ASSESSING THE EFFICACY AND PERSISTENCE OF ROSEMARY OIL AS A MITICIDE / INSECTICIDE FOR USE ON GREENHOUSE TOMATO

by

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Abstract

Efficacy of rosemary essential oil was assessed against two-spotted spider mites (*Tetranychus urticae*) and greenhouse whiteflies (*Trialeurodes vaporariorum*) as well as its effects on the tomato host plant and bio-control agents.

Laboratory bioassay results indicated that pure rosemary oil and EcoTrol™ (a rosemary oil-based pesticide) caused complete mortality of spider mites and whiteflies at concentrations that are not phytotoxic to the host plant. The predatory mite, *Phytoseiulus persimilis,* is less susceptible to rosemary oil and EcoTrol™ than twospotted spider mites both in the laboratory and the greenhouse, whereas the parasitic wasp, *Encarsia formosa,* is more susceptible to rosemary oil than whiteflies. Rosemary oil repels both spider mites and whiteflies and can affect oviposition behavior. Rosemary oil and rosemary oil-based pesticides are non-persistent in the environment and their lethal and sub-lethal effects fade within one or two days. EcoTrol ™ is safe to tomato foliage, flowers and fruits even at double the recommended label rate.

A greenhouse trial indicated that a single application of EcoTrol™ at its recommended label rate could reduce a twospotted spider mite population by 52%. At that rate, EcoTrol™ did not cause any mortality among predatory mites *Phytoseiulus persimilis* nor did it affect their eggs.

Toxicity of individual and incomplete mixtures of constituents of rosemary oil to spider mites indicated significant synergy among the constituents. Highest mortality was only obtained when all constituents were present in the mixture.

In general, EcoTrol™ was found to be a suitable option for small-scale IPM programs for controlling spider mites and whiteflies in greenhouse tomato plants.

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Chapter One: Introduction

1.1 *Major objectives*

The major objective of this research was to study the efficacy of a rosemary oil-based pesticide (EcoTrol™) for use on greenhouse tomato plants against the important pests of greenhouse crops (the two-spotted spider mite and the greenhouse whitefly). This pesticide was introduced to the US market in 2002. It has been used mainly on berries, grapes, nuts and tree fruits. The product also has been used on vegetables, but its use in the greenhouses has been limited to a few growers. Although this is a natural product and has been used in the United States, it requires regulatory approval by the Pest Management Regulatory Agency (PMRA) in order to be used by Canadian growers. Similar to all new pesticides, it requires some assessment before entering the market. The following questions need to be addressed:

- Is this product efficacious against pests?
- Can it be hazardous to human health?
- What environmental risks does it pose?
- Is it persistent in the environment?
- What effects might it have on non-target terrestrial and aquatic species?
- What are the effects of this product on the host plant?
- Is it toxic to bio-control agents?
- Are there any limitations for its application?
- Could pests evolve resistance after repeated applications?
- What is the mechanism of toxicity of this product?

- Can environmental factors affect its efficacy?

In this research, I tried to answer some of these questions. I focused mainly on the efficacy of this pesticide against the pests and its effects on their bio-control agents and host plants under laboratory conditions concluding with a small-scale greenhouse trial.

1.2 *Historical perspective*

Most present agricultural practices involve growing highly domesticated plants and animals. Animal and plant domestication involved a gradual shift in the pattern of human interaction with the environment. For thousands of years prior to domestication, humans subsisted by scavenging, gathering plants and hunting animals as they were found in the environment. In the domestication process, humans manipulated animals, plants and the environment in various ways to increase the availability of the desirable species and desired traits of these species. Over long periods of time and many generations of human selection, domesticated plant varieties and animal breeds emerged with traits that would not survive without human involvement in such tasks as selective breeding, planting, weeding, harvesting, pest control and storage.

These manipulations to the environment resulted in new problems that did not exist before. In cropping systems in particular, monoculture of plants provided an aggregated food source for pests and suitable growing conditions for their offspring.

With increasing human population the necessity of providing more food increased. They not only had to develop their agriculture practices to produce more yield, but also had to protect their products from damaging factors, most importantly pests. As a consequence, many pest control methods have been developed and this remains an important subject of research. Introduction of synthetic chemical pesticides and their

extensive use in agriculture was a new chapter in the history of pest control. Development of insecticides, such as DDT, brought hope to the agricultural industry. Many farmers took DDT as a guaranteed pest control tool that could completely solve the pest problem; however, that feeling of relief quickly faded due to a new phenomenon; pesticide resistance. Intensive application of pesticides is the most important factor in the quick build-up of resistance in most pest populations. Pesticide application removes the susceptible pests from the population and only those having resistance genes will survive, passing the resistance trait on to their offspring. The percentage of resistant pests in a population continues to multiply while pesticides eliminate susceptible ones. Eventually, resistant pests outnumber susceptible ones and the pesticide is no longer effective. In addition to the pest resistance problem, extensive application of pesticides caused damage to the environment, non-target organisms, higher animals in ecosystem and impacted human health. Numerous studies have been conducted on this subject and many books have been written to describe its detection, magnitude, mechanism, side effects and management (1,2).

Recently, some scientists have taken a different approach towards pest management by using plants as potential sources of pesticides. Plants have evolved different defense mechanism against herbivores. Secondary metabolites of plants – including a wide array of chemicals- are good examples of these mechanisms. Our ancestors were aware of the healing and medicinal properties of some plants and their extracts. They also used some plants to repel insects and other pests. In recent decades scientists started to investigate these ancient traditions in search of possible substitutes for synthetic pesticides and drugs (3). Although these natural based- products seem to be

safer than conventional synthetic products, there are many unknown aspects that need to be addressed before extensive application. In the present study, a rosemary oil (*Rosmarinus officinalis*) based- pesticide has been tested against two major pests of greenhouse tomato (*Lycopersicon esculentum*), the two-spotted spider mite (*Tetranychus urticae*), and the greenhouse whitefly (*Trialeurodes vaporariorum*).

1.3 *Greenhouse Tomato*

The greenhouse vegetable industry is an important and growing segment of Canadian agriculture. According to Agriculture and Agri-Food Canada (4), the estimated value of the greenhouse industry was $80 M in 1988, reaching $600 M in 2000. Official statistics (Statistics Canada Publ. 20-202 for 2000) value the Canadian greenhouse industry at $1711 M and the greenhouse vegetable portion at $505 M. The main greenhouse vegetable crops in Canada are tomatoes (468 ha), cucumbers (190 ha), sweet peppers (144 ha) and lettuce (21 ha) (5).

During the 1990s, the total area under glass and plastic more than doubled to nearly 1,500 hectares. By 2003, it had reached nearly 1,900 hectares. In 2003, revenue from greenhouse sales reached a record high of almost $2.1 billion; nearly double what it had been just six years earlier. Flowers accounted for about 70% of sales and vegetables the remaining 30%. In the early 1990s, revenues from the comparable greenhouse and field vegetables were roughly the same. However, since 1996, revenues from greenhouse vegetables have increased at a much more rapid pace than field vegetables. For example, in 2003, the farm gate value of the four main vegetable crops produced under glass or plastic (tomatoes, cucumbers, lettuce and peppers) amounted to $605.8 million. This was more than three times higher than the value of $171.7 million for the same four vegetable

crops produced in the field. Farmers grow more tomatoes than any other vegetable crop, whether it's in the greenhouse or in the field. Tomatoes alone, account for over one-half of revenues from the sale of greenhouse vegetables. They also cross the border in both directions. Canadian greenhouse growers have been shipping hothouse tomatoes to the United States in rising numbers. In recent years, Canada has enjoyed a trade surplus in tomatoes, shipping far more south of the border than American farmers ship north. (6)

1.4 *Two-spotted spider mite*

Spider mites belong to the family Tetranychidae of the order Prostigmata. They are so named because many members of this family produce silk webbing on host plants. Most spider mite species are polyphagous. The Tetranychidae is a large family of worldwide distribution. The family consists of two subfamilies: Bryobinae and Tetranychinae. Most pest species belong to the Tetranychinae. The two-spotted spider mite *Tetranychus urticae* Koch is the most important species in this subfamily. The two-spotted spider mite is the most common name for this species. It is also known informally by many other names (e.g. the glasshouse spider mite, the yellow spider mite). Not very appropriately, it is called the red spider mite or red spider in some literature presumably because of the red/orange color or the overwintering form, or in reference to a species complex including *T. cinnabarinus*. The two-spotted spider mite is an important pest of greenhouses throughout the world. It is a cosmopolitan species and also is the most polyphagous species of spider mite. It has been reported on about 1200 host plant species in 70 genera of which over 300 species are grown in greenhouses. Its life cycle consists of eggs, one larval stage, two nymphal stages and an adult stage.

The eggs are often laid in clusters on the under surface of leaves. They are spherical in shape and translucent, pale in color. As they develop, they become more yellowish and red eyespots inside the eggshell can be seen. Six- legged larvae are pale to yellowish when first hatched and become yellowish green after feeding. Eight-legged nymphs are yellowish green with dark spots. Their body is ovoid in shape with short legs. Adult females are about 400-500 μm in length and males are smaller with a pointed hysterosoma. The females (summer form) are yellowish to greenish in color with two black spots on the dorsolateral idiosoma, but are darker in color, often orange or red in the overwintering form. The color of mites may vary depending on the host plant and other environmental factors.

Two spotted spider mites often feed on cell chloroplasts on the under surface of the leaf. The upper surface of the leaf develops characteristic whitish or yellowish stippling, which may join and become brownish as mite feeding continues. As mites move around, their webbing can span leaves and stems. Heavy damage may cause leaves to dry and drop, and the plant may be covered with webbing and may die prematurely.

Development occurs between 12 and 40°C. Developmental time from egg to adult decreases with increasing temperature and is less than a week at optimal temperatures for development (30-32°C). Under a diurnal temperature cycle of 15 to 28°C, development time is about 16 days. Males develop slightly faster than females.

Males are attracted to a sex pheromone from dormant female deutonymphs. They guard their territory and fight against any other invading males. Mating occurs as soon as females emerge. Females start to lay eggs within a couple of days of adulthood. The rate of oviposition and fecundity varies with food plant and temperature. An average female

can lay over ten eggs per day and produce over 100 eggs during two weeks at about 25°C. The sex ratio is highly female biased, with a female to male ratio of about 3:1. *T. urticae* disperses by active walking or by passive transport in the wind, on plants, on tools or on people. Diapause is induced by short day lengths, lack of food supply and low temperature, and is normally terminated by a fixed period of chilling. Gravid females seek a protected niche at the end of summer. Diapausing adults are orange/red in color (7).

The economic threat posed by these mites is constantly increasing because of the development of resistance, and resurgence of mite populations following use of non-selective synthetic pesticides that eliminate natural enemies such as predaceous mites and spiders (8). Spider mites have evolved resistance to more than 80 acaricides to date and resistance has been reported from more than 60 countries (9). Spider mites and especially two-spotted spider mites have been on the priority lists for pest management in BC greenhouses for several years. The current control method for spider mites in BC greenhouses is based on bio-control agents that include six different predatory mites (*Phytoseiulus persimilis* Athias-Henriot, *Metaseiulus occidentalis* Nesbitt, *Amblyseius californicus* Carte, *Persimilis longipes* Evans, *Galendromus occidentalis* Nesbitt and *Amblyseius cucumeris* Oudemans), one predatory midge (*Feltiella acarisuga* Vallot) and two predatory bugs (*Deraeocoris brevis* Uhler and *Orius sp.*). Problems with bio-control include limited efficacy against high populations of spider mites, constraints, and bio-control agents' susceptibility to most pesticides.

1.5 *Greenhouse Whitefly*

The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, is another important pest of greenhouse crops worldwide. Its life cycle consists of eggs, a crawler stage, three nymphal stages, a pupa and adults. Whitefly adults are tiny, white moth-like insects. They lay eggs on the underside of leaves. Eggs hatch in 10 to 14 days. After three molts in about 14 days, they pupate and the adult emerges 6 days later. Adults begin to lay eggs 4 days after emergence. Each female is capable of laying 400 eggs over a period of up to 2 months, although usually far fewer eggs are produced. The length of the life cycle is temperature dependent. Adults live 30 to 60 days and feed by sucking sap from the plant.

Two species of whitefly infest British Columbia greenhouse crops. *T. vaporariorum* Westwood (the greenhouse whitefly) is the most common one. However *Bemesia tabaci* Gennadius (the sweet potato whitefly) was introduced to Canada in recent years on imported plant materials and has been found in some greenhouses.

Greenhouse whitefly has a wide host range and is known to develop on more than 250 ornamental and vegetable plants. Poinsettia, hibiscus, nicotiana, aster, calendula, cucumber, lantana, tomato, grape, ageratum, bean, and begonia are among the more commonly infested plants (10).

B. tabaci is more difficult to control, owing to its high egg laying capacity and wide host range. Sweet potato whitefly is smaller than the greenhouse whitefly and is more off-white or yellow-white in color. Sweet potato whitefly is typically found lower in the plant canopy than greenhouse whitefly, which is often found on the developing leaves at the growing points. Both whitefly species can cause severe damage to foliage by reducing vigor and by coating the growing points, leaves, and fruits with excreta

(honeydew). The excreta becomes a food source for fungal moulds to develop. This mould coats pepper and tomato fruit requiring extra fruit cleaning costs prior to sale. Sweet potato whitefly can also transmit viruses and cause abnormal fruit discoloration. Like the two-spotted spider mite, the greenhouse whitefly is among the most important pests of BC greenhouses. Control mostly relies on bio-control agents, such as the parasitic wasp *Encarsia formosa* (11,12). Like spider mites, whiteflies have also been evolved resistance to many pesticides (1).

1.6 *Greenhouse pest management*

As explained before, most domesticated plants cannot survive without human involvement. Greenhouse plants are among the most human-dependent ones. Greenhouses provide the ability to control environmental factors that have great impact on plants. In modern greenhouses, almost all environmental factors are under control, for instance temperature, moisture, light, nutrients and even the composition of different gases in the air that plants need for growth. In greenhouses, plants constantly grow and provide fruit throughout the year regardless of the outside conditions. These controlled conditions are not only suitable for plants but also for a wide range of pests, which normally cannot survive outside the greenhouse. Having optimal conditions for growth and an aggregated food source allows many pests to achieve epidemic populations. As the easiest control measure, pesticides have been extensively used inside greenhouses to control pests during the production season. This strong selection pressure resulted in emergence of highly resistant populations of pests to almost all pesticides that have been applied so far. Bio-control agents were introduced to greenhouses as an alternative solution. Different predators or parasitoids of insect or mite pests were identified and

released inside greenhouses to reduce their populations. This method has been successful for several pests. In Canada, the greenhouse pest management is largely based on biological control. A survey conducted in 2002 indicated that 93% of tomato growers (n= 165) use biological control for insect and mite control (12). Although biological control methods were successful for most pests, they have limitations and cannot be used in all situations. For example, it has been shown that activity and survival of the predatory mite *Phytoseiulus persimilis* can be affected by different levels of humidity (13), temperature (14) and pest density (15). Because of these limitations, bio-control agents might not be able to reduce pest populations to an acceptable level by themselves. Thus, utilization of bio-control agents for controlling greenhouse pests may only be effective when combined with other strategies (16). As an example, Nicetic *et al.* (17) found that a combination of petroleum spray oil and the predatory mite *Phytoseiulus persimilis* can be used to control two-spotted spider mites on greenhouse roses. When the use of a pesticide is necessary, materials should be selected that are least harmful to the predators and parasites released into the greenhouse. Essential oils of plants might be considered a good option for use in combination with other pest control methods.

1.7 *Plant essential oils*

Plant essential oils are odorous compounds obtained through steam distillation of herbs and medicinal plants (18). These oils have been used traditionally as healing medicines in many countries and ancient people were also aware of their pesticidal properties, however, only in recent years have these oils been commercialized as pest control products (3). Most of these oils are environmentally non-persistent, and non-toxic

to humans (with some exceptions) (19-21), fish (with some exceptions) and wildlife (22-24)

Plant essential oils are generally mixtures of mono- and sesquiterpenes (e.g., α-terpineol and pulegone) and phenolics or monophenols (e.g., thymol, carvacrol, and eugenol). They are often quite volatile and are commonly used as fragrances and as flavoring agents in food (25). They are sometimes incorporated into natural pest control products. For example, 1,8-cineole, a major component of oil of eucalyptus and rosemary is active against four stored products beetles (26). Hough–Goldstein (27) reported antifeedent effects of essential oils against the colorado potato beetle *Leptinotarsa decemlineata* L. while Sharma and Saxena (28) showed their effectiveness as growth inhibitors on houseflies *Musca domestica* L.

Many researchers have reported repellent, antifeedent and toxic properties of selected essential oils against many agriculturally important pests. Thymol showed both repellent and toxic effects against the two-spotted spider mite (29). In another study, thymol and citronellic acid were found toxic to the common housefly, western corn rootworm *Diabrotica vergifera vergifera*, and the two-spotted spider mite (30). Thyme oil was also found to be toxic to the tobacco cutworm *Spodoptera litura* (31). Choi *et al.* (32) tested a total of 53 esential oils against *T. urticae* and *P. persimilis* via fumigation. Caraway seed, citronellal, lemon, eucalyptus, pennyroyal and peppermint oils were found to be highly toxic to both mites. Rosemary oil was also found to be toxic to the predaceous mites *Amblyseius barkeri* Hughes, *A. zaheri* and *Typhlodromus athiasae* Porath (33) while it showed repellent properties against the onion aphid *Neotoxoptera formosana* Takahashi (34) and the green peach aphid *Myzus persicae* Sulzer (35). Both

contact and fumigant toxicities of eugenol and methyl eugenol were demonstrated to the American cockroach *Periplaneta americana* L. (36).

The mechanisms of toxicity of essential oils have not been fully identified. However a recent investigation using the American cockroach points to the octopaminergic nervous system as the site-of-action of some essential oils in insects (37).

1.8 *Rosemary oil*

Rosmarinus officinalis L. is an evergreen perennial woody shrub with aromatic, needle-like leaves and gray, scaly bark. Rosemary bushes can grow up to 6 ft (1.8 m) tall with a spread of 4-5 ft (1.2-1.5 m). This plant belongs to the family Lamiaceae formerly known as the Labiatae. It is used for flavoring food and beverages and it also used in cosmetics and aromatherapy. Rosemary and its constituents, have a therapeutic potential in treatment or prevention of bronchial asthma, spasmogenic disorders, inflammatory diseases and hepatotoxicity (38). Anti-cancer (39-41) and anti-viral (42) properties of rosemary have also been reported.

Beside the great medicinal value of rosemary for humans, the essential oil of rosemary has strong antibacterial activity against microorganisms such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli* O 157:H7, *Shigella dysenteria, Bacillus cereus and Staphylococcus aureus* (43).

It has been reported that the essential oil of rosemary has repellent and deterrent properties against *Thrips tabaci* and affect host plant selection and acceptance (44).
It also showed ovicidal activity against two stored-product insects (45). Essential oil of rosemary is a complex mixture of different constituents. A recent study detected 33

compounds in the oil (46). The main components of the oil are α-pinene, 1,8-cineole, camphor, β-pinene, and borneol.

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Chapter Two: Lethal and sub-lethal effects of *Rosmarinus officinalis* essential oil and three rosemary oil-based pesticides to *Tetranychus urticae* Koch, *Trialeurodes vaporariorum* Westwood, *Phytoseiulus persimilis* Athias-Henriot, and *Encarsia formosa* Gahan, on *Lycopersicon esculentum*

2.1 *Introduction*

2.1.1 *Tetranychus urticae* (Two-spotted spider mite)

Two-spotted spider mite, *Tetranychus urticae* Koch, occurs on virtually every major food crop and ornamental plant. About 1200 species of spider mites are known in the world (1). It is the most polyphagous species of spider mites and has been reported from over 150 host plants of economic value (2). The greatest problem with this mite is its ability to evolve resistance rapidly to pesticides only after few applications (3). Spider mites have evolved resistance to more than 80 acaricides to date and resistance has been reported from more than 60 countries (4). Spider mites and especially two-spotted spider mites have been a priority pest in BC greenhouses for several years. The current control method for spider mites in BC greenhouses is mostly relying on bio-control method based on six different species of predatory mites, one predatory midge and two predatory beetles. The problem with these bio-control agents is their limited efficacy against higher populations of spider mites and their susceptibility to most pesticides.

2.1.2 *Trialeurodes vaporariorum* (Greenhouse whitefly)

Greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, is another important pest of greenhouse crops worldwide. Two species of whitefly infest British Columbia greenhouse crops. *T. vaporariorum* Westwood (the greenhouse whitefly) is the more

common one. However *Bemesia tabaci* Gennadius (the sweet potato whitefly) was also introduced to Canada in recent years on imported plant materials and has been found in some greenhouses.

Greenhouse whitefly has a wide host range and is known to develop on more than 250 ornamental and vegetable plants. Poinsettia, hibiscus, nicotiana, aster, calendula, cucumber, lantana, tomato, grape, ageratum, bean, and begonia are among the more commonly infested plants (5). Like spider mites, whiteflies have also evolved resistance to many pesticides (6).

2.1.3 *Phytoseiulus persimilis* (Predatory mite)

The predatory mite, *Phytoseiulus persimilis* Athias-Henriot, has been studied extensively with respect to its potential for biological control of tetranychid mites on vegetables and ornamentals in greenhouses (7,8) *P. persimilis* is a selective predator that is able to rapidly suppress spider mites (9,10). Since juvenile development and reproduction of *P. persimilis* depends on the availability of spider mites as prey (11), it often disappears from the greenhouse after reducing pest mite populations and thus provides short-term control. Studies of functional responses of *P. persimilis* to different densities of spider mites suggested that they might not provide acceptable control for higher populations of prey (12). Thus utilization of the predatory mite for controlling spider mites in greenhouses may only be effective when combined with other strategies (13). As an example, Nicetic *et al.* found that a combination of petroleum spray oil and the *P. persimilis* can be used to control two-spotted spider mites on greenhouse roses (14).

2.1.4 *Encarsia formosa* (Parasitic wasp)

Encarsia formosa Gahan is used worldwide for commercial control of whiteflies in greenhouse crops. Commercial use began in Europe in the 1920s and it shipped to Canada in the 1930s, but by 1945 interest waned due to the development of pesticides. After 1970, use was reinitiated and has expanded from 100 hectares of greenhouse crops to 4800 hectares in 1993 (8).

In Canada, the greenhouse vegetable industry is largely based on biological controls. A survey conducted in 2002 indicated that 93% percent of the tomato growers (total of 165 growers) use biological control agents for insect and mite control (15).

More than seventy papers have been published that examine interactions between *E. formosa* and one or more pesticides, either in laboratory tests or under conditions of practical use in greenhouses. Standardized methods for determining the effects of pesticides on *E. formosa* have been developed and the effects of more than one hundred different compounds on *E. formosa* have been determined. Most of the pesticides were found to be toxic to *E. formosa*. Selective materials of interest for possible combination with *E. formosa* include insecticidal soap, buprofezin, azadirachtin, abamectin, and resmethrin (16). When the use of a pesticide is necessary, materials should be selected that are least harmful to the predators and parasites released into the greenhouse. Essential oils of plants may be a good option for use in combination with other pest control methods.

2.1.5 *Rosmarinus officinalis* (Rosemary plant)

Plant essential oils are obtained through steam distillation of herbs and medicinal plants (17) Most of these oils are environmentally non-persistent, and non-toxic to humans (with some exceptions) (18-20), fish (with some exceptions) and wildlife (21-

22). Rosemary oil has been traditionally used as a medicine for colic, nervous disorders and painful menstruation. Recent studies revealed that the rosemary oil is also an effective antibacterial agent (23-24). Rosemary oil is relatively effective against insect and mite pests. The aromatic vapor of rosemary has ovicidal and larvicidal effects on several stored product pests (25,26) and the two-spotted spider mite (27) as a fumigant. The oil can have sub-lethal effects as well, for example acting as a repellent to onion thrips, *Thrips tabaci* (28). Choi *et al.* (29) tested a total of 53 essential oils against *T. urticae* and *P. persimilis* as a fumigant. Caraway seed, citronella, lemon, eucalyptus, pennyroyal and peppermint oil were found to be highly toxic to both mite species. Rosemary oil was also found to be toxic to the predaceous mites *Amblyseius barkeri* Hughes, *A. zaheri* and *Typhlodromus athiasae* Porath (30).

In my study, lethal and sub-lethal effects of rosemary oil and three rosemary oil-based pesticides were evaluated against the two-spotted spider mite and the greenhouse whitefly and their bio-control agents.

2.2 *Materials and methods*

2.2.1 Rosmarinus officinalis essential oil and commercial pesticides

Pure *Rosmarinus officinalis* essential oil (Intarome TO, Lot# 0213142MB-100%), three commercial pesticides, Hexacide™(5% rosemary oil), EcoTrol™(10% rosemary oil) and Sporan™(17.6% rosemary oil) and a blank formulation of EcoTrol were obtained from EcoSMART Technologies Inc (Franklin, TN, USA).

2.2.2 Spider mites

Spider mites originated from a research colony maintained on tomato plants for more than five years without any pesticide exposure at Agriculture and Agri-Food Canada (Agassiz, BC, Canada). These mites were reared on three-week-old vine tomato plants (*Lycopersicon esculentum* Mill cv. Clarance) provided by Houweling's Nurseries (Delta, BC, Canada).

2.2.3 Greenhouse whiteflies

Greenhouse whiteflies obtained from Applied Bionomics Ltd. (Sidney, BC, Canada) originated from a commercial colony maintained on tobacco plants for more than 10 years without any pesticide exposure. Adult whiteflies were transferred to three-week-old tomato plants (*Lycopersicon esculentum* Mill cv. Clarance) in the greenhouse inside fine mesh cages that allowed air circulation but prevented insects from escaping.

2.2.4 *Phytoseiulus persimilis* Athias-Henriot and *Encarsia formosa* Gahan

Predatory mites and parasitic wasps were purchased from Applied Bionomics (Sidney, BC, Canada). Predatory mites were transferred to a spider mite colony maintained on tomato plants caged in the greenhouse. Parasitic wasps were introduced to a whitefly colony maintained on caged tomato plants inside the greenhouse.

2.2.5 Plant material

Three-week-old tomato plants (*Lycopersicon esculentum* Mill cv. Clarance) provided by Houweling's Nurseries (Delta, BC, Canada) were transferred to plastic pots containing a mixture of regular peat (50%), fine bark (25%) and pumice (25%) provided by West Creek Farms (Langley, BC, Canada) in greenhouse at the University of British Columbia.

2.2.6 General growing conditions for plants, mites and whiteflies

Plants infested with mites or whiteflies were kept inside isolated cages within the greenhouse at 24± 6°C, 40-60% relative humidity (RH) and under natural daylight. Plants were irrigated three times per week, two times with water and one time with water-soluble fertilizer (Peters EXCEL 15-5-15 Cal-Mag) (The Scotts Company, Marysville, OH, USA). Adult female mites were transferred to clean plants, allowed to oviposit for 48 hours, and then removed from the plant. Development of these eggs resulted in a cohort of evenly aged mites that were used for all bioassays. Adult whiteflies were used for all bioassays.

2.2.7 Calculating lethal concentration 50 (LC_{50})

Different bioassay methods were used for spider mites and whiteflies. For spider mites a leaf disc painting method was used for calculating LC_{50} of the rosemary oil and three pesticides. Tests were conducted in disposable plastic Petri dishes (3 cm diameter). Mites were treated with six nominal concentrations (2.5, 5, 10, 20, 40 and 80 ml litre $^{-1}$) of the essential oil or the commercial pesticides and their blank formulation (only for EcoTrol), using a spreader sticker adjuvant (Latron B-1956, 60 mg litre $^{-1}$) (Rohm and Haas, Philadelphia, PA, USA) diluted in distilled water. Leaf discs (3cm diameter), were

cut from leaves of greenhouse-grown plants using a cork borer. A 20 µL aliquot of each concentration was painted on the under side of the leaf disc with a micropipette giving concentrations of ≅ (6.25, 12.50, 25.10, 50.21, 100.43 and 200.87 µg/cm^2). After drying at room temperature for 5 minutes, each disc was placed in the bottom of a Petri dish atop a 3 cm diameter disc of Whatman No. 1 filter paper wetted with 50 µL distilled water (Figure 2.1). Five adult female spider mites were introduced into each Petri dish and the covered dishes were placed in a growth chamber at 26±2 °C, 55-60% RH and a 16/8h LD photoperiod. Mortality was determined under a dissecting microscope 24 hours after treatment. Mites were considered dead if appendages did not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone. All treatments were replicated five times.

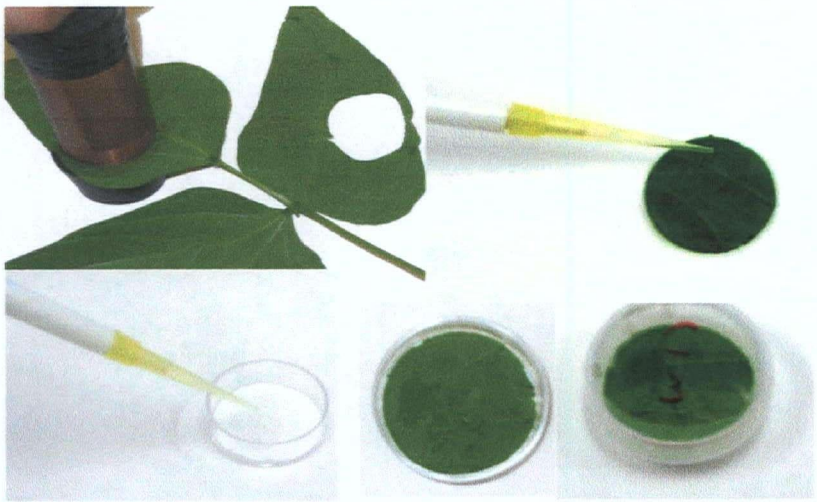

Figure 2.1. Leaf disc painting method

A fumigation chamber was used to test fumigant toxicity of rosemary oil to adult whiteflies. Tests were conducted in disposable plastic containers (4cm diameter × 6.5 cm height). Whiteflies were treated with same six nominal concentrations (2.5, 5,10,20,40

and 80 ml litre $^{-1}$) of the essential oil, using 70% aqueous methanol as the carrier solvent. A cotton pad (5cm diameter) was placed in the bottom of the plastic container and a Whatman No.1 filter paper were placed and pushed on top of the cotton pad. A 50 µl aliquot of each concentration was added to the filter paper with a micropipette producing concentrations of ≅ (1.353, 2.713, 5.432, 10.864, 21.729 and 43.459 µg/cm^3). Another filter paper was placed on top of the previous one with a 1 cm gap to prevent insects from having direct contact with the treated paper. Fifteen adult whiteflies were introduced to each container. Considering the fact that rosemary oil contains compounds that are highly volatile, bioassays were conducted in two different ways. In one group, containers were covered with a plastic lid to trap all the volatiles (closed-container). In the other group, containers were covered with a dense net to prevent whiteflies from escaping but allowed volatiles to evaporate (open-container) (Figure 2.2). A leaf disc painting method was also used to measure the contact toxicity of rosemary oil to whiteflies using plastic containers instead of Petri dishes. Containers were placed in a growth chamber at 26±2 °C, 55-60% RH and a 16/8 h LD photoperiod. Mortality was determined under a dissecting microscope 24 hours after treatment. Whiteflies were considered dead if appendages did not move when prodded with a fine paintbrush. Control whiteflies were held in containers treated with the carrier solvent alone (70% aqueous methanol). All treatments were replicated five times.

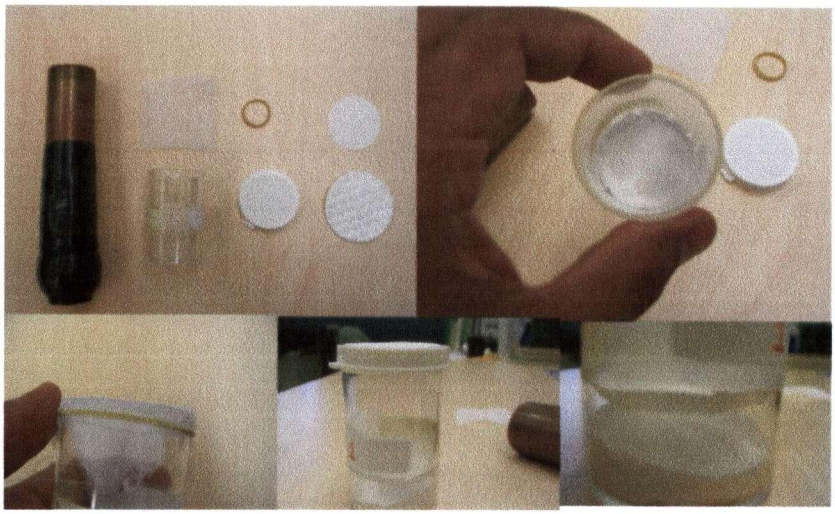

Figure 2.2. Fumigation chamber (open and closed container)

An electronic micro-sprayer was developed to measure the direct contact toxicity of the toxicants to test organism mimicking spraying practices inside greenhouses (Appendix one).

Toxicity of rosemary oil to the predatory mite *P. persimilis* and to the parasitic wasp *E. formosa* was measured using a leaf disc painting method, a direct contact method and a fumigation method. For predatory mites, ~50 spider mite eggs were placed onto each leaf disc (3cm diameter) as food source. Adult predatory mites were used for bioassays. Tomato leaves containing both spider mites and predatory mites were placed inside a Petri dish (10cm diameter) on top of an ice pack inside a Styrofoam box in order to immobilize the predators during bioassay. Adult parasitic wasps were collected by an aspirator and transferred in sealed plastic test tubes. For easier handling, they were kept at 5°C for 2 minutes prior to bioassay to immobilize them. Five adult mites and five adult wasps were then transferred to each fumigation chamber. Each treatment was replicated 5 times. Direct contact toxicity of the commercial pesticides Hexacide™ (7.5 ml litre $^{-1}$),

EcoTrol™ (7.5 ml litre $^{-1}$) or Sporan™ (7.5 ml litre $^{-1}$) against predatory mites and spider mites were measured using the electronic micro-sprayer. Five adult predatory mites were transferred to a leaf disc (3cm diameter) containing ~50 spider mite eggs and then sprayed with the sprayer. Five adult female spider mites were put on leaf discs and then sprayed with sprayer. Controls were sprayed with carrier solvent alone (70% aqueous methanol). All treatments were kept inside a growth chamber at the same conditions described above. Mortality was measured 24 hours after treatment. Mites or wasps were considered dead if they did not move their appendages when prodded with a paintbrush. Mortality in control groups was corrected by Abbott's formula. Each treatment was replicated five times.

2.2.8 Residual toxicities

Residual toxicity of rosemary oil against greenhouse whiteflies and parasitic wasps and of three rosemary oil-based pesticides against two-spotted spider mites and predatory mites was measured. Three- week-old tomato plants were sprayed individually with rosemary oil (10 ml litre $^{-1}$), Hexacide™ (7.5 ml litre $^{-1}$), EcoTrol™ (7.5 ml litre $^{-1}$) or Sporan™ (7.5 ml litre $^{-1}$)[each plant received ~ 80 ± 10 g of sprayed material]. A spreader sticker adjuvant (Latron B-1956, 60 mg litre $^{-1}$) diluted in distilled water used as the carrier solvent. Control plants were sprayed with carrier solvent alone (n= five plants for each treatment). Treated and un-treated plants then placed randomly on a greenhouse table. Leaf discs (3cm diameter) were cut from each individual plants 1 and 24 hours after spraying. Leaf discs were placed inside a Petri dish or a plastic container as described before. Five adult female spider mites or five adult predatory mite plus ~50 spider mite eggs (as a food source for predators) were placed on the sprayed surface of

the leaf disc. Fifteen adult whiteflies or fifteen adult parasitic wasps were placed inside the container. Covered Petri dishes and containers were held inside a growth chamber for 24 hours at 26±2 °C, 55-60% RH and a 16/8h LD photoperiod. Mortality was determined under a dissecting microscope 24 hours after treatment. Whiteflies, wasps, predators or mites were considered dead if appendages did not move when prodded with a fine paintbrush. All treatments were replicated five times.

2.2.9 Choice test bioassay for spider mites

Choice test bioassays were conducted for two-spotted spider mites using rosemary oil. Two leaf discs (3cm diameter) were cut from tomato plants and placed on top of a wetted Whatman No. 7 filter paper placed inside a disposable plastic Petri dish (10cm diameter). One leaf disc was painted with a 20 µL aliquot of rosemary oil (10 ml litre $^{-1}$; ≅ 25 µg/cm^{2}) dissolved in a spreader sticker adjuvant (Latron B-1956, 60 mg litre $^{-1}$) diluted in distilled water as the carrier solvent and the other one was treated with the carrier solvent alone. Thirty female adult mites were placed in the middle of the Petri dish between the two leaf discs (Figure 2.3). The number of mites found standing on each leaf disc was counted under a dissecting microscope after 1,12,24 and 48 hours. Numbers of eggs on treated and non-treated leaf discs were counted at the end of experiment at 48hours. All treatments were replicated 10 times.

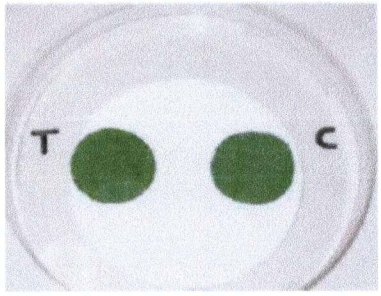

Figure 2.3.Choice test bioassay

2.2.10 Oviposition choice test bioassay for whiteflies

An oviposition choice test was conducted for whiteflies using rosemary oil (10 ml litre $^{-1}$). Styrofoam containers used for packing 4 × l solvent bottles, were used as oviposition cages for whiteflies (31). Each Styrofoam container provided four cages (cells) (24-cm length, 16.5-cm diameter). A single plastic mesh and a dense net were used to cover the top of the container and were secured by thumbtacks to prevent whiteflies from escaping. Thirty adult whiteflies were introduced to each cage containing a control and treated tomato leaf. Each leaf (approximately containing seven leaflets) was sprayed with rosemary oil (10 ml litre $^{-1}$) or carrier solvent (70% aqueous methanol) alone as control on both sides of the leaf. After drying for 30 min, leaves were placed inside a container filled with agar solution (3 gr litre $^{-1}$) (Figure 2.4). Boxes were placed inside a growth chamber at 26±2 °C, 55-60% RH and a 16/8h LD photoperiod.

Eggs were counted on each leaf at 24, 48 and 72 hours after introduction of whiteflies. Treatments were randomized among cages. All treatments were replicated eight times (total of 6 boxes).

C	B	A	C	B	A
B	A	C	A	A	B
C	B	A	C	C	B
A	C	B	C	B	A

Figure 2.4. Oviposition cages – A = after 24 hours, B = after 48 hours, C = after 72 hours

2.2.11 Trans-laminar activity of commercial pesticides

Five-week-old tomato plants were sprayed from above with Hexacide™ (7.5 ml litre $^{-1}$), EcoTrol™ (7.5 ml litre $^{-1}$) or Sporan™ (7.5 ml litre $^{-1}$) using a spreader sticker adjuvant (Latron B-1956, 60 mg litre $^{-1}$) diluted in distilled water as the carrier solvent (each plant received ~ 80 ± 10 g of sprayed material). Leaf discs (3cm) were cut from tomato plants and were placed on top of a wetted filter paper disc (as described above) with either the upper surface (sprayed) or undersurface (not sprayed) facing up (Figure 2.5). Five adult female spider mites were introduced into each Petri dish and the covered dishes were placed in a growth chamber at 26±2 °C, 55-60% RH and a 16/8h LD photoperiod. Mortality was determined under a dissecting microscope 24 hours after treatment as described above.

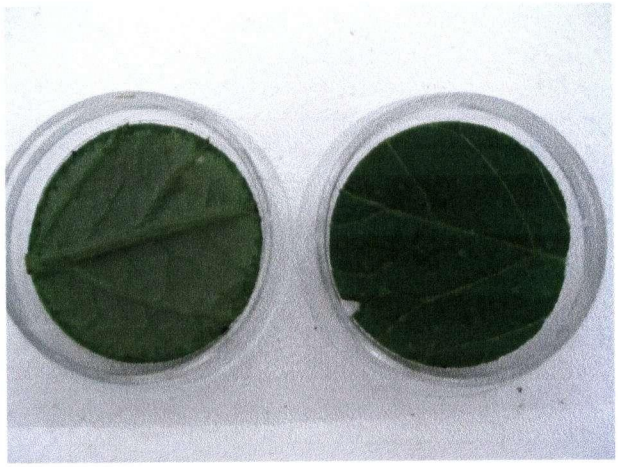

Figure 2.5. Trans-laminar effects of rosemary oil-based pesticides

2.2.12 Data Analysis

Mortality observations were analyzed using the SPSS program (Chicago, IL, USA), version 11.5 for analysis of variance (ANOVA). Tukey's test was used to compare means. Probit analysis was used to determine LC_{50}, using the EPA probit analysis program version 1.5. Abbott's formula was used to correct mortality in controls.

2.3 *Results*

2.3.1 Lethal concentration 50 (LC_{50})

LC_{50} of pure rosemary oil was 13.19 ml litre $^{-1}$ (≅ 33.09 μg/cm^2) for spider mites (Table 2.1). Hexacide (containing 5% rosemary oil) and EcoTrol (containing 10% rosemary oil) were found to be two times more active than Sporan (containing 18% rosemary oil). Although Sporan contains more rosemary oil as active ingredient it showed lower activity against mites. This might be due to difference in formulation. No mortality was observed in control mites treated with carrier solvent.

In fumigation tests against whiteflies, rosemary oil in closed containers was three times more toxic than in open containers. The results also showed that the rosemary oil is more toxic to adult whiteflies as a fumigant than as a contact toxicant.

Rosemary oil was less toxic to *P.persimilis* than to spider mites. Rosemary was more toxic to *E. formosa* through direct contact than via fumigation. Toxicity to parasitic wasps was two times greater in closed containers than in open containers (Table 2.1).

Table 2.1.Toxicity of rosemary oil and three rosemary oil-based pesticides to two-spotted spider mite, greenhouse whitefly, predatory mite and parasitic wasp. Ec-Blank= EcoTrol blank fomulation without rosemary oil, TSM= Two-spotted spider mite, WF= Whitefly, PP= *Phytoseiulus persimilis*, EF= *Encarsia formosa*, N= Number of replicates for each tested concentration (total of 6 concentrations), CI= confidence interval, N/A= not applicable

Toxicant	Organism	Bioassay method	N	LC_{50} (ml litre $^{-1}$)	χ^2 Value	95% CI	µg/cm ^{2}	µg/cm ^{3}
Rosemary	TSM	Leaf disc painting	5	13.19	2.139	10.05-17.78	33.09	N/A
Hexacide™	TSM	Leaf disc painting	5	4.01	2.583	2.36-5.46	10.05	N/A
EcoTrol ™	TSM	Leaf disc painting	5	5.51	1.358	4.03-7.06	13.79	N/A
Ec-Blank	TSM	Leaf disc painting	5	82.14	0.905	57.52-165.5	220.38	N/A
Sporan ™	TSM	Leaf disc painting	5	11.44	1.623	7.56-15.91	28.70	N/A
Rosemary	WF	Fumigation-Close	5	4.93	0.571	3.27-6.39	N/A	2.68
Rosemary	WF	Fumigation-Open	5	18.26	0.565	10.66-31.78	N/A	9.92
Rosemary	WF	Leaf disc painting	5	27.03	4.107	22.26-33.09	N/A	14.68
Rosemary	PP	Leaf disc painting	5	16.62	3.163	13.53-20.71	41.73	N/A
Rosemary	EF	Leaf disc painting	5	9.51	1.419	5.84-12.91	N/A	5.16
Rosemary	EF	Direct spray	5	5.49	0.644	3.15-7.39	N/A	N/A
Rosemary	EF	Fumigation-Close	5	5.81	1.288	4.00-7.62	N/A	3.15
Rosemary	EF	Fumigation-Open	5	10.21	1.394	7.80-13.19	N/A	5.54

Based on direct contact toxicity, three commercial pesticides at their recommended label rate produced no mortality among predatory mites indicating that two-spotted spider mites are more susceptible to rosemary oil. No mortality was observed in control mites (Figure 2.6).

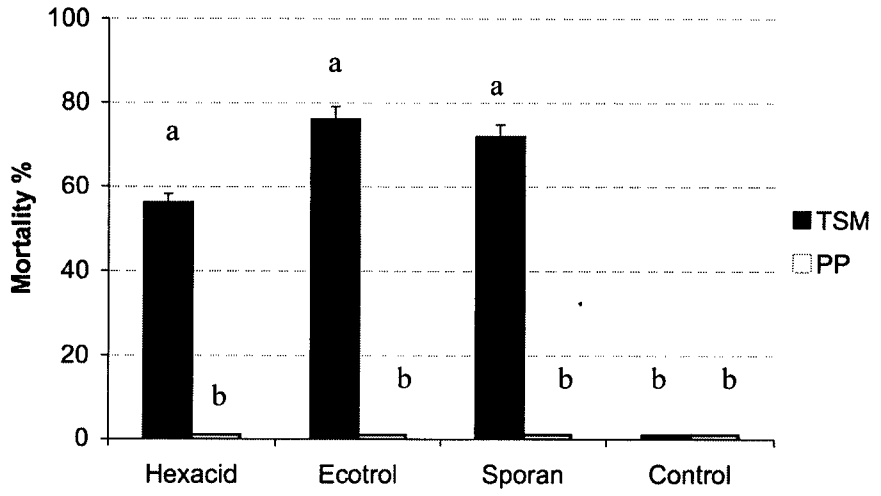

Figure 2.6. Efficacy (% mortality) of three commercial rosemary oil-based pesticides directly sprayed on *P. persimilis* (PP) and *T. urticae* (TSM) on tomato plants. TSM= two-spotted spider mite, PP= *P. persimilis*. Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(7,32)= 36.196$)

2.3.2 Residual toxicity

Residues of all three pesticides were found to be moderately toxic to spider mites within the first hour after spraying. However, toxicity decreased significantly after 24 hours. There was a significant interaction between the toxicity of residues and time [$F(3,32)= 7.203$, $p<0.05$]. This result clearly shows that rosemary oil is not persistent in the environment due to its volatile nature (Figure 2.7). No mortality was observed among controls.

Residues of three commercial pesticides did not show statistically significant toxicity to *P. persimilis* (Figure 2.8). Time had a significant main effect on the toxicity of the residues [$F(1,32) = 5.538$, $p< 0.05$] while no significant difference was found among

pesticides (F (3,32) = 1.026, $p > 0.05$ ($p = 0.394$)). There was no interaction between time and pesticides (F (3,32) = 1.026, $p > 0.05$ ($p = 0.394$)).

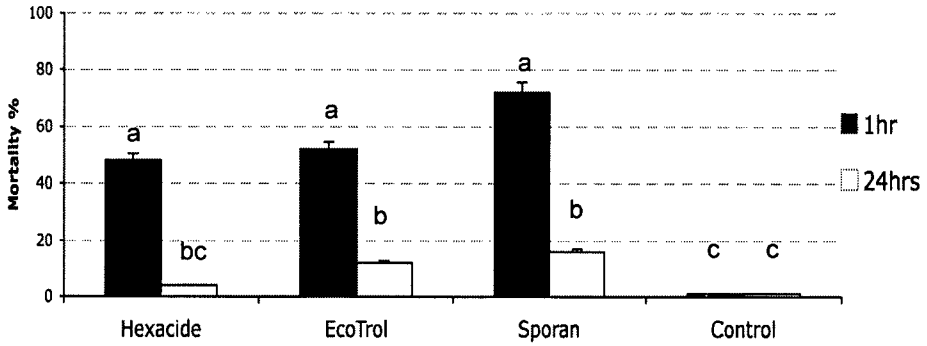

Figure 2.7. Efficacy (% mortality) via residual toxicity of three rosemary oil-based pesticide to two-spotted spider mite on tomato plants. EC=EcoTrol, HE= Hexacide, SP= Sporan. Bars representing means (± SE), *n=5* replicates with 5 adult female mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, F(7,32)= 18.836)

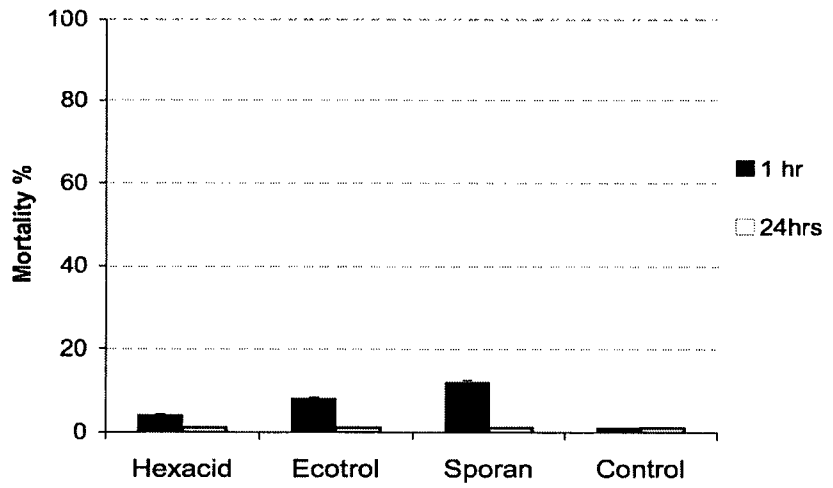

Figure 2.8. Efficacy (% mortality) via residual toxicity of three rosemary oil-based pesticides at their recommended label rate to *P. persimilis* on tomato plants. Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate.

Rosemary oil residue was slightly toxic to whiteflies within the first hour after spraying but not significantly toxic after 24 hours (Figure 2.9). No mortality was observed in control whiteflies.

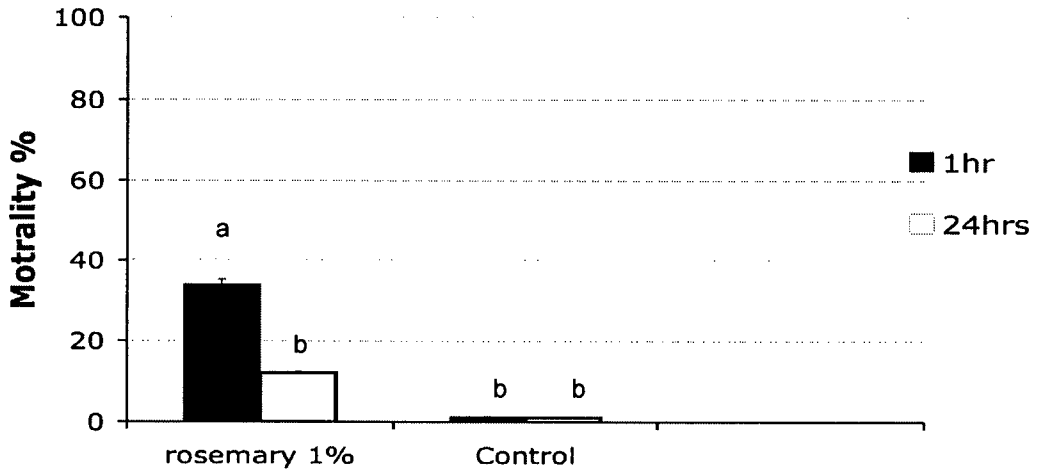

Figure 2.9. Efficacy (% mortality) via residual toxicity of rosemary oil 1% to greenhouse whitefly on tomato plants. Bars representing means (± SE), *n=5* replicates with 15 adult whiteflies per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, F (3,16)= 18.113)

The results indicate that rosemary oil residue is considerably toxic to *E. formosa* within the first hour but that toxicity decreased almost three folds after 24 hours (Figure 2.10). Both time (F (1,16)=35.588, $p<0.05$) and treatment (F (1,16) = 84.390, $p<0.05$) had significant main effects on toxicity of the residues. There was a significant interaction between time and treatment (F (1,16)= 21.844, $p<0.05$).

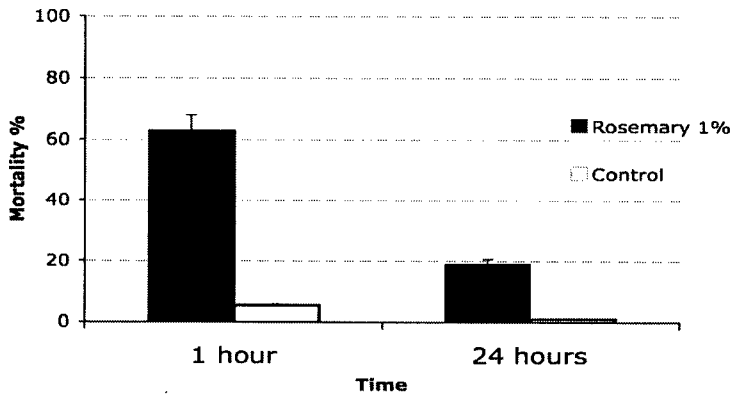

Figure 2.10. Efficacy (% mortality) via residual toxicity of rosemary oil 1% to *E. formosa* on tomato plants. Bars representing means (± SE), $n=5$ replicates with 15 adult mites per replicate.

2.3.3 Choice tests

Rosemary oil has a significant deterrent effect on mites but this effect declined over time. There was a significant interaction between the location of mites and time ($F(3,72)= 81.203$, $p< 0:05$) and treatment had a significant main effect ($F(1,72)=985.289$, $p< 0.05$). During first 12 hours mites aggregated more on the control disc or at locations far from the treated disc within the test arena. After 24 hours, they started to spread on both discs and (Figure 2.11), after 48 hours they were almost equally dispersed on both discs.

Mites laid greater number of eggs on the control discs than on treated discs. Although they started to oviposit eggs on both treated and control discs after 12 hours, the final numbers of eggs after two days was significantly higher on control discs (Figure 2.12).

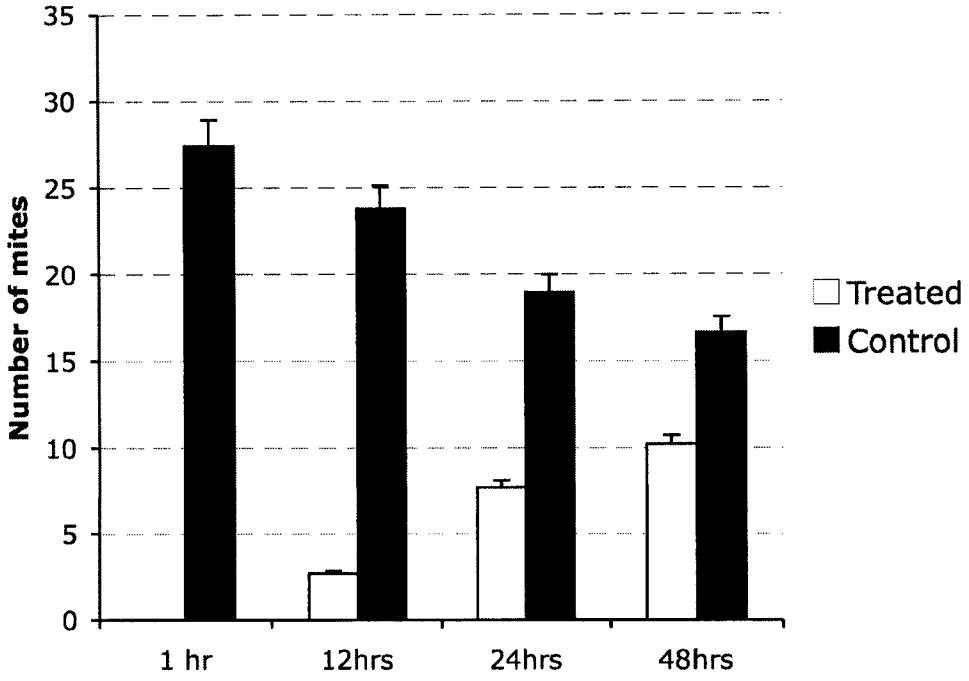

Figure 2.11. Number of two-spotted spider mites staying on leaf discs when given a choice between a treated and non-treated leaf disc with rosemary oil 1%, Bars representing means (± SE), n=10 replicates with 30 adult spider mites per replicate.

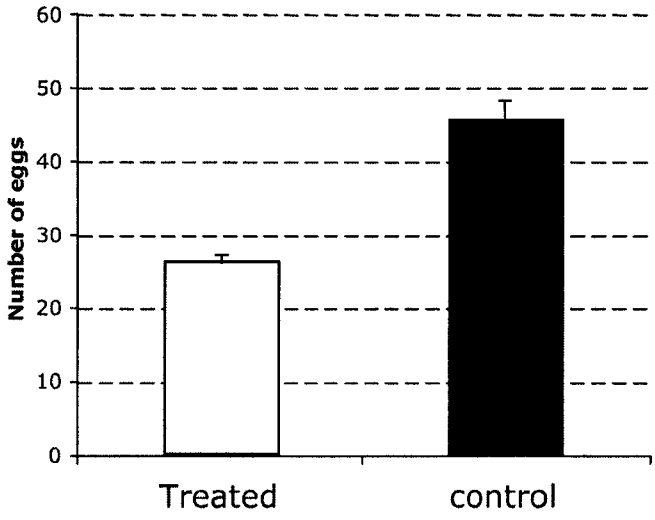

Figure 2.12. Number of two-spotted spider mites eggs on leaf discs when given a choice between a treated and non-treated leaf disc with rosemary oil 1%. (F (1,18)=34.503, p<0.05). Bars representing means (± SE), n=10 replicates with 30 adult spider mites per replicate.

Results from whitefly oviposition choice tests indicated that whiteflies laid significantly more eggs on control leaves than on treated leaves within all three intervals. Both rosemary oil ($F(1,42)=77.931$, $p<0.05$) and time ($F(2,42)=29.366$, $p<0.05$) had main effects on oviposition rate. There was a significant interaction between time and pesticide ($F(2,42)=6.273$, $p<0.05$).

Similar to spider mites, rosemary oil deters whiteflies, however, the effect does not diminish as fast is it did for spider mites. Whiteflies laid three times more eggs on control leaves than on treated leaves after 48 hours (Figure 2.13).

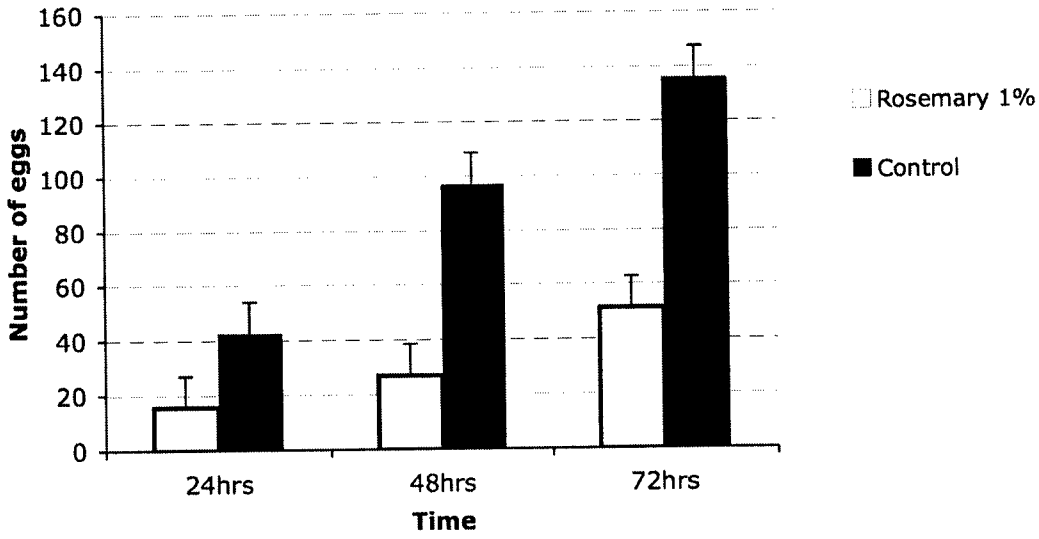

Figure 2.13. Number of greenhouse whitefly eggs on tomato leaves when given a choice between a treated and non-treated leaf with rosemary oil 1%. Bars representing means (± SE), $n=8$ replicates with 30 adult whiteflies per replicate.

2.3.4 Trans-laminar activity

No mortality was observed among mites that were placed on the un-sprayed surface of the leaf discs (Figure 2.14) while significant toxicity was observed among mites that were placed on the sprayed surface, indicating that rosemary oil does not have trans-laminar activity.

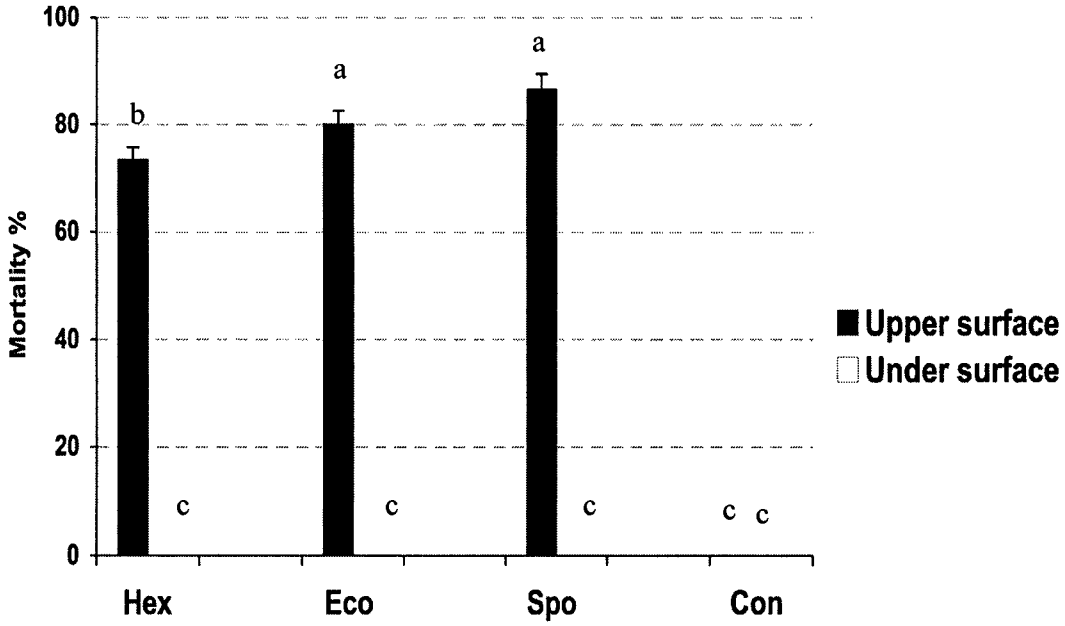

Figure 2.14. Efficacy (% mortality) of three rosemary oil-based pesticides applied at their label rate on tomato leaves when exposed to two-spotted spider mites that were placed either on upper surface or under surface of the leaf discs. Bars representing means (± SE), $n=5$ replicates with 5 adult female mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(3,16)= 36.196$).

2.4 *Discussion*

2.4.1 Efficacy against pests

My results clearly indicate that rosemary oil can be considered an acaricide /insecticide against the two-spotted spider mite and the greenhouse whitefly, causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants (Chapter three).

Rosemary oil was found to be more toxic to spider mites as a contact toxicant while it was more effective against whiteflies as a fumigant. Choi *et al.* (11) evaluated the toxicity of 53 essential oils including rosemary against eggs and adults of two-spotted spider mites as fumigants. Rosemary oil was not very toxic (mortality <60%) comparing to caraway seed, citronella java, lemon, eucalyptus, pennyroyal and peppermint oil, which were highly toxic (mortality > 90%) to the tested mites. However, Sampson *et al.* (32) tested 23 different essential oils including rosemary oil against turnip aphids and found most acted as contact toxicants causing mortality in aphids after 1 hour.

When tested against whiteflies, toxicity of rosemary oil varied significantly between open and closed containers. This result leads to the conclusion that rosemary oil activity can be affected by its volatilization and by environmental conditions. Thus, in greenhouses it might not be as toxic as it is in a laboratory setting. Tunc *et al.* (13) found that vapors of some essential oils were toxic to the cotton aphid and the two-spotted spider mite. They found that the quantity of oil needed for pest control differed in heated and cooled greenhouses compared to ambient greenhouses under plastic. They suggested that essential oil particles suspended in the air might be lost due to air circulation or adherence to surfaces inside the greenhouse.

2.4.2 Effects on bio-controls

Predatory mites *P. persimilis* are less susceptible to rosemary oil than twospotted spider mites (Table 2.1). When both mites were directly sprayed with different pesticides containing rosemary oil, no mortality was found among predators while up to 60% mortality was observed in spider mites (Figure 2.6). These results are very promising in terms of compatibility of these pesticides in an IPM program for controlling spider mites. This difference in toxicity level between spider mites and predators might be due to differential metabolism of rosemary oil –based pesticides in predatory and phytophagous mites. An important aspect of acaricides research is identification of suitable and novel target sites. Little is known about the mode and site of action of rosemary oil and other plant essential oils in the mites. The octopaminergic nervous system is considered to be the site-of-action of certain essential oils in the American cockroach (33) and fruit fly (34), but this may not be the case for two-spotted spider mite and there is a possibility that the essential oils have more than one site of action since they are complex mixtures.

Unlike predatory mites, *E. formosa* is more susceptible to rosemary oil both in fumigation and leaf disc painting bioassays compared to the greenhouse whiteflies. *E. formosa* is susceptible to more than one hundred crop protection products. However, there are some selective compounds that have fewer side effects on parasitic wasps (16). From my results, I conclude that rosemary oil can affect the adult parasitoid if hit directly by a sprayed pesticide. However, immature parasitoids developing inside whitefly nymphs might not be affected by pesticide (Appendix two). On the other hand *E. formosa* is highly mobile and might escape direct spray exposure. In order to reduce side effects of rosemary oil –based pesticides to parasitic wasps, growers can either apply the pesticide 48-72 hours prior to parasitoid release or after whitefly nymphs have been parasitized.

2.4.3 Persistence in the environment

As Dekeyser (35) mentioned, new pesticides should be safer towards non-target organisms and have shorter environmental persistence than existing products.
My results clearly indicate that the rosemary oil-based pesticides are not environmentally persistent. In all experiments, toxicity of residues significantly declined after 24 hours.

Essential oils are mixture of odorous and volatile compounds that can easily break down in the environment (27). Many environmental factors affect the breakdown of essential oils, most importantly, temperature and light. Essential oils may break down faster at higher temperatures and with direct light exposure.

Limited residual toxicity is an important advantage for these pesticides. Growers can apply them closer to harvest time. It is also important to have a safer environment for bio-control agents with fewer pesticide residues. On the other hand, quick breakdown of essential oils in the environment reduces the risk of pesticide resistance in the pest population.

2.4.4 Repellent effects

In addition to pesticidal properties, sub-lethal effects (repellent, deterrent, antifeedant) of many plant essential oils have been reported against several pests (27). Trongtokit *et al.* (36) tested 38 different essential oils on human subjects as repellents to mosquitoes. They found that diluted essential oils couldn't provide a satisfactory level of repellence while undiluted oil could provide effective repellence for up to 2 hours. Among essential oils that they tested, clove oil provided the longest duration of repellence (up to 4 hours) against mosquitoes. In another study, Traboulsi *et al.* (37) found terpineol and 1,8-cineole were very effective against *Culex pipiens molestus* bites for 1.6 and 2 hours respectively, while Zhang *et al.* (38) reported repellent effects of

ginger oil to *Bemisia argentifolii* on tomato plants. Koschier *et al.* (28) reported that essential oils from plants in the mint family could affect host plant selection and acceptance by *Thrips tabaci* Lindeman.

Plant essential oils cannot only repel arthropods, but they also can affect vertebrate behavior. Clark *et al.* (39) reported that some essential oils including rosemary, can cause violent undirected locomotory behavior in brown tree snakes, *Bioga irregularis*, therefore the oils can be used as snake repellents.

According to my choice tests results, rosemary oil is significantly repellent to two-spotted spider mites. It repelled mites for about 6 hours and then mites gradually started to move toward the treated discs. However, both mites and whiteflies preferred untreated leaves for oviposition.

Repellent effects of rosemary oil cannot be considered as a stand-alone control method but can be combined with other methods to improve pest management strategies. For instance, rosemary oil application might be combined with trap-plants as a "push-pull" tactic to repel pests from major host plants and attract them to trap-plants.

2.4.5 Rosemary oil as a pesticide: advantages and limitations

Rosemary oil can be considered a good option for small-scale pest control in greenhouses. These pesticides meet the major characteristics of an IPM-compatible pesticide. As my results indicate, under laboratory conditions, rosemary oil can provide complete mortality in pest populations at concentrations that are not harmful to host plants (Chapter four). It is less toxic to predatory mites and breaks down very fast in the environment. Other advantages of essential oil-based pesticides include low mammal toxicity, safety to terrestrial and aquatic species (with some exception) (21-22), rapid pest

mortality due to their neurotoxic mode of action and low cost, a result of their extensive worldwide use as fragrances and flavoring (40). Finally, an important characteristic of rosemary oil is its complex chemical composition.

Like other essential oils, natural rosemary oil is a complex mixture of terpenoids. Considering that target site resistance is an important problem for mite control, it is less likely that mites will evolve resistance to a mixture of different active compounds than to an acaricide based on a single active ingredient. It has been reported that green peach aphids *Myzus persicae* Sulzer developed resistance to pure azadirachtin (the major ingredient of neem insecticide) but not to a refined neem seed extract containing the same absolute amount of azadirachtin but with many other constituents present (41).

Although rosemary oil-based pesticides meet most requirements of IPM-compatible pesticides, they have some disadvantages that must be considered before extensive application. For example, rosemary oil does not have trans-laminar activity. Both spider mites and whiteflies aggregate on the under-surface of leaves so they might not be affected by rosemary oil sprays to the upper-surface of the plants. On the other hand, the sprayers themselves might modify the efficacy of the rosemary oil. Ebert *et al.* (42) reported differences in the efficacy of spinosad and azadirachtin when applied with different application equipment (carbon dioxide powered high-volume sprayer, DRAMM cold fogger or an electrostatic spraying system). As described before essential oils might breakdown faster at high temperatures so, some application equipment might damage the oil during spraying and reduce its efficacy. In order to be effective, the pesticide must hit the target, so application must be conducted in such a way that it provides complete coverage to the whole plant.

In addition to application methods, effects of environmental factors on the efficacy of the pesticide must be addressed. For instance, temperature, moisture and light effects on toxicity and degradation of pesticides should be studied. Toxicity of the oil might also vary on different host plants due to synergistic or antagonistic effects of plant secondary metabolite with the constituents of the oil (Chapter three).

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Chapter three: Comparative toxicity of *Rosmarinus officinalis* L. essential oil and blends of its major constituents against *Tetranychus urticae* Koch (Acari: Tetranychidae) on two different host plants[1]

3.1 *INTRODUCTION*

3.1.1 Two-spotted spider mite

The two-spotted spider mite, *Tetranychus urticae* Koch, is one the most important pests of fruit, vegetable and ornamental plants worldwide (1). The mite has been reported from about 1200 species of plants (2), of which more than 150 are economically important (3). The economic threat posed by these mites is constantly increasing because of the development of pesticide resistance, and resurgence of mite populations following use of non-selective synthetic pesticides that eliminate natural enemies such as predaceous mites and spiders (4). Spider mites have evolved resistance to more than 80 acaricides to date and resistance has been reported from more than 60 countries (5). In the U.S.A. in 2001, spider mites control programs cost approximately USD 8 million in cotton alone (National Cotton Council of America). Spider mites impose a great expense to greenhouse growers worldwide in terms of damage and control cost and are therefore considered one the most important pests of greenhouses production.

3.1.2 *Rosmarinus officinalis* essential oil

Plant essential oils are obtained through steam distillation of herbs and medicinal plants (6). These oils have been used traditionally as healing medicines in many countries and ancient people were also aware of their pesticidal properties, however, only in recent

[1] Miresmailli, S., Bradbury, R., and Isman, M.B. (2006). *Pest Management Science*: In press (Accepted on 27 September 2005)

years these oils have been commercialized as pest control products (7). Most of these oils are environmentally non-persistent, and non-toxic to humans (with some exceptions) (8-10), fish (with some exceptions) and wildlife (10-13). Rosemary (*Rosmarinus officinalis* L.) oil has been traditionally used as a medicine for colic, nervous disorders and painful menstruation. Recent studies revealed that the rosemary oil is an effective antibacterial agent, which can control many food micro-organisms such as *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli* O157:H7, *Shigella dysenteria, Bacillus cereus and Staphylococcus aureus* (14). It also can inhibit the activity of food spoilage bacteria and yeast strains (15).

Rosemary oil is relatively effective against insect and mite pests. It has been shown that the aromatic vapor of rosemary has ovicidal and larvicidal effects on several stored product pests (16-17) and the two-spotted spider mite (7) as a fumigant. The oil can have sub-lethal effects as well, for example acting as a repellent to onion thrips, *Thrips tabaci* Lind (18).

Synthetic acaricides usually contain a single active compound; however, botanical pesticides such as plant essential oils are complex mixture of several constituents. In the present study, we characterize the toxicity of rosemary oil and its major constituents as residual acaricides against *T. urticae*.

3.2 MATERIALS AND METHODS

3.2.1 Rosmarinus officinalis essential oil[2]

Pure *Rosmarinus officinalis* essential oil (Intarome TO, Lot# 0213142MB-100%) was obtained from EcoSMART Technologies Inc. (Franklin, TN, USA). Major constituents of the essential oil were identified by gas chromatography/mass spectroscopy on a Varian 3900 system with a Saturn 2100T ion trap mass selective detector (Walnut Creek, CA, USA and using a WCOT fused silica 30m x 0.25 mm ID column with a CP-Sil 8 CB low bleed MS coating, a 1 µl injection volume and pure helium as the carrier at 1.0 ml/min. The temperature program used was 80°C for 0.5 min, an increase of 8.0°C/min for 8.0 min, followed by an increase of 50°C/min for 3.2 min. Cinnamic alcohol (Sigma, St. Louis, MO, USA) was used as an internal standard.

3.2.2 Spider mites

Two colonies of *T. urticae* were used in this study. The first colony was collected from the UBC horticulture greenhouse and reared on three-week-old green bush bean plants (*Phaseolus vulgari* cv. Speculator #24A Stokes). The second colony originated from a research colony maintained on tomato plants for more than five years without any pesticide exposure at Agriculture and Agri-Food Canada, Agassiz, BC. These mites were reared on three-week-old vine tomato plants (*Lycopersicon esculentum* Mill var. Clarance) provided by Houweling's Nurseries (Delta, BC, Canada).

3.2.3 General growing conditions for plants and mites

Plants contaminated with mites were kept inside an isolated greenhouse section at 24± 3°C, 45-60% relative humidity (RH) under natural daylight. Plants were irrigated three times per week, two times with water and one time with water-soluble fertilizer

[2] Analyzed by Rod Bradbury at Ecosafe Natural Products Inc. (Saanichton, BC, Canada)

(Peters EXCEL 15-5-15 Cal-Mag) (The Scotts Company, Marysville, OH, USA). Adult female mites were transferred to clean plants, allowed to oviposit for 48 hours, and then removed from the plant. Development of these eggs resulted in a cohort of evenly aged mites that were used for all bioassays.

3.2.4 Calculating lethal concentration 50 (LC_{50}) of the oil

A leaf disc painting method was used to determine LC_{50} of the rosemary oil. Tests were conducted in disposable plastic Petri dishes (3 cm diameter). The bean colony of mites was treated with six nominal concentrations [(2.5, 5,10,20,40 and 80 ml litre $^{-1}$) ≅ (6.25, 12.50, 25.10, 50.21, 100.43 and 200.87 μg/cm^2)] of the essential oil in 70% aqueous methanol as the carrier solvent. The tomato colony was treated with the same six concentrations, using water plus a spreader sticker adjuvant (Latron B-1956, 60 mg litre^{-1}) as the carrier solvent. Leaf discs (3cm diameter), were cut from leaves of greenhouse-grown plants using a cork borer. A 20 μL aliquot of each concentration was painted on the under side of the leaf disc with a micropipette. After drying at room temperature for 5 min, each disc was placed in the bottom of a Petri dish atop a 3 cm diameter disc of Whatman No 1 filter paper wetted with 50 μL of distilled water. Five adult female spider mites were introduced into each Petri dish and the covered dishes were placed in a growth chamber at 26±2 °C, 55-60% RH with a 16/8h LD photoperiod. Mortality was determined under a dissecting microscope 24h after treatment. Mites were considered dead if appendages did not move when prodded with a fine paintbrush. Control mites were held on leaf discs painted with the carrier solvent alone (70% aqueous methanol, or Latron B-1956 60 mg litre^{-1}). All treatments were replicated five times.

3.2.5 Comparative toxicities

Based on the 100% lethal concentration and following the natural composition of the oil indicated by GC/MS (Table 3.1), individual constituents were tested at levels equivalent to those found in the LC_{100} of the oil (20 ml litre $^{-1}$ for beans and 40 ml litre $^{-1}$ for tomatoes) (Figures 3.2 and 3.3). Individual constituents (α-pinene 98%, β-pinene 99%, 1,8-cineole 99%, p-cymene 99%, α-terpineol 97%, bornyl acetate 97%, borneol 99%, camphor 96%, d-limonene 97% and camphene 95%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). In order to identify the contribution of each constituent to the toxicity of the oil, we made a blend of all major constituents as well as blends each lacking one of the ten major constituents (Figure 3.1). We compared the toxicity of the complete and incomplete blends to that of pure rosemary oil. In the next step, we made blends of those constituents, which contributed to the toxicity of the oil (active constituents) and compared them with those which did not affect the toxicity (inactive constituents). The leaf disc painting method was used for all bioassays.

3.2.6 Data Analysis

Mortality observations were analysed using the SPSS program (Chicago, IL, USA), version 11.5 for analysis of variance (ANOVA). Tukey's test was used to compare means. Probit analysis was used to calculate LC_{50}, using the EPA probit analysis program version 1.5.

3.3 *RESULTS*

3.3.1 Essential oil constituents

GC-MS analysis indicated that there are ten major constituents in the oil, comprising 92.8% of the total weight. 1, 8-Cineole was the most abundant compound (31.5%), followed by camphor (20.0%) and α-pinene (17.5%) (Table 3.1). There are more than 40 other compounds in the rosemary oil, which are mostly monoterpenes, but their concentration in the oil is very low (19).

Table 3.1. Major constituents of rosemary oil (Intarome TO, Lot# 0213142MB-100%)

Constituent	% v/v
Camphene	8.0
1, 8-Cineole	31.5
β-Pinene	6.8
Camphor	20.0
p-Cymene	0.9
Borneol	1.2
d-Limonene	3.7
α-Terpineol	1.1
Bornyl acetate	2.2
α-Pinene	17.5
Other compounds	7.2

3.3.2 Lethal concentration 50 of the oil

The LC_{50} of rosemary oil was 1% (10 ml litre $^{-1}$) (95% confidence interval (CI) = 6.95 – 13.11) for adult female spider mites reared on bean plants and 1.3% (13.0 ml litre $^{-1}$) (95% CI = 10.05 – 17.78) for those reared on tomato plants. Complete mortality

(100%) of mites was obtained with a 2% (20 ml litre $^{-1}$) concentration of the oil on bean plants and 4% (40 ml litre $^{-1}$) on tomato plants. No mortality was observed in the controls.

3.3.3 Comparative toxicities of individual constituents and blends thereof

For the bean host strain of mites, bioassay of single constituents revealed that two constituents (1,8-cineole and α-pinene) were significantly toxic at the tested concentration ($P< 0.05$), one (β-pinene) was slightly but not significantly toxic and the remaining seven (p-cymene, borneol, bornyl acetate, camphor, d-limonene, camphene and α-terpineol) were slightly or non-toxic to mites (Figure 3.1)

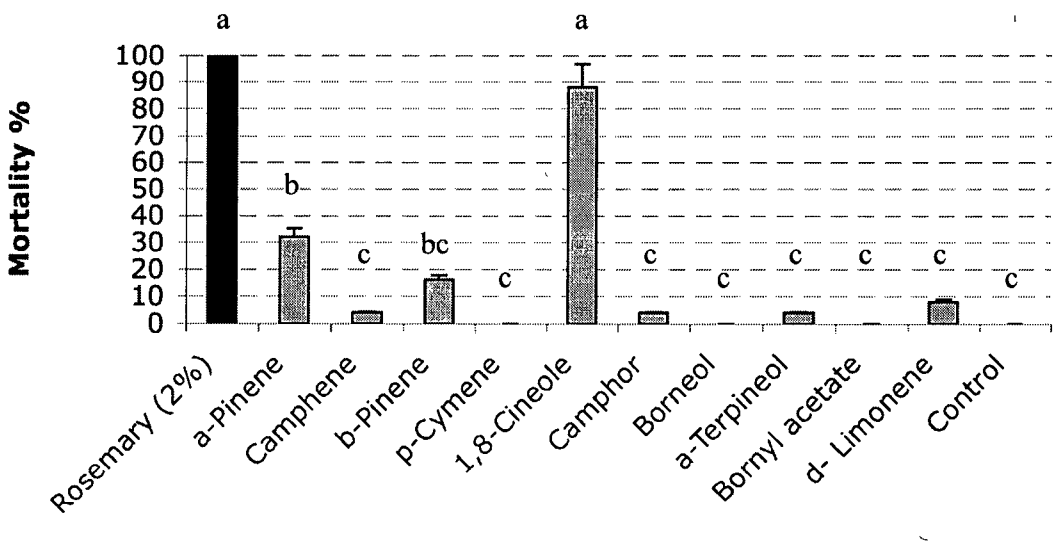

Figure 3.1. Efficacy (% mortality) of pure constituents of rosemary oil to two-spotted spider mite on bean plants at concentration equivalent to their proportion at 100% lethal concentration of the oil (2%). Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(11,48)= 82.364$)

When tested against tomato host strain of mites, three constituents (camphor, p-cymene and camphene) were found non-toxic to mites, five (bornyl acetate, β-pinene, d-limonene, α-terpineol and borneol) were moderately toxic and two (α-pinene, 1, 8-cineole) were highly toxic (Figure 3.2).

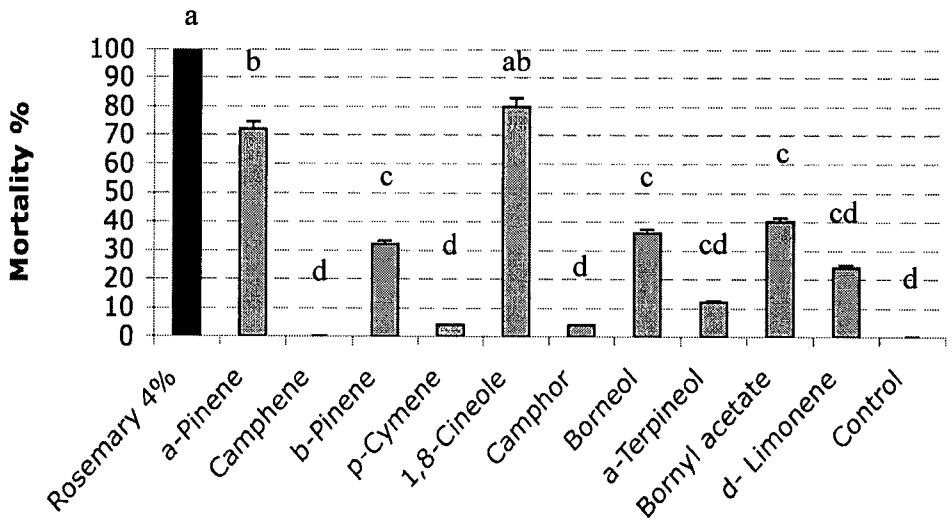

Figure 3.2. Toxicity of pure constituents of rosemary oil to two-spotted spider mite on tomato plants at concentration equivalent to their proportion at 100% lethal concentration of the oil (4%). Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(11,48)= 23.196$)

Bioassays with artificial mixtures showed that the greatest mortality was obtained when all ten constituents were present (full mixture). The mortality caused by the artificial mixture of all ten constituents did not differ significantly from that caused by pure rosemary oil for either strain of mites ($P>0.05$ ($p= 0.989$, Figures 3.3 and 3.4).

In the bean host strain, component-elimination assays indicated that the absence of 1,8-cineole or α-pinene caused the largest decrease in toxicity of the blend. Removal of

p-cymene, α-terpineol or bornyl acetate also had a significant effect (P< 0.05) on the toxicity of the blend but less so than for 1, 8-cineole or α-pinene. Excluding five remaining constituents (camphor, camphene, borneol, d-limonene and β-pinene) from the mixtures did not significantly affect the toxicity of the blends (Figure 3.3).

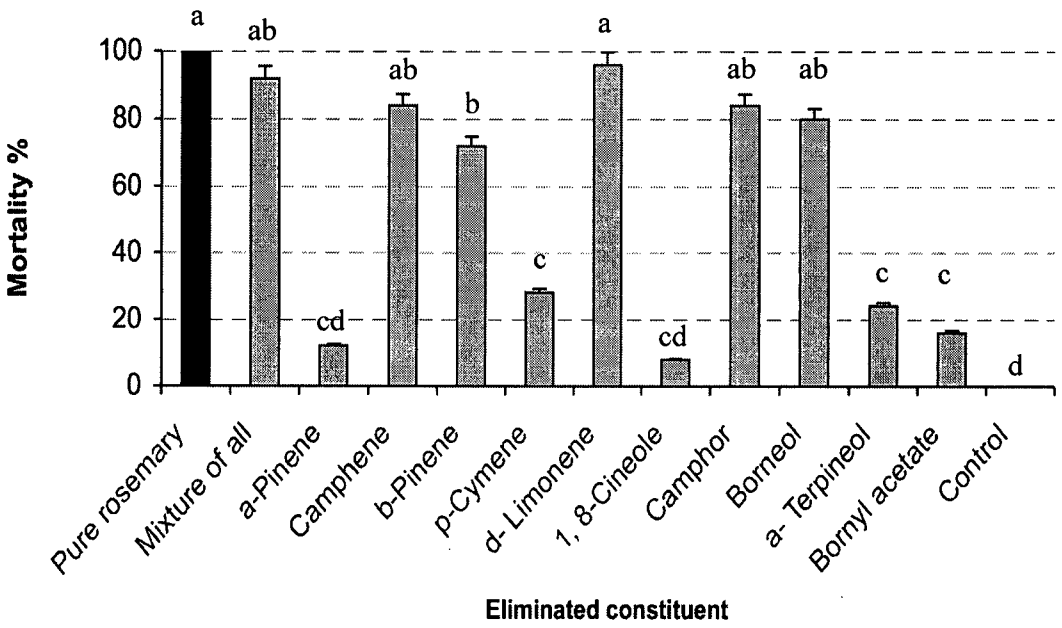

Figure 3.3. Efficacy (% mortality) of different blends of pure constituents of rosemary oil to two-spotted spider mite on bean plants at concentration equivalent to their proportion at 100% lethal concentration (2%)- Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(12,52)= 57.787$)

In the tomato host strain, α-pinene, 1, 8-cineole, α-terpineol and bornyl acetate were found to contribute to the toxicity of the oil whereas β-pinene, p-cymene and borneol had only a moderate influence on the toxicity. Camphor, camphene and d- limonene were found to be inactive when tested individually and their absence did not have any effect on the toxicity of the mixture (Figure 3.4)

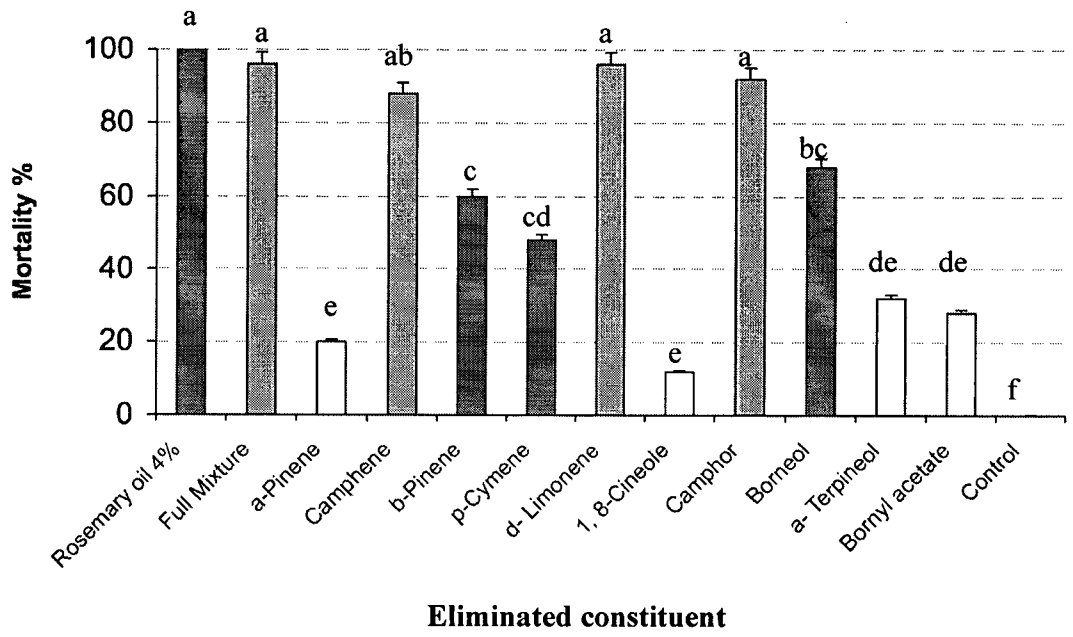

Figure 3.4. Efficacy (% mortality) of different blends of constituents of rosemary oil to two-spotted spider mites on tomato plants at concentration equivalent to their proportion at 100% lethal concentration (4%)- Bars representing means (± SE), $n=5$ replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(12,52)= 46.312$)

Our comparison between the toxicity of a mixture of effective constituents versus a mixture of non-effective constituents showed that the effective constituents alone are not as toxic as the full mixture of all constituents (both actives and inactives). The inactive constituent blend did not cause any mortality in either strain, but when added to the active constituents blend, toxicity became equivalent to the natural oil (Figures 3.5,3.6 and 3.7).

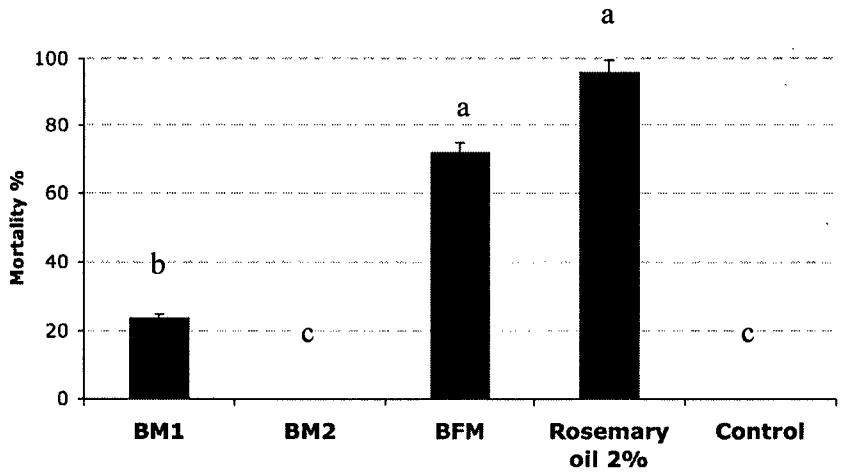

Figure 3.5. Efficacy (% mortality) of different blends of rosemary oil constituents to two-spotted spider mites on bean plants at concentration equivalent to their proportion in the 100% lethal concentration (2%)- **BM1** (Active) = a-Pinene + 1,8 Cineole + a- Terpineol + Bornyl acetate + p-Cymene , **BM2** (Inactive) = b-Pinene + Borneol + Camphor + Camphene+ d- Limonen, **BFM** = Full mixture of all constituents, Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(4,20)= 43.636$

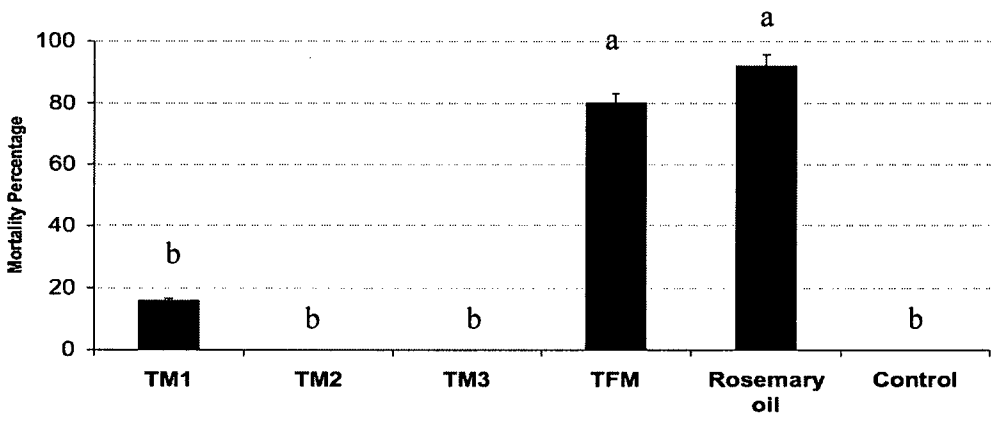

Figure 3.6. Efficacy (% mortality) of different blends of rosemary oil constituents to two-spotted spider mites on tomato plants at concentration equivalent to their proportion in the 100% lethal concentration (4%)- **TM1** (Active) = a-Pinene + 1,8 Cineole + a- Terpineol + Bornyl acetate, **TM2** (Moderately active) = b-Pinene + Borneol + p-Cymene, **TM3** (Inactive) = Camphor + Camphene+ d- Limonen, **TFM** = Full mixture of all constituents, Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(5,24)= 83.867$)

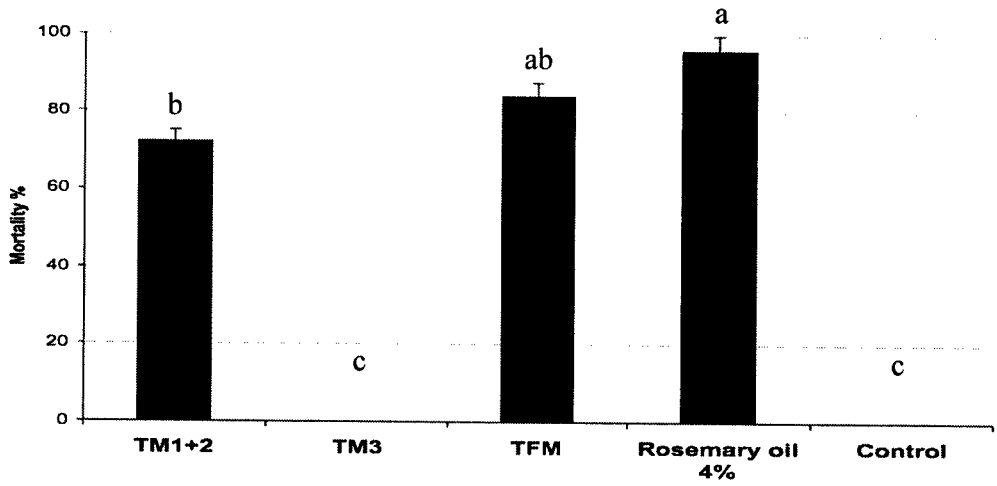

Figure 3.7. Efficayc (% mortality) of different blends of rosemary oil constituents to two-spotted spider mites on tomato plants at concentration equivalent to their proportion in the 100% lethal concentration (4%)- **TM1** (Active) = a-Pinene + 1,8 Cineole + a- Terpineol + Bornyl acetate, **TM2** (Moderately active) = b-Pinene + Borneol + p-Cymene, **TM3** (Inactive) = Camphor + Camphene+ d- Limonen, **TFM** = Full mixture of all constituents, Bars representing means (± SE), *n=5* replicates with 5 adult mites per replicate. Bars marked with the same letter do not differ significantly, Tukey ($P<0.05$, $F(4,20)= 54.706$)

3.4 *DISCUSSION*

3.4.1 Rosemary oil as an acaricide

Our results clearly indicate that rosemary oil can be considered an acaricide against the two-spotted spider mite, causing complete mortality in the laboratory at concentrations that cause no phytotoxicity to host plants (Chapter four). Rosemary oil and most of other plant essential oils are environmentally non-persistent and break down easily in presence of light. Some essential oils are not toxic to non-target organisms and can be used in conjunction with biological control. Furthermore, most plant essential oils including rosemary oil are safe for humans and other mammals and many of them are used as flavorings in foods, beverages and medicines. In some rare cases, chronic exposure to rosemary oil in high concentration has caused contact dermatitis (8-10) but acute toxicity of rosemary oil to humans or other mammals has not been reported.

Like other essential oils, natural rosemary oil is a complex mixture of terpenoids. Considering that targetsite resistance is an important problem for mite control, it is more probable that mites will evolve resistance faster to an acaricide based on a single active ingredient than to a mixture of different active compounds. It has been reported that green peach aphids *Myzus persicae* Sulzer developed resistance to pure azadirachtin (the major ingredient of neem insecticide) but not to a refined neem seed extract containing the same absolute amount of azadirachtin but with many other constituents present (20). Little is known about the exact site of action of rosemary oil and other plant essential oils on the two-spotted spider mites. The octopaminergic nervous system is considered to be the site-of-action of essential oils in the American cockroach (21), but this may not be the case for two-spotted spider mite and there is the possibility that the essential oils have

more than one site of action since they are complex mixtures. Further studies need to be done to find the exact mechanism(s) of action of the essential oils in spider mites.

3.4.2 Synergy among constituents

We observed that individual constituents differ in their toxicity to the two host strains of mites, and it seems that they are more toxic to mites that feed on tomato than on bean plants. We found that some constituents (viz. borneol, bornyl acetate) that were not toxic to mites feeding on beans were relatively toxic to mites on tomatoes. Similar results were reported when major constituents of two other essential oils were used alone against two post-harvest insect pests (22). To corroborate the role of individual constituents in the toxicity of rosemary oil to spider mites, we eliminated each individual constituent from a synthetic mixture that simulated natural rosemary oil. We found that the absence of some constituents (1,8-cineol or α-pinene) in the artificial mixture caused a significant decrease in toxicity (84% and 80% respectively), which tempted us to conclude that these constituents are the major contributors to the oil's toxicity. However, when we mixed these active constituents together we found that their toxicity level was not as high as we expected. The toxicity of our artificial mixtures only reached the level of the natural rosemary oil when we mixed the blends of active constituents with inactive ones. This indicates that the 'inactive' constituents have some synergistic effect on active constituents, and although not active individually, their presence is necessary to achieve full toxicity. Active constituents on the other hand might have an antagonistic effect on each other since their toxicity level is significantly greater when tested individually and not in a mixture with other active constituents. The highest mortality rates were obtained

in both strains when all the constituents were present in the mixture (96% in tomato mites and 92% in bean mites).

Knowing the role of each constituent in the toxicity of the oil gives us the ability to screen different rosemary oils and choose the most effective one for pest control proposes. It might be also possible to artificially create a blend of different constituents, base on their activity and their effect on the pest.

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Chapter four: Integrated control of two-spotted spider mites on greenhouse tomato: studying the field efficacy of a rosemary oil-based acaricides combine with predatory mite *Phytoseiulus persimilis* and assessing the phytotoxic effects of the pesticide on tomato plants

4.1 *Introductiuon*

4.1.1 Two-spotted spider mite

Two-spotted spider mite, *Tetranychus urticae* Koch is one of the most important pests of the greenhouse crops. The current control method for spider mites in BC greenhouses is mostly relying on bio-control method.

4.1.2 *Phytoseiulus persimilis*

The predatory mite *Phytoseiulus persimilis* Athias-Henriot is a selective predator that is able to rapidly suppress spider mites. However, it might not provide acceptable control for higher populations of prey particularly on greenhouse tomato (1).

4.1.3 Rosemary oil

Rosemary oil is relatively effective against many insect and mite pests (2-4). In this study, the efficacy of EcoTrol ™- a rosemary oil-based pesticide- combined with *Phytoseiulus persimilis* against two-spotted spider mites on tomato plants was evaluated under the greenhouse condition.

4.1.4 Phytotoxic effect

The impetus on the use of plant essential oils for insect pest and pathogen control originates from the need for control with reduced environmental and health impacts in comparison to the highly effective synthetic pesticides. Although essential oil-based

pesticides are considered low-risk pesticides, phytotoxicity to greenhouse crops constitutes one possible obstacle for their use in practice. In a few cases essential oil-treated plants have become attractive to plant damaging insects and phytotoxic effects on cultivated plants have been observed. Ibrahim *et al.* (5) reported phytotoxicity in limonene treated plants, while Chiasson *et al.* (6) did not observe any phytotoxicity among lettuce, roses and tomatoes that were treated with a *Chenopodium*-based pesticide. The active ingredients in pesticides do not necessarily cause phytotoxicity. Plant damage can result from the solvents in a pesticide formulation, impurities in the spray water, using more pesticide than prescribed on the label, or poorly mixing spray emulsion. Blank *et al.* (7) found phytotoxicity in 25% of kiwifruits treated with mineral oil mixed with diazinon which resulted in up to 17% reduction in the yield.

Although low levels of phytotoxicity might not be a physiological threat for plant, cosmetic damages can reduce marketability of the product. In this study phytotoxic effects of three rosemary oil-based pesticides to foliage, fruits and flowers of greenhouse tomato plants have been investigated.

4.2 *Materials and methods*

4.2.1 Commercial pesticide

Commercial pesticides, Hexacide™(5% rosemary oil), EcoTrol™(10% rosemary oil) and Sporan™(17.6% rosemary oil) were obtained from EcoSMART Technologies Inc. (Franklin,TN, USA).

4.2.2 Plant materials

Three week- old tomato plants (*Lycopersicon esculentum* Mill cv. Clarance) were provided by Houweling's Nurseries (Delta, BC, Canada) (for phytotoxicity tests) and Bevo Farms Ltd. (Langley, BC, Canada) (for the efficacy test). Plants were transferred to plastic pots containing a mixture of regular peat (50%), fine bark (25%) and pumice (25%) provided by West Creek Farms (Langley, BC, Canada). For efficacy tests, plants were kept inside isolated cages within the greenhouse and for phytotoxicity tests plant were kept on a regular bench at the same greenhouse at 24± 3°C, 45-60% RH under natural daylight. Plants were irrigated three times per week, two times with water and one time with water-soluble fertilizer (Peters EXCEL 15-5-15 Cal-Mag) (The Scott Company, Marysville, OH, USA).

4.2.3 Two-spotted spider mites & *Phytoseiulus persimilis*

Spider mites originated from a research colony maintained on tomato plants for more than five years without any pesticide exposure at Agriculture and Agri-Food Canada (Agassiz, BC, Canada). Predatory mites were purchased from Applied Bionomics (Sidney, BC, Canada).

4.2.4 Efficacy test

A 2 x 2 factorial design was used with two factors (pesticide or predator) and two levels (absence or presence) in each factor. Treatments were randomly assigned inside cells of cages. Data was analyzed by two-way ANOVA (SPSS, Chicago, IL, USA). Two tomato plants were placed inside each cell (total of 40 plants). Eight extra plants were randomly placed inside some cells as indicators to estimate initial density of mites prior to pesticide application or predator release. Three HOBO data-loggers (Contoocook, NH, USA) were randomly installed inside cells to collect temperature and moisture data at 10 minutes intervals (Figure 4.1)

▲C	B ◍$_1$	▲D	A▲	D	▲B	C	◍$_3$ A▲
A	▲D	C▲	B	◍$_2$ C	A	▲B	D

Figure 4.1. Experiment layout. A= Control, B = Predator, C= Pesticide, D= Predator + Pesticide▲ = extra plant, ◍ = HOBO Data Logger.

Approximately 15-20 adult spider mites were placed on each plant. After one week, extra plants were removed from the cells and numbers of spider mites on their foliage counted under a stereomicroscope. Based on the initial density of the spider mites, *Phytoseiulus persimilis* was introduced to treatments D and C at ratio of 1:20 predators to prey. Two hours after releasing the predators, treatments B and D were sprayed with EcoTrol ™ at 7.5 ml litre $^{-1}$ (Label rate- water was used as carrier solvent as recommended by manufacturer). After 7 days, tomato plants were fully harvested and placed inside paper bags. Bags then were put inside a cold room (4°C) to prevent further development of mites during data collection. Numbers of spider mites and predatory mites were counted on all foliage under a stereomicroscope. The single apical leaflet at

the end of each leaf was selected as a sub-sample for counting the number of spider mite and predatory mite eggs (Figure 4.2).

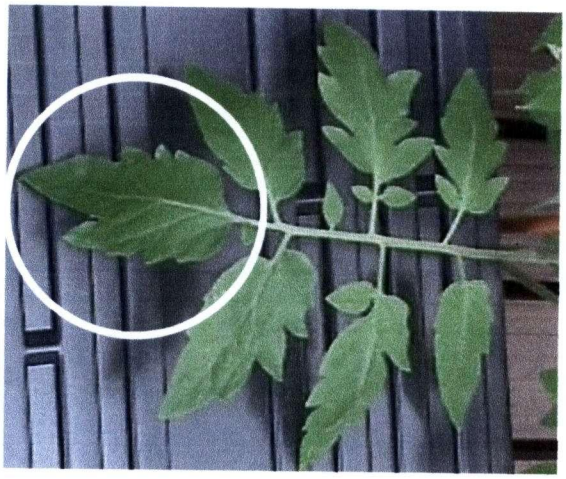

Figure 4.2. Sub-sampling for counting number of eggs

4.2.5 Phytotoxicity tests

Pesticides were tested at three concentrations (One half of label rate [3.75 ml litre $^{-1}$], label rate [7.5 ml litre $^{-1}$] and doubled label rate [15 ml litre $^{-1}$]). Five plants of equal age were used for each concentration. Control plants were sprayed with carrier solvent (water) alone. Sprayed plants were randomly placed on a greenhouse bench (Figure 4.3).

Cuttings made from stems containing flowers or fruits were transferred to new pots placed inside a steam room for one week. Plants were then transferred to regular benches where they remained for one week prior to the experiment. Fruits and flowers were sprayed with the same pesticides at the same concentrations.

In order to determine the phytotoxic effect of pesticides on foliage, medium size leaflets were selected as sub-samples. Damage was defined in five grades from grade 0

(no damage) to grade 5 (completely burned leaflet). Five leaflets of the same size from each plant were selected randomly from all tested plants for damage assessment. Damage to flowers was defined as no damage (grade 0), minor burning sign on petals (grade 1) and major burning sign or flower abortion due to sever damage (grade 2). Damage to fruits was defined as no damage (grade 0) or any particular burning sign, which might cause cosmetic damage to fruit (grade 1). Effects were recorded 24 hours, 48 hours and 72 hours after spraying. Five flowers and five fruits from each plant were used for damage assessment.

Figure 4.3. Phytotoxicity tests

4.2.6 Data Analysis

Observations were analyzed using the SPSS program, version 13 for analysis of variance (ANOVA). Data were transformed to logarithm values when necessary.

4.3 *Results*

4.3.1 Efficacy test

Approximately 100 ± 8 mites were found on each plant (i.e. on eight extra plants randomly placed inside cages) prior to spraying pesticide or releasing predators. Temperature within cages during the experiment was 24 ± 6°C and relative humidity was 60± 15%. Numbers of mites in the blocks treated with pesticide or predator was significantly decreased compared to controls (Table 4.1). Pesticide application showed significant effect on the number of mites. A significant effect was also found with predator introduction. No significant interaction between the two factors was observed (Table 4.2).

Table 4.1. Average number of spider mites on each block, TSM= two-spotted spider mite, LogTSM= Log transferred number of spider mites.

Pesticide	Predator	Mean TSM	Std. Deviation	Mean LogTSM	Std. Deviation LogTSM	N
absent	absent	183.3750	65.67264	5.1616	.36771	4
	present	47.7500	4.17333	3.8630	.08998	4
	Total	115.5625	84.32862	4.5123	.73707	8
present	absent	87.8750	29.41194	4.4365	.31937	4
	present	42.5000	21.42429	3.6480	.52862	4
	Total	65.1875	33.99573	4.0422	.58405	8
Total	absent	135.6250	69.46158	4.7991	.50191	8
	present	45.1250	14.56206	3.7555	.36938	8
	Total	90.3750	67.33981	4.2773	.68677	16

Table 4.2. Results from analyze of variance of number of spider mites (Log transferred) following pesticide and predator treatment. (a) R Squared = 0.777 (Adjusted R Squared = 0.777)

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.500 (a)	3	1.833	13.976	.000
Intercept	292.721	1	292.721	2231.313	.000
Pesticide	0.884	1	0.884	6.738	.023
Predator	4.356	1	4.356	33.206	.000
Pesticide * Predator	0.260	1	0.260	1.984	.184
Error	1.574	12	.131		
Total	299.795	16			
Corrected Total	7.075	15			

Numbers of spider mite eggs were not significantly affected by pesticide or predators (Table 4.3), although predators alone appeared to suppress spider mite eggs. No interaction was found between the two factors (Table 4.4)

Table 4.3. Average number of spider mite's eggs on each block, Log Eggs= Log transferred number of spider mite's eggs.

Pesticide	Predator	Mean Egg	Std. Deviation	Mean LogEggs	Std. Deviation LogEggs	N
absent	absent	159.7500	90.92625	4.9228	.66619	4
	present	54.8750	22.26498	3.9092	.56090	4
	Total	107.3125	83.05546	4.4160	.78651	8
present	absent	73.8750	35.33972	4.2288	.42463	4
	present	75.5000	44.43160	4.1056	.87568	4
	Total	74.6875	37.17616	4.1672	.64051	8
Total	absent	116.8125	78.64792	4.5758	.63646	8
	present	65.1875	34.35210	4.0074	.68883	8
	Total	91.0000	64.40471	4.2916	.70472	16

Table 4.4. Results from analyze of variance of number of spider mites eggs (Log transferred) following pesticide and predator treatment. (a) R Squared = 0.313 (Adjusted R Squared = 0.141)

Source	Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.33 (a)	3	0.778	1.824	.196
Intercept	294.688	1	294.688	691.127	.000
Pesticide	0.248	1	0.248	0.581	.461
Predator	1.292	1	1.292	3.031	.107
Pesticide * Predator	0.793	1	0.793	1.859	.198
Error	5.117	12	0.426		
Total	302.137	16			
Corrected Total	7.450	15			

No significant difference was found among the number of predators ($F(1,6) = 0.00$, $P > 0.05$ (1.00)) or their eggs ($F(1,6) = 1.720$, $P > 0.05$ (0.238)) on blocks sprayed with pesticide compared to those not sprayed.

4.3.2 Phytotoxicity test

Hexacide and EcoTrol were not phytotoxic to foliage, flowers or fruits (grad 0). Sporan (containing 18% rosemary oil) caused first grade damage (burning signs on less than 1/5 of leaflet) to 12% of foliage at the recommended label rate and second grade (burning signs on between 1/5 to 2/5 of the leaflet) damage to 32% of leaflets after 24 hours. No additional damage was found at day two or day three of data collection (Figure 4.4).

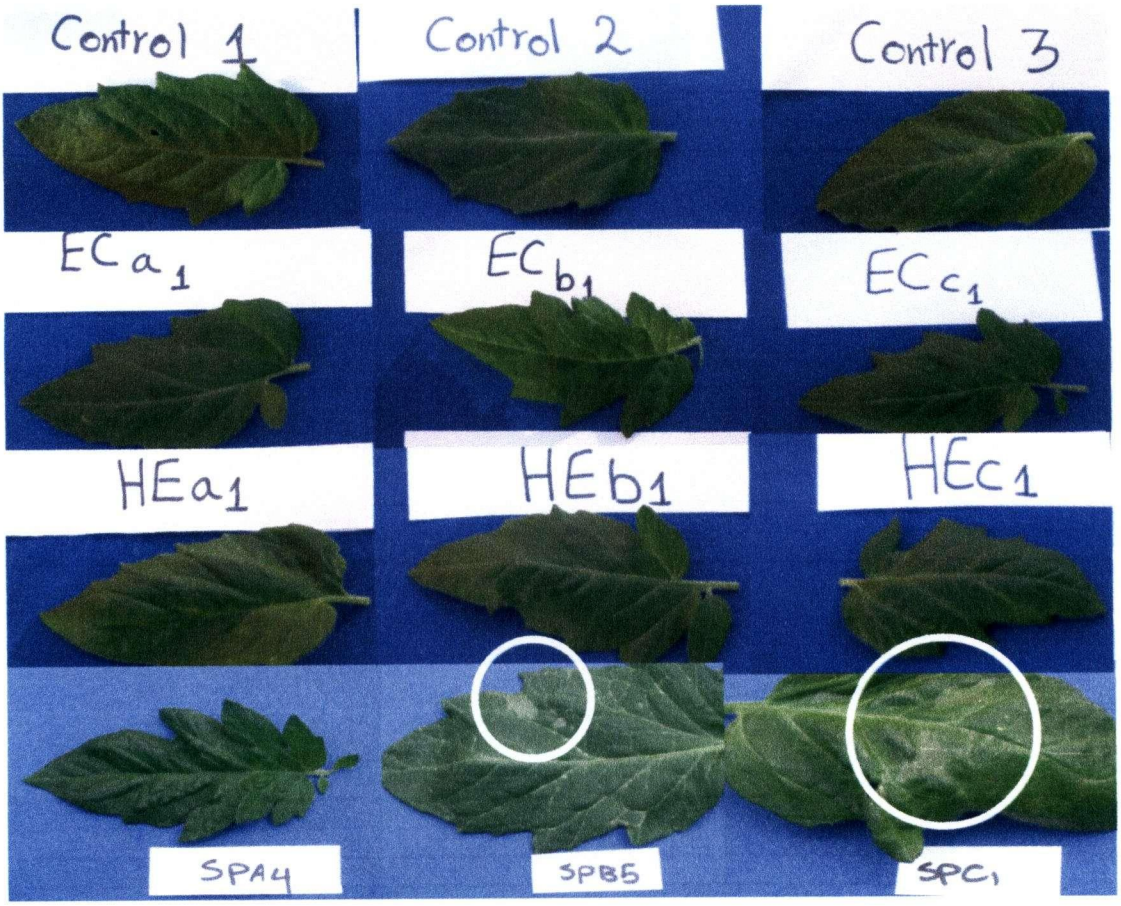

Figure 4.4. Phytotoxicity to foliage. EC = EcoTrol, HE= Hexacide, SP= Sporan, a=one half of label rate, b= label rate, c = doubled label rate.

Hexacide and EcoTrol were not phytotoxic to flowers. Sporan caused second grade damage (some petals of flowers demonstrated minor burning sings) in 40% of tested flowers at its label rate, and second grade damage (complete burning or flower abortion) in 56% of flowers and third grade damage (3/5 of flowers demonstrated burning signs) in 36% of flowers at double the label rate after 24 hours (Figure 4.5). No additional damage was found at day two or day three of data collection. None of the pesticides were found to be phytotoxic to fruits (Figure 4.6).

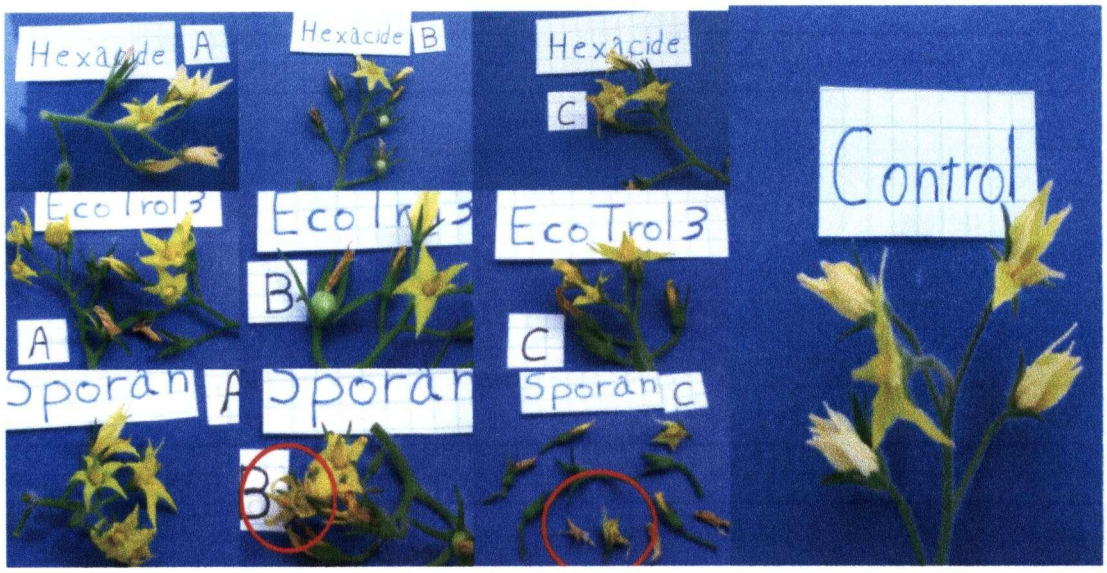

Figure 4.5. Phytotoxicity to flowers. EC = EcoTrol, HE= Hexacide, SP= Sporan, a=one half of label rate, b= label rate, c = doubled label rate.

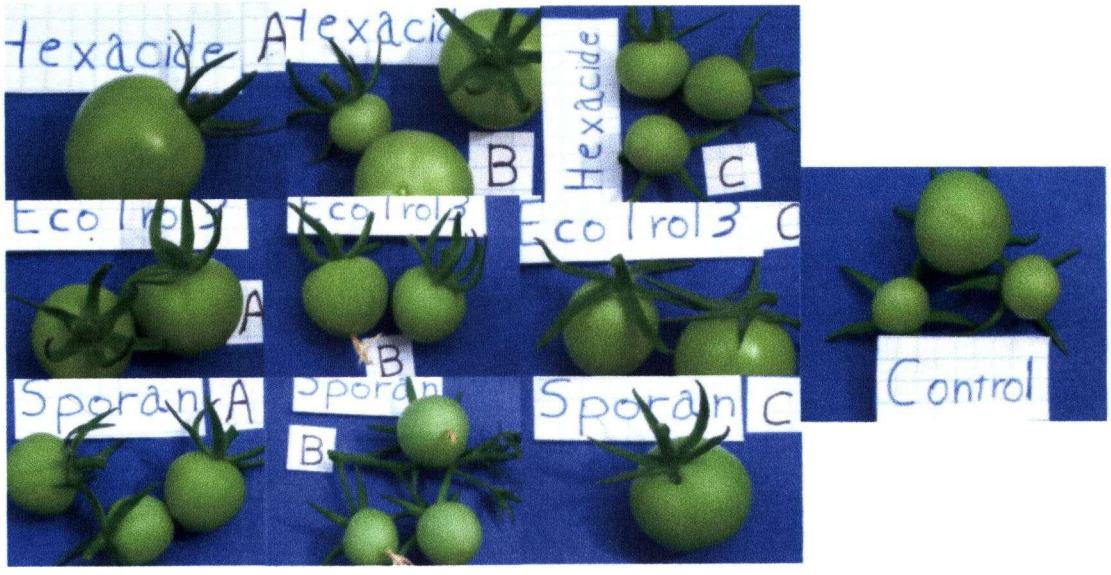

Figure 4.6 Phytotoxicity to fruits. EC = EcoTrol, HE= Hexacide, SP= Sporan, a=one half of label rate, b= label rate, c = doubled label rate.

4.4 *Discussion*

4.4.1 Efficacy of EcoTrol™

In this study, the efficacy of EcoTrol™ (10% rosemary oil) at its recommended label rate (7.5 ml litre $^{-1}$ for spider mites on tomato) was evaluated against two-spotted spider mites on greenhouse tomato plants, both individually and in combination with predatory mites, *Phytoseiulus persimilis* under greenhouse conditions.

In previous laboratory bioassays, contact toxicity of rosemary oil based-pesticides was observed with two-spotted spider mites. However, in the present study, the same degree of mortality was not observed. There was considerable variation among observations requiring transformation of the data to logarithmic form. Based on the average number of mites on each block (Log-transformed), we can conclude that pesticide application, predator release and the combination of pesticide and predators, suppress the mite population by 52 ± 16%, 74 ± 2% and 76 ± 12% respectively (Table 4.1). Several factors may have lead to this difference. As mentioned before (Chapter two), this product does not have trans-laminar activity and cannot affect mites that move deep within the canopy or remain at parts of the plant that pesticide cannot reach. In addition, environmental conditions such as temperature and light may accelerate the degradation of the oil. There was a fluctuation of 12 degrees in temperature and 30% in relative humidity within the greenhouse during the experiment. This fluctuation might be a reason for differences in efficacy in different blocks and cages. Moreover, it was shown that this product has repellent effects (Chapter two) so there might be another scenario. Mites that were not directly hit by pesticide might move to the non-sprayed parts of the plants enhancing their survival. However, this should not affect numbers of eggs of spider mites and predatory mites.

According to the final number of mites we might conclude that the combination of pesticide and predators was twice as effective as the pesticide alone. However, statistical analysis did not show a significant interaction between pesticide and predators (Table 4.2). This difference (15%) was not statistically significant but in this experiment may be biologically significant. There are many factors that affect the statistical significance of the results such as sample size or number of replicates or number of repeated trials. In this study, because of time and space limitations, I could only conduct the trial once with four replicates for each treatment (which is the minimum requirement by PMRA) (8). Further experiments are needed to clarify the efficacy of this pesticide against spider mites in the greenhouse. According to my results, I conclude that EcoTrol™ can effectively suppress (not control) the spider mite population on greenhouse tomato and it is safe for predatory mites and their eggs at the tested concentration.

4.4.2 Phytotoxicity trials

According to PMRA efficacy guidelines for plant protection products (8) three concentrations of EcoTrol™ were used for phytotoxicity tests. Effects were recorded for three continuous days. Phytotoxicity tests were performed on foliage, flowers and fruits. No sign of phytotoxicity was found among tested tomato plants.

Certain plant essential oils have recently been used as least-toxic herbicides (9-10). Unlike essential oil-based pesticides, the herbicides contain higher amounts of essential oils. For instance, Matran EC ™, a contact, non-selective, broad spectrum, foliar herbicide developed by EcoSMART Technologies Inc. contains 50% clove oil. It might

be possible for EcoTrol to cause phytotoxicity if used at higher concentrations, however, my results show that EcoTrol is safe to tomato even when applied at double the recommended label rate. This is a very important aspect of this pesticide which makes it a favorable option for IPM programs in greenhouse tomato plants.

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Chapter five: Summary and Conclusion

The major objective of this research was to study the efficacy of a rosemary oil-based pesticide (EcoTrol™) to be used on greenhouse tomato plants against important pests of this crop (two-spotted spider mite and greenhouse whitefly). According to PMRA efficacy guidelines for plant protection products (1) there are some issues that need to be addressed before making a decision about this product. Some of these questions have been answered based on the results of my study.

1) ***Is this product efficacious against pests?***

The laboratory results showed that rosemary oil can cause contact and fumigant toxicity in two-spotted spider mites and greenhouse whiteflies. EcoTrol at its recommended label rate in particular, caused >80% mortality among spider mites when tested under laboratory conditions. However, when tested in a greenhouse, lower mortality was observed but suppression of the mite population was observed. The answer to this question depends on our definition of efficacy. According to the results (considering the limitations of greenhouse trial) it is clear that EcoTrol alone might not control a large mite population inside a greenhouse. However, it might effectively restrain the population either below the economic threshold or make it more manageable with other control measures such as predatory mites.

2) ***Is it hazardous to human health?***

Humans have used plant essential oils as flavors and food additives from ancient times (2). These oils have been used traditionally as healing medicines in many countries and ancient people were also aware of their pesticidal properties (3). Rosemary in

particular plays a great role in modern medicine as anticancer (4-7), antiviral (8) and antibacterial (9) agent. It has even been used recently as a treatment for snoring (10). Although some essential oils in very high concentration and over long periods of time can cause chronic toxicity or some dermal problems (11-13), in general, essential oils are safe for humans and other mammals in low concentrations.

3) ***Is it persistent in the environment?***

The short answer to this question is no. My results indicate that both pure rosemary oil and EcoTrol disappear quickly in the environment and there is a significant decrease in the toxicity of their residues after 24 hours. In most cases, no toxicity was detected after 48 hours.

4) ***What are the effects of this product on the host plant?***

I did not find any sign of phytotoxicity on tested plants (foliage, flowers and fruits) with tested concentrations (one-half, recommended and doubled label rate). According to my results EcoTrol is safe for greenhouse tomato plants (*Lycopersicon esculentum* Mill cv. Clarance) at its label rate within the environmental conditions that I used for my experiment.

5) ***Is it toxic to bio-control agents?***

In both laboratory experiments and the greenhouse trial, Ecotrol was not found to be toxic to predatory mites when directly sprayed on plants. Contact toxicity bioassays showed that predatory mites are less susceptible to rosemary oil than spider mites. However, unlike predatory mites, parasitic wasps were found to be more susceptible to rosemary oil both as a contact and fumigant toxicant compare to greenhouse whiteflies.

Residual toxicity assays indicate a significant decrease in toxicity of residues to bio-control agents after 24 hours. Thus, the best strategy for protecting bio-control agents from side-effects of EcoTrol is to either release them with a short delay (48-72 hours) after the pesticide application or applying the pesticide after the bio-control agents are established on the pest population.

6) ***Are there any limitations for its application?***

Rosemary oil cannot pass through the plant tissues and does not have trans-laminar activity. It cannot affect the pests that aggregate on the under-side of the leaves. To be effective, it should directly hit the target and this is an obstacle for its use in tomato greenhouses where the plant canopy get dense during the season and pesticides can not penetrate deep enough to reach the pests.

7) ***Is there potential for pests to evolve resistance to this product?***

It is hard to make a solid statement about this issue but I have a theory. Like other essential oils, natural rosemary oil is a complex mixture of terpenoids. Considering that targetsite resistance is an important problem for mite control, it is more probable that mites will evolve resistance faster to an acaricide based on a single active ingredient than to a mixture of different active compounds. It has been reported that green peach aphids *Myzus persicae* Sulzer developed resistance to pure azadirachtin (the major ingredient of neem insecticide) but not to a refined neem seed extract containing the same absolute amount of azadirachtin but with many other constituents present (14). Little is known about the exact site of action of rosemary oil and other plant essential oils on the two-spotted spider mites. The octopaminergic nervous system is considered to be the site-of-

action of essential oils in the American cockroach (15), but this may not be the case for two-spotted spider mite and there is the possibility that the essential oils have more than one site of action since they are complex mixtures.

As mentioned before, EcoTrol is not persistent in the environment so fewer pests will encounter the pesticide and selection pressure will be lessened.

8) ***What is the mechanism of toxicity of this product?***

I observed that individual constituents differ in their toxicity to the two host strains of mites, and it seems that they are more toxic to mites that feed on tomato than on bean plants. I found that some constituents (viz. borneol, bornyl acetate) that were not toxic to mites feeding on beans were relatively toxic to mites on tomatoes. Similar results were reported when major constituents of two other essential oils were used alone against two post-harvest insect pests (16). To corroborate the role of individual constituents in the toxicity of rosemary oil to spider mites, I eliminated each individual constituent from a synthetic mixture that simulated natural rosemary oil. I found that the absence of some constituents (1,8-cineol or α-pinene) in the artificial mixture caused a significant decrease in toxicity (84% and 80% respectively), which tempted me to conclude that these constituents are the major contributors to the oil's toxicity. However, when I mixed these active constituents together I found that their toxicity level was not as high as I expected. The toxicity of my artificial mixtures only reached the level of the natural rosemary oil when I mixed the blends of active constituents with inactive ones. This indicates that the 'inactive' constituents have some synergistic effect on active constituents, and although not active individually, their presence is necessary to achieve full toxicity. Active constituents on the other hand might have an antagonistic effect on

each other since their toxicity level is significantly greater when tested individually and not in a mixture with other active constituents. The highest mortality rates were obtained in both strains when all the constituents were present in the mixture (96% in tomato mites and 92% in bean mites).

Knowing the role of each constituent in the toxicity of the oil gives us the ability to screen different rosemary oils and choose the most effective one for pest control proposes. It might be also possible to artificially create a blend of different constituents, base on their activity and their effect on the pest.

9) ***Can environmental factors affect its efficacy?***

In this study, I did not test the effect of environmental factors on the toxicity or degradation level of rosemary oil but it is obvious that any factor that can accelerate the volatilization of the oil, can affect its toxicity or degradation level. Further studies are needed to find the effect of factors such as temperature, moisture or light on toxicity level of the oil.

Abiotic environmental factors can change the toxicity of the oil, but biotic factors might also play a role in their toxicity. For instance, toxicity of the rosemary oil to the same species of pest might be different on various host plants. I observed a small difference in toxicity of rosemary oil to spider mites on bean plants and tomato plants. Host plant chemicals can affect (either as a synergist of antagonist) the essential oil (i.e. phenolics in tomato plants). Further studies are needed to indicate the effect of host plant on the toxicity of rosemary oil.

10) ***What are the most effective ways of using this product?***

According to the results of this study, I can make some suggestions for effective use of EcoTrol as part of an IPM program for mite and whitefly management in the tomato greenhouse.

A) As seen in the greenhouse trial, EcoTrol cannot provide complete control of pests and must be combined with other control measure such as bio-control agents. An effective monitoring system can detect pests before they become a major problem. EcoTrol can then be used for spot spraying and small-scale control of pests in lower populations following bio-control agent release.

B) In order to reduce the side effect of EcoTrol on the bio-control agents, predators and parasitoids should be released with a short delay (48-72 hours) after pesticide application. If necessary, additional applications should be made after the bio-controls are established on the plants.

C) EcoTrol application can also be combined with cultural control methods such as trap plants. Considering the repellent effect of the EcoTrol, for mobile pests like whiteflies, it can be use to repel the adults from major host plants and push them toward trap plants (i.e. tobacco) where they can be controlled locally (push-pull strategy).

In general, EcoTrol can be considered a favorable option for a chemical control portion of a greenhouse IPM program.

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Appendix

I. Electronic micro-sprayer[1]

Electronic micro-sprayer was developed to measure the direct contact toxicity of the toxicants to test organism mimicking spraying practices inside greenhouses. A mechanical switch was installed on an airbrush (Badger 200NH- IL, USA) linked to a dishwasher solenoid controlled electronically by a digital timer.

The timer is constructed using a NE555 chip. NE555 chip is a highly stable controller capable of producing accurate timing pulses. This chip has 8 input terminals as shown in the diagram bellow (Figure A.1). When the low signal input is applied to the reset terminal (pin 4), the timer output remains low regardless of the threshold voltage (pin 6) or the trigger voltage (pin 2). Only when the high signal is applied to the reset terminal, the timer's output changes according to threshold voltage and trigger voltage.

When the threshold voltage exceeds 2/3 of the supply voltage (pin 8) while the timer output (pin 3) is high, the timer's internal discharge turns on, lowering the threshold voltage to below 1/3 of the supply voltage. During this time, the timer output is maintained low. Later, if a low signal is applied to the trigger voltage so that it becomes 1/3 of the supply voltage, the timer's internal discharge turns off, increasing the threshold voltage and driving the timer output again at high.

The start and stop buttons are held at the "+" potential by the resistors R4 and R6. At the rest position, C2 is short circuited with the ground. By pressing the start button, this short-circuit is annihilated, the output is supplied via T1 and C2 starts to be loaded via R1 and RV1. RV1 is a variable resistor. As value of RV1 increases, it takes longer for

[1] Digital timer designed and developed by Maryam Antikchi, Department of Engineering Physics, UBC

C2 to reach the same value as the cross point between R2 and R3, which is connected to the reference voltage (pin 5). Once C2 is loaded, the output goes to zero and C2 will discharge. If the Stop button is pressed during this process, IC will be reset, and the output goes to zero immediately. The timer can turn the power on and off within the intervals between 0.1 second to 1 minute. When the power is on, the solenoid pull the mechanical switch in and that push the airbrush bottom so it sprays. The longer the power is on, the longer it sprays (Figure A.2).

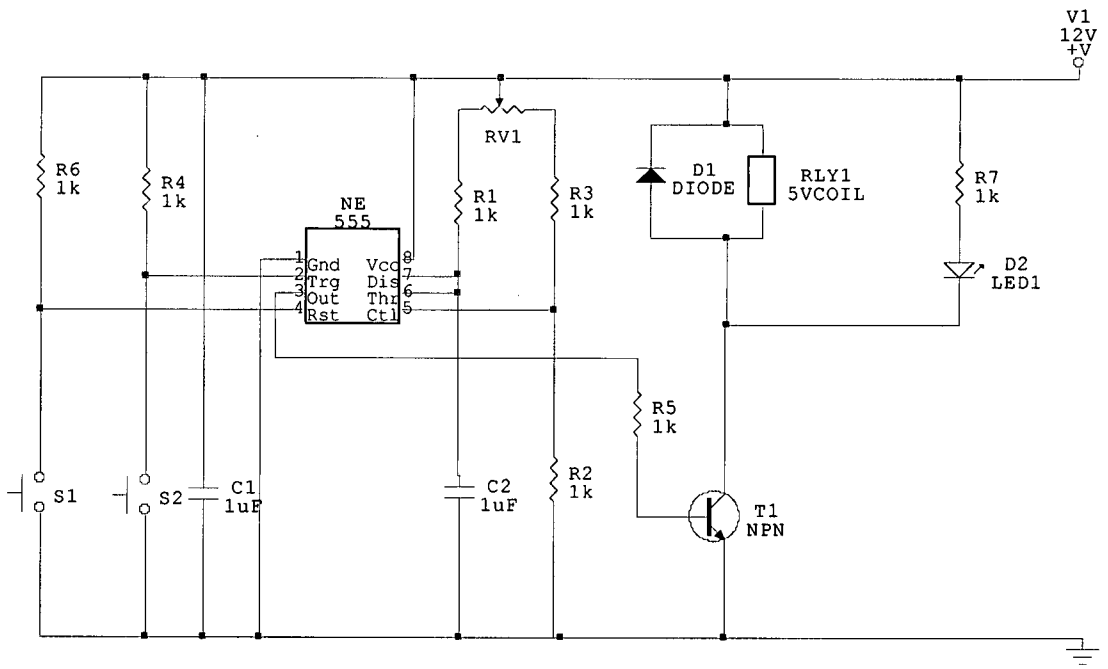

Figure A.1. Digital timer circuits

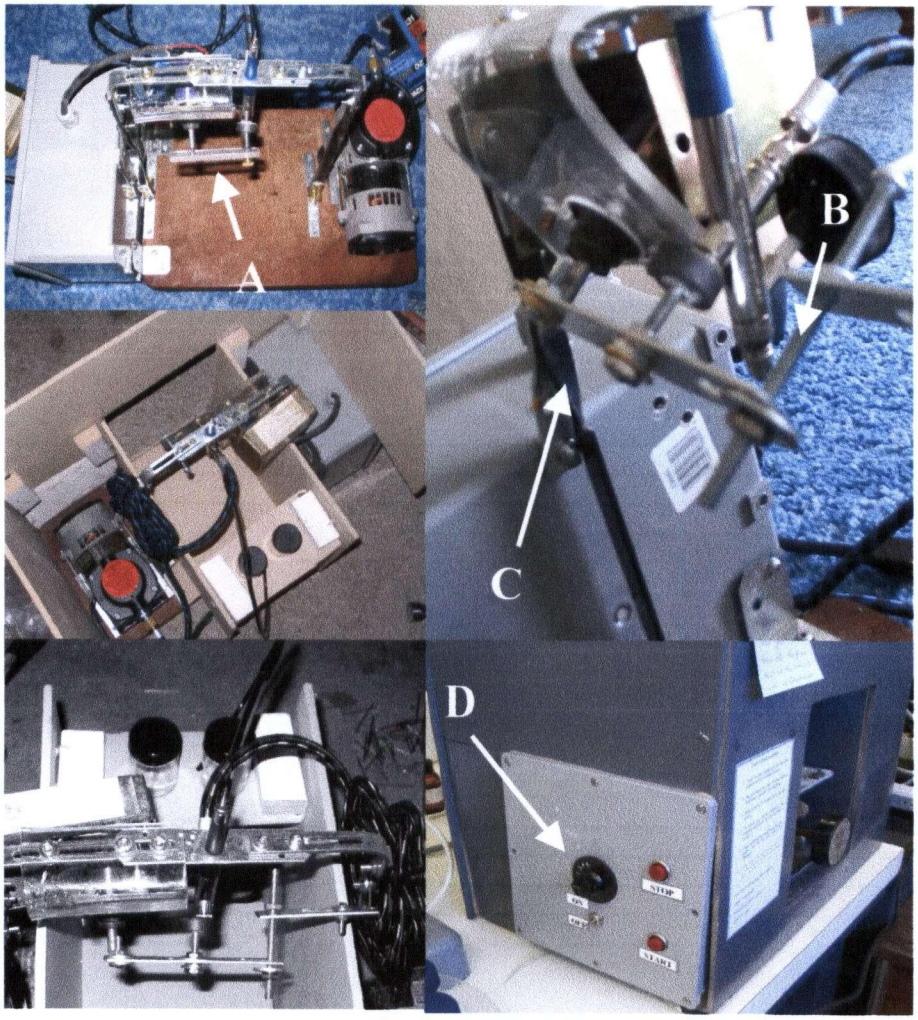

Figure A.2. Electronic micro-sprayer. A= mechanical switch, B = Airbrush, C = Solenoid, D = digital timer

The timer was calibrated in five stages base on the amount of aliquot that spray. In stage one it can deliver 20μgr ± 5 and in following stages this amount will be doubled up to 100μgr. In order to reduce errors and increase the accuracy of the tests, the timer was calibrated once prior to each experiment.

II. Sub-lethal effects of rosemary oil

The effect of rosemary oil on parasitization by *E. formosa* was investigated. Leaflets of tomato containing whitefly nymphs were placed inside eppendorf tubes filled with MS (M5524) standard tissue culture media (Figure A.3). Thirty whitefly nymphs were selected on each leaflet and remaining nymphs were removed. The leaflet was placed in Petri dishes (10cm diameter) and sprayed with rosemary oil (10 ml litre $^{-1}$) or 70% aqueous methanol as carrier solvent using electronic micro sprayer or not sprayed at all. Five adult *E. formosa* were introduced to each Petri dish and the sealed dishes were place inside a growth chamber at 26±2 °C, 55-60% RH and a 16:8 LD photoperiod. After 72 hours numbers of parasitized nymphs were counted. All treatments were replicated five times.

Figure A.3. Sub-lethal effect of rosemary on parazitation by of *E. formosa*

In order to find the effect of rosemary on emergence of *E. formosa* adults, tomato leaflets containing parasitized whitefly nymphs were picked and prepared as described above inside Petri dishes with 30 parasitized nymphs on each leaflet. Leaflets were sprayed with rosemary oil (10 ml litre $^{-1}$), 70% aqueous methanol by micro-sprayer or not sprayed at all. All leaflets were placed inside the growth chamber at the conditions mentioned above. Numbers of emerged wasps were counted after one week.

Rosemary oil did affect parasitization (Figure A.4) or emergence (Figure A.5) of *E. formosa.* Because of fungal infection of many leaflets and resource limitations, the experiments had inadequate sample size and lacked statistical power.

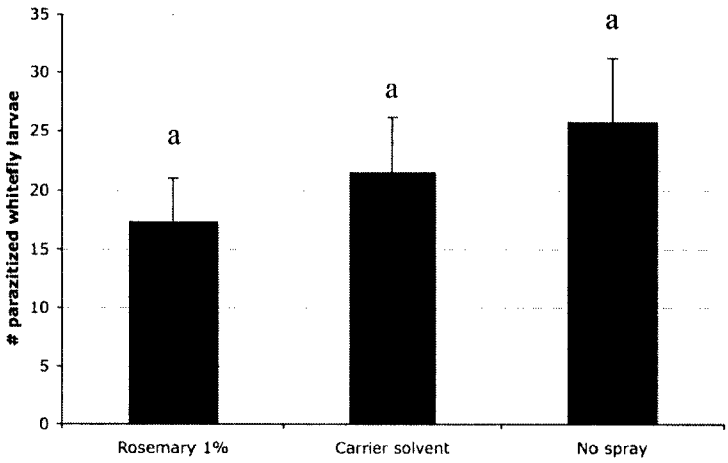

Figure A.4. Effect of rosemary oil 1% on parasitizing pattern of *E. formosa.* Bars representing means (± SE), *n=5* replicates with 30 whitefly larva and 5 parasitic wasp per replicate. Bars marked with the same letter do not differ significantly, Tukey (*P*>0.05 (0.305), F(2,12)= 1.314)

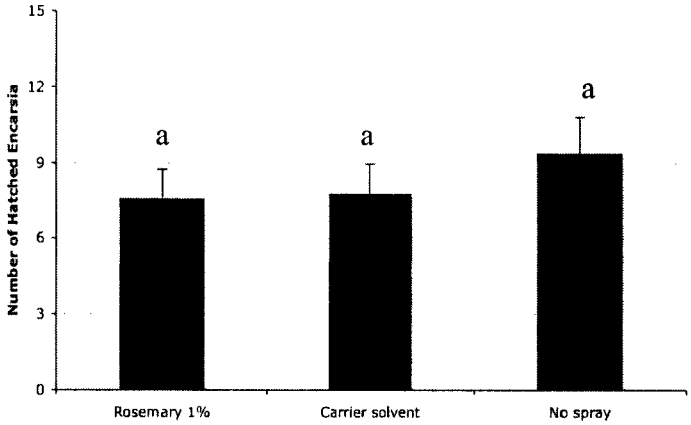

Figure A.5. Effect of rosemary oil 1% on emergence of *E. formosa.* Bars representing means (± SE), *n=5* replicates with 30 parasitized whitefly larva per replicate. Bars marked with the same letter do not differ significantly, Tukey (*P*>0.05 (0.464), F(2,12)= 0.820)