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Toxicity and Repellent Activity of Plant Essential Oils and Their Blending Effects Against Two Spotted Spider Mites, *Tetranychus urticae* Koch

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식물정유 및 혼합물의 점박이응애(*Tetranychus urticae* Koch)에 대한 살비 및 기피활성

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ABSTRACT: Miticidal and repellent activity of twenty plant essential oils against the adults of two spotted spider mites, *Tetranychus urticae*, were examined. Sandal wood oil was the most potent one in mortality, whereas clary sage oil exhibited the greatest repellent activity. On those twenty essential oils tested, no apparent correlation between toxicity and repellency was observed. When the most active oils were blended, the combinations tend to exhibit antagonistic interactions in both toxicity and repellent activity. The chemical compositions of sandal wood oil and clary sage oil were identified via GC/MS analyses, and the major constituents of sandal wood oils were sesquiterpene compounds, whereas the major ones for clary sage oil were monoterpenes. Among the major components in clary sage oil, linalyl acetate was not only the most abundant constituent, but also the most responsible one for its repellent activity.

Key words: Two spotted spider mites, *Tetranychus urticae*, Plant essential oil, Toxicity, Repellency

초 록: 본 연구에서는, 20종의 식물정유를 이용하여 점박이응애 성충에 대한 살비활성 및 기피활성을 확인하였다. 살비활성 평가에서는 샌달우드 오일이, 기피활성 평가에서는 클라리 세이지 오일이 가장 높은 효과를 나타내었고, 평가에 사용한 20종의 식물정유간에는 살비활성과 기피활성간의 상관관계가 매우 낮게 나타났다. 높은 활성을 나타낸 정유들의 혼합시험에서는, 거의 대부분의 조합이 서로간에 저해효과를 갖는 것으로 확인되었다. 샌달우드 및 클라리 세이지 오일의 구성성분은 GC/MS 분석을 통해 확인하였으며, 샌달우드는 세스퀴터펜류가, 클라리 세이지 오일은 모노터펜류가 주종을 이루었다. 클라리세이지 오일의 구성성분 중에서는 linalyl acetate가 가장 높은 함량을 갖고 있을 뿐만 아니라, 해당정유가 기피효과를 갖는 주된 효능물질임을 확인하였다.

검색어: 점박이응애, *Tetranychus urticae*, 식물정유, 기피효과, 살비효과

Two spotted spider mites, *Tetranychus urticae* Koch, is one of the most severe agricultural pests worldwide. Owing to their extraordinary ability to adapt to harsh conditions such as short life cycle and unique sex determination system, many efforts to control them by conventional pesticides often fail, which requires new methods for the pest control (Agut et al., 2018).

Due to their less environmental residual and relatively low toxicity to mammals (Chae et al., 2014; Isman, 2006), plant essential oils have been considered to be prominent alternatives to conventional pesticides. Especially, plant essential oils have broad spectrum of their bioactivity including insecticidal, acaricidal, nematicidal, antifungal and antimicrobial activity

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(Burt, 2004). Although we still know little about their specific modes-of-action, many essential oils have been used to protect stored foods and crop plants (Koul et al., 2008). Thus, there has been notable increase of research on botanical pesticides as 'green pesticide'.

Plant essential oils generally extracted via a steam distillation method, and there are more than 3,000 essential oils are known to date (Burt, 2004). Essential oils are stored in various plant organs, such as leaves, flowers, wood, roots, rhizomes and seed, and oil-bearing plant species are belong to a few families, including the Asteraceae, Lamiaceae, Lauraceae, and Myrtaceae (Regnault-Roger et al., 2012). In the regard to the chemical structure, they tend to be composed with relatively low-molecular weight compounds, more particularly monoterpenes and sesquiterpenes with various functional groups including ethers, pheneols, aldehydes, carboxylic acids, ketones, or hydrocarbons.

In the present study, we examined the miticidal activity of twenty plant essential oils which are generally known to pose insecticidal or miticidal activity (Choi et al., 2003; Choi et al., 2004; Pavela, 2005) as well as their repellent activity against the adult mites of *T. urticae* to see whether there is any correlation between the two activity. Moreover, blending effect of selected oils was evaluated to search for synergistic combinations. At last, the contribution of each major compound in clary sage oil was tested to determine the main active compound in the repellent activity of the oil.

Materials and Methods

Essential oils and individual components

Twenty plant essential oils (organic grades) were purchased from Klimtech (Dimitrovgrad, Bulgaria), and their scientific names and origins are listed in Table 1. The individual monoterpene compounds in the present study were obtained from Sigma-Aldrich (Darmstadt, Germany; linalool, a-terpineol, geraniol, a-terpinyl acetate, geranyl acetate) or Tokyo Chemical

Table 1. List of essential oils tested in the present study

Name	Species name	Extracted from	Harvested at
Bergamot	*Citrus bergamia*	Peel*	Italy
Clary sage	*Salvia sclarea*	Flower	Bulgaria
Cypress	*Cupressus sempervirens*	Leaf	Greece
Eucalyptus	*Eucalyptus Globulus*	Leaf	Sri Lanka
Fennel sweet	*Foeniculum vulgare*	Seed	Bulgaria
Frankincense	*Boswellia carteri*	Bark	Bulgaria
Geranium	*Pelargonium graveolens*	Flower	Bulgaria
Lavender	*Lavandula angustifolia*	Flower	Bulgaria
Lemon	*Citrus limonum*	Peel	Greece
Lemongrass	*Cymbopogon citratus*	Leaf	Sri Lanka
Mandarin	*Citrus retidulata*	Peel*	Greece
Marjoram	*Origanum majorana*	Leaf	Bulgaria
Narrow-leaved peppermint	*Eucalyptus radiata*	Leaf	Madagascar
Orange sweet	*Citrus sinensis*	Peel*	Greece
Patchouli	*Pogostemon patchouli*	Leaf	Indonesia
Peppermint	*Mentha piperita*	Leaf	Bulgaria
Rosemary	*Rosmarinus officinalis*	Leaf	Bulgaria
Sandal wood	*Santalum album*	Wood	India
Teatree	*Melaleuca altemifolia*	Leaf	Australia
Ylang ylang	*Cananga odorata*	Flower	Madagascar

*Prepared via a cold-press extraction. All the other essential oils were extracted via a steam distillation method.

Industry CO. (Tokyo, Japan; linalyl acetate). All the chemicals tested were in technical grades (>90%).

Mites and plants

The colony of test mites (obtained from National Academy of Agricultural Science, South Korea) were maintained at 24 ± 1℃, 50 ± 10% relative humidity with 16:8 h L:D photoperiod in the insectary at Seoul National University without any exposure to known insecticides since 2004. Kidney bean plants were grown in a separate space under the same condition above. To maintain the mites colony healthy, pots of new beans (one-week-old) were provided to the mites for their habitat and nutrient sources twice a week.

Bioassays

Toxicity and repellent activity of individual essential oils

A disc (23 mm diameter) was cut from a bean leaf by using a cork borer. The disc was placed on an agar medium (2%) in a plastic petri dish (60 mm diameter)(Pavela, 2015a). Test solutions were prepared by dissolving essential oils in 95% ethanol at 10 mg/ml. Twenty µL of the test solution was carefully applied on the disc by using a micropipette as not touching the leaf surface with the tip to avoid any direct physical damage, and each droplet of the test solution was applied on different spots on the leaf to cover as large area as possible (Miresmailli et al., 2006). The leaf disc was sit for 3 minutes as allowing the solvent fully evaporated, and then 20 adult mites were introduced on the disc within 3 mins, then a lid was tightly covered and sealed with parafilm.

All the test petri dishes were incubated for 24 h under the same condition above (for the colony maintenance), and mortality was recorded. A mite which was insensitive when prodded and lifted by using a small painting brush was considered dead. EtOH alone was used as a negative control for toxicity test, which showed no mortality after 24 hrs.

Repellent activity to the essential oils was evaluated by a bridge assay with a minor modification (Tak and Isman, 2017a). A 2% agar medium was hollowed out by a cork borer to make an island by filling water to prevent mites from escaping, and leaf disc was placed on top of the island to make a 'leaf island'. Two leaf islands were interconnected by LDPE (Low Density Polyethylene) bridge. Each island was painted with 95% EtOH and test solutions with essential oils. Before evaporation, 10 starved adult mites (for 12 hrs) were introduced on the center of the bridge. The bridge was removed when all mites made their choices for which leaf to settle, then the number of the mites on each disc was recorded. Repellent activity was calculated by the equation below;

$$\text{Repellency}\ (\%) = \frac{N_c - N_t}{N_c + N_t} \times 100$$

where N_c in the equation represents the number of mites on the EtOH-treated leaf island, while N_t represents the number of mites on the oil-treated leaf island (Tak and Isman, 2017a). All the bioassay was conducted three times, using the mites from different pots to avoid pseudoreplication.

Toxicity and repellent activity of essential oil mixtures

To examine the mixture effect of the plant essential oils, binary mixtures of sandal wood oil (which was the most toxic) and five other oils (which showed the greatest repellent activity) were prepared at two different doses [(5 mg + 5 mg)/ml and (10 mg + 10 mg)/ml]. The same bioassay methods were employed to each of the toxicity and repellent activity test.

To determine the blending effects of the mixture, an equation below was used (Pavela, 2014);

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

where O_a and O_b in the equation are the observed repellent activity and toxicity of individual essential oils, while E represents expected repellency or toxicity of a binary mixture. By a χ^2 test, the interaction between the two oils were determined either additive ($\chi^2 < 3.84$) or interacting ($\chi^2 > 3.84$).

$$\chi^2 = \frac{(O_m - E)^2}{E}$$

where O_m in the equation are the observed repellency or toxicity of the mixture; χ^2 with degree of freedom = 1 and α = 0.05 is 3.84. The interaction of two oils were determined either synergetic (when $O_m > E$) or antagonistic ($O_m < E$).

Chemical composition of most active oils

The chemical composition of sandal wood (the most toxic) and clary sage (the most repellent) oils were analyzed on ISQ gas chromatograph mass spectrometer (Thermoscientific, USA) operating in EI mode fitted with a VF5ms column (60 m, × 0.25 mm ID, 0.25 µm thickness). The injection volume was 1.0 µL, and the initial temperature for the oven was set at 50℃ for 5 min, then increased to 65℃, 120℃, 180℃, 210℃ and 325℃ with each rate of 10℃/min, 50℃/min, 5℃/min, 50℃/min and 20℃/min, respectively. Each stage was held for 30 min, 10 min, 10 min, 0 min, 10 min and 10 min. The total runtime was 101.25 min, and Helium (99.999%) was used as a carrier gas with the constant flow of 1.0 mL/min. The data were analyzed using NIST Mass Spectral Search software (version 2.0), and the major constituents were determined by matching the spectra against the NIST/EPA/NIH Mass Spectral Library.

Compound elimination assay

To determine the major active compound for the repellent activity of clary sage oil, a compound elimination assay was used (Kim et al., 2016). All the six major compounds which comprised more than 2% in the composition of the oil were blended to make an artificial essential oil (whole mixture), and one compound each was excluded from the whole mixture to prepare a series of artificial mixtures as following the composition of them. Repellent activity of the seven artificial oils was examined to determine which compound is the most responsible for the repellent activity of clary sage oil by using the bridge assay.

Data analysis

ANOVA test was conducted to compare the miticidal and repellent activity of the test oils as well as the comparison among the major constituents of clary sage oil, and Student T-test was performed for the correlation analysis between the two activity by using a SPSS software (version 2.5) for the statistical analyses.

Results and Discussion

Toxicity and repellent activity of single essential oil

The miticidal and repellent activity of twenty essential oils were evaluated against the adult *T. urticae* (Table 2). Among the oils tested, only sandal wood oil showed the notable performance for miticidal effect (82 ± 2%). Regarding repellent activity, although clary sage oil showed the greatest repellent activity (93 ± 7%), it did not differ to many other oils, partially due to the wide variation of repellent activity (from 93 ± 7% to -40 ± 60%). Some essential oils including mandarin, peppermint, eucalyptus, and teatree oils rather attracted the mites (-20 ± 20, -33 ± 27, -27 ± 7, -40 ± 60, respectively).

Several different bioassay methods to screen the toxicity of plant essential oils against the two spotted spider mites have been proposed, which makes sometimes a little challenging to compare the efficacy of them directly. For example, when a slide dipping method was employed, rosemary and clary sage oils reached to total death of the test population within an hour (Laborda et al., 2013). In the meantime, several oils including lemongrass, peppermint, geranium and bergamot oils showed adequate miticial activity in *T. urticae* adults via a filter paper diffusion assay (Choi et al., 2004), whereas they failed to show any notable toxicity in the present study. Even if the same test method is used for the bioassay, the type of the test surface, or which/how much solvent they use can affect the determination. For instance, when the miticidal activity was tested on either a bean leaf or a cabbage leaf disc, 14 out of 20 test compounds (70%) showed significant differences in their toxicity. Likewise, 9 out of 20 compounds (45%) showed statistical difference when the application volume was different (Tak and Isman, 2017a). Since there is no strict rule in which method the researchers ought to use for the screening for the candidates in the pest control, we may continue to observe these types of discrepancy in the future as well. To avoid this, or at least to reduce the deviance, a field trial with proposed formulated products can be performed along the lab-based tests. Meanwhile,

Table 2. Miticidal and repellent activity of 20 essential oils against adults of the two spotted spider mites, *Tetranichus urticae*

Test oil	Mortality (% ± SE)	Repellency (% ± SE)
Bergamot	15.0 ± 3.3CDEF	53.3 ± 6.7abcd
Clary sage	11.7 ± 4.4DEFG	93.3 ± 6.7a
Cypress	11.7 ± 1.7DEFG	80.0 ± 0.0ab
Eucalyptus	10.0 ± 2.9EFG	-26.7 ± 6.7cd
Fennel sweet	25.3 ± 5.3BCD	46.7 ± 13.3abcd
Frankincense	6.7 ± 1.7EFG	46.7 ± 6.7abcd
Geranium	35.0 ± 0.0B	60.0 ± 20.0abcd
Lavender	8.3 ± 1.7EFG	80.0 ± 11.5ab
Lemon	0.0 ± 0G	26.7 ± 17.6abcd
Lemongrass	26.7 ± 4.4BC	73.3 ± 6.7abc
Mandarin	8.3 ± 3.3EFG	-20.0 ± 20.0bcd
Marjoram	8.3 ± 3.2EFG	73.3 ± 17.6abc
Narrow-leaved peppermint	13.3 ± 4.4CDEFG	33.3 ± 13.3abcd
Orange sweet	6.7 ± 1.7EFG	53.3 ± 13.3abcd
Patchouli	18.3 ± 3.3CDE	60.0 ± 11.5abcd
Peppermint	6.7 ± 1.7EFG	-33.3 ± 26.7d
Rosemary	3.3 ± 1.7FG	0.0 ± 0.0abcd
Sandal wood	81.7 ± 1.7A	40.0 ± 11.5abcd
Teatree	5.0 ± 0.0EFG	-40.0 ± 60.0d
Ylang ylang	35.0 ± 0.0B	60.0 ± 11.5abcd

*In each column, different letters are statistically different at P = 0.05 (Tukey's test), d.f = 19, F = 42.334, P < 0.001 for mortality, and d.f = 19, F = 4.718, P < 0.001 for repellency, respectively.

a further study focused on the correlation between the lab and field results can illuminate more suitable bioassay methods for the botanical insecticides screening.

When comparing each oil's toxicity and repellent activity, no correlation between the two bioactivity was observed (R^2 = 0.0537 with P = 0.031, Fig. 1). Even the exclusion of the most outlying oil (sandal wood) only increased the R^2 value to 0.1661, showing lack of the relationship between them.

A similar trend which showed low correlation between the two activity was observed not only in the other essential oils (Roh et al., 2013) but also in the major compounds of essential oils (Tak and Isman, 2017a) as well. However, in spite of low R^2 value in the present study, these observations do not necessarily mean that the toxicity and the repellent activity are entirely indifferent. When an insect or arthropod pest can detect a concentration gradient of a potentially toxic compound in the atmosphere around, it can determine to avoid the toxicant and move away from the source, which can show spatial repellency. In a broad point of view, repellent activity may include biting deterrence in the blood-sucking pests such as mosquitoes and ticks, and behavioral avoidance such as oviposition disincentiveness in the stored insects or agricultural pests.

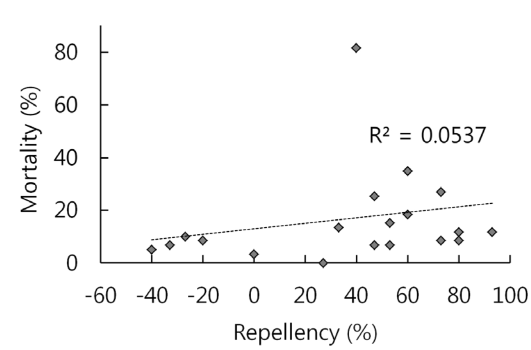

Fig. 1. Correspondence between mortality and repellent activity of tested plant essential oils against two spotted spider mites, *Tetranychus urticae* adults. Twenty μL of each oil was applied on a bean leaf disc at 10 mg/mL. No apparent correlation between the two bioactivity was observed with the R^2 value of 0.0537 with P = 0.031.

Mixture effects of selected oils on toxicity and repellent activity

Combination effects in the binary mixtures of the most active oils in either toxicity or repellent activity were examined (Table 3). When sandal wood oil (the most toxic) was blended with five of the most repellent oils including majoram, cypress, lemongrass, clary sage and lavender oil, no apparent increase in the toxicity was observed. Rather, all of the five combinations were determined to pose antagonistic interactions, as showing significantly less toxicity compared to the expected (calculated) mortality. Among the combinations tested, the mixture of sandal wood and clary sage oil revealed the most notable decrease in the toxicity (χ^2 value of 30.7), and the lower concentration (5 + 5 mg/mL each) failed to show any mortality.

On the repellent activity of the binary mixtures, a similar trend in antagonistic relationship was observed except the sandal wood + lavender oil, which was additive. Interestingly, three out of five combinations (sandal wood + majoram, cypress, or lemongrass oil) showed reversed activity, as showing greater repellent activity at lower concentration (5 + 5 mg/mL each) than higher concentration (10 + 10 mg/mL each).

Although no synergistic interaction was found in the present study, synergy in toxicity and repellent activity of plant essential oils or their major compounds is not rare. For example, Pavela (2014) tested 435 binary mixtures of monoterpene compounds against *Spodoptera littoralis* larvae, and found 135 combinations were synergistic, while 150 combinations had anatagonistic interactions. In his another study, more than a half of the combinations (249 out of 435 mixtures) showed a significant synergistic effect, while 74 combinations were antagonistic against *Culex quinquefasciatus* larvae (Pavela, 2015b). If contact of essential oil and mite is necessary to show toxicity, perhaps mixture of toxic oil and attractant oil can be expected to show synergetic effect.

When the test compounds directly contact the integument of insect pest via a topical application, it seems that the synergistic or antagonistic effects are governed by the increase or decrease of surface tension of a combination, later affects the penetration of the active compound into the insects' cuticle (Tak and Isman, 2017b). However, when the test compounds or the combinations of them are applied on a certain surface first and then the test arthropods are release such as in the present study, much more factors must be considered to understand the underlying mechanism of synergistic/antagonistic effects. For instance, physico-chemical affinity of the test compounds (test oils in this case) can influence how much chemicals are absorbed or strongly attached to the wax layer of the leaves, as limiting the compounds released into the air. Moreover, an addition of other compounds can accelerate the speed of evaporation of the active compounds, as hampering the residual effects of the active ones. A comprehensive

Table 3. Miticidal and repellent activity of binary mixtures of selected essential oils against the adults of two spotted spider mites, *Tetranychus urticae*

Mixture		Concentration (mg/mL)	Miticidal activity				Repellent activity			
			Observed (% ± SE)	Exp.*	χ^2	Interaction	Observed (% ± SE)	Exp.*	χ^2	Interaction
Sandal wood +	Marjoram	10 + 10	51.7 ± 8.3	83.4	12.1	Antagonistic	46.7 ± 12.0	83.8	16.5	Antagonistic
	Marjoram	5 + 5	18.3 ± 1.7				53.0 ± 7.0			
	Cypress	10 + 10	51.7 ± 4.4	84.2	12.5	Antagonistic	13.3 ± 6.7	88.0	63.3	Antagonistic
	Cypress	5 + 5	51.7 ± 7.3				47.0 ± 7.0			
	Lemongrass	10 + 10	38.3 ± 1.7	86.9	27.1	Antagonistic	60.0 ± 12.0	83.8	6.8	Antagonistic
	Lemongrass	5 + 5	41.7 ± 4.4				80.0 ± 12.0			
	Clary sage	10 + 10	33.3 ± 3.3	84.2	30.7	Antagonistic	53.0 ± 13.0	95.8	19.2	Antagonistic
	Clary sage	5 + 5	0.0 ± 0.0				53.3 ± 24.0			
	Lavender	10 + 10	31.7 ± 3.3	83.4	32.1	Antagonistic	86.7 ± 6.7	88.0	0.02	Additive
	Lavender	5 + 5	30.0 ± 2.9				47.0 ± 13.0			

*Expected miticidal or repellent activity of the binary mixture assuming an additive interaction.

understanding as considering for all the possible factors is required to fully elucidate the synergistic/antagonistic phenomena when the test chemicals or plant essential oils are applied on certain test surfaces.

Analyzing main components of selected oils by GC/MS

The major constituents of sandal wood and clary sage oils were identified via GC/MS analyses (Table 4). In sandal wood oil, a-santalol was the most abundant compound (20.30%), followed by other sesquiterpene compounds. Unlike other typical plant essential oils which are usually obtained from flowers or leaves, sandal wood oil was extracted from the wood part. Partially due to the difference in the origin of the sources, relatively heavier sesquiterpenes (C15) were the main components of the oil, whereas monoterpene compounds (C10) are generally the major ones in other essential oils. In clary sage oil, linalyl acetate (44.29%) was the most abundant constituent, followed by linalool, geranyl acetate, a-terpinyl acetate, and a-terpineol (31.63, 4.70, 4.24, and 4.19%, respectively).

Sandal wood oil as well as its major constituent, a-santalol, has risen some interests in research not only due to its insecticidal property (Roh et al., 2015), but also its medicinal utility as well including antitumor, cancer preventive property, anti-inflammatory, or antihyperglycemic properties (Bommareddy et al., 2017). In a previous study, sandal wood oil not only exhibited a strong miticidal activity but also significantly greater repellent activity against the adults of *T. urticae* in no-choice test (Roh et al., 2012). The difference in the bioassay method might influence the different result, but the difference in the composition of the test oils, particularly the composition of the major active compound, a-santalol, may also contribute significantly in terms of the bioactivity. In their study, it revealed that a-santalol has a strong repellent activity, and the composition in the oil was 45.8% (Roh et al., 2011), whereas in the present study, the composition of a-santalol was 20.3%. The bioactivity including pesticidal activity of plant essential oils can vary significantly based on their chemical composition (Isman et al., 2008), due to their complex interactive nature.

Table 4. Major compounds (>2%) of sandal wood and clary sage essential oils identified via a GC-MS analysis

	Retention time	Constituent	%Area
Sandal wood	38.41	Benzyl alcohol	7.28
	74.26	a-santalol	20.30
	74.56	bergamotol	2.13
	75.31	trans-a-santalol	5.90
	77.18	corymbolone	4.25
	77.53	6-Isopropenyl-4,8a-dimethyl-4a,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one	17.17
	77.66	d-Norandrostane	3.64
	78.39	1-Cyclohexene-1-butanal, à,2,6,6-tetramethyl-	19.61
	78.83	1,4-Methanoazulene-9-methanol, decahydro-4,8,8-trimethyl-, [1S-(1à,3aá,4à,8aá,9R*)]-	3.40
	Total (%)		83.68
Clary sage	44.82	linalool	31.63
	50.98	a-terpineol	4.19
	54.63	linalyl acetate	44.29
	54.93	geraniol	2.30
	62.02	a-terpinyl acetate	4.24
	62.56	neryl acetate	3.38
	63.6	geranyl acetate	4.70
	Total (%)		94.73

Major active component for repellent activity in clary sage oil

A compound elimination assay was conducted to determine the major active component for repellent activity in clary sage oil. As shown in Fig. 2, an exclusion of linalyl acetate (44.29% in its concentration of the oil) showed substantial decrease of repellency in the artificial essential oil (P = 0.002), indicating the main role of the compound in the oil for its repellent activity against *T. urticae*.

However, the full mixture of the six major constituents in clary sage oil which comprise 91.3% of the total composition only gave 47% of repellent activity, whereas the authentic oil showed 93% of repellent activity. This difference may suggest the notable contribution of the remaining compounds to the overall activity, which may synergize the activity of the major constituents. Since plant essential oils are very complex mixture, it is not rare to observe complex interactions among the chemical components in the oils. In a previous study, Miresmailli et al. (2006) found that the mixture of active compounds in rosemary oil against *T. urticae* only gave less than 30% of mortality when the compounds were applied at the equivalent amount present in the oil, whereas the combination of inactive constituents enhanced the toxicity of the active portion of rosemary oil up to >90%, even though the 'inactive' constituents failed to exhibit any mortality when they were individually applied to the mites. On the other hand, it is also common to find that a single compound is largely responsible to the overall insecticidal activity of a whole oil (Tak et al., 2017). The present study suggest although linalyl acetate seems to be the most responsible compound for the repellent activity of clary sage oil against the adults of *T. urticae*, the contribution of the minor constituents also cannot be neglected. A further study on more comprehensive understanding in the role of those minor constituents in clary sage oil is required to fully understand the contribution of each compound to the overall repellent activity.

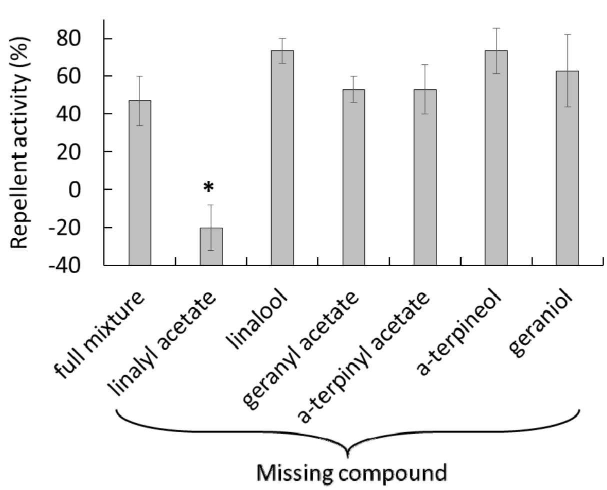

Fig. 2. Repellent activity of artificial mixtures of the major compounds in clary sage essential oil via a compound elimination assay. When linalyl acetate (the most abundant compound, 44.3%) was excluded among the major compounds, the repellent activity of the artificial mixture oil decreased significantly (P < 0.05), indicating the major contribution of the compound to the overall activity of clary sage oil.

Conclusion

In the present study, we examined the miticidal and repellent activity of twenty plant essential oils and their mixture effect were appraised. Sandal wood oil and clary sage oil showed most notable effect in toxicity and repellency, but there was no correlation between two parameters. Binary mixtures of Sandal wood oil and five most repelling oils showed antagonistic interactions, which differs from previous studies. Lastly, the main active constituent for clary sage oil's repellent activity was identified as linalyl acetate, with synergetic interactions with minor constituents. The studies about veiled mechanisms in phenomena of synergetic and antagonistic interactions between essential oil constituents will further be needed.

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