Biological activities of essential oils from *Anethum graveolens* L. and *Allium sativum* L. for controlling *Tetranychus truncatus* Ehara and *Tetranychus urticae* Koch

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ABSTRACT

The essential oils of *Anethum graveolens* L. and *Allium sativum* L. were investigated for the biological activities against *Tetranychus urticae* Koch and *Tetranychus truncatus* Ehara. The results indicated that *A. sativum* oil at 20% (v/v) had high contact toxicity (73.50% mortality) on adult females of *T. truncatus*. The LC_{50} value was 12.71% for 24 h after treatment. Eggs of both spider mites were also susceptible to 5-20% and 20% concentrations of *A. sativum* and *A. graveolens* oil, respectively. *Allium sativum* oil caused 97-100 and 84% mortality against *T. truncatus* and *T. urticae* eggs with LC_{50} values of 1.62 and 11.58% at 7 d after exposure, respectively. In repellency test, *A. graveolens* oil at 5-20% concentrations showed a high percentage of repellency on *T. truncatus* (62-96%) and *T. urticae* (98-100%) during 1-5 h after treatment. On the other hand, 5-20% and 15-20% concentrations of *A. sativum* oil appeared high percentage of repellency on *T. truncatus* (98-100%) and *T. urticae* (86-100%) during 1-5 h after treatment, respectively. In a fumigant toxicity test, *A. sativum* and *A. graveolens* oils were toxic to both spider mites. *Allium sativum* oil (LC_{50} = 4.94 µL/L in air) and *A. graveolens* oil (LC_{50} = 13.67 µL/L in air) were toxic to adult females of *T. urticae* whereas *T. truncatus* adult females were responsible to *A. graveolens* oil (LC_{50} = 5.24 µL/L in air) and *A. sativum* oil (LC_{50} = 5 µL/L in air). The results suggested that essential oils of *A. sativum* had potential for ovicidal activity on *T. truncatus* and *T. urticae* and acaricidal activity on *T. truncatus*. Both oils showed repellent activity against both spider mites. Oils of *A. sativum* and *A. graveolens* were toxic in fumigant effect on both spider mites. Their biological activities could be useful in promoting of integrated mite management.

Keywords: Plant extract, Contact toxicity, Egg-hatching, Repellency, Fumigant, Mite control.

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INTRODUCTION

Many spider mites, especially in the genus *Tetranychus* are economic importance in many regions of the world such as *Tetranychus urticae* Koch, two-spotted spider mite. This species infests ca. 1200 plants species, of which more than 150 are economically significant (Zhang, 2003) and it is a serious pest of strawberry, shrub and ornamental trees in the northern part of Thailand (Charanasri *et al*., 1988). *Tetranychus truncatus* was first described from mulberry in Japan (Ehara, 1956). To date, it is known from many regions in Southeast and East Asia such as China, Guam Island, Hainan Island, Indonesia, Japan, Korea, Mariana Island, Philippines, Taiwan, Thailand and Vietnam (Migeon and Dorkeld, 2013; Ullah *et al*., 2014). *Tetranychus truncatus* is also recorded as a serious pest of many plants in Thailand includes peach, papaya, cassava, yard-long bean, peanut and castor bean (Ehara and Wongsiri, 1975). Large populations of these mites can cause webbing, as well as spotty yellowing and curling in leaves and thus reduce the quality of yields (Kumral *et al*.,

2010). The host plants can be affected in different ways, including a decrease in photosynthesis or an injection of phytotoxic substances when feeding (Johnson and Lyon, 1991).

Control of these spider mites with conventional acaricides is particularly difficult because of their ability to quickly develop resistance and the resistance to more than 80 acaricides has been reported in *T. urticae* (Martinson *et al*., 1991; Kim *et al*., 1999; Badawy *et al*., 2010). Selection for resistance is increased by its high fecundity, inbreeding, arrhenotokous reproduction and very short life cycle resulting in many generations per year, especially in warmer conditions (Van Leeuwen *et al*., 2009). Farmers responded to the resistance problem by increasing the dosage and frequency of acaricide sprays, but this irrational use of acaricide only speeded up the development of resistance in spider mites (Guo *et al*., 1998). In order to solve the problem in widespread use of synthetic pesticides and threat of these chemicals to non-target organisms and human health, plants extracts may be valuable materials for natural pesticides because their mammalian toxicity is low and environment persistence is short as compared to many chemical pesticides (Ahmed *et al*., 1984; Prakash and Rao, 1997; Nauen *et al*., 2001; Kim *et al*., 2004; Regnault-Roger *et al*., 2012).

Plant essential oils are produced commercially from several botanical sources, they can be synthesized by all plant organs such as buds, flowers, leaves, stems, twigs, seeds, fruits, roots, woods or barks. For some species, essential oils are stored in secretary cells, cavities, canals, epidermal cells or glandular trichomes (Isman, 2001) and commonly referred to as botanical insecticides, show insecticidal and acaricidal properties at different levels that regulate pest populations (Venzon *et al*., 2005). They contain secondary metabolites that have the potential to provide protection against phytophagous organisms and pathogens (Alves, 1998).

The acaricidal activity of plant essential oils is often enhanced by the presence of several chemical compounds, and they exhibit more than one mode of actions including repelling the pest or prohibiting feeding activity and pest physiology, molting and respiratory inhibition, growth and fecundity reduction and also cuticle disruption (Isman, 2000; Gokce *et al*., 2011). Moreover, exposure to varying mixtures of biosynthetically different compounds found in plant extracts can delay the development of resistance (Isman, 2000).

Anethum graveolens L., (an annual herb (Umbelliferae)), is probably native to South West Asia or South East Europe (Bailer *et al.,* 2001). This plant is one of the most plants that can be used in several functions such as spices and medicines (Said Al Ahl *et al*., 2015). Its medicine uses as an antispasmodic, carminative, diuretic, stimulant and stomachic (Simon *et al*., 1984) as well as an inhibit of spouting in stored potatoes (Sorce *et al*., 1997). The essential oil and extracts of *A. graveolens* seeds have shown varying degrees of antimicrobial activities (Kaur and Arora, 2009). Their insecticidal activities against beetles, cockroaches and mosquitoes have been reported (Arnason *et al.,* 1989; Babri *et al.,* 2012; Conti *et al*., 2010). *Allium sativum* L. is an ancient medicinal plant originated from Central Asia, belonging to family Alliaceae, and beside the use of food, it has been used as medicinal plant for a variety of ailments including headache, bites, intestinal worms and tumors (Block, 1985). In order to protect itself from insects and fungi, garlic produces allicin by enzymatic reaction when it is injured. Thus, allicin is mother-nature's insecticide (Rahman, 2007). Garlic is also an alternative to synthetic chemical pesticides, it has been used for antibacterial, antifungal purposes (Dancewicz and Gabrys, 2008).

This study aimed to investigate the biological activities of essential oils from *Anethum graveolens* Linn. (Umbelliferae) and *Allium sativum* Linn. (Alliaceae) for controlling the two-spotted spider mite (*Tetranychus urticae* Koch) and the cassava red mite (*Tetranychus truncatus* Ehara) under laboratory conditions.

MATERIALS AND METHODS 3

Culture of Spider Mites

The stock colonies of *T. urticae* and *T. truncatus* used in this study were obtained from Acarology laboratory, Department of Entomology, Kasetsart University, Bangkok, Thailand. The adult females of mites were transferred with a fine paint brush to lower surface of mulberry leaf (*Morus alba* L.) placed on moistened cotton pads resting on sponges in the plastic box (17.5 cm width x 25 cm length x 4 cm height) and removed after 24 hrs. Recently emerged adult females (1 day old) were used in all experiments. The colonies were maintained at room temperature under laboratory conditions (Auamcharoen and Chandrapatya, 2015a).

Preparation of Essential Oils

Dry seeds of *Anethum graveolens* L. and fresh bulbs of *Allium sativum* L. were used for extraction of the essential oils. Each plant material was separately used for extraction. One kilogram of each plant was placed in a round bottom flask and 3 L of distilled water was added. The essential oils were obtained by water distillation for 8 hrs using Clevenger-type apparatus (Torres *et al*., 2014). The oils were separated, dried over anhydrous sodium sulphate and kept at 10-12 ^{o}C until used.

Contact Toxicity Bioassay

A direct spray bioassay was used to evaluate the toxicity of *A. graveolens* and *A. sativum* oils against *T. urticae* and *T. truncatus* adult females. Leaf discs (2 cm in diameter) were punched from mulberry leaves using a cork borer and then placed on moistened cotton pads in glass Petri-dishes (9 cm in diameter) (2 leaf discs/replication, 5 replications/treatment). Twenty adult females of the same age were transferred with a fine paint brush to each leaf disc and sprayed with 500 µL of different concentrations (5, 10, 15 or 20% (v/v)) of tested essential oils by using a plastic atomizer spray. In each test, a control was sprayed with Tween® 20 plus water (1%). Mortality of adult mites was observed at 24, 48 and 72 hrs after treatment. The adult mites were considered dead if no movement was apparent after probing with a fine paint brush (Auamcharoen and Chandrapatya, 2015b).

Egg Hatching

Leaf discs (2 cm in diameter) were punched from mulberry leaves and then placed on moistened cotton pads in glass Petri-dishes (9 cm in diameter) (2 leaf discs/replication, 5 replications/treatment). Twenty adult females of the same age were transferred with a fine paint brush to each leaf disc for laying eggs and then removed after 24 hrs. Eggs were sprayed with 500 µL of different concentrations (5, 10, 15 or 20% (v/v)) of tested essential oils by using a plastic atomizer spray. In each test, a control was sprayed with Tween® 20 plus water (1%). Number of larvae that emerged from eggs and unhatched eggs was monitored at 7 days after treatment.

Repellent Activity

We developed a novel laboratory method for the evaluation of the repellent potentiality of *A. graveolens* and *A. sativum* oils to impede the migration of *T. urticae* and *T. truncatus*. Two leaf discs were punched from mulberry leaves and then placed on moistened cotton pads in glass Petri-dishes (9 cm in diameter). The filter paper disc (Whatman No.1) (2 cm in diameter) was sealed with adhesive tape and placed between mulberry leaf discs. This filter paper was connected to two mulberry leaf discs by filter paper bridges (0.5 cm width, 1 cm length) served as bridges for the mites cross from the middle filter paper disc to the mulberry leaf disc on each side of the Petri dish. Twenty five microliters of different concentrations (5, 10, 15 or 20% (v/v)) of essential oils was applied to filter paper bridge and Tween® 20 plus water (1%) was used as control bridge. Both the treatment and control bridges were allowed to dry for 10 minutes. Twenty adult females were transferred to filter paper disc. The number of mites on the mulberry leaf discs was monitored at 1, 2, 3, 4 and 5 hrs after treatment. Five replications were used per treatment. Percentage repellency was calculated using the formula:

$$\% \text{ repellency} = [(C-T)/(C+T)]x100,$$

where C and T are the number of mites in the control and treatment, respectively (Akhtar *et al*., 2012).

Fumigant Toxicity

Leaf discs (2 cm in diameter) were punched from mulberry leaves and then placed on moistened cotton pads in plastic box (10 cm in diameter and 4 cm height) (2 leaf discs/replication, 5 replications/treatment). The methodology for transferring the spider mites to plastic box was the same as in the contact toxicity bioassay. The filter paper (Whatman No.1) (2 cm in diameter) placed on small tumbler (2 cm in diameter) was dropped with 100% concentration of tested essential oil at the volumes of 1, 2, 3, 4 or 5 µL corresponding to 3.03, 6.06, 9.09, 12.12 or 15.15 µL/L in air and allowed to dry for 10 minutes. The small tumbler containing tested filter paper was placed onto plastic box and the lid was covered to prevent the tested oils from leaking out of the box. Mortality of adult mites was observed every 2 hrs until 24 hrs after treatment.

Statistical Analyses

All data were analysed using analysis of variance (ANOVA) and Tukey's test was employed to compare the treatment means (p = 0.05) using the R program (R Development Core Team, 2016). The lethal concentration (LC_{50}) was calculated under probit analysis using SPSS program.

RESULTS

Contact Toxicity

The contact toxicity activity of essential oils from *A. graveolens* and *A. sativum* against adult females of *T. urticae* and *T. truncatus* was investigated in this study. The results showed that low mortality rates were observed at low concentrations of both tested oils. The acaricidal activity enhanced with increasing concentrations (Figs. 1, 2). The highest concentration of *A. sativum* oil showed the highest mortality to adult females of *T. truncatus*. The accumulated mortality rates were 73.50 and 80.50% at 24 and 48 hrs after treatment, respectively. However, mortality rates were not significantly different from mortality rates of *T. truncatus* treated with 15% concentration of this oil for 48 hrs (p > 0.05). The LC_{50} value of *A. sativum* oil on *T. truncatus* for 24 hrs was 12.71%. On the other hand, *A. graveolens* oil at 20% concentration caused the lower mortality (< 70%) than the *A. sativum* oil on both tested spider mites at 48 hrs after treatment.

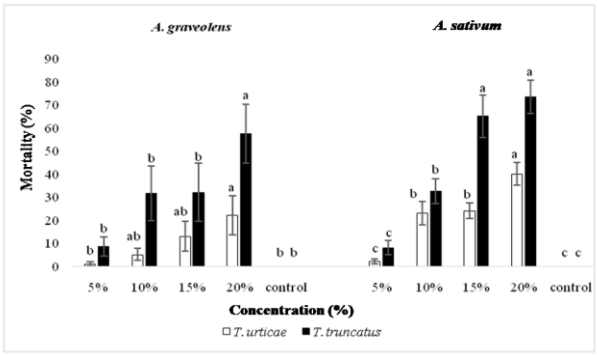

Fig. 1 Mortality of *Tetranychus urticae* Koch and *Tetranychus truncatus* Ehara adult females at 24 hour after treated with different concentrations of *Anethum graveolens* L. and *Allium sativum* L. under contact toxicity bioassay. For each oil and mite, means followed by the same letter are not significantly different (p = 0.05; Tukey's test).

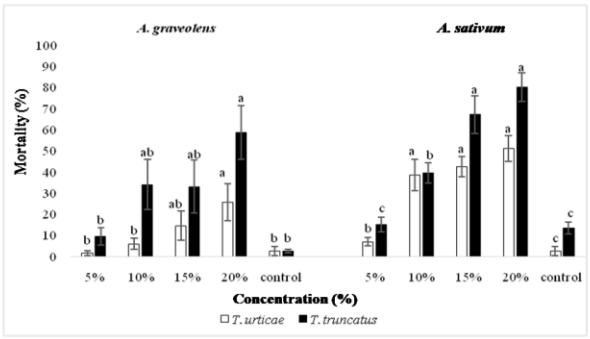

Fig. 2 Accumulated mortality of *Tetranychus urticae* Koch and *Tetranychus truncatus* Ehara adult females at 48 hour after treated with different concentrations of *Anethum graveolens* L. and *Allium sativum* L. under contact toxicity bioassay. For each oil and mite, means followed by the same letter are not significantly different (p = 0.05; Tukey's test).

Egg Hatching

The effect of essential oils on egg hatching of *T. urticae* and *T. truncatus* was shown in Table 1. After 7 days, egg mortality rates were recorded on *T. truncatus* treated with 5-20% concentrations of *A. sativum* oil. The percentages of unhatched eggs were not significantly different from each other (p > 0.05). The LC_{50} of *T. truncatus* eggs at 7 days was 1.62%. The high *T. urticae* egg mortality appeared at 15-20% concentrations of *A. sativum*. The LC_{50} for *T. urticae* eggs exposed to *A. sativum* at 7 days was 11.58%. On the contrary, *A. graveolens* oil caused low egg mortality of both tested spider mites.

Table 1. Percentage of unhatched eggs of *Tetranychus urticae* Koch and *Tetranychus truncatus* Ehara at 7 days after treated with different concentrations of *Anethum graveolens* L. and *Allium sativum* L.

Essential oil	Spider Mite	Concentration (%)	Unhatched egg (%) (Mean±SE)[1/]
A. graveolens	*T. urticae*	5	7.66±1.05b
		10	9.57±2.04ab
		15	17.31±7.10ab
		20	27.73±4.27a
		Control	2.18±0.65b
		$F_{(4,45)}$	6.60
		p	0.00
	T. truncatus	5	0.60±0.22c
		10	3.59±1.31c
		15	16.06±3.38b
		20	36.30±3.93a
		Control	0.30±0.09c
		$F_{(4,45)}$	41.07
		p	0.00
A. sativum	*T. urticae*	5	5.88±0.53c
		10	39.43±6.55b
		15	69.15±5.84a
		20	83.67±4.53a
		Control	2.05±0.49c
		$F_{(4,45)}$	68.35
		p	0.00
	T. truncatus	5	96.83±2.34a
		10	99.94±0.05a
		15	100.00±0.00a
		20	100.00±0.00a
		Control	0.30±0.09b
		$F_{(4,45)}$	1.78
		p	0.00

[1/]Means±SE within the same column followed by the same letters are not significantly different ($p = 0.05$; Tukey's test).

Repellent Activity

The results of repellency test showed that 15-20% concentrations of *A. sativum* and *A. graveolens* oils had high effect on *T. truncatus* and *T. urticae* adult females, respectively (Table 2). The percentages of repellency of 15 and 20% concentrations of oils were not significantly different from each other at every tested hour. On the contrary, the percentages of repellency were low at 5 and 10% of *A. sativum* oil on *T. urticae* and increased when concentrations increased, while percentage of repellency in *A. graveolens* oil at 5, 10 and 15% concentrations against *T. truncatus* increased when time passed. At 20% concentration of *A. graveolens*, it showed the highest percentage repellency which was not significantly different from percentage repellency of lower concentrations (10 and 15%) from 1 h until 5 hrs after treatment ($p > 0.05$).

Fumigant Toxicity

The fumigant toxicity of essential oils from *A. graveolens* and *A. sativum* on *T. urticae* and *T. truncatus* was observed in this study. The *A. sativum* oil showed the strongest activity against both adult females of spider mites (Fig. 3a, b). At 5 µL of 100% concentration of this oil, it caused 100% mortality of *T. truncatus* and *T. urticae* from 2 -24 hrs after treatment. The LC_{50} values at 24 hrs were 1.65 (5) and 1.63 (4.94) µL (µL/L in air) for *T. truncatus* and *T. urticae*, respectively. *Anethum graveolens* oil also had toxicity to *T. truncatus* and *T. urticae* (Fig. 4a, b). At 24 hrs, LC_{50} value (1.73 µL or 5.24 µL/L in air) was obtained for *T. truncatus*, where LC_{50} (4.51 µL or 13.67 µL/L in air) value was obtained against *T. urticae*. Adult females of *T. truncatus* fumigated with 5 µL of *A. graveolens* died 100% from 2-24 h after exposure, while this oil affected 76.50%

mortality on *T. urticae* at 16 hrs after exposure. At 1, 2 and 1, 2, 3, 4 µL of 100% concentrations of *A. graveolens*, they showed low fumigant toxicity on *T. truncatus* and *T. urticae*, respectively.

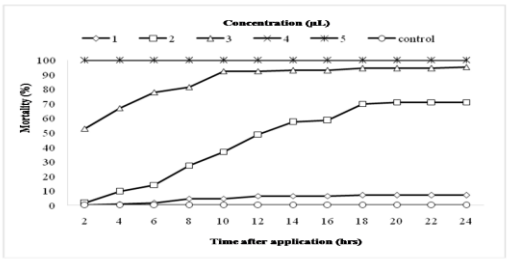

Fig. 3a Fumigant toxicity of *Allium sativum* L. essential oil against *Tetranychus truncatus* Ehara adult females.

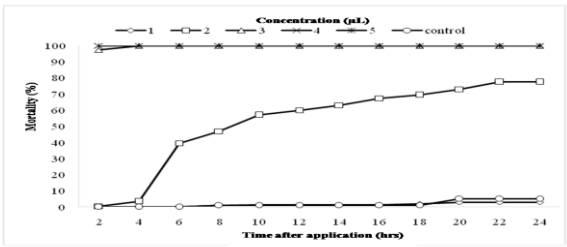

Fig. 3b Fumigant toxicity of *Allium sativum* L. essential oil against *Tetranychus urticae* Koch adult females.

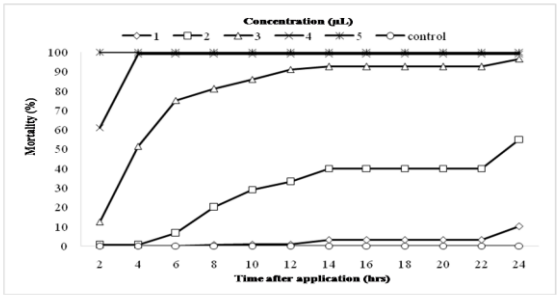

Fig. 4a Fumigant toxicity of *Anethum graveolens* L. essential oil against *Tetranychus truncatus* Ehara adult females.

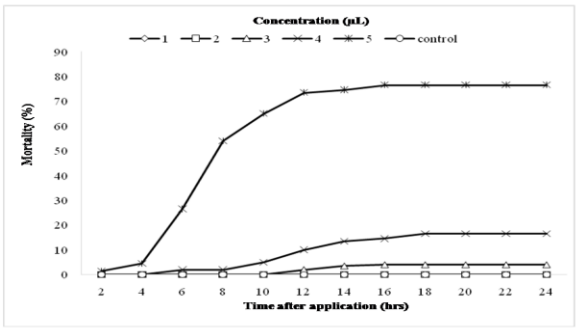

Fig. 4b Fumigant toxicity of *Anethum graveolens* L. essential oil against *Tetranychus urticae* Koch adult females.

Table 2. Percentage of repellency of *Tetranychus urticae* Koch and *Tetranychus truncatus* Ehara at 1, 2, 3, 4 and 5 hours after treated with different concentrations of *Anethum graveolens* and *Allium sativum*.

Essential oil	Spider Mite	Concen-tration (%)	Percentage of repellency (Mean ± SE)[1/]				
			1 h	2 h	3 h	4 h	5 h
A. graveolens	*T. urticae*	5	98.0±2.0a	98.0±2.0a	98.0±2.0a	98.0±2.0a	98.0±2.0a
		10	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a
		15	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a
		20	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.0±0.0a
		$F_{(3,16)}$	1	1	1	1	1
		p	0.418	0.418	0.418	0.418	0.418
	T. truncatus	5	62.0±14.6b	64.0±23.6a	66.0±14.7a	82.0±5.8a	90.0±3.2a
		10	90.0±3.2ab	92.0±3.7a	96.0±2.5a	96.0±2.5a	96.0±2.5a
		15	88.0±2.0ab	92.0±3.7a	94.0±4.0a	96.0±2.5a	96.0±2.5a
		20	96.0±4.0a	96.0±4.0a	96.0±4.0a	96.0±4.0a	96.0±4.0a
		$F_{(3,16)}$	3.716	1.458	3.402	3.161	0.947
		p	0.33	0.26	0.44	0.053	0.4441
A. sativum	*T. urticae*	5	34.0±10.77b	34.0±10.8b	34.0±10.8b	34.0±10.8b	34.00±10.8b
		10	32.0±8.00b	32.0±8.0b	32.0±8.0b	32.0±8.0b	32.00±8.0b
		15	100.0±0.00a	100.0±0.0a	100.0±0.0a	100.0±0.0a	100.00±0.0a
		20	86.0±8.72a	86.0±8.7a	86.0±8.7a	86.0±8.7a	86.00±8.7a
		$F_{(3,16)}$	19.27	19.27	19.27	19.27	19.27
		p	0.00	0.00	0.00	0.00	0.00
	T.truncatus	5	98.00±2.00a	98.00±2.00a	98.00±2.00a	98.00±2.00a	98.00±2.00a
		10	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
		15	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
		20	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a	100.00±0.00a
		$F_{(3,16)}$	1	1	1	1	1
		p	0.418	0.418	0.418	0.418	0.418

[1/]Means±SE within the same column followed by the same letters are not significantly different ($p = 0.05$; Tukey's test).

DISCUSSION **7**

Plant essential oils have been widely used against several species of mites (Sertkaya *et al*., 2010; Hussein *et al*., 2013; Sohrabi and Kohanmoo, 2017). There are many studies on insecticidal and acaricidal activities (Ebadollahi *et al*., 2012; Sousa *et al*., 2015; Song *et al*., 2016). The results of present study showed similarity with the results of previous studies, which revealed that *Anethum graveolens* and *Allium sativum* oils had several acaricidal activities on two species of spider mites, *Tetranychus urticae* and *Tetranychus truncatus*. The biological activities of plant-derived essential oils are due to their chemical compositions (Singh *et al*., 2001). The essential oils have more than one site of action because they are complex mixtures (Dhifi *et al*., 2016). Our results indicated that *A. graveolens* oil had high repellency and fumigant activities against *T. urticae* and *T. truncatus*. However, this oil was lightly toxic under contact toxicity test to adults and eggs of both mites. Choi *et al*. (2004) reported that many of the tested essential oils were effective against eggs and adults of *T. urticae* without direct contact. The volatile phase of the essential oils was reported to be more toxic than the contact phase to the microorganisms (Soylu *et al*., 2006).

Kim *et al*. (2013) characterized the major constituents of *A. graveolens* oil, found the most abundant compounds of *A. graveolens* oil containing (+)-carvone (40.77%), limonene (22.83%), dill ether (5.04%) and α-phellandrene (3.90%) and evaluated the insecticidal activities of *A. graveolens* oil and their constituents on the adult rice weevil, *Sitophilus oryzae* L. under laboratory conditions. The result showed strong fumigant toxicity (LC_{50}= 3.29 mg/L air). Among the tested compounds, fumigant toxicity of the individual compound showed that (+)-carvone exhibited the strongest activity against rice weevil (LC_{50}= 0.61 mg/L air). This result indicated that (+)-carvone is a major contributor to the fumigant toxicity of *A. graveolens* oil. On the contrary, the chemical analyses from cultivated *A. graveolens* had component of α-phellandrene (59%). The oil derived from *A. graveolens* was determined as the most toxic (LC_{50}= 52.74 mg/L) against *Culex pipiens* L. 3^{rd}-4^{th} instars larvae, while the authentic α-phellandrene exhibited toxicity (LC_{50}= 38.20 mg/L) on 3^{rd}-4^{th} larvae (Evergetis *et al*., 2013).

In our study, *A. graveolens* oil had higher residual toxicity than *A. sativum* oil (data not shown). Our result is similar to the result of Ismail *et al*. (2011) evaluating the acaricidal activity of *A. sativum* oil 4 days post-treatment at 27±2˚C and reporting that no mortality was recorded on *T. urticae*. The persistence of toxicity varied according to their chemical components (Obeng-ofori *et al*., 1997). *Anethum graveolens* oil proved the most persistence, oils rich in oxygenated monoterpenes having lower volatility and most of the monoterpenes were lethal to the mite at high concentrations (Lee *et al*., 1997). Essential oil of *A. graveolens* is comprised of 91.1% oxygenated monoterpenes, while the equivalent compounds in *A. sativum* have sulphide groups. These difference could explain the variations observed in the persistence of the pesticidal activity of these oils (Mikhaiel, 2011).

Allium sativum oil had contact toxicity to adult females of *T. truncatus* and showed strong contact toxicity to eggs of *T. truncatus* and *T. urticae*. The mortality of mites increased with increasing concentration. *Allium sativum* distillate was used against *T. urticae* under laboratory conditions and few mortality rates were observed at low concentrations. The mortality of *T. urticae* females increased with increasing concentration, however fecundity was clearly affected by low concentration of *A. sativum* distillate (Attia *et al*., 2012). Sarmah *et al*. (2009) reported that strong ovicidal action against red spider mite, *Oligonychus coffeae* (Neitner) was observed with *Xanthium strumarium* L. (87.09%) and *Acorus calamus* L. (70.62%) and over 50% mortality was found at higher concentrations under laboratory conditions. Mozaffari *et al*. (2013) reported that *Mentha pulegium* L. had high toxicity on two stages of *T. urticae* (LC_{50} = 2.25 and 2.75 μL/air on eggs and adults,

respectively). Eggs were more susceptible than adults.

For the contact toxicity using different techniques, effects of *A. sativum* oil on eggs showed no ovicidal effect by leaf-dipping technique while mortality of *T. urticae* larvae treated with *A. sativum* ranged between 66-78% and 29-49% when they used leaf-spraying and leaf-dipping techniques, respectively (Erdogan *et al*., 2012). The foregoing results cleared that *A. sativum* oil was the most effective compound on the contact toxicity of spider mites. *Allium sativum* extract which extracted by different techniques of extraction from garlic bulbs showed different acaricide activity against *T. urticae* (Hincapie *et al*., 2007). Bhuyan *et al*. (1974) reported that a formulation of 1% garlic oil in petroleum jelly and beewax provided protection for approximate 8 hrs against *Culex fatigans* Wiedemann. Trongtokit *et al*. (2005) found that 100% garlic oil offered 70 minutes of protection against yellow fewer mosquito, *Aedes aegypti* L.

In fumigant toxicity test, *A. sativum* showed the strongest activity against both spider mite adult females. Yang *et al*. (2012) studied the fumigant activity of garlic oil and its major components, diallyl disulphide, diallyl trisulphide and diallyl sulphide against the stored product insect, *Tribolium castaneum* (Herbst) and reported that garlic oil and diallyl trisulphide had strong fumigant activities against the adult insect, meanwhile, garlic oil, diallyl disulphide and diallyl trisulphide had significantly fumigant activity to adult grain moth, *Sitotroga cerealella* (Olivier) with 50% lethal concentration values at 1.33, 0.99 and 1.02 µL/L air space, respectively. On the other hand, Mikhaiel (2011) reported that garlic oil achieved 100% mortality of *T. castaneum* and *Ephestia kuehniealla* (Zeller) larvae when contacted to 30% dilution-treated paper within 24 hrs and 72 hrs, respectively but had the lowest fumigant toxicity against this insect. The toxicity effects of *A. sativum* may be explained by its high concentrations of organosulphur components (Singh *et al*., 2001), because some organosulphur compounds have been shown to reduce the population densities of mite pest (Prischamann *et al*., 2005). Diallyl disulphide was the most abundant compound (10.8%), followed by sulphur (cyclooctasulphur) (10.1%), isomenthone (isomer 1) (8%), 1,8-cineole (6%) and pulegone (5.7%) of *A. sativum* distillate (Attia *et al*., 2012).

Our results demonstrated that *A. graveolens* and *A. sativum* oils at 15-20% concentrations had > 80% repellency against *T. urticae* and *T. truncatus* from 1-5 hrs after exposure, which is similarity to that of Geng *et al*. (2014) who reported that the repellency of *T. urticae* was 95.6% after 24 h at 10 g/L of garlic-straw extract. Dabrowski and Seredynska (2007) reported that first hour after transferring the mite on treated leave, 50% of mite have escaped the treated leaf surface by 50 g/L garlic concentration and a high repellent effect already after 2 hrs of observation. Hanifah *et al*. (2012) conducted a study on essential oil of *A. sativum* under laboratory condition and found that *A. sativum* extract provided 80% repellency against *Leptotrombidium deliense* Walch in 5 minutes.

The results of our study indicated that essential oils of *A. graveolens* and *A. sativum* could be useful as acaricide candidates for *T. urticae* and *T. truncatus*. Further study is needed to identify the active compounds of these plant essential oils based on their activities and their effects on the pests.

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