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Antifeeding, settling inhibitory and toxic activities of labiate essential oils against the green peach aphid, *Myzus persicae* (Sulzer) (Homoptera: Aphididae)

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Abstract

Labiate plants, such as spearmint, thyme and rosemary, inhibit the settling of *Myzus persicae* (Sulzer) on them. The effects of their essential oils on the aphids' behaviors are little known thus far. In this study, the antifeeding, settling inhibitory and insecticidal activities of 10 labiate oils were investigated. On EMIF (electronic measurement of insect feeding behavior), 8 of the ten oils (rosemary and sage oils were exceptions) significantly reduced the total penetration time, average penetration time, and maximum penetration time, and increased the frequency of penetration. In the no-choice test, aphids rarely settled on the sealing film which covered the diet containing spearmint or thyme oil and most died. In the toxicity test, pennyroyal and thyme oils indicated relatively high toxicities while toxicities of the other oils were low. In the choice test, all 10 oils exhibited settling inhibitory activities. Spearmint, thyme, pennyroyal, mint and peppermint oils had especially high activities. Although the mechanisms of the settling inhibition differed among the oils tested, all the oils inhibited the aphids from settling under the choice condition. Labiate essential oils may play a role in deterring aphids from settling on these plants by effecting the aphids' gustatory and/or olfactory sense.

Key words: *Myzus persicae*, labiate essential oil, antifeeding activity, settling inhibitory activity, toxicity

INTRODUCTION

The green peach aphid, *Myzus persicae* (Sulzer), is polyphagous, and has secondary hosts in over 40 different plant families, including many important agricultural plants (Blackman and Eastop, 1984). The aphids, however, are unable to reproduce or settle on labiate plants and *Allium* species; they disappear within a few days when released on these plants (Hori and Harada, 1995). In the previous paper, the author reported that garlic and onion oils had settling inhibitory and insecticidal activities and that the volatile constituents of their essential oils might inhibit the settling by *M. persicae* on these plants (Hori, 1996). In this paper, feeding and settling behaviors were investigated to analyze the disappearance mechanisms of aphids from labiates with a system consisting of four assays: EMIF (electronic measurement of insect feeding behavior), no-choice-, choice- and toxicity tests.

MATERIALS AND METHODS

The apparatuses and methods used in the assay series of the four experiments, nos. 1–4, were essentially the same as those described in the previous paper (Hori, 1996). Except for during toxicity tests in Exp. 3, the aphids were confined in a small plastic vessel having a bottom of sealing film, and their behaviors were recorded with an EMIF or video system. Diets with or without each of the essential oils were given to the aphids. The details of the apparatuses, particularly the vessels confining aphids, are shown in Figs. 1–3 in Hori, 1996.

Aphids. Adult alatae of *Myzus persicae* (Sulzer), derived from a stock culture maintained on *Nicotiana tabacum* at 20°C in a greenhouse, were used for all experiments.

Diets for experiments. In Exp. 1, a 20% sucrose solution was used as the basal diet. In Exps. 2 and 4, however, a meridic artificial diet (Mittler and Koski, 1974) was used to ensure good settling of the aphids. In all experiments except for Exp. 3, the aphids were fed with absorbent cotton pads (1 g in Exp. 1; 1.5 g in Exps. 2 and 4) soaked in the diets (10 ml in Exp. 1; 15 ml in Exps. 2 and 4). The absorbent cotton pads were first soaked in a diethylether solution (10 ml in Exp. 1; 15 ml in Exps. 2 and 4) containing essential oil (100 μl in Exp. 1; 150 μl in Exps. 2 and 4), and dried in a draft chamber for 1 h. In the control, diethylether only was applied.

Essential oils. The essential oils tested were all supplied from Soda Aromatic Co. (Tokyo).

Experiment 1: Feeding behavior. In this EMIF experiment, a wingless aphid tethered with a fine gold wire was released on a sealing film which covered the diet cotton pad (9 cm in diameter) and its behavior was recorded by EMIF for 2 h at 20–23°C.

Experiment 2: No-choice test. The settling inhibitory activity of each oil was evaluated under circumstances in which the aphids were not free to choose diets. The aphids were fed through a sealing film which covered the diet cotton pad (6.7 cm in diameter). Aphid behavior was recorded with a video system at 20–23°C.

Experiment 3: Toxicity test. Twenty aphids were confined in a small plastic vessel ($\phi 60 \times 22$ mm) with a piece of filter paper (10×20 mm) treated with 40 μl essential oil-acetone solution in two–four doses. Mortality of aphids was investigated 4 h after the aphids were released into the vessel. Experiments were conducted at 22–26°C. Five replications were performed for each oil evaluation.

Experiment 4: Choice test. The settling inhibitory activity of each of the oils was evaluated when the aphids were freely allowed to choose diets with or without each oil. One hundred aphids released in a vessel were allowed to choose between the control and oil-containing diets. The behavior of the aphids was recorded with the same video system as used in Exp. 2, and the numbers of aphids on the sealing film were counted as settling individuals from 15 to 300 min in regular intervals after the start of the test at 20–23°C. This was replicated six times.

The settling inhibition expressed as a percentage was as follows (Table 2):

Settling inhibitory percentage

$$=100(\bar{n}_C-\bar{n}_T)/(\bar{n}_C+\bar{n}_T).$$

$\bar{n}_C$: mean number of aphids on film of the control area

$\bar{n}_T$: mean number of aphids on film of the treatment area

RESULTS

Experiment 1. Feeding behavior

The time to first penetration, the penetration frequency, the total and the average penetration times, and the maximum penetration time were obtained by the measurement of 10 aphids for each oil test (Table 1).

Most of the oils did not significantly affect the time to register the first penetration, but the time for spearmint and mint oils were over twice that of the control. A significant difference from the control was obtained for spearmint oil but not for mint oil due to large standard errors.

The eight essential oils excluding rosemary and sage oils significantly increased the penetration frequency and reduced the total penetration time and maximum penetration time. Consequently, the average penetration times were significantly reduced in these eight oils.

In particular, the penetration frequencies for pennyroyal, marjoram, basil, spearmint, peppermint, thyme and mint oils were increased to about twice that of the control. The total penetration time for mint oil was reduced to about half that of the control; for spearmint, peppermint and thyme oils to ca. 60–65%; for pennyroyal, marjoram and basil oils to ca. 70%. The average penetration times for spearmint, peppermint, thyme and mint oils were reduced to ca. 30% that of the control; for marjoram and basil oils to ca. 40%; for lavender and pennyroyal oils to ca. 60% and 50%, respectively. In spearmint, peppermint, thyme and mint oils the maximum penetration times were reduced to ca. 25%; in pennyroyal, marjoram and basil oils they were reduced to ca. 35%; in lavender oil it was reduced to ca. 45%.

The penetration frequency tended to increase with reductions in the penetration time. The increase of penetration frequency implies unstable feeding of the aphid.

Experiment 2. No-choice test

The oils were grouped into two as shown in Fig. 1. Thyme, spearmint and pennyroyal oils notably affected the aphid settling (Group A),

Table 1. Effects of labiate essential oils on feeding behavior of *Myzus persicae* in EMIF

Diets	Time to first penetration (min)	Penetration frequency	Total penetration time (min)	Average penetration time (min)	Maximum penetration time (min)
20% Sucrose (Control)	4.1±1.0	5.7±0.6	101.4±2.8	19.7±2.2	71.6±5.6
20% Sucrose+rosemary oil	3.5±0.3	6.0±0.8	97.6±3.9	21.3±4.8	67.1±9.0
20% Sucrose+sage oil	5.3±1.3	8.0±1.5	94.4±3.6	20.3±5.9	53.8±8.9
20% Sucrose+lavender oil	5.7±1.3	8.7±0.9*	85.3±4.8**	11.6±2.1*	32.9±4.1**
20% Sucrose+pennyroyal oil	2.9±0.5	10.9±1.5**	74.9±4.8**	9.7±2.9*	22.9±3.7**
20% Sucrose+marjoram oil	4.7±1.5	10.3±1.1**	74.1±6.5**	8.3±1.4**	27.1±4.8**
20% Sucrose+basil oil	5.2±1.1	11.7±1.3**	72.8±8.3**	7.5±1.3**	27.0±5.4**
20% Sucrose+spearmint oil	8.3±1.6*	12.2±1.1**	66.9±6.1**	6.2±1.3**	17.3±3.3**
20% Sucrose+peppermint oil	2.6±0.6	13.7±1.9**	64.5±8.3**	5.3±0.8**	18.2±3.2**
20% Sucrose+thyme oil	3.8±1.0	10.6±1.1**	61.1±9.4**	5.8±0.9**	17.5±3.2**
20% Sucrose+mint oil	11.5±6.3	11.6±2.5*	50.7±10.2**	6.1±1.7**	17.8±3.8**

Values are mean±standard error/10 aphids.
*, **Significant difference at $p<0.05$, 0.01, respectively, compared with control, by *t*-test.
Recording: 2 h, 20–23°C.

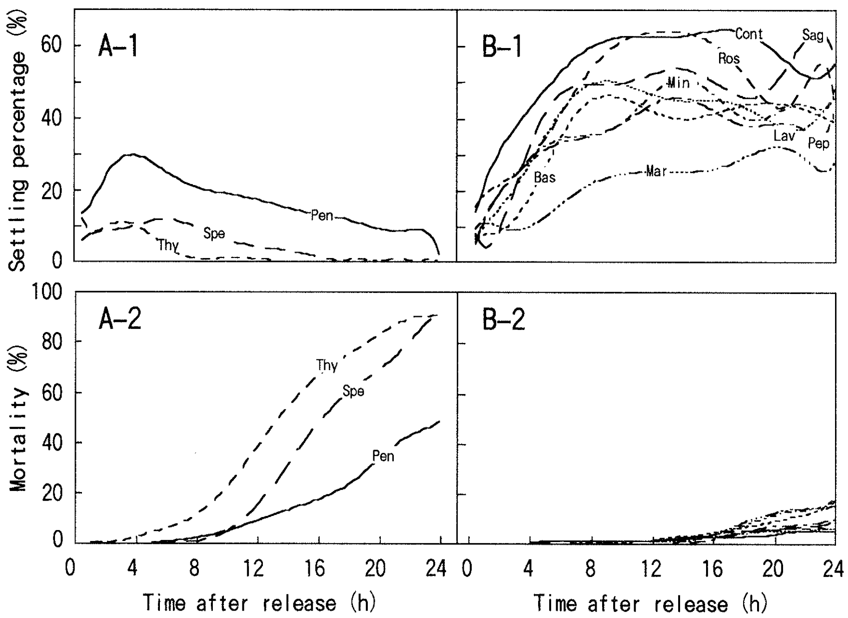

Fig. 1. Effect of labiate essential oils on *Myzus persicae* settling in no-choice test. Cont: basal diet (BD); Ros: BD+rosemary oil; Sag: BD+sage oil; Bas: BD+basil oil; Lav: BD+lavender oil; Min: BD+mint oil; Pep: BD+peppermint oil; Mar: BD+marjoram oil; Pen: BD+pennyroyal oil; Spe: BD+spearmint oil; Thy: BD+thyme oil. Recording: 10 aphids/replicate, 8 replicates, 20–23°C.

but the other seven oils did not (Group B). The three oils of Group A lowered the aphid settling percentage on the sealing film and decreased it further with the passage of time (Fig. 1, A-1).

In the other six oils of Group B except marjoram oil, the settling percentages of aphids were high (Fig. 1, B-1). The settling percentage on the marjoram oil-diet was low but it increased gradually with time, showing a similar trend to those of the other oils in Group B.

Furthermore, the aphid mortalities on the three oils in Group A increased notably with the passage of time (Fig. 1, A-2). The mortality of aphids on the diet with thyme or spearmint oil reached over 90%, and over 40% for the pennyroyal oil-diet 24 h after release. The mortality of the aphids on the Group B oils was as low as that of the control (Fig. 1, B-2).

Experiment 3. Toxicity test

In the above no-choice test, the aphids showed high mortalities on the thyme, spear-

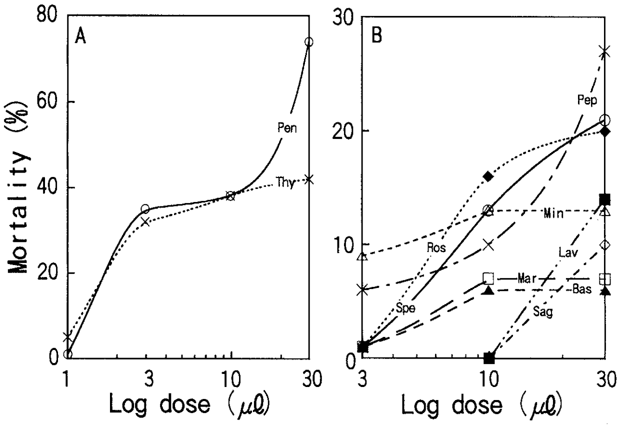

Fig. 2. Toxicity of labiate essential oils against *Myzus persicae*. Pen: pennyroyal oil; Thy: thyme oil; Pep: peppermint oil; Ros: rosemary oil; Spe: spearmint oil; Min: mint oil; Lav: lavender oil; Sag: sage oil; Mar: marjoram oil; Bas: basil oil. Recording: 20 aphids/replicate, 5 replicates, 22–26°C.

Table 2. Effect of labiate essential oils on settling of *Myzus persicae* in choice test

Essential oils	Settling inhibitory percentage (%)[a]								
	Time (min) after release								
	15	30	45	60	90	120	180	240	300
Pennyroyal oil	18.1	31.5	47.3**	46.8**	70.1**	78.8**	80.5**	80.5**	73.9**
Spearmint oil	25.6	30.2*	30.9*	41.0**	65.8**	79.5**	89.6**	89.5**	83.8**
Peppermint oil	3.5	16.7	25.4*	37.5**	53.3**	58.1**	68.1**	70.0**	65.5**
Thyme oil	−3.8	3.8	12.2	21.6	44.8*	48.8**	62.4**	71.0**	69.1**
Mint oil	1.2	24.4*	27.3*	39.6*	63.9**	66.7**	62.8**	60.7**	60.4**
Marjoram oil	−5.6	10.5	17.0	35.3*	48.6**	51.1**	55.7**	53.6**	57.5**
Rosemary oil	−1.1	4.9	9.7	23.5	34.4**	38.8**	41.3**	44.4**	45.1**
Sage oil	0.0	6.3	17.9	17.2*	34.7**	42.4**	47.0**	46.9**	42.5**
Basil oil	34.6**	22.0*	22.7	25.7	33.8*	39.7*	31.3*	32.1*	24.1*
Lavender oil	21.4**	14.7	14.7	21.8	35.4*	37.2*	43.2**	41.0*	45.6*

[a] Settling inhibitory percentage $=100(\bar{n}_C-\bar{n}_T)/\bar{N}$; $\bar{n}_C$, $\bar{n}_T$: mean number of aphids on sucking film of control and treatment area, respectively, and $\bar{N}=\bar{n}_C+\bar{n}_T$.
*, ** Significant difference at $p<0.05$ and 0.01, respectively, by paired *t*-test (six replicates).
Recording: 100 aphids/replicate, 20–23°C.

mint or pennyroyal oil-diet which notably inhibited the aphids from settling. In the previous study, aphids in the vessel with no diet (no water) showed a high mortality of ca. 50% at 14 h and over 95% at 24 h after the release of aphids, while the mortality of aphids fed water alone was also moderately high and ca. 70% at 24 h after the aphids were released (Hori, 1996). The vapors of essential oils emanated through the sealing films. Therefore, it was thought that starvation, oral toxicity and/or fumigant toxicity caused the death of the aphids in the case of thyme, spearmint and pennyroyal oils in the no-choice test. Toxicity tests were made to evaluate fumigant and contact toxicity of the essential oils.

The oils were grouped into two types according to their toxic levels, as shown in Fig. 2. The mortalities of pennyroyal and thyme oils were similar at doses below 10 μl (Fig. 2, A). However, at a higher dose of 30 μl, pennyroyal oil showed a much higher mortality than thyme oil. The toxicities of eight other essential oils were much lower, the mortalities being lower than 30% at a dose of 30 μl (Fig. 2, B).

Experiment 4. Choice test

All the tested essential oils inhibited the aphids from settling on the film of the oil-diet semicircular area (Table 2). Pennyroyal and spearmint oils had the highest settling inhibitory activity. The settling inhibitory percentage of these oils reached ca. 30% at only 30 min after release. As time elapsed, aphids concentrated on the control area and the settling inhibitory percentage was ca. 80% at 120 min after release. Peppermint, thyme and mint oils had high settling inhibitory activities. The settling inhibitory percentages of these three oils were ca. 60–70% at 180 min after release. The last five essential oils, marjoram, rosemary, sage, basil and lavender, had low settling inhibitory activities with a settling inhibitory percentage of below 60% at most.

DISCUSSION

It is thought that the settling inhibition of essential oils is mainly caused by their repellency and probing inhibitory, sucking inhibitory and locomotion stimulatory activities. In the EMIF for the antifeeding activity, aphid-tethering restrained the aphids from leaving the sealing film and prompted probing of the test diet. Therefore, the antifeeding activity measured by EMIF may be correlated to the inhibitory activity against aphid sucking, which follows probing, and was mainly caused by the effect on the gustatory senses of the aphids.

In the previous study using a linear track olfactometer, the five essential oils of rosemary, thyme, peppermint, spearmint and lavender repelled the aphids (Hori, 1998). In the olfactometer test, the aphids chose directions by chemotaxis. Therefore, the repellency as shown in the olfactometer test is caused by the effect on the olfactory senses of the aphids.

In Table 3, the activities of tested essential oils were compared. These essential oils are divided into the following four types based on sucking inhibitory activity and repellency: Group 1) Essential oils having both activities (thyme, spearmint, peppermint and lavender oils); Group 2) Essential oils having only sucking inhibitory activity (pennyroyal, mint, marjoram and basil oils); Group 3) Essential oils having only repellency (rosemary oil); and Group 4) Essential oils having neither sucking inhibitory activity nor repellency (sage oil).

The essential oils having sucking inhibitory activity and/or repellency naturally inhibited the aphids from settling. In fact, all the tested essential oils except sage oil had a sucking inhibitory activity and/or repellency, and inhibited the aphids from settling.

Thyme, spearmint, peppermint and lavender oils had a sucking inhibitory activity and repellency against the aphids. Thus, these oils inhibit the aphids from settling by affecting their gustatory and olfactory senses. Thyme oil also showed fumigant and/or contact toxicity effect in the test. The toxicity of thyme oil vapor, which emanates through the sealing films, may not only cause the aphids to die but also causes avoidance by the aphids. As a result, thyme oil strongly inhibited the aphids from settling by its sucking inhibition, repellency and fumigant insecticidal activities. The cause of death of the aphids in the no-choice test was attributed to starvation, oral toxicity and/or fumigant toxicity. Spearmint, peppermint and lavender oils had neither contact nor fumigant toxicity. In the case of spearmint oil, the cause of death of the aphids in the no-choice test was attributed to starvation and/or oral toxicity. While spearmint oil inhibited the

Table 3. Investigated parameters of the inhibitory activities of tested oils against *M. persicae*

Essential oils	Items of investigation[a]				
	AFA	REP	SIC	SIN	TOX
(Group 1)					
Thyme oil	O	O	O	O	O
Spearmint oil	O	O	O	O	×
Peppermint oil	O	O	O	×	×
Lavender oil	O	O	O	×	×
(Group 2)					
Pennyroyal oil	O	×	O	O	O
Mint oil	O	×	O	×	×
Marjoram oil	O	×	O	×	×
Basil oil	O	×	O	×	×
(Group 3)					
Rosemary oil	×	O	O	×	×
(Group 4)					
Sage oil	×	×	O	×	×

[a] AFA: antifeeding activity in the EMIF; REP: repellency in the olfactometer test; SIC: settling inhibition in the choice test; SIN: settling inhibition in the no-choice test; TOX: toxicity in the toxicity test.
O: the activity was clearly observed in the test; ×: the activity was not clearly observed in the test.

aphids from settling even under the no-choice condition, the other two oils did not. Spearmint oil may not only have repellency and sucking inhibition but also probing inhibitory or locomotion stimulatory activities. This oil may strongly inhibit the aphid from settling by the concerted effect of these activities.

Pennyroyal, mint, marjoram and basil oils had a sucking inhibitory activity but no repellency against the aphids. These oils inhibited the aphids from settling mainly from the effects on their gustatory senses. Pennyroyal oil also had fumigant and/or contact toxic effects in the toxicity test. In the no-choice test, the settling percentages of the aphids for this oil had a peak about 4 h after the aphids' release and decreased gradually with time. Contrary to this, the mortalities of the aphids increased with time and about 50% of the aphids died at 24 h after their release. The cause of the death of the aphids in pennyroyal oil was attributed to starvation, oral toxicity and/or fumigant toxicity as in the case of thyme oil. Although mint oil strongly inhibited the aphids from sucking, it only slightly inhibited the aphids from settling under the no-choice condition. When the aphids have no opportunity to choose diets, they may feed on even the unpreferred diets.

Although rosemary oil strongly repelled the aphids in the olfactometer test, it did not inhibit the aphids from sucking in EMIF. It seems that the effect on the aphids' olfactory sense is the main factor of the settling inhibition in the case of rosemary oil.

Although sage oil inhibited aphids from settling in the choice test, it neither inhibited the aphids from sucking notably in EMIF nor repelled them in the olfactometer test. The avoidance by the aphids of this oil in the choice test may be caused by the concerted effect of these activities or other inhibitory activities, for example, probing inhibitory and the locomotion stimulatory activities, which could not be detected in these assays.

It is known that spearmint and thyme oils have *l*-(−)-carvone and thymol, respectively (Moroe (ed.), 1989). Comparison of GC retention times to those of the standards and GC-MS data showed that *l*-(−)-carvone occupied 75.6% of spearmint oil and thymol occupied 25.2% of thyme oil (Hori, unpublished). Both *l*-(−)-carvone and thymol inhibited aphid settling and penetration to the same degree as spearmint and thyme oils in the EMIF, no-choice- and choice tests (Hori, unpublished). *l*-(−)-Carvone and thymol may be the main factors causing antifeeding and settling inhibitory activities in spearmint and thyme oils, respectively.

The pattern and levels of inhibitory activities of the essential oils against the aphids differed from one oil to the other, though they belong to the same plant family. Although the mechanisms of the settling inhibition differed among the tested oils, all the oils inhibited the aphids from settling under the choice condition. Labiate essential oils may play a role in deterring aphids from settling on these plants by affecting the aphids' gustatory and/or olfactory senses.

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*translation by author.