Diana Sammataro

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Department of Entomology
556 ASI Building
University Park, PA 16802

Final Report for the California Almond Board

The Effect of Some Volatile Plant Oils on Varroa Mites (Acari: Varroidae) in Honey Bee Colonies (Hymenoptera: Apidae)

DIANA SAMMATARO[1], GLEN NEEDHAM[2], GLORIA DEGRANDI-HOFFMAN[3], and GORDON WARDELL[4]

ABSTRACT

Beekeepers and researchers have been experimenting with plant ethereal or essential oils to control varroa mites on honey bees. Currently, there are some products that appear to work well under some conditions. We first screened oils in a lab assay to see if mite mortality could be achieved. Once lab tested, field applications were evaluated. Oils that killed mites in the lab were origanum, a thymol mixture, clove, bay and tea tree. Origanum, the thymol mixture, cineole and the commercial product Bee Calm all dislodged varroa mites, but Apistan strips (fluvalinate), applied after the oils, resulted in additional mites being removed from bees.

KEYWORDS: *Varroa jacobsoni* Oud., Varroa Mites, Honey Bees, *Apis mellifera*, volatile oils.

INTRODUCTION

Varroa mite *Varroa jacobsoni* (Oudemans), a parasite on honey bees *Apis mellifera* (L.), has had a catastrophic effect on the population of both managed and feral bee colonies, beekeepers and the beekeeping industry as a whole. In 1995-1996, the U.S. reported epidemic losses of managed bee colonies, from 25 to 80% (Finely et al. 1996), reaching 90% or more in some regions (Flottum, pers. comm.). In addition, feral bee colonies, hidden in crevices, tree boles and houses are virtually gone. This has left a void of 'wild bees' noticed even by homeowners and especially by growers who rely on bees for pollinating their fruits and vegetables. The end result has been a nationwide loss of beekeepers (35 to 50%), fewer beehives (25%) and an increased cost of leasing hives for pollination (up 50% in some regions).

Varroa is an external parasite on bees and was first described and discovered in 1904 in Java. Originally confined to Asia on *Apis cerana*, varroa has spread to all continents as a result of human movement of bees The only areas still free of varroa are Australia, New Zealand and the state of Hawaii. In 1986, varroa was first reported in the United States.

Adult female mites are phoretic on adult bees and are thus inadvertently carried to uninfested colonies or apiaries. The movement of the Africanized honey bees north from Central America into the southern states where queen and package bee breeders raise and ship bees, has accelerated the mite's spread throughout the United States.

Only adult female varroa mites are found on adult bees, where they feed on bee hemolymph by piercing the soft tissues of the intersegmental membranes between the abdominal segments of the bee. Adult mites are 1.2 mm by 1.6 mm and can be seen, after close examination, with the unaided eye. Females are attracted to the odor of the drone brood pheromone—but they will also invade worker brood if there are not enough drones—and enter prepupal cells prior to being capped. Female mites hide at the bottom of the cell and emerge from the brood food after the pupa forms and the cell is capped over with wax. The female lays her eggs on the pupa, which develope into protonymphs and later deutonymphs; all active stages feed on pupal hemolymph (Donzé & Guerin 1994). Daughter mites mate with their brother in the cell, after which the new females will emerge with the callow bee.

Females varroa are attracted to the odor of the drone brood pheromone, but they will also invade worker brood if there are not enough drones. The female mites enter cells prior to capping, and hide on the bottom. The mite emerges from the liquid brood food after the pupa forms and the cell is capped with wax. The female lays her eggs on the pupa, and after eclosion the mites develop into protonymphs and later deutonymphs; all active stages feed on pupal hemolymph (Donzè & Guerin 1994). Daughter mites mate with their brother in the cell, and the mated females emerge with the callow bee.

Parasitism of worker larvae and adults soon overwhelms a colony if it is not treated. Even if treated once, colonies can be lost within a year if the beekeeper is not diligent; re-infestation occurs due to the robbing of colonies that are dying within the flight distance of the home apiary. Viral and bacterial infections within parasitized colonies are common, and may be transmitted by mites or opportunistically invade weakened colonies (Sammataro 1997). Once these infections are established, worker population and replacement brood will rapidly decline and the colony will die within three to six weeks of infection.

Controlling mites

Legal control of varroa is by an EPA registered plastic strip impregnated with the contact pesticide fluvalinate (Apistan strips). Apistan is placed into the colony in the spring and again in the fall, if mite numbers are high. One of these strips per five frames of bees is inserted for 42-56 days (two to three complete bee brood cycles) to kill mites as they emerge with the brood. To keep the chemical from contaminating honey, strips are removed before the honeyflow.

This single treatment option for varroa is of grave concern to beekeepers and researchers alike because of the recent report of fluvalinate-resistant mites in Italy (Loglio and Plebani 1992, Lodesani et al. 1995, Milani 1996) Austria, (Moosbeckhofer pers. comm.) and Israel (U. Gerson, pers. comm.). New World varroa is under the same pressure and resistance has been reported in some states (Eischen 1998). Not only is this a potential disaster for beekeepers, but the use and misuse of Apistan can contaminate hive products. Fluvalinate contamination has bee reported in European beeswax and in some U.S. wax at low concentrations (K. Flottum, pers. comm.). While some researchers

are selecting strains of bees that tolerate or resist mites, beekeepers need immediate relief from Varroa infestations that already exist in their colonies.

Recently, work has been published reporting that some volatile plant ethereal oils control bee mites (Amrine et al. 1996; Calderone et al. 1995a & b, Imdorf et al. 1995, Murillo-Yepes 1998) and other bee diseases (Carpana et al. 1996, Floris et al. 1996). This is a new area of research and many beekeepers are already trying their own experiments in an attempt to disrupt mite life cycles and keep their bees alive. Plant oils are complex compounds that may have unwanted side-effects on bees and beekeepers (Schaller & Korting 1995), while contaminating 'natural' hive products. To help beekeepers select safe mite control alternatives, we wanted to evaluate the use of some oils, determine their short- and long-term effects on bees, and to develop application techniques that would protect hive products from contamination.

Plant Ethereal or Essential Oils

Plants produce chemicals to help ward off or kill insects or other pests. These chemicals are very complex and are still being studied for their usefulness to humans. We selected oils reported to be beneficial to bees and repel or kill mites.

Origanum or oregano essentail oil or *2-Methyl-5-[1-methylethyl)phenol* is steam distilled from several plants. The herb thyme, *Thymus capitatus* L. Hoffmanns. et Link is the source of Spanish Origanum Oil, and other carvacrol-rich plants in the *Origanum* genus (Labiateae, or Lamiaceae family) (Budavari 1996, Leung & Foster 1996) are also used. The oils obtained from these plants have over 20 compounds whose ratios are highly variable, but most contain high percentages of phenols such as thymol and carvacrol. Most Origanum oil is of Spanish origin, becauseit has a high phenolic content. The molecular formula of carvacrol is $C_{10}H_{14}O$ (an isomer of thymol) with a molecular weight of 150.22. This chemical is a liquid with a thymol odor and while insoluble in water, is freely soluble in alcohol. Carvacrol is used as a disinfectant, anti-fungal, anti-infective, anti-viral, or an anthelmintic agent (Menasherov et al. 1995, Mueller et al. 1995, Siddiqui et al. 1996, Sivropoulou et al. 1996). Oral LD_{50} in rabbits is 100 mg/kg and it is a skin irritant to them (Leung & Foster 1996) and to humans. Karpouhtsis et al. 1998 found insecticidal and mutagenic activity (on Drosophila fruit flies) of the oil varied by plant species.

Thymol, comes from the herb thyme, *Thymus vulgaris* L., and also from *Monarda punctata* L.; it has the same molecular weight and formula as carvacrol (Leung & Foster 1996, Prakash 1990). The chemical name for thymol is *5-Methyl-2-[1-methylethyl)phenol.* Thymol is a white or colorless crystal or powder and one gram is soluble in alcohol (1 ml), olive oil (1.7 ml) or water (1L) (Budavari 1996). Thymol is used as an antioxidant and has antimicrobial activity against bacteria, molds and fungi (Panizzi et al. 1993, Shapiro et al. 1994). It also has larvicidal (mosquitoes) activity, antiseptic and anthelmintic properties (especially for hookworms and nematodes), and is used as a meat preservative at 0.003% (Leung & Foster 1996). Thymol is a powerful mutagenic (causes mutations) to fruit flies (Karpouhtsis et al. 1998).

Cineole, or eucalyptol (*1,2,2-Trimethyl-2-oxabicyclo[2.2.2]-octane*) is steam distilled from *Eucalyptus* trees, primarily *E. globulus* Labillardière, called the Tasmanian Blue Gum. It has a molecular formula of $C_{10}H_{18}O$ and the molecular weight is 154.25 (Gildemeister & Hoffman 1900, Leung & Foster 1996). The young leaves of the eucalyptus tree have a white wax coat from which the oil is obtained; it is a colorless liquid with a camphor-like odor that is used primarily in flavorings.

Eucalyptol contains numerous constituents including: aliphatic compounds (20), monoterpenes (15), sesquiterpenes (10), monoterpenoid ethers (5), monoterpenoid alcohols (15), monoterpenoid carbonyls (15), monoterpenoid esters (5), sesquiterpenoid alcohols (10), and benzenoid compounds (5) (Bolens 1984). Leung & Foster (1996) describe the medicinal uses of eucalyptol as an a expectorant (for bronchitis) and a mild anesthetic, but is also a cockroach repellent (Budavari 1996) and an antiseptic against some bacterial strains (Meena et al. 1994, Pattnaik et al. 1995). The flavonoids quercitrin and hyperoside killed influenza type A virus in mouse tissue (Leung & Foster 1996). Eucalyptol has promoted tumors if absorbed through the skin, and a fatal dose in humans, if taken internally is 3.5 ml (Leung & Foster 1996).

Cajeput or Tea Tree Oil, also called niaouli oil or cajeputol, is a colorless or yellowish liquid with a camphor odor; it is slightly soluble in water (Budavari 1996). It's molecular formula is $C_{10}H_{18}O$ and it comes from eucalyptus relatives called *Melaleuca leucadendron* L. and *M. alternifolia* (Maiden & Betche) Cheel (Family: Myrtaceae); the trees are native to Australia and SE Asia. Early in this century melaleuca was introduced into Florida to drain swampy land, and although an important honey source, is now

classified as a noxious weed because it is highly invasive and prolific, threatening native plant species in the Everglades National Park.

Major components of cajeput oil are 1,8 cineole (see above), nirolidol and terpinen-4-ol, among others. The latter product is best for clinical uses as a carminative, stimulant, antimicrobial and antiseptic, and as a non-toxic acne treatment. Cajeput is also used in dentistry, perfumes, lotions (at 0.4%), and as flavoring for foods, condiments (at a 0.001% concentration). Nerolidol has been used to control gypsy moth due to its properties as a feeding deterrent (Leung & Foster 1996).

MATERIALS AND METHODS

Laboratory Bioassay

Essential oils were first screened for efficacy as miticides by exposing adult varroa to the volatiles of the oils. Adult mites were removed from sealed worker and drone cells, and placed in glass petri dishes (4-5 mites per dish). The dishes contained a bottom layer of damp tissue paper, covered with a sheet of Parafilm 'M' laboratory film. Small holes were punched in the film to allow evaporation of the water from the tissue paper below. A piece of filter paper equal in size to the diameter of the dish (9.0 cm) was placed on top of the Parafilm sheet. Forty microliters of a 50% solution of each plant essential oil diluted in olive oil were placed on a second piece of filter paper (4.25 cm diameter) attached to the lid of the petri dish. Control dishes contained only olive oil on the filter paper.

Eight dishes assembled as described above were used for testing each plant oil (4 treatment and 4 control dishes). All petri dishes were placed in an incubator set at 34.4°C for 6 hours. The number of live mites at each 2 hr interval were counted. The procedure was repeated three times for each essential oil. A t-test was performed to determine if mortality in dishes with the essential oils differed from that of control.

Field Tests

Promising oils identified in the lab assays were tested in two locations; bee colonies at the OARDC Extension Honey Bee Lab, in Wooster, OH (1997) and an apiary in Medina, OH. In both yard, colonies were split to form one deep super hives, provided with a new queen and normally managed. The parent colonies all had been treated for varroa that

spring or the fall before and the OARDC test colonies were not isolated, but were part of the apiary.

To monitor varroa populations, grid-line printed sheets of a synthetic paper called kimdora were smeared with petroleum jelly (Fischer P-66) and placed beneath a wire 8-mesh sheet (cut to fit inside a hive's bottom board) supported with wooden lathe to keep the wire off the paper. This 'sticky board' was pushed inside the hive's bottom board under the wire/wood frame, where falling mites were trapped in the jelly; the wire mesh kept the bees from removing the oil and mites. After a five to seven days, the sticky boards were removed, and the mites counted and recorded. Then, the sticky boards were scraped clean, re-covered with the petroleum jelly and reused.

The oils selected were origanum, cineole, a thymol mixture and a commercial mixture Bee Calmer (Tuttle Apiaries); olive oil was used as the control. To apply the oils, a 50% solution was made by diluting the oils with an equal amount of food grade olive oil (DaVinci Extra Virgin). Under a hood, 20-30 ml was placed in double-layered zippered plastic freezer bags in which ten new paper cardboard candle separators measuring 4.5 by 22 cm (A.I. Root Co., Medina, OH) were placed. Each strip absorbed approximately 2-3 ml of the oil mixture. The bags were stored in the freezer until needed.

In the field trials, we used cineole at the Medina site and Bee Calm, thymol mix and origanum at the OARDC site; the control was olive oil. The few number of colonies and available help limited our field trials. The efficacy of the essential oils as miticides was tested in 4-8 colonies per oil. Test colonies were examined and frames of brood and bees were recorded and overall fitness observed, before and after the experiment. On 24 July 1997, sticky boards were installed; they were removed on 5 August 1997. New boards were inserted on 6 August, after which the oil strips were installed. Boards were collected on 7 August and replaced with new ones. Boards were recovered and replaced on 11, 15, 18, 22 and 26 August. Eight days after the 26th, any remaining cardboard strips were removed and Apistan strips were placed in all test colonies with a new sticky board. The final boards were removed on 12 September.

Mite counts on the sticky board after the essential oil treatment was applied were divided by the number of mites counted prior to the treatment (mites after treatment / mites before treatment = mite ratio). Mite ratio values greater than one indicate a greater

number of mites counted on the sticky boards after applying the essential oil than before. An analysis of variance (ANOVO) was performed on the average mite ratios from each colony to determine if the treatments differed from each other or the controls. The variance was not homogeneously distributed among the means, so a [logt (x + 1)] transformation was performed prior to the ANOVO.

RESULTS

The results of the lab assay are summarized in Table 1. Of the oils tested, origanum, thymol, clove, bay, and tea tree were the most effective miticides. That is, they killed the greatest number of mites in the petri dish test. Bee Calm, cineole, and cinnamon were moderately effective, and patchouli was the least effective.

The temperatures and precipitation on each treatment dates were also noted. In colder weather, the effectiveness of some oils may not be enough to kill or repel mites; menthol, a miticide used for tracheal mites, is temperature dependent (menthol trade label.)

Of the four essential oils tested only BeeCalm differed from the olive oil control in its effect as a miticide (Table 3). Thymol and origanum were as effective as BeeCalm, but not significantly different from the control. Cineole was the least effective essential oil. The mortality rate of the essential oils over time indicate that thymol and origanum killed slightly more mites than the olive oil control while the cineole did not differ from the control until the fourth sampling interval

As shown on Fig. 1, BeeCalm killed 10 times more mites than the control after sample interval-2. All the essential oil except the Cineole killed more mites than the controls until the seventh sampling interval (about 20 days after application). Only BeeCalm had higher counts of mites on the sticky boards than the controls after sample interval-7. In all cases, counts on the sticky boards were the greatest after Apistan was inserted into the colonies.

DISCUSSION AND OBSERVATIONS

The petri dish bioassay indicated that several plant essential oils have the potential to be effective miticides. Origanum and thymol, which were lethal to most if not all mites in six hours showed some efficacy in the colonies. None of the oils remained effective

for the entire experimental interval nor did they kill all mites in the colonies. All the cardboard strips were chewed up, in one to 14 days depending upon colony strength. More populous colonies destroyed the strips sooner than weaker ones. This suggests that the delivery system of the oil in the colony is the next hurdle to clear in the implementation of plant essential oils for control of varroa mites.

Surprisingly, BeeCalm was one of the least effective miticides in the petri dish bioassays, but most effective in the colony. It is possible that Bee Calm either repelled the mites or narcotized them so that they could no longer cling to their hosts. If there was no sticky board in place, the mites may have been able to walk back up and reinfest the bees later.

It is important to note that Apistan strips killed more mites they did any of the oils, and these were applied after the oil treatment of three weeks. The oil strips were applied one time to see if they had long lasting effects like Apistan strips.

Table 4 summarizes the effect oils had on bees over winter. In general, we found the colonies treated with Bee Calm were much weaker coming out of the winter than the other oil-treated colonies. The thymol and origanum oils are more promising, but in many cases the queens were superseded (data not available) and honey production was not measured.

In conclusion, some essential plant oils decrease the overall populations of varroa mites in a colony. The effect of these oils on bees, worker and queen behavior, drone production and overall productivity of the colony remains unknown. That the oils were not well-received by the bees was evident in their removal of the treatment strips. These produce powerfully pungent odors that, even at a 50% concentration, may have adverse effects on bees and hive products. We plan to continue screening oils and assessing the effects they have on bees and mites.

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Table 1. Average percent mortality ± S.E. of varroa mite mortality from exposure to the volatiles of essential oils for 6 hours in the lab assay.

Essential oil	Control	Treatment
Bay	12.5 ± 9.1	75.5± 8.9
Bee Calm	28.6 ± 8.8	32.6 ± 7.2
Cineole	12.5 ± 5.8	29.9 ± 7.4
Cinnamon	12.5 ± 5.7	29.8 ± 7.4
Clove	23.3 ± 7.0	87.2 ± 5.1
Origanum	19.4 ± 5.7	100.0
Tea Tree	20.0 ± 7.1	59.4 ± 10.0
Thymol	13.9 ± 4.7	95.2 ± 3.2
Patchouli	6.7 ± 2.8	8.3 ± 3.9

Table 2. Temperatures on treatment dates.

Treatment Dates 1997	Temperature
24 July	79.5 F
6 August	74.1 F
11 August	80.9 F
18 August	72.0 F
26 August	79.6 F
5 August	71.5 F
7 August	80.7 F.
15 August (precip 0.26 in)	83.8 F
22 August	64.0 F
Sept 3	65.6 F

Table 3. Average ratios of Varroa mites counted on sticky boards in a colony after exposure to plant essential oils. Ratios were calculated using the equation / mites after treatment / mites before.

Essential oil	Total Counts	Average ratio
Olive oil control	28	2.3 ± 0.3 a
Thymol	42	3.9 ± 0.4 ab
BeeCalm	35	13.3 ± 3.9 b
Cineole	39	3.1 ± 0.8 ab
Origanum	49	3.7 ± 0.2 ab

Means followed by the same letter are not significantly different as determined by a Tukey's W Procedure (Ott 1977).

Table 4. Condition of test colonies in February 1998. All were treated with Apistan strips in the fall of 1997.

Treatment	No. colonies	No. alive spring 1998	Condition
Thymol	7	7	2 weak
Origanum	6	5	very strong
Bee Calm	4	3	2 very weak
Cineole	8	7	2 weak, others good
Control	3	2	good

Figure 1. Counts of mites on sticky boards over time after the plant essential oil was placed in honey bee colonies. The number of mites on the sticky boards were counted prior to applying the essential oil (BEFORE), and this value was divided by counts of mites on the sticky board after the essential oil treatment (AFTER TREATMENT). Counts were made at 1-4 day intervals after treatment.

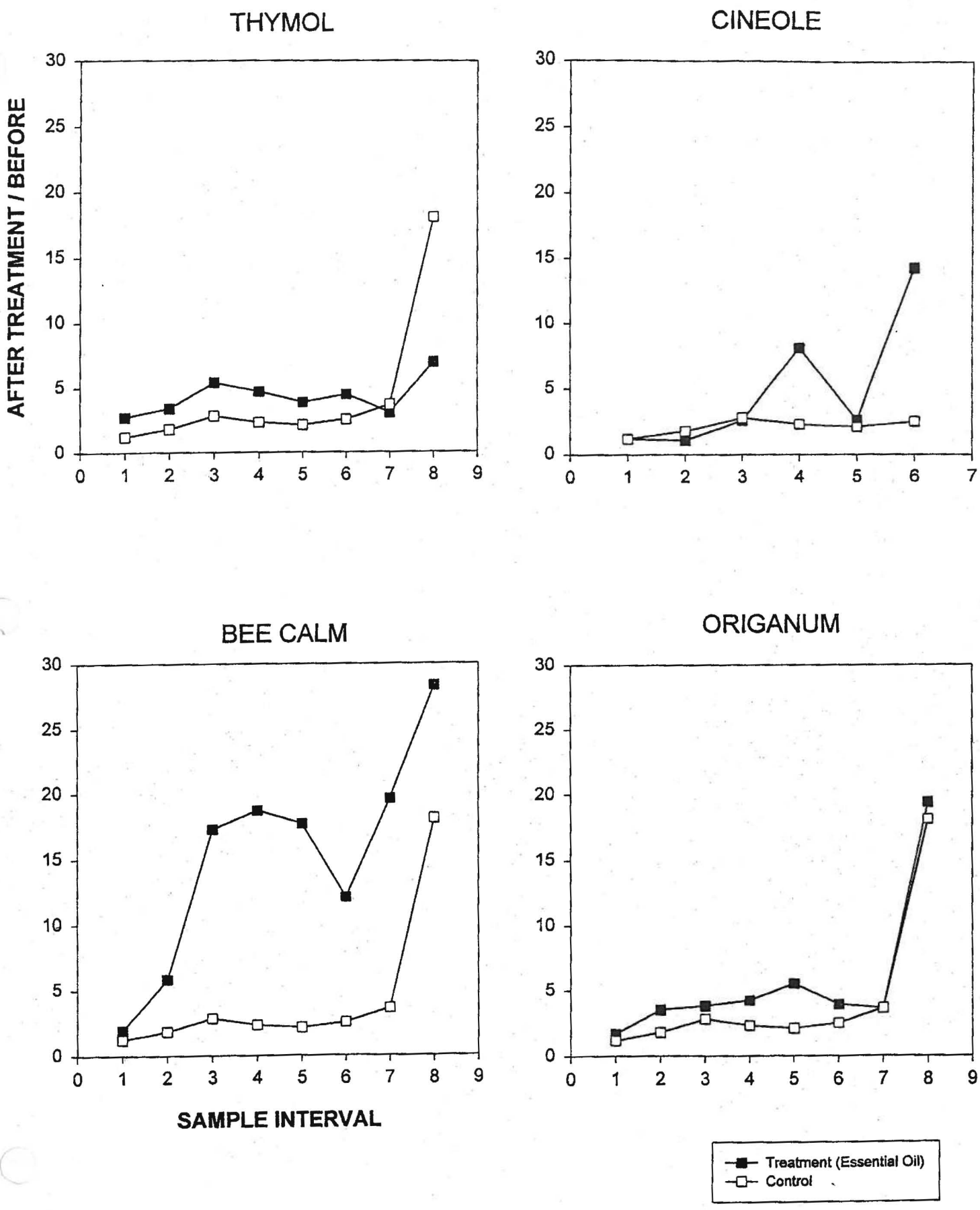
THYMOL
CINEOLE
BEE CALM
ORIGANUM
AFTER TREATMENT / BEFORE
SAMPLE INTERVAL
Treatment (Essential Oil)
Control

Fig. 1.