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Original article (Orijinal araştırma)

Effect of Fungatol and Gamma-T-ol from *Melaleuca alternifolia* (Maiden & Betche) Cheel on *Aphis gossypii* Glover (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae)[1]

Aphis gossypii Glover (Hemiptera: Aphididae) ve *Tetranychus urticae* Koch (Acari: Tetranychidae) üzerine *Melaleuca alternifolia* (Maiden & Betche) Cheel'dan elde edilen Fungatol ve Gamma-T-ol'ün etkisi

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Summary

In this study, the effect of Fungatol and Gamma-T-ol extracted from *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae) on *Aphis gossypii* Glover and *Tetranychus urticae* Koch was determined using leaf dipping method. In laboratory tests, the leaf discs (50 mm diameter) taken from bean (*Phaseolus vulgaris* L.) and eggplant (*Solanum melongena* L.) and were dipped in five different concentrations of Fungatol (1.25, 1.90, 2.20, 2.50 and 3.50%) and Gamma-T-ol (0.25, 0.50, 1.00, 1.50 and 3.60%) for 5 s. The tests were repeated five times. Mortality was recorded after 1, 24, 48 h in tests with *A. gossypii* adult females and after 1, 24, 48, 72 h in tests with *T. urticae* adult females. After 1, 24 and 48 h, the highest concentrations of Fungatol (3.50%) and Gamma-T-ol (3.60%) had caused 0, 18, 42% and 0, 20, 48.9% mortality of *A. gossypii*, respectively. After 1, 24, 48 and 72 h the same concentrations of these extracts had caused 0, 52, 74, 94% and 0, 52, 78, 93.3% mortality of *T. urticae* adult females, respectively. The results showed that Fungatol and Gamma-T-ol extracts offer good potential to be used to control *A. gossypii* and *T. urticae*. Their use in pest management could be considered after validation in the field.

Keywords: *Aphis gossypii, Tetranychus urticae,* Fungatol, Gamma-T-ol, botanical extract

Özet

Bu çalışmada *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae)'dan elde edilen Fungatol ve Gamma-T-ol ekstraktlarının *Aphis gossypii* Glover ve *Tetranychus urticae* Koch üzerine etkisi yaprak daldırma yöntemi kullanılarak belirlenmiştir. Laboratuvar testlerinde patlıcan (*Solanum melongena* L.) ve fasulye (*Phaseolus vulgaris* L.) bitkilerinden alınan 50 mm çapındaki yaprak diskleri Fungatol'un %1.25, 1.90, 2.20, 2.50, 3.50; Gamma-T-ol'ün %0.25, 0.50, 1.00, 1.50, 3.60'lık konsantrasyonlarına 5 sn daldırılmıştır. Testler 5 tekerrürlü olarak yapılmıştır. Ölüm kontrolü *A. gossypii* ergin dişileriyle yapılan testlerde 1, 24, 48 saat ve *T. urticae* ergin dişileriyle yapılan testlerde 1, 24, 48, 72 saat sonra yapılmıştır. Fungatol (%3.50) ve Gamma-T-ol (%3.60)'ün en yüksek dozları *A. gossypii*'de 1, 24 ve 48 saat sonra sırasıyla %0, 18, 42 ve %0, 20, 48.9 oranında ölüme sebep olmuştur. Ekstraktların aynı konsantrasyonları *T. urticae* ergin dişilerinde 1, 24, 48, 72 saat sonra sırasıyla %0, 52, 74, 94 ve % 0, 52, 78, 93.3 ölüm meydana getirmiştir. Sonuçlar Fungatol ve Gamma-T-ol ekstraktlarının *A. gossypii* ve *T. urticae* ile mücadelede iyi bir potansiyele sahip olduğunu göstermektedir. Zararlılar ile mücadelede bu ekstraktların kullanımı arazi çalışmalarından sonra değerlendirilebilir.

Anahtar sözcükler: *Aphis gossypii, Tetranychus urticae,* Fungatol, Gamma-T-ol, bitkisel ekstrakt

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Introduction

Chemical application is the most widely used method for control in agricultural production throughout the world, due to its simplicity and effectiveness. However, synthetic pesticides used in agriculture have caused serious short and long-term damage to the environment and human health. Long-term use of synthetic pesticides has caused water and soil pollution leading to serious health problems such as abortion, diarrhea, hepatitis A and typhoid in humans exposed to this pollution (Cutler & Miller, 2005; Grant et al., 2012).

Botanical pesticides can provide a viable alternative to synthetic chemicals. The first control using plant extracts dates back to the ancient Romans who used *Helleborus* plants and olive oil as natural insecticides (Smith & Secoy, 1975). Likewise, *Balanites* plant extracts were used by the ancient Egyptians against species belong to the Diptera family (Thacker, 2002). By 2005, about 2000 botanical extracts had been reported to have insecticidal effects on agricultural pests (Koul, 2005). Tea tree, *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae), is a native to Australia. Tea tree oil has become important in recent years due to its antimicrobial and anti-inflammatory properties (Carson et al., 2006). Furthermore, this oil has toxic and repellent effects on agricultural pests (Halbert et al., 2009). Tea tree oil has repellent and fumigant effects on sheep blowfly [*Lucilia cuprina* (Wiedemann)] and sheep lice [*Bovicola ovis* (Schrank)] (Callander & James, 2012). Both andiroba, *Carapa guianensis* Aubl. and *M. alternifolia* showed insecticidal activity against adult flies of *Haematobia irritans* L. and *Musca domestica* L. (Klauck et al., 2014). Common thyme, cinnamon, lemongrass, geranium, peppermint, tea tree and sweet basil caused >94% mortality against *Tetranychus urticae* Koch 10 μl/l air at 25°C (Lim, 2011).

Aphids not only suck plant sap but also excrete honeydew on plant leaves. As a result, saprophytic fungi grow the leaves impeding photosynthesis. Furthermore, aphids carry phytopathogenic viruses (Uygun et al., 2010). The cotton aphid, *Aphis gossypii* Glover, is a polyphagous pest of cultivated cotton and other hosts (Minks & Harrewijn, 1987). This aphid damages plants by feeding on fruits and leaves (Blackman & Eastop, 2000). The two-spotted spider mite, *T. urticae* is a serious pest on many crops and ornamental plants. This mite feeds on more than 150 species of host plants, including most deciduous fruit trees and many vegetables (Jeppson et al., 1975; Zhang et al., 2003). Control of *A. gossypii* and *T. urticae* is mainly dependent on the application of pesticides due to their ease of application and effectiveness. However, these pests can become resistant to the pesticides used to control them, resulting in lower efficacy.

The aim of this study was to determine the insecticidal effect of Fungatol and Gamma-T-ol, products based on extracts of *M. alternifolia,* on *A. gossypii* and *T. urticae*.

Materials and Method

Botanical extracts

Fungatol and Gamma-T-ol, the botanical products used in this study, were derived from *M. alternifolia*. The Fungatol is a commercial blend of three mono terpene alcohols solubilised in nonionic surfactants. The Gamma-T-ol is a commercial blend of three non oxygenated monoterpenes solubilised in nonionic surfactants. Both material produce from the steam distilled essential oil of *M. alternifolia* using super critical carbon dioxide extraction. Fungatol and Gamma-T-ol were supplied by BioAust Pty Ltd (Brisbane, Qld, Australia).

Pest rearing

Aphis gossypii Glover adults used in the experiments were reared on eggplant (*Solanum melongena* L.) plants in laboratory conditions (25±2°C, 65±10% humidity and 16L:8D photoperiod). *Tetranychus urticae* adults were reared similarly on bean (*Phaseolus vulgaris* L.) plants.

Leaf dipping method

The tests were carried out in 50 mm diameter Petri dishes. A layer of moistened cotton was placed in the dishes under a sheet of blotting paper, and after treatment 50 mm diameter disc of eggplant or bean leaf placed on top. A 10-mm-diameter hole was made in each dish lid in order to prevent moisture accumulation inside and these holes were covered with insect net. Fungatol and Gamma-T-ol were diluted with distilled water to concentrations of 1.25, 1.90, 2.20, 2.50, 3.50% and 0.25, 0.50, 1.00, 1.50, 3.60%, respectively (Iramu, 2012). Leaf discs were dipped in these solutions for 5 s. The leaf discs were allowed to dry for about 15 min. Leaf discs were then placed into the dishes. Distilled water was used as a control. Ten adult females (5-6 d old) of *A. gossypii* and *T. urticae* were placed on the leaf discs in each dish. Assessment was performed 1, 24, 48 and 72 h after treatment and the number of alive and dead individuals recorded. In experiments with *A. gossypii* adults, the mortalities were recorded after 1, 24 and 48 h only, because the products had caused phytotoxic effects on eggplant leaf discs by 72 h. The tests were repeated five times.

Statistical analyses were performed on data obtained from the tests using SAS software package (SAS Institute, 1998). Mortalities were calculated by dividing the initial number of individuals by the number alive at the assessment time. Mortalities were corrected by the Abbott's formula (Abbott, 1925). Analysis of variance was applied to the Abbott values, and different concentrations of each product were evaluated in compared with each other. Duncan's Test was performed to compare mean values. Probit analysis was performed by Polo-PC™ software using concentrations of Fungatol and Gamma-T-ol, total number of insects and number of dead insects obtained from tests and LC_{50} values were determined (LeOra Software, 1994). Probit analysis was only done for the tests with 20-80% mortality (Yu, 2008). This study was carried out at Çanakkale Onsekiz Mart University in 2015.

Results and Discussion

For both Fungatol and Gamma-T-ol, the lowest mortality of *Aphis gossypii* Glover occurred at the lowest concentration of the products and the highest mortality at the highest concentrations. For Fungatol, the highest mortality occurred at a concentration of 3.50% after 48 h and the lowest at 1.25%, likewise after 48 h. For Gamma-T-ol, the highest mortality occurred at a concentration of 3.60%, while the lowest mortality occurred at 0.50% also after 48 h. For both products mortality increased with increasing product concentration (Table 1).

Table 1. Mortality of *Aphis gossypii* caused by Fungatol and Gamma-T-ol (mean ± SE)

Products	Concentration (%)	24 h Mortality (%)		48 h Mortality (%)	
Fungatol	1.25	4.0±2.45	a*	8.9±4.16	a
	1.90	10.0±3.16	ab	18.0±3.74	ab
	2.20	12.0±4.90	ab	22.0±5.83	ab
	2.50	16.0±2.45	b	26.7±4.44	b
	3.50	18.0±3.74	b	42.0±4.90	c
Gamma-T-ol	0.25	6.0±2.45	a	12.0±2.00	a
	0.50	6.0±2.45	a	16.0±5.10	a
	1.00	8.0±2.00	a	20.0±4.47	a
	1.50	12.0±2.00	a	22.2±6.09	a
	3.60	20.0±3.16	b	48.9±5.67	b

*Means in the same column followed by the same letters are not significantly different (P<0.05).

For *A. gossypii* adult females, there was no statistically significant difference in mortality after 1 h at any concentration of either product. However, the difference between mortalities after 24 and 48 h for Fungatol and Gamma-Tol were statistically significant at all concentrations (P<0.05).

Similarly for *Tetranychus urticae,* Fungatol and Gamma-T-ol caused the lowest mortality at the lowest concentration and the highest mortality at the highest concentration. As with *A. gossypii*, as concentration of both products increased, mortality increased (Table 2). LC_{50} values of Gamma-T-ol for *T. urticae* after 48 and 72 h are presented in Table 2.

Table 2. Mortality of *Tetranychus urticae* caused by Fungatol and Gamma-T-ol (mean ± SE)

Products	Concentration (%)	24 h Mortality (%)		48 h Mortality (%)		72 h Mortality (%)	
Fungatol	1.25	2.0±2.00	a*	32.0±3.74	a	44.4±4.97	a
	1.90	14.0±7.48	ab	52.0±5.83	b	62.2±10.30	b
	2.20	26.0±5.10	b	66.0±5.10	c	75.6±2.22	bc
	2.50	44.0±5.10	c	68.0±3.74	c	88.9±3.51	cd
	3.50	52.0±3.74	c	74.0±4.00	c	94.0±2.45	d
Gamma-T-ol	0.25	0.0±0.00	a	10.0±5.48	a	28.0±3.74	a
	0.50	4.0±2.45	a	20.0±4.47	a	53.3±8.17	b
	1.00	4.0±2.45	a	42.0±5.83	b	64.0±7.48	b
	1.50	18.0±3.74	b	60.0±4.47	c	66.7±6.09	b
	3.60	52.0±3.74	c	78.0±3.74	d	93.3±4.44	c
LC_{50} Values				1.3±0.14		0.8±0.23	

*Means in the same column followed by the same letters are not significantly different (P<0.05).

In tests with *T. urticae* adult females, there was no statistically significant difference in mortality after 1 h at any concentration. However, the difference between mortalities after 24, 48 and 72 h for Fungatol and Gamma-Tol were statistically significant for all concentrations (P<0.05; Table 2).

Mortalities of *A. gossypii* and *T. urticae* at 3.50% concentration of Fungatol and 3.60% concentration of Gamma-T-ol were compared. For 3.50% Fungatol, the mortality of *T. urticae* was higher than *A. gossypii*. Likewise, 3.60% Gamma-T-ol was more effective against *T. urticae* (Table 3).

In tests with *A. gossypii* and *T. urticae* adult females, there was no statistically significant difference in mortality after 1 h at any concentration. However, the difference between mortalities of *A. gossypii* and *T. urticae* after 24 and 48 h for 3.50% Fungatol and 3.60% Gamma-T-ol were statistically significant at all concentrations (P<0.05; Table 3).

Table 3. Mortality of *Aphis gossypii* and *Tetranychus urticae* caused by Fungatol (3.50% concentration) and Gamma-T-ol (3.60% concentration) (mean ± SE)

Product	Pests	24 h		48 h	
		Mortality (%)		Mortality (%)	
Fungatol (3.50%)	*Aphis gossypii*	18.0±3.74	a*	42.0±4.90	a
	Tetranychus urticae	52.0±3.74	b	74.0±4.00	b
Gamma-T-ol (3.60%)	*Aphis gossypii*	20.0±3.16	a	48.9±5.67	a
	Tetranychus urticae	52.0±3.74	b	78.0±3.74	b

*Means in the same column followed by the same letters are not significantly different (P<0.05).

Although botanical extracts can be used as effective pest control, the effects of Fungatol and Gamma-T-ol on agricultural pests have not been fully investigated. Only a single study has investigated the effect of Fungatol and Gamma-T-ol on *A. gossypii* (Iramu, 2012). It showed that Fungatol, Gamma-T-ol, Fungatol + neem and Gamma-T-ol + neem were toxic to *A. gossypii* under laboratory conditions. Fungatol + neem and Gamma-T-ol + neem LC_{50} rates were 2.78 and 0.76%, respectively, using the leaf dipping method. Furthermore, it was reported that these products had no effect on development and reproduction of *A. gossypii*. Bayindir et al. (2015) examined the effects of Gamma-T-ol, Fungatol, Fungatol + neem spray (50.0-001) and Fungatol + neem spray (50.0-002) on the third or fourth stages of tomato leaf miner using the dipping method under laboratory conditions. They reported Fungatol + neem spray (50.0-001) was the most effective combination against *Tuta absoluta* (Meyrick). Kök & Kasap (2015) investigated toxic effects of Fungatol and Gamma-T-ol on adult females of *Myzus persicae* Sulzer under laboratory conditions. They reported that concentrations of 3.50 and 3.60% of caused mortality of 72 and 80%, respectively after 72 h. They argued that further studies of Fungatol and Gamma-T-ol on *M. persicae* under field conditions are needed before these products can be recommended for pest control in agricultural production.

Birgücü et al. (2015) investigated the effects of extracts derived from *Cassia angustifolia* Vahl, *Melia azedarach* L., *Ocimum basilicum* L., *Satureja hortensis* L., *Schinus molle* L. and *Thevetia peruviana* L., on *A. gossypii* and *Bemisia tabaci* (Gennadius). They reported that extracts of *C. angustifolia*, *O. basilicum*, *T. peruviana* were potentially useful. Durmuşoğlu et al. (2011) examined efficacy of commercial products made from annona (*Annona squamosa* L.), karanj [*Derris indica* (Lam.)], neem (*Azadirachta indica* A. Juss), alone and in mixtures, on larvae of *T. absoluta* using the leaf dipping method. They concluded that annona, karanj and neem, alone and in mixtures, can be used as alternative pesticides. Durmuşoğlu et al. (2003) tested mortality of NeemAzal T/S and neem oil on different stages of *Nezara virudula* (L.) under laboratory conditions. They reported that 0.5% NeemAzal T/S and 2% neem oil were highly effective in controlling nymphs of *N. virudula*. Balcı et al. (2014) tested the effect of unprocessed and nanoformulated of neem oil and tea tree oil, and two commercial neem formulations on larvae of *T. absoluta* under laboratory conditions. Pavela (2016) examined the acaricidal effect of aqueous extracts obtained from 28 plant species on *T. urticae*. Twenty four of these extracts showed effects higher than 50% and 16 of them demonstrated mortality higher than 90%. The toxic and repellent effects of leaf, flower and seed extracts of *Prunus laurocerasus* L. on *T. urticae* were investigated by Akyazı et al. (2015) under laboratory conditions. They reported that the mortality from seed extracts was higher than from flower and leaf extracts. Akyazı et al. (2014a) investigated the ovicidal effect of a tobacco (*Nicotiana tabacum* L.) leaf extract on *T. urticae* eggs under laboratory conditions.

They reported that the tobacco extract at 10% was highly effective on *T. urticae* eggs for a period of 10 d after treatment. Akyazı et al. (2014b) examined ovicidal effect of garlic bulb extract, soft soap and garlic + soft soap mixture on *T. urticae* eggs using the leaf dipping method under laboratory conditions. They reported that garlic extract (1, 5 and 10 ml) and garlic + soft soap mixture (25 g soft soap + 125 ml garlic stock solution) had an ovicidal effect on *T. urticae* eggs. The efficacy of 8-hydroxydihydrochelerythrine and 8-methoxydihydrosanguinarine, the principal components isolated from *Macleaya cordata* (Willd.), on *A. gossypii* was investigated by Baek et al. (2013) under laboratory conditions. They indicated that these alcohol extracts caused mortalities of 76.1 and 73.6% in *A. gossypii*, respectively. Tunç & Şahinkaya (1998) investigated the insecticidal effect of *Eucalyptus camaldulensis* Dehnh., *Cuminum cyminum* L., *Origanum syriacum* var *bevanii* L. and *Pimpinella anisum* L., extracts on *Tetranychus cinnabarinus* Koch and *A. gossypii*. They reported that a concentration of 0.5% for the first three extracts caused 99% mortality of the pest 2-3 d after application.

New control methods that are less harmful to the nature and human health are needed to replace chemicals used in agriculture. The importance of natural insecticides is increasing year by year as negative effects on both human health and environment of synthetic chemicals used in agriculture are uncovered. In conclusion, Fungatol and Gamma-T-ol show moderate to high level insecticidal effect under laboratory conditions against *A. gossypii* and *T. urticae.* If these effects can be confirmed in field experiments, the use of these products in pest management should be considered.

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