suggest that both the crystalline extract of the crude sesame oil extract and dillapiole are synergists of natural pyrethrum against all of the experimental insects including the greenhouse thrip, LBAM, tomato fruit worm, and passionvine hopper for all of their tested application ratios.

2) The use of the synthetic synergist PBO could be dispensed with by the use of all of the spray formulations other than the crystalline extract of the crude sesame oil extract at the ratio of pyrethrum:SX 1:1 against the passionvine hopper and LBAM.

3) Further research needs to be carried out in order to determine which of the natural synergist spray formulations could replace the use of PBO against the greenhouse thrip and tomato fruit worm.

4) Whilst these preliminary laboratory results indicate the strong potential that dill and sesame oil extracts have as organic synergist replacements for PBO in natural pyrethrum spray applications, further field work is necessary in order to determine whether these compounds would be useful in agricultural situations.

GENERAL DISCUSSION

As has been mentioned previously, the use of organic pesticides is a valuable tool in the development of a sustainable agriculture.

With the current increasing emphasis by consumers and agricultural export markets towards sustainability and organic production, there is a growing need to research and develop new organic insecticides, which maintain a level of insect control sufficient for both grower and consumer needs, in order to match this demand.

This dissertation has revealed a range of soft insecticides and natural pyrethrum synergists that can be used effectively against various insect species under laboratory conditions.

However, whilst these preliminary laboratory results indicate the strong potential that some of the soft insecticides as well as oil extracts examined in this dissertation have for use in organic pesticides, further field work is necessary in order to determine whether these compounds would be useful in agricultural situations.

Further, even if these compounds are found to be useful in agricultural situations, their adoption is limited by many factors including importantly their cost and desirability. Changing future demands for organic as opposed to non-organic pesticides is therefore of ultimate importance in the future adoption of organic pesticides.

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APPENDIX 1

SAS programme for probit analysis

PPENDIX 1.

```
*pesticide data for insects
     rate =50% or 100% of recommended dose
     pestic 1= yates pyrethrum
            2= eco oil
            3= defender
            4= orchex692
            5= confidor
            6= agrimec
            7= encap pyreth
            8= encaps pyret + neem
            9= azatin and eco oil
            10= azatin
            11= neemazal;

 ata daphid;
 filename ina 'a:\aphid.dat';
   infile ina;
   input repl control rate corrmort day pestic;
   insect='aphid';
   * corrmort is mortality corrected for control
     by Abbot's formula;

 ata dcod;
 filename inc 'a:\codling.dat';
   infile inc;
   input repl control rate corrmort day pestic;
   insect=' codl';   * coddling moth;
   * corrmort is mortality corrected for control
     by Abbot's formula;

data ddiam;
 filename ind 'a:\diamond.dat';
   infile ind;
   input repl control rate corrmort day pestic;
   insect=' diam';   * diamond backed moth;
   * corrmort is mortality corrected for control
     by Abbot's formula;

 data dmealy;
   filename inm 'a:\mealybug.dat';
   infile inm;
   input repl control rate corrmort day pestic;
   insect='mealy';
   * corrmort is mortality corrected for control
     by Abbot's formula;

 ata dmites;
 filename inm1 'a:\mites.dat';
   infile inm1;
   input repl control rate corrmort day pestic;
   insect='mites';
   * corrmort is mortality corrected for control
     by Abbot's formula;
```

```
data dthrips;
 filename ina 'a:\thrips.dat';
   infile ina;
   input repl control rate corrmort day pestic;
   insect='thrip';
   * corrmort is mortality corrected for control
     by Abbot's formula;

data dlbam;
 filename inl 'a:\lbam.dat';
   infile inl;
   input repl control rate corrmort day pestic;
   insect='lbam'; * light brown apple moth;
   * corrmort is mortality corrected for control
     by Abbot's formula;


data comb;
set daphid dcod ddiam dmealy dmites dthrips dlbam;
 if corrmort>=1 then corrmor=0.99;
 else if corrmort<=0 then corrmor=0.01;
 else corrmor=corrmort;
 logtrans=log(corrmor/(1-corrmor));

 to see if corrected mortality is significantly different
  from zero. ie different from control;
 note that given corrmort has used control in the calculation,
 all corrmort figures have been corrected to lie in the range
 0 to 1, this may be of only marginal use;


proc sort; by insect pestic rate day;

proc univariate ; var corrmort; by insect pestic rate day;


proc sort; by insect pestic rate repl day;

data comb2;
array combine(day)  logtr1 logtr2;
array comb(day)     cormort1 cormort2;
do over combine;
 set comb; by insect pestic rate repl;
  combine=logtrans; comb=corrmort;
  if last.repl then return;
 end;

       * rough version of a logistic regression type model;
       * note no iteration to solution;

proc glm data=comb2;
class insect pestic rate;
model logtr1 logtr2 = insect pestic rate
                      insect*pestic insect*rate pestic*rate;
repeated day;
output out= dstat r=resid1 resid2 p=pred1 pred2;

proc univariate normal plot;
```

```
 var resid1 resid2;

proc print;
 var insect pestic rate repl
   cormort1 cormort2 logtr1 logtr2 resid1 resid2;
run;

proc means data=comb;
 class insect pestic rate day;
 var corrmort;
run;
```

APPENDIX 2

Extraction of Sesamin, Sesamolin, and Dillapiole

Extraction of Sesamin, Sesamolin and Dillapiole, May 1996

A Falshaw, Industrial Research Limited, P O Box 31-310, Lower Hutt

Summary

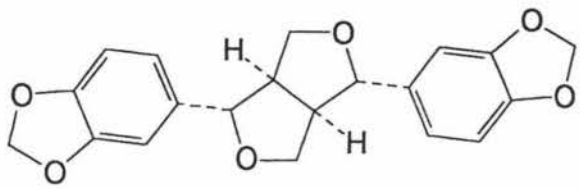
A mixture of sesamin and sesamolin was isolated in a yield of 0.3% from cold pressed sesame oil by extraction, chromatography and recrystallisation.

Dillapiole was isolated in 6.5% yield from dill oil by high vacuum distillation.

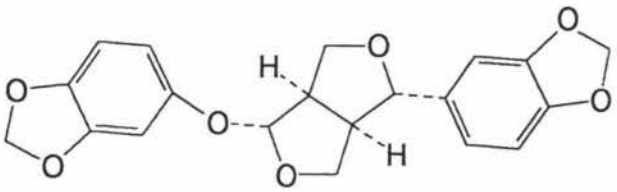
Discussion

(i) Target compounds

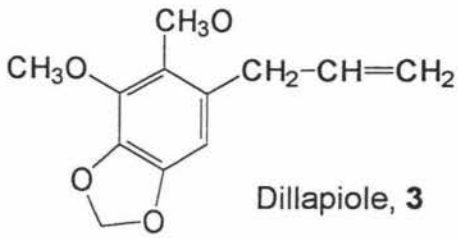
Sesamin (**1**) and sesamolin (**2**) are components of sesame oil. Dillapiole (**3**) is found in dill oil and a number of other plant species. Dillapiole can be reduced to dihydrodillapiole (**4**) by hydrogenation. The aim of this work was to prepare sufficient material for use by Miss S Wright in trials as pyrethrum synergists.



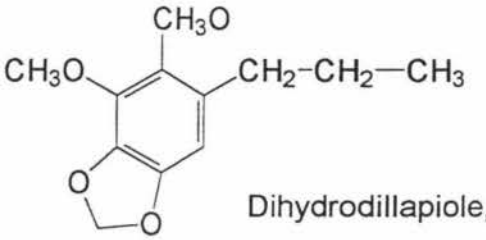
Sesamin, **1**



Sesamolin, **2**



Dillapiole, **3**



Dihydrodillapiole, **4**

(ii) Commercial Sources

On-line catalogue searching on the Chem Cats and CS Chem databases produced the results shown in table 1. The high costs of the targets meant that extraction was a viable option.

Table 1 Commercial availability of **1-4**

Compound	Commercial Source	Price
Sesamin, **1**	Cayman Chemicals 690 KMS Place Ann Arbor MI 48108, USA	$75/5 mg; $120/10 mg; $480/50 mg; $775/100 mg (Presumably US$)
	APIN Chemicals Ltd Unit 29D, Wilton Park Abingdon Oxon, UK	US$350/50 mg
Sesamolin, **2**	None	
Dillapiole, **3**	Fluka Chemie	US$70.75/0.5 g US$151.60/1.0 g

(iii) Extraction of Sesamin and Sesamolin

From literature supplied by Miss Wright it appeared that these compounds could be isolated by (a) acetonitrile extraction of sesame oil to give a crude extract; (b) chromatography of the crude extract; and (c) recrystallisation of the sesamin/sesamolin containing fractions. The results of following this approach are summarised in Table 2. Sesamin and sesamolin co-eluted on thin layer and column chromatography, and separating them was deemed impractical. Hence the final recrystallised material is a mixture of the two compounds.

Extract number	Yield of crude extract / g (%)	Yield of impure **1** and **2** after chromatography/g	Yield of mixed **1** and **2** after recrystallisation/g
1	7.80 (0.78)	2.22 (from 4 g crude extract; 55%)	1.23 (31% overall from 4 g crude; 0.24% from oil)
2	4.48 (0.45)	1.36 (30% from crude extract)	0.87 (19% from crude extract; 0.09% from oil)
3	2.99 (0.30)	1.30 from extracts 3 and 4 combined, (18% from crude extract)	0.47 (6.7% from crude extract; 0.05% from oil)
4	4.04 (0.40)		
Total	19.31 (1.9)	4.88 (from 15.5 g crude; 31%)	2.57 (16% of crude extract)

Clearly most **1** and **2** were in the first extract, and this extract contained around 55% **1** and **2**.

Recrystallisation from ethanol caused significant loss of material but gave a much cleaner product.

(iv) Isolation of Dillapiole **3**

3 has been isolated by vacuum distillation of dill oil. Such a distillation at ca 1 mm Hg gave 5 fractions, shown by NMR to not contain dillapiole, and a residue containing dillapiole. A second distillation of the residue at higher temperature (oil bath 160°C) and much better vacuum (0.1 mm Hg) gave 2 fractions. One fraction contained **3** and unidentified material, whilst the latter fraction was almost pure dillapiole. This fraction was obtained in 6.5% yield. Insufficient time was available to attempt reduction of **3** to **4**.

Experimental

(i) Sesamin/Sesamolin

Crude extract

Sesame oil (Healtheries cold pressed, no batch numbers, 5 x 200 mL) was placed in a 2 L flask and acetonitrile (2L) added. The mixture was stirred magnetically and heated in a water bath (50-65°C) for 1 hour. After cooling the 2 phase system was poured into a 2 L separating funnel and the lower (sesame oil) layer removed. The cloudy upper layer was transferred to a 1 L separating funnel and left to settle overnight. Fresh acetonitrile (750 mL) was added to the sesame oil and stirred at room temperature for 1 hour. The mixture was treated as for the first extract.

After standing overnight any lower layer was decanted, and the acetonitrile removed under vacuum to give crude extract - 7.80 g of biphasic yellow oil from the first extract, 4.48 from the second.

The extraction was repeated a third time with *ca* 800 mL of acetonitrile, giving 2.99 g oil. A fourth extraction with 750 mL of solvent gave 4.04 g oil.

Thin layer chromatography

Using ethyl acetate/petroleum ether mixtures (1:1, 1:4; 1:9) four distinct components were visible in crude extracts 1 and 2. These components were only weakly fluorescent under UV light but showed up clearly using ammonium molybdate/ceric sulphate dip.

Column chromatography

Crude extract 2 (4.46 g) was chromatographed on flash grade silica in a 5 cm diameter column 35 cm tall. The solvent used was 4:1 petroleum ether:ethyl acetate. The 4 components detected on TLC were collected and examined by NMR. The material with R_F 0.25 (1.36 g) was shown to contain **1** and **2**. The majority of the sample (2.34 g) had R_F 0.7. Two other components (0.06 g and 0.92 g) had R_F 0.3 - 0.4.

The mixture of **1** and **2** was recrystallised from boiling absolute ethanol (*ca* 10 mls). The crystals were collected by filtration and sucked dry to give 0.76 g pale yellow solid. NMR showed this to be a ~5:1 mixture of **1** and **2** (by peak heights of ^{13}C at 50 ppm).

Similarly, combined crude extracts 3 and 4 (7.03 g) gave 1.30 g of the R_F 0.25 material. This was recrystallised to give 0.46 g of crystals of **1** and **2**.

Finally, 4 g of crude extract 1 gave 2.22 g of the desired material, which yielded 1.23 g mixed **1** and **2** after recrystallisation.

The mixtures of **1** and **2** were combined and blended to yield the final sample. This was shown by NMR (spectra attached) to be a roughly 2:1 mixture of **1:2**.

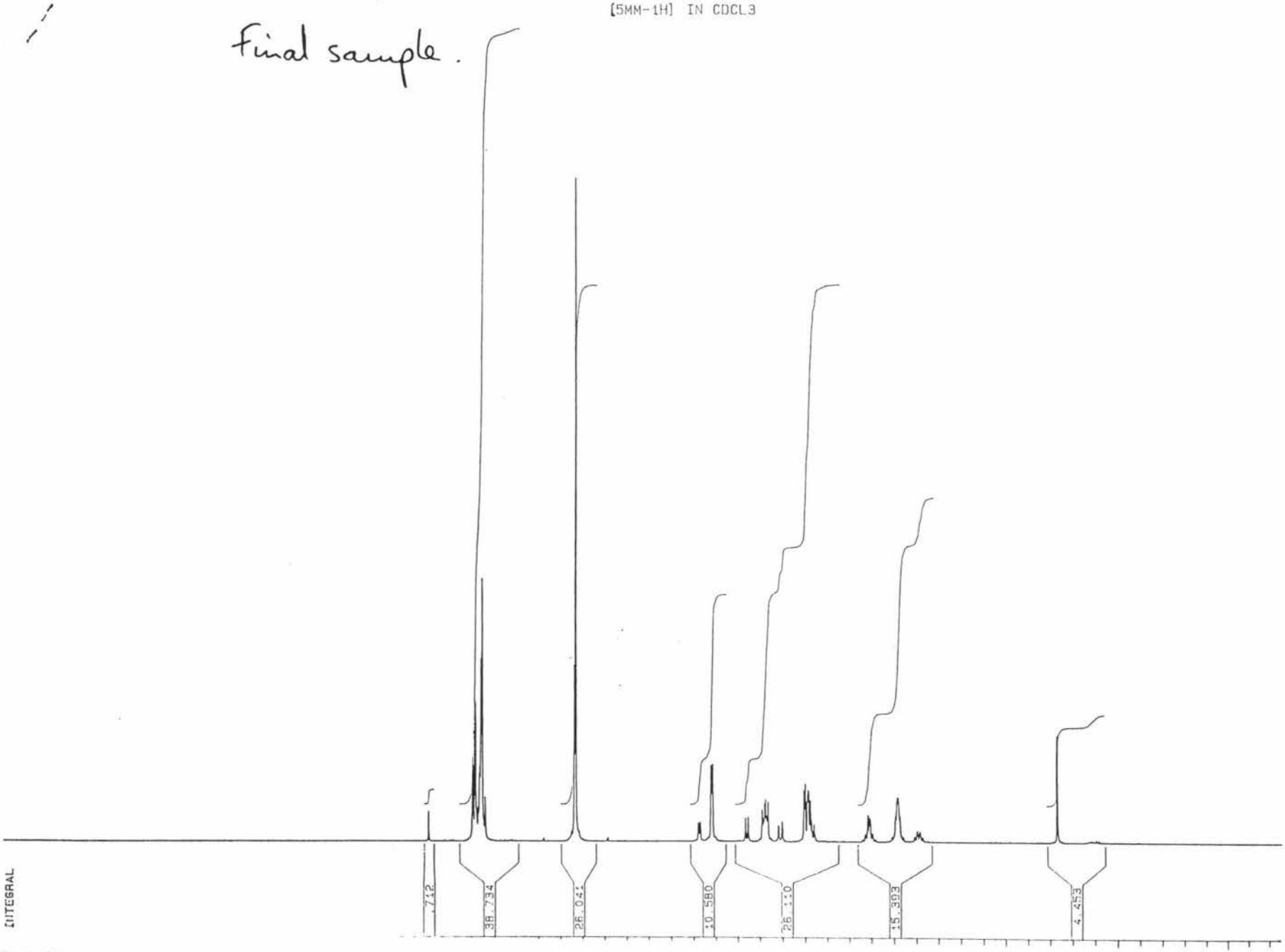
(ii) Dillapiole, **3**

Dill oil (Auroma, batch #2902/3575; 49.25 g) was distilled at >1.0 mm Hg to give 5 fractions (total 40.95 g), (bp's ranging from 50°C to 110°C) and a residue. NMR showed only the residue contained significant amounts of **3**.

The residue was redistilled at 0.1 mm Hg and two fractions collected. The first (2.4 g) was a mixture of **3** and another compound. The second fraction, collected at an oil bath temperature of 160°C, was shown by NMR to be virtually pure **3**. Yield 3.2 g. NMR spectra attached.

Sesamin / sesamolin

final sample.



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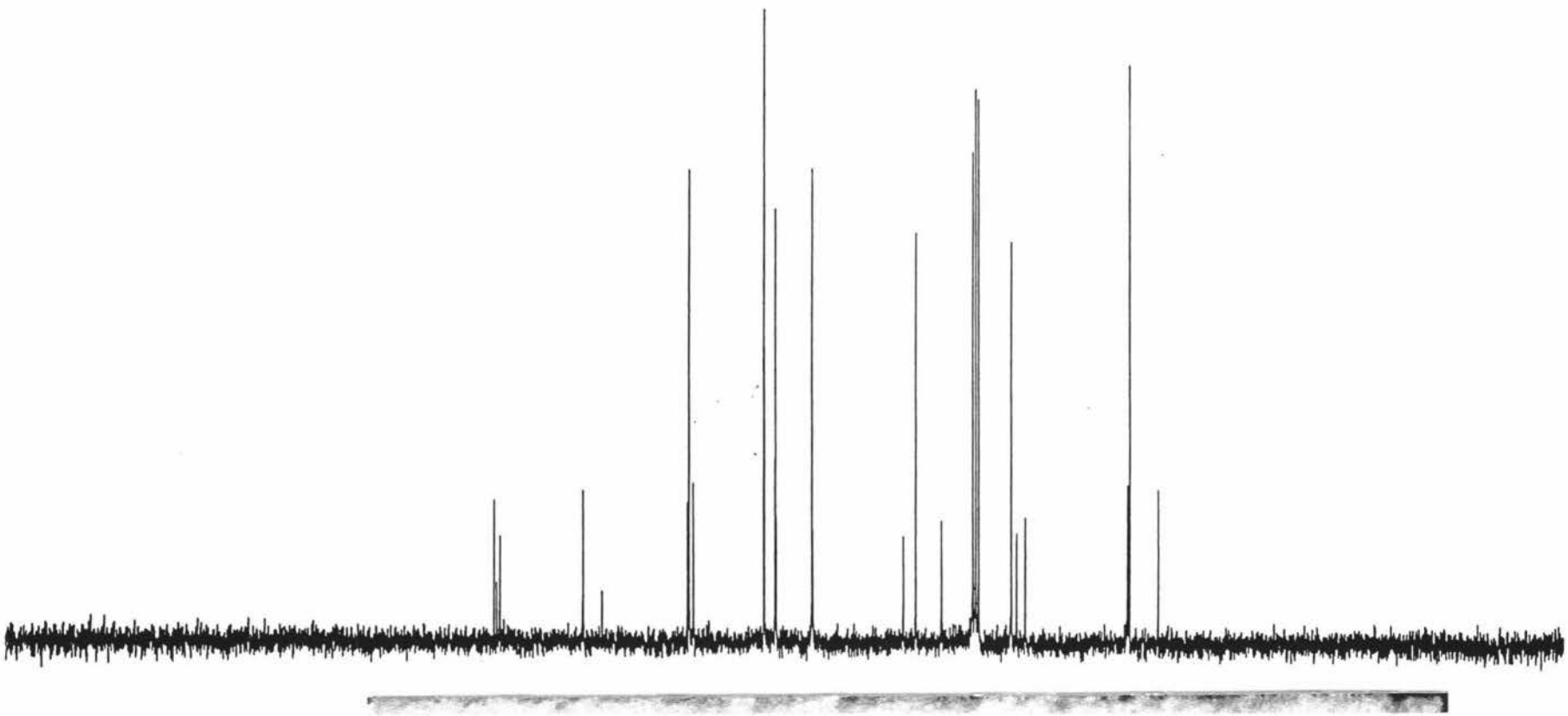

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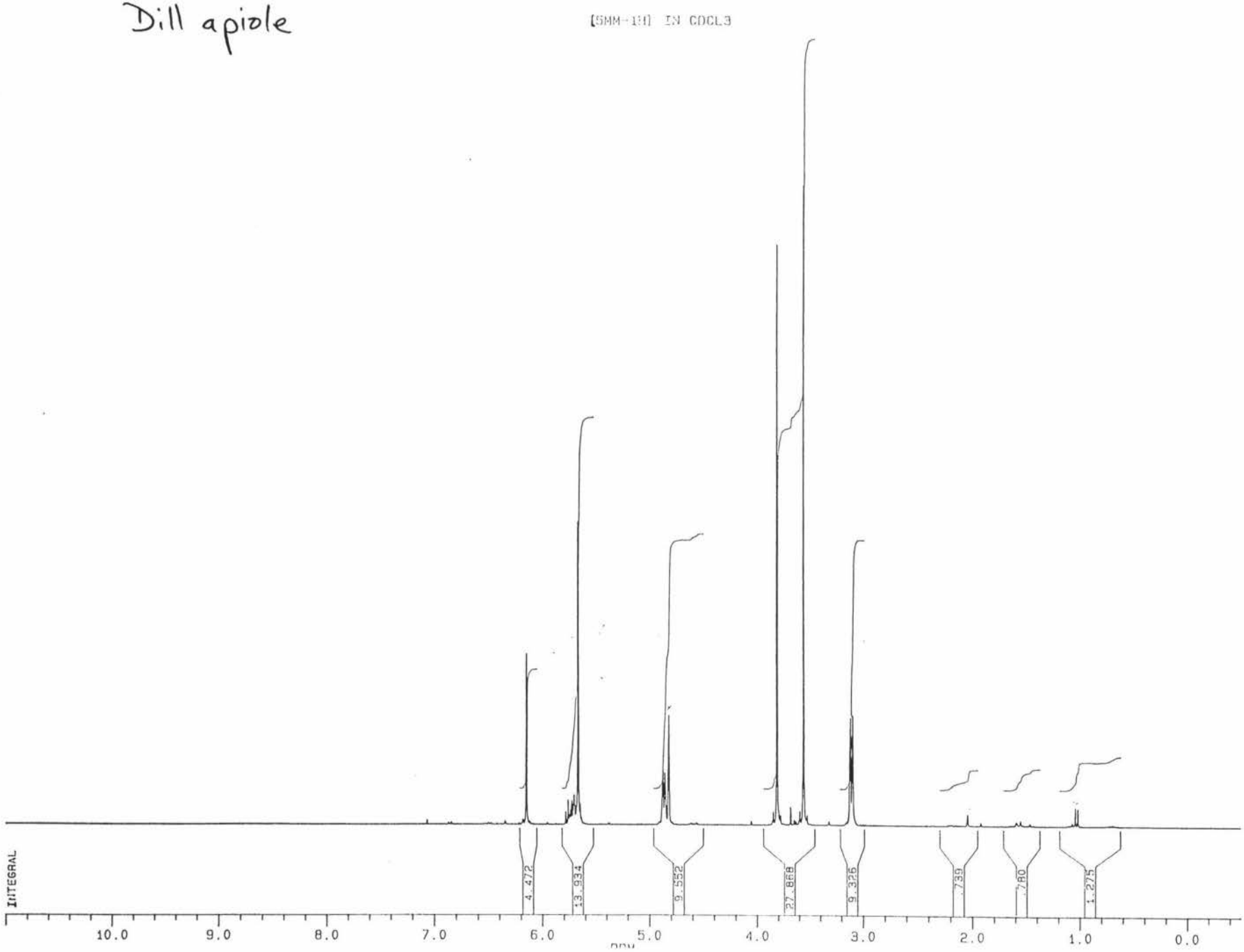
SA.NO MY10 108

NS 960

LB 1.000
SR -1391.14

Dill apiole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.780
1.275
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9.0
8.0
7.0
6.0
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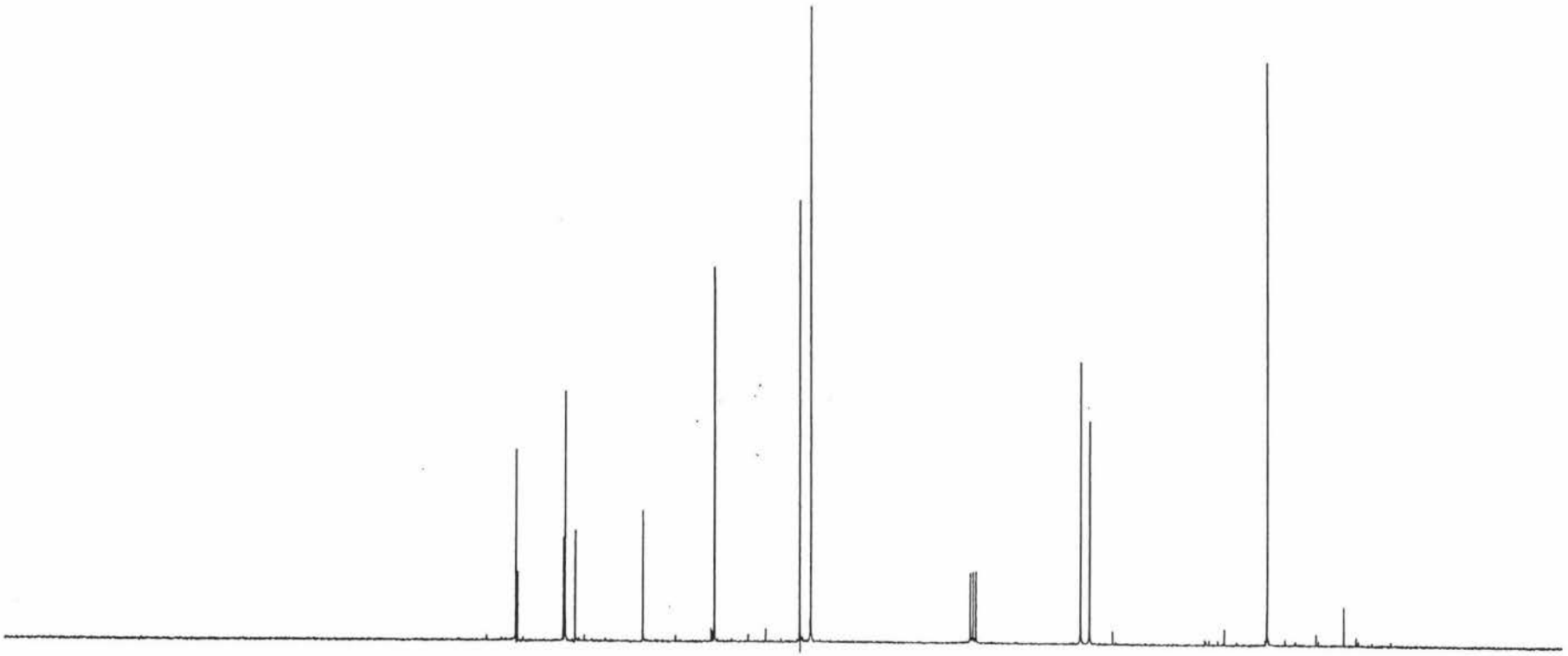
PPM

(5MM-13C) IN CDCL3 : POWGATE WITH WALTZ DECOUPLING



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SA.NO MY09 119

NS 5120

LB 1.000
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APPENDIX 3

SAS programme for probit analysis

```
/* SAS PROGRAM FOR  */

options ls=78 ps=60 nodate;

data d1;

       input insectsd $ dose ratio nodead totno ;
        phat = nodead/totno;
       ldose = log(dose) ;
cards;



 ;

proc logistic;
 model nodead/totno = dose /ctable  ;
output out=d2 pred = propdead ;
 by insectsd ratio ;
  title 'Output from logistic procedure';
run;

proc plot data = d2; by insectsd ratio;
plot propdead*dose ;
run;


 title 'complimentary log-log link';

proc logistic data=d1;
 model nodead/totno = dose / link=cloglog ctable  ;
output out=d3 pred = propdead ;
by insectsd ratio ;
  title 'Output from complimentary log-log link procedure';
run;

proc plot data = d3; by insectsd ratio;
plot propdead*dose ;
run;

  title;

data d5;
       input insectsd $ dose ratio nodead totno ;
        phat = nodead/totno;
       ldose = log(dose) ;
cards;



;

proc probit data=d5;
   model nodead/totno=dose/lackfit inversecl;
```

```
   output out=b1 p=prob std=std xbeta=xbeta ;
  Title 'Output from Probit linear dose proceedure' ;
 run ;

Proc plot ;
   plot phat*dose = 'X' prob*dose = 'P' / overlay ;
  Title 'Plot of observed and fitted probabilities' ;
run ;


   /*
proc probit log10 data=d5;
   model nodead/totno=dose/lackfit inversecl;
   output out=b1l p=prob std=std xbeta=xbeta ;
  Title 'Output from Probit log dose proceedure' ;
 run ;

Proc plot ;
   plot phat*ldose = 'X' prob*ldose = 'P' / overlay ;
  Title 'Plot of observed and fitted probabilities' ;
run ;
   */


proc probit data=d5;
   model nodead/totno=dose/lackfit inversecl d=logistic;
   output out=b2 p=prob std=std xbeta=xbeta ;
  Title 'Output from logistic dose proceedure' ;
 run ;

Proc plot ;
   plot phat*dose = 'X' prob*dose = 'P' / overlay ;
  Title 'Plot of observed and fitted probabilities' ;
run ;

proc print;
run;

 proc probit data=d5 ;
   model nodead/totno=dose/lackfit inversecl d=gompertz;
   output out=b3 p=prob std=std xbeta=xbeta ;
  Title 'Output from Gompertz dose proceedure' ;
 run ;

Proc plot ;
   plot phat*dose = 'X' prob*dose = 'P' / overlay ;
  Title 'Plot of observed and fitted probabilities' ;
run ;
```