# Acute, Sublethal, Antifeedant, and Synergistic Effects of Monoterpenoid Essential Oil Compounds on the Tobacco Cutworm, *Spodoptera litura* (Lep., Noctuidae)

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Monoterpenoids (terpenes and biogenically related phenols) commonly found in plant essential oils were tested for acute toxicity via topical application to tobacco cutworms (*Spodoptera litura* Fab.). The most toxic among 10 such compounds was thymol ($LD_{50} = 25.4$ μg/larva) from garden thyme, *Thymus vulgaris*. The compounds were then tested for sublethal effects, specifically inhibition of larval growth after topical application of low doses. Among 6 compounds tested, an $LD_{10}$ dose reduced growth by 20% on average 3 days after administration. Feeding deterrence was determined using a cabbage leaf disk choice test. The most deterrent compound was thymol, with a $DC_{50}$ of 85.6 μg/cm$^2$ leaf disk area. Because minor constituents in complex essential oils have been suggested to act as synergists, binary mixtures of the compounds were tested for synergy vis à vis acute toxicity and feeding deterrence. *trans*-Anethole acted synergistically with thymol, citronellal, and α-terpineol, in terms of both acute toxicity and feeding deterrence. On the basis of these findings, several complex mixtures were developed and tested as leads for effective control agents. Candidate mixtures demonstrated good synergistic effects. The observed $LD_{50}$ of mixture 3 was 40.6 μg/larvae compared to an expected value of 74.6 μg/larvae. The result of this research is a proprietary product suitable for commercial production.

**Keywords:** *Monoterpenes; phenols; thymol; natural insecticides; feeding deterrents; synergy; Spodoptera litura*

## INTRODUCTION

Plant essential oils are the odorous components and secondary metabolites that can be separated from other plant tissues through steam distillation. Most are mixtures, some quite complex, of mono- and sesquiterpenes (e.g., α-terpineol and pulegone) and biogenically related phenolics or monophenols (e.g., thymol, carvacrol, and eugenol). They are often quite volatile and are commonly used as fragrances and as flavoring agent food additives. More recently, they have become the focus of interest in developing "ecologically sensitive" pesticides (*1*).

Various essential oils are documented to exhibit acute toxic effects against insects. Lee et al. (*2*) demonstrated the toxicity of a number of essential oil constituents against the western corn rootworm, *Diabrotica virgifera virgifera*, the two-spotted spider mite, *Tetranychus urticae*, and the common house fly, *Musca domestica*. Eugenol has demonstrated contact toxicity to the American cockroach, *Periplanata americana* (*3*), and carvacrol is acutely toxic to the German cockroach, *Blattella germanica*, and to many other insects (*4*). The toxicity of anethole has been demonstrated against a number of species, including various beetles, weevils, mosquitoes, and moths (*5*−*8*).

Compounds lacking acute toxicity may still confer protection to crops by reducing fitness of insect herbivores via inhibition of larval growth, disruption of larval development, or failure in pupal eclosion. Deterrence of feeding and repellency can also fulfill a protective role. Inazuka (*9*) demonstrated repellency of several monoterpenoids to the German cockroach, and several species of aphids that are greenhouse pests are affected (*10*). The mosquito, *Aedes aegypti*, is repelled by various essential oil compounds, including eugenol, cineole, and citronellal. Citronellal is actually more effective than the commercial insect repellent DEET in deterring oviposition (*11*).

Largely as a result of registration difficulties, synthetic control agents have been developed and marketed as pure compounds, yet there are a variety of reasons to suggest that complex mixtures would be more effective, chief among them synergy. Because plants usually present defenses as a suite of compounds, not as individual ones, it is thought that the minor constituents found in low percentages may act as synergists, enhancing the effectiveness of the major constituents through a variety of mechanisms (*12*). Complex mixtures are also likely to be more durable with respect to insects evolving resistance and developing behavioral desensitization (*13, 29*). The status of many essential oil compounds as exempt from EPA registration (as food grade ingredients) makes them good candidates for use in developing complex mixtures.

*Spodoptera litura* is an economically important pest of vegetable and tobacco crops in southeast Asia, India, China, and Japan. For insecticide discovery, it is a conservative model, in that this species seems to require higher doses for acute toxic effects relative to other insect species, including German cockroaches, house flies and diamondback moths (e.g., *4*).

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## MATERIALS AND METHODS

**Insects.** Bioassays were conducted using larvae of the tobacco cutworm, *Spodoptera litura* (Fab), obtained from an established laboratory colony (>50 generations; out-crossed once). Insects were reared on an artificial diet (No. 9795, BioServ Inc., Frenchtown, NJ), supplemented with finely ground alfalfa to improve acceptability, and vitamins (No. 8045, BioServ Inc.). The colony was reared at room temperature (19−24 °C) under an L16:D8 photoperiod.

**Chemicals.** Pure compounds were purchased from Sigma Chemical Co., St. Louis, MO (thymol, carvacrol, *trans*-anethole, citronellal, and *d*-limonene), Arylessence Inc., Marietta, GA (*d*-pulegone, α-terpineol, eugenol, and cinnamic alcohol), and Acros Organics, NJ (terpinen-4-ol) and used as received. White thyme oil was obtained from Liberty Natural Products, Portland, OR, and technical grade pyrethrum was a gift from Later Chemical Co., Richmond, BC. Analytical grade acetone was used as the carrier. Compounds and other test materials were dissolved in acetone (for topical adminstration) or methanol (for application to leaf disks) as required.

**Bioassays.** *Acute Toxicity of Pure Compounds.* Acute toxicity (measured as mortality after 24 h) of essential oil compounds was determined by topical application to early fourth-instars (15−20 mg body weight). Initial screening to approximate the active dose range determined a range of five doses that were used to establish the $LD_{50}$. Four replicates of 10 larvae were tested per dose.

Larvae were individually weighed prior to treatment. Essential oil compounds were prepared using acetone as a carrier such that each larva received 1 μL of oil solution per treatment, with acetone alone as the control. Doses were applied to the dorsum using a repeating topical dispenser attached to a 50 μL syringe. All 10 treated larvae from each replicate were transferred onto a 2 $cm^3$ block of diet placed in a 5 cm diameter plastic Petri dish (each replicate was transferred to a separate dish). Treatment groups were then placed in sealed plastic boxes lined with moistened paper towels and held for 24 h in a growth chamber (L16:D8, 26 °C). Mortality was recorded after 24 h. Death was recorded if larvae did not respond to prodding with forceps. Dishes were returned to the growth chamber and rechecked after 48 h to confirm mortality.

Probit analysis (*14*) was used to determine $LD_{50}$, $LD_{90}$, and the corresponding 95% confidence intervals. Experiments were repeated at least twice.

*Sublethal Growth Effects of Pure Compounds.* Sublethal growth effects of essential oil compounds were investigated using fourth-instar larvae (15−20 mg body weight) treated topically and then placed on standard artificial diet and weighed at 3 and 6 days post-treatment. Treatment groups were held in large plastic boxes lined with moistened paper towels and placed in a growth chamber (L16:D8, 26 °C).

Sublethal dosages of $LD_{10}$ and $LD_{30}$ were calculated on the basis of the $LD_{50}/LD_{90}$ values determined through probit analysis. For each treatment, 25 larvae were weighed and then treated in a glass Petri dish using acetone as a carrier. Controls received acetone only. Larvae were then placed on standard artificial diet placed in individual cells of injection-molded plastic trays. Diet was removed and replaced after 3 days. Larvae were weighed after 3 and 6 days. Data were analyzed for mean, variance, and standard deviation using Microsoft Excel, and values for control, $LD_{10}$, and $LD_{30}$ were then compared using single-factor ANOVA.

*Antifeedant Effects of Pure Compounds.* Antifeedant effects were investigated using leaf-disk choice bioassays. Fresh leaf disks (cv. Stonehead hybrid cabbage 61A, Stokes Seeds Ltd., St. Catherines, ON) were cut from greenhouse-grown cabbage plants ∼4 weeks old. Leaf disks were made using a cork borer ($^7/_{16}$ in.), yielding disks ∼1.1 cm in diameter. Increasing amounts of essential oil compounds were "painted" onto one side of leaf disks with a pipet using methanol as a carrier. The dose of essential oil was determined as the amount of essential oil per square centimeter of leaf disk area. Controls were treated with methanol alone. The dose range was determined from pilot trials which indicated the upper and lower limits for the concentrations of the most active compounds. Twenty larvae were tested per dose.

Late fourth-instar larvae were placed on fresh cabbage overnight to eliminate any effects of novel foods. Insects were removed from the cabbage and starved for 4 h prior to testing. Freshly molted fifth-instar larvae (2−4 h post-molt) were then individually placed in cells of injection-molded plastic trays with one treated and one untreated leaf disk and a small piece of moistened cotton to protect leaf disks from desiccation. Larvae were allowed to feed for 4−6 h, after which time the leaf disks were removed for analysis. Leaf disks were placed on plate glass slides, and a digital picture was captured using an imaging system (IS-500 Digital Imaging System, Alpha Innotech Corp.) and saved as electronic files. NIH software (Scion Image for Windows, Beta 3b release) was utilized to determine amounts of treated leaf disks consumed versus control disks, and a feeding deterrence index was calculated according to the formula from Isman et al. (*15*):

$$\%\ \text{deterrence} = (\text{control} - \text{treated})/(\text{control} + \text{treated}) \times 100$$

For each compound tested, a $DC_{50}$ (concentration required to produce 50% feeding deterrence compared to untreated disks) was determined using Probit analysis (*14*).

*Acute Effects of Binary Mixtures.* The acute effects of binary mixtures of essential oil compounds were determined as in the $LD_{50}$ experiments described above. Three test groups were run concurrently for each binary combination tested: the binary mixture and each of the pure compounds. The compounds were combined in a 1:1 ratio. Initially, the $LD_{50}$ value of the most active compound of the pair was chosen as the concentration for each in the mixture.

Further experimentation included variation of the dosage around this value, with values somewhat lower and higher than the $LD_{50}$ of the most active compound. Owing to the large numbers of larvae required to run concurrent trials, larvae were chosen by age (early fourth instar) and approximate size, such that weight varied between 15 and 35 mg per larvae (compared to the earlier method used to determine $LD_{50}$ of pure compounds, where each individual larvae was weighed, adhering to the strict 15−20 mg body weight). Actual mortalities were compared to expected mortalities based on the formula

$$E = O_a + O_b(1 - O_a)$$

where $E$ is expected mortality and $O_a$ and $O_b$ are observed mortalities of pure compounds at the given concentration (*16*).

The effects of mixtures were designated either antagonistic, additive, or synergistic by analysis using $\chi^2$ comparisons

$$\chi^2 = (O_m - E)^2/E$$

where $O_m$ is observed mortality from the binary mixture and $E$ is expected mortality; $\chi^2$ with df = 1 and $a = 0.05$ is 3.84.

A pair with $\chi^2$ values >3.84 and having greater than expected mortality were considered to be synergistic, with $\chi^2$ values <3.84 representing additive effects.

*Antifeedant Effects of Binary Mixtures.* Compounds were mixed in a 1:1 (w/w) ratio, such that each represented half of the total dose tested (i.e., 50 μg of eugenol and 50 μg of citronellal would be found in a 100 μg dose). Choice tests were then carried out and analyzed as described for pure compounds, again using 20 larvae per dose. $DC_{50}$ values were determined using probit analysis as for pure compounds.

*Acute and Synergistic Effects of Complex Mixtures.* Complex mixtures were prepared from a number of essential oil constituents that previous experiments suggested would be appropriate for development of an insect control product. Ratios of compounds were manipulated on the basis of efficacy in binary mixtures and several other factors, including cost and input from associated laboratories. Actual $LD_{50}$ values

**Table 1. Acute Toxicity of Essential Oil Compounds (Micrograms per Larva) to Early Fourth-Instar *S. litura***

| | LD$_{50}$ | 95% ci[a] | LD$_{90}$ | 95% ci |
|---|---|---|---|---|
| pyrethrum[b] | 1.6 | 1.3–1.9 | 3.0 | 2.2–6.1 |
| thymol | 25.4 | 23–28 | 46.8 | 38–75 |
| carvacrol | 42.7 | 38–48 | 73.8 | 56–142 |
| pulegone | 51.6 | 49–54 | 69.7 | 62–91 |
| *trans*-anethole | 65.5 | 62–70 | 98.8 | 88–129 |
| citronellal | 111.2 | 104–119 | 153.4 | 131–224 |
| terpinen-4-ol | 130.4 | 122–140 | 205.8 | 180–284 |
| α-terpineol | 141.3 | 128–155 | 206.4 | 190–250 |
| eugenol | 157.6 | 150–166 | 212.9 | 195–263 |
| *d*-limonene | 273.7 | 234–320 | 744.1 | 584–1336 |
| cinnamic alcohol | 311.4 | 242–299 | 1590 | 758–9000 |
| thyme oil | 43.7 | 41–47 | 60.9 | 55–77 |

[a] ci denotes confidence interval. [b] Contains 20% pyrethrins as active ingredients.

were compared to expected mortalities (assuming additive mortality) calculated from

$$E = (A_1Z_1) + (A_2Z_2) + (A_3Z_3) + (A_4Z_4) + (A_5Z_5)$$

where $A_1$ is the proportion of compound **A** in the mix and $Z_1$ is the LD$_{50}$ of compound **1** (Hewlett and Plackett, 1979, in *17*).

## RESULTS

**Acute Toxicity of Pure Compounds.** Of the essential oil compounds tested for acute toxicity, the most potent were thymol, carvacrol, pulegone, and *trans*-anethole, with LD$_{50}$ values <100 $\mu$g per larva (Table 1). Citronellal, terpinen-4-ol, α-terpineol, and eugenol were of intermediate toxicity. The activities of all tested compounds were significantly less than that of pyrethrum, based on nonoverlap of confidence intervals for any compound with those for pyrethrum. Thyme oil, a complex mixture obtained from garden thyme, *Thymus vulgaris* (Labiatae), containing thymol and carvacrol as major constituents, was similar in toxicity to the most active pure compounds tested.

There were notable behavioral effects following topical application to larvae. Most of the compounds elicited symptoms diagnostic for neurotoxicity: extreme agitation and hyperactivity, followed by tremors, forced diuresis, and convulsions, ending finally in paralysis and death. Citronellal was notable for causing the most extreme examples of hyperactivity. In individuals that survived, recovery from paralysis took from 2 to >8 h.

**Sublethal Growth Effects of Pure Compounds.** Chronic effects of topical application were demonstrated in growth assays. A good dose response was obtained, with a greater degree of growth inhibition occurring at LD$_{30}$ than at LD$_{10}$ (Table 2). At the LD$_{10}$ dose, larval growth was inhibited by ~20%, on average, in comparison to control weights. For most of the compounds, growth inhibition was significant compared to controls after 3 days.

Although significant differences between LD$_{10}$ or LD$_{30}$ and controls occurred at 3 days, larvae in the LD$_{10}$ treatments recovered (with the exception of those treated with eugenol) to approach the weights of the controls by the sixth day. At the higher dose (LD$_{30}$), larval growth was inhibited by ~38% on average, compared to the controls. Although recovery was not as complete as at LD$_{10}$, over time, larval growth at LD$_{30}$ also appears to increase between days 3 and 6. Curiously, thymol, carvacrol, and pulegone, compounds that are the most acutely toxic to *S. litura* (Table 1), were the least effective in this bioassay, whereas thyme oil, consisting largely of thymol and carvacrol, was the most effective.

**Table 2. Inhibition of Larval Growth Following Topical Administration of Sublethal Doses of Essential Oil Compounds to Fourth-Instar *S. litura***

| | mean larval growth (% of controls) | | | |
|---|---|---|---|---|
| | LD$_{10}$ | | LD$_{30}$ | |
| compound | day 3 | day 6 | day 3 | day 6 |
| thyme oil | 59.8*[a] | 83.3* | 41.4* | 48.7* |
| *trans*-anethole | 66.3* | 97.5 | 53.0* | 76.3* |
| eugenol | 71.5* | 84.7* | 72.6* | 86.0* |
| α-terpineol | 74.7* | 96.4* | 58.6* | 64.9* |
| citronellal | 75.0* | 94.4 | 54.2* | 70.0* |
| carvacrol | 92.7* | 108.6 | 57.3* | 81.7* |
| pulegone | 97.7 | 102.9 | 84.2* | 106.1 |
| thymol | 99.4 | 105.6 | 78.5* | 94.4 |
| average | 79.6 | 96.9 | 62.4 | 78.5 |

[a] Asterisks indicate means significantly different from controls, $p < 0.05$.

**Table 3. Feeding Deterrence of Pure Compounds to Fifth-Instar Larvae of *S. litura***

| | DC$_{50}$[a] ($\mu$g/cm$^2$) | 95% ci |
|---|---|---|
| thymol | 85.6 | 69.2–105.8 |
| carvacrol | 115.1 | 109.3–121.2 |
| *trans*-anethole | 103.1 | 82.3–129.2 |
| α-terpineol | 130.2 | 104.4–162.3 |
| eugenol | 141.8 | 122.8–163.8 |

[a] Concentration required to deter consumption by 50%.

**Antifeedant Effects of Pure Compounds.** Most compounds demonstrated a good dose response in the leaf disk choice bioassay (Table 3). There was minor deterrence at 50 $\mu$g/cm$^2$, approaching or reaching 100% deterrence at 200 $\mu$g/cm$^2$. Thymol and *trans*-anethole were the most effective feeding deterrents. Citronellal did not demonstrate any significant feeding deterrence.

**Acute Toxicity of Binary Mixtures.** LD$_{50}$ values reported in Table 4 for pure compounds are somewhat higher than those reported in Table 1. This is explained by the broader size range of larvae tested as per the experimental methods. Bioassays using binary mixtures of compounds revealed that one of the ingredients, *trans*-anethole, strongly synergized the toxicity of the other compounds (Table 4). The citronellal/α-terpineol mix also demonstrated synergy, whereas the remaining combinations operated in an additive fashion only. Tests conducted at half-concentration continued to demonstrate synergistic effects.

**Antifeedant Effects of Binary Mixtures.** Similar to the results for acute toxicity, *trans*-anethole demonstrated synergistic effects for feeding deterrence when combined with other compounds (Table 5). Citronellal also demonstrated this in combination with thymol. Comparison of DC$_{50}$ values (Table 5) for pure compounds used in binary mixtures demonstrates synergism for several of the pairs, including *trans*-anethole with thymol and α-terpineol.

**Acute Toxicity of Complex Mixtures.** A number of complex mixtures (compositions are proprietary) containing five or six of the compounds or oils in Table 1 were prepared. Synergy was clearly demonstrated in two of the complex mixtures, with observed mortality significantly greater than expected for additive effects. A six-component mixture with an expected LD$_{50}$ value of 72.8 $\mu$g/larva (based on additive toxicity of the

**Table 4. Acute Effects of Binary Mixtures of Compounds to Early Fourth-Instar Larvae of *S. litura* and Measures of Interactions**

| | | | larval mortality (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | pure compounds | | binary mix | | | |
| compd **a** | compd **b** | dosage ($\mu$g/larvae) | obsd **a** | obsd **b** | expected | obsd | $\chi^2$ | effect |
| thymol | *trans*-anethole | 35 + 35 | 37.5 | 12.5 | 45.3 | 100 | 66.0 | synergy |
| thymol | *trans*-anethole | 17.7 + 17.7 | 5 | 2.5 | 7.4 | 55 | 307.5 | synergy |
| thymol | $\alpha$-terpineol | 35 + 35 | 32.5 | 5 | 35.9 | 32.5 | 0.3 | additive |
| thymol | citronellal | 40 + 40 | 80 | 0 | 80.0 | 90 | 1.3 | additive |
| citronellal | $\alpha$-terpineol | 110 + 110 | 10 | 15 | 23.5 | 65 | 73.3 | synergy |
| citronellal | *trans*-anethole | 70 + 70 | 15 | 60 | 66.0 | 100 | 17.5 | synergy |
| $\alpha$-terpineol | *trans*-anethole | 60 + 60 | 32.5 | 37.5 | 57.8 | 95 | 23.9 | synergy |
| $\alpha$-terpineol | *trans*-anethole | 30 + 30 | 0 | 5 | 5.0 | 47.5 | 361.3 | synergy |

**Table 5. Comparison of DC$_{50}$ for Binary Mixtures with Pure Compounds**

| | DC$_{50}$ ($\mu$g/cm$^2$) | 95% ci |
|---|---|---|
| *trans*-anethole + thymol[a] | 66.77 | 56.42−79.01 |
| *trans*-anethole | 103.13 | 82.34−129.16 |
| thymol | 85.59 | 69.24−105.79 |
| *trans*-anethole + $\alpha$-terpineol | 94.59 | 81.24−116.13 |
| *trans*-anethole | 103.13 | 82.34−129.16 |
| $\alpha$-terpineol | 130.18 | 104.40−162.3 |
| $\alpha$-terpineol + eugenol | 115.78 | 96.38−139.09 |
| $\alpha$-terpineol | 130.18 | 104.40−162.3 |
| eugenol | 141.81 | 122.78−163.79 |
| thymol + citronellal | 77.77 | 63.83−94.75 |
| thymol | 85.59 | 69.24−105.79 |
| citronellal | none | |

[a] 1:1 mixtures (w/w).

constituents) had an observed LD$_{50}$ of 45.8 $\mu$g/larva (synergy statistically significant, $p < 0.05$ based on $\chi^2$ analysis), and a five-component mixture with an expected LD$_{50}$ value of 74.6 $\mu$g/larva had an observed LD$_{50}$ of 40.6 $\mu$g/larva (synergy statistically significant, $p < 0.05$).

## DISCUSSION

Many of the individual essential oil constituents proved to be acutely toxic to larvae of *S. litura* to some degree. In general, the pure compounds tested were considerably less efficacious (15−50 times) than pyrethrum, the predominant botanical insecticide in current use. On the basis of toxicity data for conventional insecticides applied topically to larvae of *S. litura* reported by Armes et al. (*18*), thymol is 4 times less toxic than the organophosphate monocrotophos, 21 times less toxic than the organochlorine endosulfan, 55 times less toxic than the carbamate methomyl, and 875 times less toxic than the pyrethroid cypermethrin. On the other hand, a mixture of five essential oil terpenes and phenols (see Results) is more acutely toxic to *S. litura* larvae than other botanical insecticides (e.g., rotenone, neem, toosendanin) except pyrethrum (*19*). Unlike many conventional synthetic insecticides (organophosphates, carbamates, pyrethroids), however, essential oils generally have quite favorable vertebrate toxicities, with rat oral LD$_{50}$ values ranging from 2 to 5 g/kg (*20*). A mixture of $\alpha$-terpineol, eugenol, and thyme oil was found to be ~300 times less toxic to fish than either azadirachtin, rotenone, or pyrethrum and 3000 times less toxic than some common synthetic insecticides, namely, azinphos-methyl and carbofuran (*21*). This could be due in part to differing pharmacokinetics and detoxicative metabolism but may also be a result of a biorational mode of action.

Several essential oil compounds have been demonstrated to block octopamine (*22*), a neurotransmitter unique to insects that functions similarly to epinephrine (adrenaline) and norepinephrine found in vertebrates. Because it is unique to insects, the octopaminergic system is of considerable interest as a target site for control agents. Octopamine agonists and antagonists act as antifeedants and can have profound adverse effects on insect behavior, with symptoms including knockdown, agitation, hyperactivity, tremors, forced diuresis, convulsions, and death (*23*).

The behavioral effects of sublethal topical doses of the tested compounds are significant and are consistent with octopamine agonists. The hyperactivity manifested as extreme agitation and rolling would likely result in larvae dropping off the plant. When this happens, the probability of mortality increases greatly, particularly as a result of predation from ground beetles and ants, although other factors such as wandering and exposure would contribute. These behavioral effects are similar to those of formamidine insecticides, which have been referred to as “pestistatic” because they function at doses far below the acute lethal doses of conventional insecticides (*24*).

Synergistic effects of complex mixtures are thought to be important in plant defenses against herbivory. Plants usually present defenses as a suite of compounds, not as individual ones, and it is thought that the minor constituents may act as synergists, enhancing the effect of the major constituents through a variety of mechanisms. It is frequently noted that the “original” complex essential oils are considerably more efficacious than the pure compounds derived from them. Examples include oil of anise (from which *trans*-anethole is derived), rosemary oil, and various citrus oils (*8*, *10*, *17*). Identifying such synergist compounds within complex mixtures may allow for the development of more effective control agents as well as the use of smaller absolute amounts in the mixture to achieve satisfactory levels of efficacy. Among the most active compounds, we found that *trans*-anethole strongly synergized the toxicity of other essential oil monoterpenes and phenols, whereas the others (with the exception of $\alpha$-terpineol and citronellal mixed) operated in an additive fashion only.

In the search for new pesticides, acute toxic effects, as demonstrated by LD$_{50}$ values, are usually the yardstick by which products are measured, yielding a spectrum of fast-acting, potent products. However, compounds with little immediate toxicity may still confer protection to crops through a reduction of fitness in insect herbivores, and, combined with other effects such as feeding deterrence, may be sufficient to protect a crop through its vulnerable stages of growth. This is borne out by the situation in nature, in which chemical defenses are usually present only in sublethal concentrations (*25*). Prolonging the duration of the developmental stages of insect herbivores likely exposes them to increased mortality, particularly if the bulk of larval mortality occurs during feeding.

Topical exposure to several of the tested compounds delayed larval development through decreased growth rates. Although the effect appears to be transitory, with recovery occurring over the duration of 6−8 days, this may still represent a significant period that could enhance abiotic and biotic mortality factors. Life table studies using lepidopterans indicate that 80% of larval mortality occurs before the third instar (reached in this species by the sixth day of development), with parasitoids and predators accounting for the majority of deaths (*26*). The growth effects of binary mixtures were not tested, but thyme oil, comprising largely thymol and carvacrol, demonstrated greater sublethal effects on growth than either of the pure compounds.

Several of the essential oil compounds demonstrated feeding deterrence in a dose-dependent manner, including *trans*-anethole, eugenol, and α-terpineol, although they are an order of magnitude less active than some triterpenoids we have previously tested (e.g., toosendanin) (*27*) and 3−4 orders of magnitude less active than the outstanding antifeedant azadirachtin (*28*). Under no-choice laboratory conditions, test insects may actually starve to death because of the absence of a perceived acceptable food source, whereas in the field insects are able to leave unacceptable hosts to seek viable food sources. However, increased search times for acceptable food would significantly increase exposure to both biotic and abiotic mortality factors.

There was notable synergy in terms of feeding deterrence in some of the binary mixtures. *trans*-Anethole plus thymol, thymol plus citronellal, and α-terpineol plus citronellal were more deterrent than the pure compounds.

Comparison of $LD_{50}$ values to feeding deterrence values yields a generally positive correlation ($r = 0.75$). Thymol, carvacrol, and *trans*-anethole were the most active in terms of $LD_{50}$ and also demonstrated the greatest degrees of feeding deterrence. This is a positive relationship in terms of effectiveness as control agents.

Given the above observations, several candidate compounds were chosen from which a unique complex control agent could be developed. Some served as effective acute toxicants, others as effective growth inhibitors, and still others as potent feeding deterrents and synergists. The inclusion of a number of compounds is more desirable in that the insecticidal spectrum of action is increased, because different species have different responses to individual compounds.

A number of complex mixtures based on these compounds were tested. Candidate mixtures demonstrated good synergistic effects, with acute toxicities at much lower doses than those expected for additive effects. Concentrations of individual compounds were varied with several factors in mind, including cost of individual compounds and any potential phytotoxicity issues, and additional compounds were inserted on the basis of previous experimentation.

A resulting proprietary product was field tested for control of lepidopteran pests on cabbage (*1*). Two foliar sprays of the product at 0.9% active ingredient gave control of diamondback moth larvae (*Plutella xylostella*) and cabbage loopers (*Trichoplusia ni*) comparable to that with two sprays of formulated pyrethrum (0.07% active ingredient), although pyrethrum gave better control of imported cabbageworms (*Artogeia rapae*). Although it may require a relatively high rate of active ingredient to achieve control, this is not an important concern for home and garden (consumer) use or for use in organic agriculture, where the only competing products are other botanicals and microbial insecticides. This product is undergoing further development and refinement before entering commercial production.

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