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Insecticidal activity of 23 essential oils and their major compounds against adult *Lipaphis pseudobrassicae* (Davis) (Aphididae: Homoptera)

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Abstract: Essential oils from 23 species of plants comprising 14 genera and 4 plant families were obtained by Clevenger-type water distillation. The major compounds in these essential oils were identified with GC-MS and their insecticidal activity against adult turnip aphids, *Lipaphis pseudobrassicae* (Davis), tested with dosage-mortality bioassays. We examined mortality only for viviparous adults because sizeable aphid populations on crucifer (Brassicaceae) hosts are largely produced by these wingless, parthenogenic females. Twenty-two of the oils were directly applied to aphid females in randomized blocks at concentrations of 0.0, 1.0, 2.5, 5.0 and 10.0 mg ml^{-1}. Essential oils mixed with a non-toxic emulsifying agent, dimethyl sulfoxide (DMSO), more easily penetrated the waxy insect cuticle. Probit analysis and LC_{50} at three different exposures showed aphids were quickly incapacitated and killed by aliphatic aldehydes, phenols and monocyclic terpenes contained in *Bifora* and *Satureja* oils and at applied concentrations as low as 0.3 to 1.0 mg ml^{-1}. Only enough *Pimpinella isaurica* oil and its three phenylpropanoid fractions were available for testing at a single concentration of 10 mg ml^{-1}. We could not spare any additional *P. isaurica* oil for testing at other concentrations. Phenylpropanoids isolated from *P. isaurica* oil when recombined or left naturally blended in the oil were highly bioactive against *L. pseudobrassicae* at 10 mg ml^{-1}.

Keywords: biopesticides; natural products; Brassicaceae; bioassay; phenylpropanoids

1 INTRODUCTION

Insecticides which contain essential oils are derived from plants naturally toxic to insects. Few have been evaluated or commercially developed for managing important pests of agricultural or medical importance.[1–7] Plant oils are generally considered broad-spectrum and safe for the environment because the array of compounds they contain quickly biodegrade in the soil.[8] The smallest pests, for instance, immature, soft-bodied or delicate arthropods, can be killed or weakened once the lipophilic plant oils penetrate their waxy cuticle and the aliphatic and aromatic components of the oils begin to block specific neurotransmitters, growth hormones or digestive enzymes.[1,2,4,9–17] Acute insecticidal activity is common in essential oils derived from Lamiaceae plants containing carvacrol, cymene or thymol, as well as mono- and sesquiterpenoids.[3,18] A monoterpenoid, 1,8-cineole, also makes up a large portion of *Eucalyptus* and *Laurus* oils, and can be an effective component of a contact insecticide, miticide, feeding deterrent or repellent.[19] Among the more noxious herbs in the Apiaceae (= Umbelliferae), those in the genus *Pimpinella* have insecticidal compounds derived mainly from pseudoisoeugenol-type phenylpropanoids: 2-hydroxy-5-methoxy-1-(*E*)-propenylbenzene.[20–23]

As part of our ongoing search for natural insecticides useful for fruit and vegetable plant protection, probit analysis and other appropriate statistical tests evaluated the insecticidal activities of 23 water-distilled essential oils and their principal compounds. The chemical composition of partially

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purified oil samples or their isolated components was analyzed with gas chromatography-mass spectroscopy (GC-MS) and tested for biological activity against viviparous females of turnip aphids *Lipaphis pseudobrassicae* (Davis). Aphids are excellent candidates for testing essential oil extracts because some species are resistant to conventional organophosphate insecticides and many species are economically important pests, but difficult to control in greenhouses, nurseries and on vegetables, fruits and nuts. Turnip aphids, in particular, are serious crop pests attacking many closely related vegetables, including broccoli, cabbage, collards, mustard and radish. They occasionally feed on non-cruciferous hosts such as tomato and zucchini.[9,24,25] Turnip aphids occur all over the World and they damage or kill leaves and flowers, thereby reducing oilseed production. *Lipaphis pseudobrassicae* also vectors *ca* 10 non-persistent crucifer viruses. Natural enemy activity cannot prevent or hinder virus transmission, thus earlier aphid control and faster knockdown are preferred, and essential oils with their novel, highly bioactive compounds can be developed as insecticides.

2 MATERIALS AND METHODS

2.1 Plant material and essential oil extraction

Oils were obtained from leaves, stems (aerial parts) or fruit of 23 herb species gathered at 26 sites throughout Turkey (Table 1). Extraction required *ca* 100 g of crushed plant tissue per species from which essential oils were water-distilled for 3 h in a Clevenger-type apparatus. Oil yields (mg ml^{-1}) were calculated on a moisture-free basis for each plant species. Air-dried plant materials, collection information, and essential oil yields are listed in Table 1. Voucher plant specimens were authenticated and deposited into a Herbarium at the Faculty of Pharmacy of Anadolu University in Eskisehir, Turkey (ESSE).

2.2 Gas chromatography-mass spectrometry

Major compounds constituting each essential oil were identified and quantified from mass spectra produced on a GC-MS Hewlett-Packard GCD system. An HP-Innowax fused silica column 60 m long with a 0.25 mm internal diameter and 0.25 μm thick film was used with helium as a carrier gas (1 ml min^{-1}). Experimental conditions included an oven temperature kept at

Table 1. List of essential oils: species, family, common name, plant tissues, collection sites and oil yield

Plant species	Plant family	Common name	Plant tissues used	Collection site	Oil yield from plant tissue (mg ml^{-1})
Bifora radians Bieberstein	Apiaceae	Wild bishop	Aerial part	Turkey: (Eskisehir) Seyitgazi	7
Coriandrum sativum L.	Apiaceae	Coriander	Fruit	Turkey: Herbalist shop	7
Coridothymus capitatus (L.) Reichb. Fil.	Lamiaceae	Thyme	Aerial part	Turkey: (Izmir) Cesme	29
Eucalyptus camaldulensis Schlect.	Myrtaceae	Redgum eucalyptus	Leaves	Turkey: Tarsus Forestry Res. Ins.	22
Foeniculum vulgare Miller	Apiaceae	Sweet fennel	Fruit	Turkey: Herbalist shop	10
Laurus nobilis L.	Lauraceae	Sweet bay	Leaves	Turkey: Antalya	48
Mentha citrata Ehrh.	Lamiaceae	Orange mint	Leaves	Turkey: Balikesir, Erdek, Ocaklar	28
Mentha piperita L.	Lamiaceae	Peppermint	Aerial part	USA: Herbalist shop	—
Mentha pulegium L.	Lamiaceae	Pennyroyal	Aerial part	Turkey: Antalya	36
Micromeria fruticosa (L.) Druce	Lamiaceae	Tea hyssop	Aerial part	Turkey: Artvin, Oruclu	21
Origanum minutiflorum Schwarz & Davis	Lamiaceae	Small-flowered oregano	Aerial part	Turkey: Antalya, Finike	38
Pimpinella anisum L.	Apiaceae	Anise	Fruit	Turkey: Mersin, Incom A.S.	34
Pimpinella isaurica Matthews	Apiaceae	Not available	Aerial part (without fruits)	Turkey: Karaman	3
Rosmarinus officinalis L.	Lamiaceae	Rosemary	Leaves	Turkey: Tarsus Forestry Res. Ins.	24
Salvia aramiensis Rechb. Fil.	Lamiaceae	Sage	Aerial part	Turkey: Hatay	21
Salvia sclarea L.	Lamiaceae	Clary sage	Aerial part	Turkey: Anadolu University: TBAM garden	7
Salvia tomentosa Miller	Lamiaceae	Sage	Aerial part	Turkey: Balikesir	21
Satureja aintabensis Davis	Lamiaceae	Not available	Aerial part	Turkey: Gaziantep, Dulukbaba	28
Satureja hortensis L.	Lamiaceae	Summer savory	Aerial part	Turkey: Maras: Andirin	36
Satureja thymbra L.	Lamiaceae	Goat oregano, barrel sweetener	Aerial part	Turkey: Icel, Murt	43
Satureja wiedemanniana (Lallem) Velen	Lamiaceae	Not available	Aerial part	Turkey: Balikesir, Ayvacik	30
Thymbra sintenisii Bornm. & Aznav	Lamiaceae	Thyme	Aerial part	Turkey: Urfa	29
Thymbra spicata L.	Lamiaceae	Thyme	Aerial part	Turkey: Batman, Kurtalan	42

60 °C for 10 min, programmed to 220 °C at a rate of 4 °C min^{-1}, then kept constant at 220 °C for another 10 min, and then programmed to 240 °C at a rate of 1 °C min^{-1}. Split ratio was adjusted at 50:1. MS were recorded at 70 eV. Mass range was from *m/z* 35 to 425. *n*-Alkanes were used as a reference point for calculating relative retention indices (RRI). A chemical library search was carried out using the Wiley GC-MS Library, the Mass Finder Library and an in-house (Baser) Library of Essential Constituents (Table 2).

2.3 Aphid culture and biological assay for insecticidal activity

The insecticidal activities of 23 essential oils were evaluated using dosage-dependent bioassays previously described and later modified by Sampson *et al*.[26] Natural populations of turnip aphids were used to collect test subjects. Winter aphid colonies were field-raised on 150 row-meters of mustard, collard, turnip and broccoli (*Brassica* spp.) near the USDA-ARS Small Fruit Research Station, Poplarville, MS (30°50.208′N, 89°32.647′W). Sizeable aphid colonies first appeared on mustard leaves and petioles in early November 2002. Leaves with large colonies of nymphs and wingless adults were detached from plants at the petiole, placed into a clean glass beaker or plastic sorting tray, and taken indoors before starting bioassays. A total of 14 000 adult aphids were used in all bioassays run for six months from November 2002 to April 2003. Adult Aphididae were identified by Gary L Miller at the USDA-ARS Systematic Entomology Laboratory.

Stock solutions of test materials were prepared by dissolving essential oils in dimethyl sulfoxide (DMSO; 1 + 9 by volume). The stock solutions of oil in DMSO were diluted with deionized water to give emulsions containing 1.0, 2.5, 5.0 and 10.0 mg ml^{-1} oil. Equivalent quantities of DMSO alone were used as negative controls. The higher concentrations contained increasing quantities of DMSO, which was necessary to maintain emulsion stability, but the highest level, 90 mg ml^{-1}, was shown to be non-toxic to aphids ($F_{1,298} = 0.94$, $P = 0.3334$). Each bioassay used 25 aphids and was replicated 5–8 times depending on the availability of aphids and essential oil solution.

Bioassays were carried out at room temperature (∼21 °C) in 96-well plastic microtiter plates. Insecticidal pads composed of circular 33 mm^2 Whatman #1 filter paper discs were dropped into each well. Healthy, vigorous adult female aphids were gently dislodged from the undersides of leaves with a metal probe onto the dry pads. Sick or molting aphids were not transferred or were immediately removed from wells upon discovery. Twenty microliters of essential oil emulsion were then pipetted onto the pads at the appropriate concentration according to a randomized block design. Mean percentage mortality was calculated from the total number of insects for the five wells per treatment and expressed as the proportion (p) of insects that exhibited no outward signs of life at 10, 30 and 60 min of exposure. Aphids were considered alive if their appendages moved or flinched, including tremors induced by the test materials. Otherwise, aphids were judged to have died, which was often easy to confirm by gently prodding them with an insect pin and watching for any post-mortem color change.

2.4 Data analysis

LC_{50} values and confidence limits for each essential oil bioassayed were produced by probit analysis[27] with the natural response used to correct for control mortality.[27] However, only raw aphid mortality (means ±1 standard error) were plotted. Control values in Fig 1 are actually a pool of data for all non-treated controls at different DMSO concentrations, because DMSO was non-toxic to turnip aphids in our tests (see Section 2.3). Probit analysis permitted us to rank our essential oils by relative bioactivity using multiple *t*-tests for homogeneity, which compared slope estimates in a pair-wise manner at a critical $P \leq 0.05$. We decided to organize mortality plots by the oils' major constituents because moieties in the oil more strongly affected aphid mortality and we wanted to reduce the number of figures. A relationship between percentage oil yield and insecticidal activity based on slope estimate was tested using Pearson rank correlation.[27]

We bioassayed *Pimpinella isaurica* Matthews oil at only 10 mg ml^{-1} because of low plant yield, a smaller volume of purified oil (Table 1) and the need to use some of the purified oil for GC-MS. Pure *P. isaurica* oil, its three novel phenylpropanoid fractions and their combination were bioassayed for aphidicidal activity at four different exposure times (Fig 2) and analyzed using one-way ANOVA and Tukey's HSD at a critical $P \leq 0.05$ (PROC GLM).[27] *Pimpinella isaurica* fractions were subsequently identified as 4-(2-propenyl)-phenylangelate (PPA), 4-(1-propenyl)-phenyltiglate (PPT) and 4-methoxy-2-(1-propenyl)-phenylangelate (MPPA).[23]

3 RESULTS

Oil yields varied among our 23 plant sources (Table 1), but were not necessarily coupled with aphidicidal activity ($r = -0.407$, $N = 21$, $P = 0.067$). Mean mortality in the DMSO controls was 4% (range: 2–7%) after omitting aphids later found to be parasitized by a braconid wasp, *Diaeretiella rapae* (McIntosh). With the exception of *Foeniculum vulgare* Mill and DMSO controls, essential oils acted as rapid contact poisons with most adult aphids succumbing after 1 h (Table 2, Figs 1, 2). Aphid mortality was affected by the types of compound constituting essential oils, as well as the concentration, and exposure (Table 2). The most aphidicidal oil came from *Bifora radians* Bieb. An LC_{50} of 0.3 mg ml^{-1} demonstrated the potency of (*E*)-2-tridecenal and (*E*)-2-tetradecenal in *B. radians* tissues (Table 2, Fig 1b),

Table 2. Summary of probit analyses for 23 essential oil extracts directly applied to adult turnip aphids *Lipaphis pseudobrassicae*; percentage composition for the major chemical constituents in each extract tested is provided in column 2

Essential oil source	Major constituents of the tested extracts based on in-house GC-MS analysis	Activity[d] ranking ($P < 0.05$)	n	Slope (±SEM)	LC_{50} (mg ml^{-1}) (95% confidence limits) exposure time: 10 min	30 min	60 min	X^2	P
B. radians	(*E*)-2-Tridecenal (47%), (*E*)-2-Tetradecenal (23%)	1	379	5.76 (±0.58)	1.7 (0.9–2.4)	0.4 (0.0–0.8)	0.3 (0.0–0.7)	100.18	<0.0001
S. wiedemaniana	Carvacrol (40%), thymol (14%)	1–2	366	4.72 (±0.59)	3.4 (2.8–4.0)	1.9 (1.6–2.1)	1.0 (0.8–1.2)	63.93	<0.0001
S. thymbra	Carvacrol (37%), γ-terpinene (29%)	2	366	4.49 (±0.58)	3.5 (3.0–4.0)	1.9 (1.4–2.3)	1.2 (0.9–1.4)	60.49	<0.0001
C. capitatus	Carvacrol (27%), *p*-cymene (19%)	2	399	4.39 (±0.51)	3.4 (2.5–4.5)	1.7 (1.4–2.1)	1.2 (0.8–1.5)	73.99	<0.0001
T. sintenisii	Carvacrol (27%), thymol (17%)	2–3	379	4.32 (±0.52)	4.7 (3.9–5.6)	1.7 (1.2–2.2)	0.9 (0.6–1.2)	68.41	<0.0001
O. minutiflorum	Carvacrol (72%), *p*-cymene (7%)	2–3	399	4.27 (±0.51)	3.6 (2.8–4.5)	1.8 (1.4–2.2)	1.3 (1.1–1.5)	70.85	<0.0001
S. aintabensis	*p*-Cymene (33%), thymol (32%)	2–3	366	4.00 (±0.55)	4.1 (2.8–5.1)	2.5 (2.1–2.8)	1.7 (1.4–2.0)	52.53	<0.0001
S. hortensis	Carvacrol (24%), γ-terpinene (45%)	2–3	399	3.81 (±0.50)	4.1 (2.7–0.9)	2.5 (2.0–3.0)	1.7 (1.5–1.9)	58.50	<0.0001
T. spicata	Carvacrol (30%), γ-terpinene (38%)	2–3	379	3.74 (±0.51)	6.0 (5.6–6.5)	2.2 (1.4–3.0)	0.9 (0.6–1.2)	54.11	<0.0001
M. pulegium	Pulegone (81%), 3-octanol (2%)	3	399	3.49 (±0.49)	3.1 (1.7–4.9)	1.9 (1.1–2.7)	1.8 (0.1–2.6)	50.39	<0.0001
S. sclarea	Linalyl acetate (49%), germacrene D (13%)	3–4	366	3.29 (±0.53)	7.3 (4.7–17.30)	2.4 (1.7–3.1)	1.5 (0.9–2.1)	38.65	<0.0001
M. fruticosa	Isomenthone (25%), pulegone (20%)	3–4	393	3.06 (±0.49)	4.6 (3.8–5.5)	2.7 (2.3–3.2)	2.5 (1.9–3.0)	38.43	<0.0001
C. sativum	Linalool (72%), geranyl acetate (10%)	3–4	379	3.03 (±0.49)	4.6 (4.1–5.2)	3.3 (2.2–4.1)	2.9 (2.2–3.6)	37.42	<0.0001
M. citrata	Linalool (32%), linalyl acetate (21%)	4	379	2.57 (±0.49)	5.5 (4.9–6.1)	4.0 (3.1–4.8)	3.2 (1.8–4.1)	27.36	<0.0001
P. anisum	(*E*)-Anethole (85%), methyl chavicol (6%)	4–5	393	1.94 (±0.49)	6.9 (5.2–10.2)	4.9 (3.6–7.0)	4.6 (3.3–6.7)	15.82	<0.0001
P. isaurica	4-(2-Propenyl)-phenylangelate (43%), 4-(1-Propenyl)-phenyltiglate (13%)								
S. aramiensis	1,8-Cineole (49%), β-pinene (10%)	4–5	366	1.82 (±0.50)	7.7 (6.6–9.2)	6.4 (5.6–7.2)	4.9 (2.9–6.4)	13.15	0.0003
E. camaldulensis	1,8-Cineole (49%), spathuleneol (10%)	5	393	1.70 (±0.50)	10.1 (8.1–14.9)	6.1 (4.8–8.3)	4.1 (3.1–5.3)	11.63	0.0006
S. tomentosa	Camphor (30%), borneol (14%)	5–6	393	1.26 (±0.50)	8.0 (6.2–11.9)	6.9 (4.9–11.2)	6.6 (4.5–10.2)	6.35	0.0117
R. officinalis	1,8-Cineole (38%), α-terpineol (19%)	5–6	393	0.84 (±0.51)	10.2 (8.4–15.4)	7.8 (6.2–10.4)	7.4 (5.4–11.3)	2.67	0.1013
L. nobilis	1,8-Cineole (45%), α-terpineol/α-terpinyl acetate (13%)	6	393	0.69 (±0.52)	11.7 (9.2–21.6)	9.3 (6.5–28.0)	7.5 (4.7–17.0)	0.74	0.1875
M. piperita	Menthol (29%), menthone (17%)	6	399	0.28 (±0.50)	—[a]	11.2[b]	8.8[b]	0.31	0.5795
F. vulgare	Anisaldehyde (46%), anisketone (13%)	6	379	0.00	18.0[b]	12.0[b]	9.9[b]	—[c]	—[c]
Other effects									
$\log_e$concentration		.	2114	2.15 (±0.12)	.	.	.	337.7	<0.0001
Exposure time		.	2114	0.09 (±0.01)	.	.	.	128.7	<0.0001

[a] LC_{50} too low or high to reliably calculate for *L. pseudobrassicae* due to limits on the concentrations that were used (1.0–10.0 mg ml^{-1} oil).
[b] Estimates of LC_{50} or 95% confidence limits too far above 10 mg ml^{-1} oil concentration.
[c] *Foeniculum vulgare* used as a baseline control for testing the relative biological activity (H_o: slope = 0) of the other essential oils.
[d] Activity rankings based on multiple step-wise slope comparisons ($P < 0.05$), overlapping rankings shown as a range (1: highly active, 6: least active).

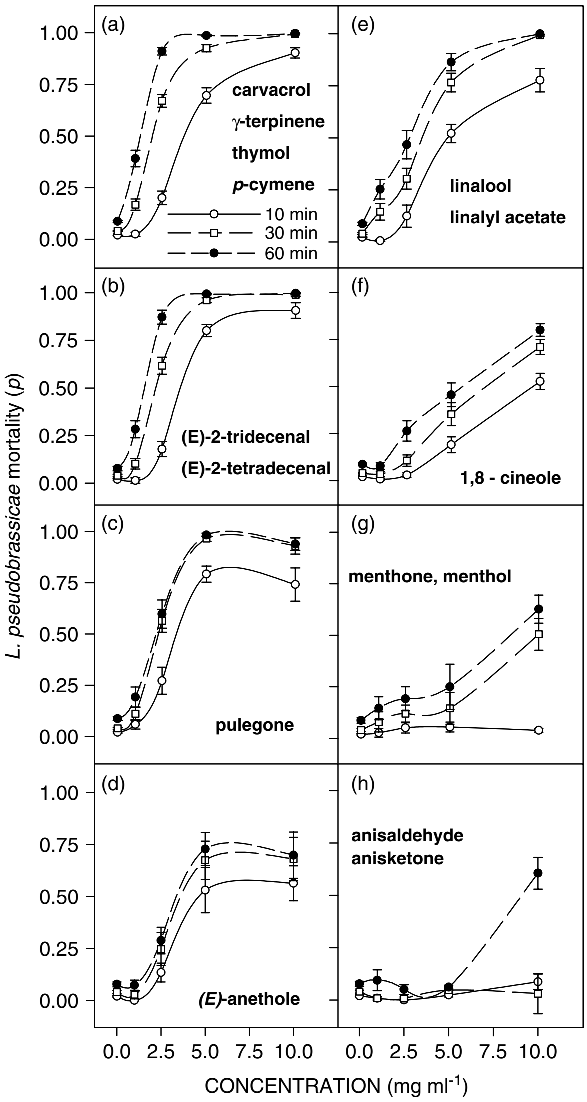

Figure 1. Mortality (p of 0.00 = 0% mortality; p of 1.00 = 100% mortality) for adult *Lipaphis pseudobrassicae* after they were exposed to a control and four concentrations of essential oils for (○) 10, (□) 30 and (●) 60 min. Aphid mortality responses are grouped by the 15 major active compounds (a–h) in the oils.

which also caused rapid darkening of the aphids' integument. Color change in aphid cadavers was less intense with oils containing phenolics like carvacrol, thymol, γ-terpinene and p-cymene, but oils with these compounds still induced mortality at the very low concentrations (Table 2, Fig 1a). Oils containing pulegone, linalool, citronellal, 1,8-cineole and (E)-anethole were the next most insect-toxic (Table 2, Figs 1c–f). A reduction over time in aphid mortality to oils from *Mentha pulegium* L. and *Micromeria fruticosa* (L.) Druce showed that some individuals overcame paralysis induced by pulegone and its analog isomenthone (Table 2). Oil of *F. vulgare* was slower acting and insecticidal activity was detected only after exposing aphids for 60 min to 5 mg ml^{-1} (Fig 1h).

Essential oils of *Pimpinella anisum* L. and *P. isaurica* contained phenylpropanoids moderately active against *L. pseudobrassicae* (Figs 1d, 2). Pure oil of *P. anisum* and oils with linalool and 1,8-cineole shared a similar level of aphidicidal activity (Table 2, Figs 1d–f). *Pimpinella isaurica* oil mostly contained the phenylpropanoids and appeared more active than *P. anisum* oil at the same concentration (10 mg ml^{-1}, see Figs 1d, 2a). The phenylpropanoids PPA, PPT or MPPA isolated from *P. isaurica* oil showed no detectable aphidicidal activity (Tukey's HSD, $P >$ 0.05, Fig 2) unless they were combined. However, the pure oil was still faster acting; killing >90% of aphids in 10 min.

4 DISCUSSION

Our results show quantitative and qualitative differences in the chemical composition and insecticidal activities of our essential oils. All oils became insect-toxic as concentration was increased. However, susceptibility of adult female aphids to the essential oils was also consistently and qualitatively dependent on the major chemical classes and analogous compounds constituting ~50% or more of the oils based on GC-MS. Turnip aphids were most susceptible to (E)-2-tridecenal, a simple aliphatic aldehyde concentrated in *B. radians* leaves and proven lethal to a few other insect species.[9,28] An oil containing both carvacrol and thymol usually extracted from oregano and thyme (*Satureja*, *Coridothymus*, *Thymbra*, and *Origanum*) has similarly been found to be lethal to turnip aphids.[29] Leaf oils containing pulegone and linalool showed moderate activity against turnip aphids and some adult aphids even fully recovered after a day. Thus, oils with pulegone and linalool can effectively control smaller, immature insects, and their bioactivity against adult insects might be enhanced by adding a synergist or using another biopesticide.[7,30] The least toxic oils at the concentrations tested contained 1,8-cineole, menthone, menthol, or anisketone/anisaldehyde (*F. vulgare*). A weaker response of turnip aphids to *F. vulgare* oil was in stark contrast to other studies consistently showing a much higher sensitivity to the oil in other organisms.[26,31]

Insecticidal activity of essential oils, even from the same source, can be inherently variable for many reasons. The chemical composition and broad spectrum of biological activity for essential oils can vary with plant age, the plant tissues or organs used in the distillation process, the type of distillation and the species and age of a targeted pest organism.[2,29,32] Biological activity is also affected by interactions among structural components in the essential oil. Even minor compounds can have a critical function due to coupled effects, additive action between chemical classes and synergy or antagonism.[29] Plants in the Apiaceae such as *Pimpinella* were found to have a rare class of simple but biologically active phenylpropanoids.[33] Individually, the three major phenylpropanoids PPA, PPT, MPPA of *P. isaurica* oil, were benign to *L. pseudobrassicae*. When combined, however, they killed aphids. The pure extract killed aphids faster than the phenylpropanoid

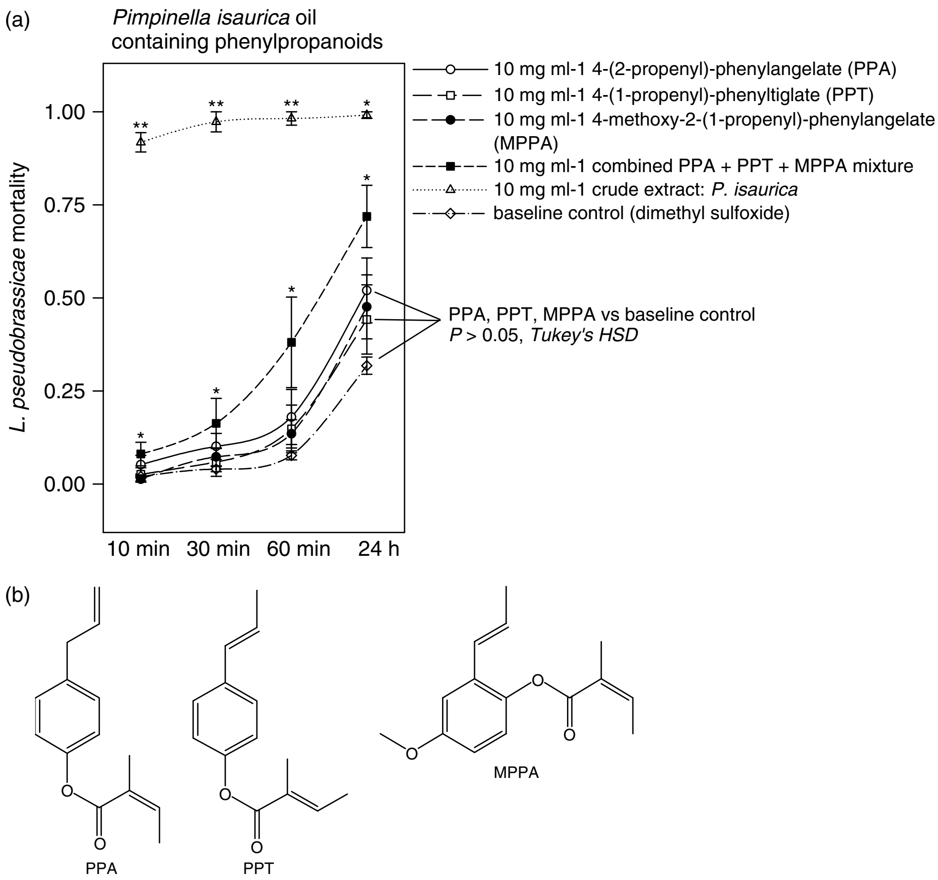

Figure 2. **a** Mortality of *Liphaphis pseudobrassicae* exposed to (◊) dimethyl sulfoxide control, (△) essential oil of *Pimpinella isaurica*, its three major phenylpropanoid compounds (○) PPA, (□) PPT, (♦) MPPA and (■) their combination. Asterisks that are the same indicate no difference between mean mortality for the crude extract and the combined phenylpropanoid mixture for each exposure time. **b.** Structures of the three major phenylpropanoid compounds of *P. isaurica* oil that were bioassayed: 4-(2-propenyl)-phenylangelate (PPA), 4-(1-propenyl)-phenyltiglate (PPT), 4-methoxy-2-(1-propenyl)-phenylangelate (MPPA).

combination (Fig 2a) implying that we still need to find an even rarer, but critical initiator or activator compound.

Essential oils from *Bifora, Satureja, Coridothymus, Thymbra* and *Pimpinella* plants could be developed into consistently effective insecticides with additional research into essential oil chemistry, entomology and plant breeding. We are now examining the molecular configurations of various lipophilic monoterpenes and phenols from Lamiaceae oils, as well as various phenylpropanoid analogs derived from *Pimpinella* oils to determine optimum structure activity relationships in the pharmacophore from which new, perhaps more powerful, natural product-based insecticides can be developed with lower mammalian and environmental toxicity.[2,34] Field and laboratory research is focusing on isolating, identifying and evaluating other natural product leads from the most bioactive oils from higher yielding plant species and clones. Thus, insecticidal bioassays are very useful for identifying which clones and plant organs yield the most oil with acceptable and consistently high levels of bioactivity. Our aphid bioassay can be one way to quickly test oils extracted from wild or selectively bred plants of *Bifora, Satureja* and *Pimpinella* to ensure a reliable insecticidal product.

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