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Larvacidal Activity of Some Medicinal Plants Naturally Growing in Turkey Against *Aedes albopictus* (Diptera: Culicidae)

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Abstract: Massive use of chemical based insecticides led to detrimental effects on the public health and environment. Environment friendly and biodegradable natural products of plant origin alternatives to synthetic larvacids have received attention as agents for vector control. Thus, many researchers have studied on alternative methods for pest control instead of chemical ones.

In this study, some medicinal valuable plants naturally growing in Turkey were selected for this purpose and the larvicidal effect against Asian tiger mosquito *Aedes albopictus* larvae was investigated. Bioassays were carried out by appliying late 3th to early 4th instar larvae of *Aedes albopictus* to two different dose of plant extracts. The larval mortality was counted after 24 and 48 h after experiments. Bioassay results revealed that *Leucanthemum vulgare* was the most effective for ethyl acetate extract in 500 ppm concentration, 100 % for 24 h, 100 % for 48 h. *Salvia vertisillata* induced a second high mortality after 24 h treatment for two doses of both extracts. *Inula vulgaris* and *Matricaria chamomilla* produced high and moderate mortality for 500 ppm dose of both extracts, respectively.

Our results evidenced that the high larvicidal activity was detected products obtained from crude extracts with ethyl acateta and methyl alcohol of *Salvia verticillata*, *Leucanthemum vulgare*, *Inula vulgaris* and *Matricaria chamomilla*. But there is a need for further studies about possible candidate species for new and safer control products against mosquito control.

Keywords:. *Aedes albopictus*, larvicidal activity, mosquito control, plant extracts.

Türkiye'de Doğal Olarak Yetişen Bazı Tıbbi Bitkilerin Aedes albopictus (Diptera: Culicidae)'a Karşı Larvasidal Aktiviteleri

Öz: Kimyasal insektisitlerin yoğun kullanımı halk sağlığı ve çevre üzerinde olumsuz etkilere sebep olmuştur. Bu durum, sentetik larvasitlere alternatif, bitki kökenli çevre dostu ve biyobozunur doğal ürünler vektör kontrolünde dikkat çekmiştir. Bu nedenle birçok araştırmacı, kimyasal olanlar yerine haşere kontrolü için alternatif yöntemler üzerinde çalışmıştır.

Bu çalışmada, Türkiye'de doğal olarak yetişen bazı tıbbi bitkiler, bu amaç için seçilmiş ve Asya kaplanı sivrisinek *Aedes albopictus* larvalarına karşı larvasit etkileri araştırılmıştır. Biyoassayler, *Aedes albopictus*'un geç 3. ile erken 4. evre larvalarının iki farklı bitki ekstraktına uygulayarak gerçekleştirilmiştir. Larva ölümleri, deneylerden 24 ve 48 saat sonra sayılmıştır. Biyoassay sonuçları, *Leucanthemum vulgare*'nin etil asetat özütünün 500 ppm'lik konsantrasyonda 24 saatte % 100, 48 saatte % 100 oranında en etkili olduğunu gösterdi. *Salvia vertisillata,* her iki ekstraktın iki dozu için 24 saat uygulamadan sonra ikinci yüksek ölüm oranı yaratmıştır. *Inula vulgaris ve Matricaria chamomilla*, her iki ekstraktın 500 ppm'lik dozu için sırasıyla yüksek ve orta ölüm oranı göstermiştir.

Sonuçlarımız, yüksek larvisidal aktiviteye, *Salvia verticillata, Leucanthemum vulgare, Inula vulgaris ve Matricaria chamomilla* 'nın etil asetat ve metil alkollü ham özütlerinden elde edilen ürünlerin sahip olduğunu göstermiştir. Ancak, sivrisinek kontrolüne karşı yeni ve daha güvenli kontrol ürünleri için olası aday türler hakkında daha fazla araştırma yapılması gerekir..

Anahtar sözcükler: *Aedes albopictus*, larvasidal aktivite, sivrisinek kontrolü, bitki ekstraktı.

INTRODUCTION

Mosquitoes are responsible for transmitting several infectious diseases like malaria, yellow fewer, dengue, West nile encephalitis, filariasis, zika, chikungunya (Nauen, 2007; Kovendan et al., 2012; Govindarajan et al., 2013). For this reason, public health is becoming a challenging problem and mosquitoes and their transmitting diseases have a serious social and economic impact for many countries in tropical and subtropical areas (Bossche & Coetzer, 2008). The distribution of mosquitoes is dynamic in space and time and their life cycles are influenced by environmental conditions (Crans, 2004). Climate change seems to be a driving force for establishing new invasion areas for invasive *Aedes* mosquitoes (Caminade et al., 2012, Campbell et al., 2015). Mosquito-borne diseases are endemic in more than 100 countries all over the world, and new ones are added every year (Ogden 2018). They are causing serious public health problems and mortality of two million people every year. More than two billion people are at risk around the world related to mosquito borne diseases (Klempner et al., 2007).

In Turkey, various genera of mosquitoes, such as *Aedes*, *Anopheles*, *Culex* are important for mosquito borne diseases. Recently *Aedes albopictus* and *A. aegypti* introduced and established populations in Eastern Blacksea part and raises concerns about a possible resurgence of the pathogens that can be transmitted by this vector species (Akıner et al., 2016). Increase of the human population and movement are raising the transmission risk of the pathogens especially yellow fewer, dengue, chikungunya, West Nile fewer and zika. Limited funds of mosquito control, lack of awareness among the residents and highly competent behaviour of the invasive vector species resulted in an increase in mosquito borne diseases (Gubler, 1998). Mosquito control efforts are the best strategy for protection against mosquito-borne diseases. Usage of the syntetic insecticides is the most important method for controlling mosquito and other pests for human and animal health. But, extensive usage of synthetic insecticides causes environmental problems and development resistance. Furhermore, it causes toxic and detrimental/lethal effects on non-target organisms (Roberts & Andre, 1994; Milam et al., 2000; Dinesh et al. 2015, Sakthivadivel et al., 2015). Pyhtochemicals obtained from plants are mainly biodegradable, non-phytotoxicity and more importantly, they are renewable (Çalmaşur et al., 2006; Boulogne et al., 2012). In this respect, plant and plant derived substances regain interest to control of insect pests.

Plant secondary products as terpenoids, flavonoids and phenolic compounds which serve as storage compounds in plants are important for plant defence mechanisms against insects and used as an excellent alternative to synthetic or chemical insecticide in many parts of the World (Luthria et al., 1993; Ghosh et al 2012; Ali et al., 2015; Hikal et al., 2017). Especially, plants with high terpenoid, phenolic compound and alkaloid content are known to be used for this purpose. Therefore, the insecticidal, larvicidal, ovicidal, antifeedant and repellent activity of many plants belongs to Asteraceae and Lamiaceae families due to the rich terpenoid content in the crude extract or their components have been described in several studies (Çalmaşur et al., 2006; Pavela 2008, Boussaada et al., 2008;.Çetin et al., 2009; Govindarajan & Karuppannan, 2011, Boulogne et al., 2012; Cheah et al., 2013).

Salvia vertisillate L., *Phlomis lychitis* L., *Salvia tomentosa* Mill., are belonging to the Lamiaceae family. Many species of the genera *Salvia* and *Phlomis* over the Mediterranean region native to Turkey, North Africa, Europe and Asia are used as herbal tea for many decades in folk medicine as sore throats, colds, gastrointestinal disorder among others (Orhan et al., 2007; Tepe et al., 2007; Amor et al., 2009; Lopez et al., 2010; Aşkun et al., 2010). The other five plants (*Matricaria chamomilla* L., *Leucanthemum vulgare* (vall.) Lam., *Triploaraspermum caucasicum* (Wild.) Haya, *Erigeron annuus* (L.) Pers, *Inula vulgaris* (Lam.) Trevisan) belonging to the Asteraceae family are also subject of chemical screening and bioactivity studies and used for folk medicine (Singh et al., 2011; Kumar et al., 2014; Magharri et al., 2015; Assi et al., 2017). There are a number of reports on the isolation of various secondary metabolites and phytochemical analysis from all of these plants, e.g., monoterpenoids, sesquiterpenoids, diterpenoids, alkaloids, flavonoids and other phenolics coumpounds (Sefidkon et al., 1999; Chalchat et al., 2001; Pitarokili et al., 2006; Amor et al., 2009; Nazaruk and Kalemba, 2009; Aşkun et al., 2010; Ayoughi et al., 2011; Singh et al., 2011; Kumar et al., 2014; Magharri et al., 2015; Hatipoğlu et al., 2016; Assi et al., 2017; Kim et al., 2018). *Salvia verticillate* L., *Salvia tomentosa*, *Matricaria camomilla*, *Leucanthemum vulgare (vall.)* Lam., *Erigeron annuus* (L.) Pers plant extracts have been determined by some researchers in recent years with phytochemical analyses by using classic or spectral methods. According to these studies, the major chemical compositions of Turkish *S. verticillate* L. and *S. tomentosa* plant extracts are monoterpenes such as β-pinene, α-pinene, borneol, camphor and sesquiterpenes such as caryophyllene oxide, α- / β-caryophyllene, γ-muurulone (Chalchat et al., 2001; Pitarokili et al., 2006; Aşkun et al., 2010). The essential oils of *M. camomilla, L. vulgare (vall.)* Lam. and *E. annuus* (L.) Pers extracts contain α-bisabolol, α- / β-farnesene, guaiazulene, β-caryophyllene, caryophyllene oxide as major components (Ayoughi et al., 2011; Kumar et al., 2014; Magharri et al., 2015; Kim et al., 2018). These components are found in these plants but with different amounts. Differences among chemical compositions of the crude oils or essetial oils widely depend on conditions such as climate, variety, origin, time and soil factors.

Many *Salvia* species have been reported to have insecticidal activities. According to these records, methanolic ectract of *Salvia vertisillate* L. and hexane ectract of *Salvia tomentosa* Mill. have moderate larvacidal activity against *Culex tritaeniorhynchus* and *Culex pipiens* (Diptera:

Culicidae), respectively (Pavela, 2008; Gün et al., 2011). Also, essential oils of *Salvia tomentosa* Mill. have insecticidal activity against *Lipaphis pseudobrassicae* (Aphididae: Homoptera), *Spodoptera exigua* (Lepidoptera) (Sampson et al., 2005; Polatoğlu et al., 2017). Khodadad et al. (2007) reported that acaricidal potential against female *Rhipicephalus annulatus* (Ixodidae) of *Matricaria chamomile* ethanolic extract. In another report, ethanolic extract of *Matricaria chamomile* has showed oviposition deterrent, larvacidal and repellent activity against *Culex quinquefasciatus* (Al–Mehmadi & Al- Khalaf 2008; Gad & El-DaKheel 2009). Literature survey has shown that there are no such studies for our other studied plants.

The present study aims to evaluate potential larvacidal activity of eight different plants (Lamiaceae and Asteracea), are grown naturally in Turkey against *Aedes albopictus* larvae.

MATERIALS and METHODS

Plant material: Fresh aerial parts of *Salvia vertisillate* L. and *Salvia tomentosa* Mill. were collected from Antalya in southwest part of Turkey in April 2018 and May 2018, respectively (Table 1). Dried aerial parts of *Phlomis lychitis* L. were purchased from a market in Konya, southwest part of Turkey in May 2018. *Matricaria chamomilla* L., *Leucanthemum vulgare* (vall.) Lam., *Triploaraspermum caucasicum* (Wild.) Haya, *Erigeron annuus* (L.) Pers, *Inula vulgaris* (Lam.) Trevisan, were collected from Rize in northeast part of Turkey in May-August 2018 (Table 1) and transported to the laboratory. The identification of the plants was performed by Prof. Vagıf Atamov (Faculty of Science and Arts, Recep Tayyip Erdogan University in Rize, Turkey).

Plant Extracts: The solvents (*n*-hexane, $CHCl_3$, MeOH, AcOEt) used were either of analytical grade or bulk solvents distilled before use. Electric blender was used as a Waring Commercial (CT, USA). Ultrasonic bath was Bandelin Sonorex (Berlin, Germany).

Fresh plant materials were cleaned and washed with distillated water and ethyl alcohol mixture (1:1, v/v). Then, these materials were dried for one week at room temperature with reduced humidity (25 %) and whole plants were powdered using an electric blender. The resulting powder of each plant samples was divided into two flasks, 100g / flask. Extraction procedure is the method developed by Selvi et al., (2018). Each sample was defatted with 100 mL of chloroform at 25 °C for 30 minutes. Two different solvent extractions of each sample were performed with 2 x 400 mL of solvent at room temperature for 2 h in a shaker. The most preferred solvent for phenolic coumpounds extraction is methanol, because the phenolic composition at plant samples is mostly soluble and stable in this solvent. Ethyl acetate is mostly suitable for flavonoid extraction (Dmitrienko et al. 2012; Mokrani and Madani 2016; Sukeksi and Sarah 2016). Therefore, plant extractions were performed using two different polarities: methanol and ethyl acetate. The crude extracts were filtrated and dried under vacuum in a rotary evaporator at 40°C and then lyophilized. A stock solution of each crude extract was prepared in DMSO and stored below 4 °C until testing for bioassay. Crude oil yields obtained from extractions with different solvents of studied plant have been given in Table 1. In this study, larvacidal activities of 50 and 500 ppm doses of crude extracts obtained from eight plants against *Ae albopictus* (Diptera: Culicidae) have been tested.

Table 1. Plants used in this study, their parts used, origins and crude oil yields.

Plant species	Plant family	Common name	Plant part	Origin and date	Crude oil yield (%)	
					MeOH	EtOAc
Salvia vertisillate L.	Lamiaceae	lilac sage	aerial part	Antalya, April 2018	2.58	1.72
Phlomis lychitis L.	Lamiaceae	lampwick plant	aerial part	Konya, May 2018	3.77	2.46
Salvia tomentosa Mill.	Lamiaceae	balsamic sage	aerial part	Antalya,May 2018	2.07	4.73
Matricaria chamomilla L.,	Asteraceae	chamomile	aerial part	Rize, May 2018	2.62	1.97
Leucanthemum vulgare (vall.) Lam.	Asteraceae	ox-eye daisy	aerial part	Rize, July 2018	2.94	2.47
Triploaraspermum caucasicum (Wild.) Haya	Asteraceae	vulture weed	aerial part	Rize, July 2018	4.62	4.03
Erigeron annuus (L.) Pers	Asteraceae	fleabane	aerial part	Rize, August 2018	4.55	2.47
Inula vulgaris (Lam.) Trevisan	Asteraceae	ploughman's spikenard	aerial part	Rize, August 2018	2.78	3.33

Test organisms: *Aedes albopictus* was collected from Rize province in July 2018 in around whole sales market (41,0416 Lat 40,5771 Lan) / small industirial area (41,0453 Lat 40,5784 Lan) and used for the larvicidal activity. Whole samples collected larval stages in inside used tires. Alive collected larvae transferred to the laboratory and colonised in the laboratory condition. Mosquitoes were held at 26 ± 2°C, 65 ± 10 % relative humidity and a photoperiod regime of 14:10 h (L:D) in the insectarium. The larvae were fed on larval food. Adult mosquitoes were periodically fed with 10% glucose solution (Gerbert et al., 1994, Imam et al., 2014). F1 and F2 generation larvae were used for tests.

Larvicidal bioassay: The larvacidal activity against *Ae albopictus* was analysed with minor modifications adopted for the study in standard protocol recommended by the World Health Organization (WHO) (WHO, 2005). The tests were conducted in 250 mL plastic container. Tested third or early fourth instar mosquito larvae were obtained from laboratory colonized mosquitoes of F1 and F2 generation. From the stock solution, different concentrations 50 and 500 ppm with distillated water were prepared. Twenty healthy larvae were released into each 250 mL plastic container containing 200 mL of water and two hours after test extracts (50-500 ppm) were added the plastic container. Controls made with DMSO solution were performed simultaneously. Larval mortality was observed for 24 and 48 hours. The larval percent mortality was calculated. All tests were done in two replications and control mortality was corrected using Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

The results of larvacidal affects of *S. verticillate* L., *S. tomentosa* (Mill.) extracts showed low level effects on *Ae albopictus* except *S. verticillate* L. methanol and ethyl acetate extracts 500 ppm dose. *S. verticillate* L. bioassay results showed high larval mortality (up to 85 % for 500 ppm dose) but *S. tomentosa* extracts showed low larval mortality (under 15 % for 50 and 500 ppm doses). Similarly, *P. lychitis* L. extracts showed low level mortality for two doses (under 20 %) (Table 2). Gün et al. (2011) showed larvicidal effects of some *Salvia* species hexane extracts on *Culex pipiens* larvae. They used four different *Salvia* species (*S. tomentosa*, *S. scleerae*, *S. argentea*, *S. syriaca*) and tested against *Cx pipiens* 3^{th} and 4^{th} instar larvae. They indicated that *S. tomentosa* is the most effective *Salvia* species against *Cx pipiens* larvae. In contrast to these results, *S. vertisillate* species showed higher larvicidal effect against *Ae albopictus* larvae and gived high mortality rate after 24 and 48 h in our study. *S. tomatosa* mortality rates were very low and also found 15 % after 48 h. Çetin et al. (2006) tested larvicidal activities some Lamiaceae species against *Cx pipiens* larvae. In that study, ethanol etxract of the *Salvia sclerae* showed high larvicidal activity and 200 ppm ethanol extract gived 90 % mortality after 24h. Kaliopoulos et al. (2010) reported the larvacidal activity of *Salvia fruticosa* Mill., *Salvia pomifera* L. subsp. *calycina* (Sm.) Hayek and *Salvia pomifera* L. subsp. *pomifera* essential oils against *Cx pipiens* larvae. All these studies have shown different/similar larvicidal activity of many *Salvia* species related to the polar/apolar solvent extracts and mosquito species.

Different daisy extracts results showed moderate and high mortaliy especially in methanol and ethyl acetate high dose experiments. *M. camomilla* bioassay revealed that the low level mortality of methanol extracts low dose for two test duration. But, the high dose of this extract gived 75 % mortality for 48 h duration. Ethyl acetate extracts bioassay results showed moderate (ethyl acetate 50 ppm 48 h) and high mortality for two test duration. A similar study of Gad & El-DaKheel (2009) showed that the larvicidal activity of *M. chamomilla* against *Cx quinquefastiatus* larvae and determined 0.310 mg/L LC_{50} value for 4^{th} instar larvae. Al–Mehmadi (2011) also reported that the larvacidal activity of *M. chamomilla* extracts against *Cx quinquefasciatus* and indicated that the duration is important for the LC_{50}. They showed 0.505 mg/L after 28 h LC_{50} value and 0.301 mg/L after 48 h from treatment. Another study of Al–Mehmadi & Al- Khalaf (2008) reported that ethanolic extracts of *M. chamomilla* had oviposition deterrent and skin repellent against *Cx quinquefasciatus*. Mahyoub et al. (2014) reported that larvicidal effects of *M. chamomilla* extract against *Ae aegypti* larvae. They indicated that the concentration is important for the incrasing larval mortality against 4^{th} instar *Ae aegypti* larvae. Candido et al. (2013) reported that the high larval and pupal mortality with *R. communis* and *C. phyllacanthus* oils against *Ae aegypti* larvae. They also indicated that the lethality was enhanced with increasing time of exposure to plant products. Our results also indicated that mortality increasing with time.

L. vulgare (vall.) Lam. extracts also showed similar results as *M. chamomilla,* but methanol 500 ppm and ethyl acetate 50 ppm results showed moderate mortality for two test duration. The highest mortality result for this plant was seen at 500 ppm of ethyl acetate extract (100 %, 24 h and 48 h).

T. caucasicum (Wild.) Haya extracts showed low level mortality for whole extracts and duration except ethyl acetate 500 ppm 48h duration (75 % mortality). Almost similar results were found in *E. annuus* (L.) Pers and ethyl acetate 500 ppm 48h duration showed 67.5 % mortality.

I. vulgaris (Lam.) Trevisan methanol and ethyl acetate low doses showed low level mortality for two test duration. In contrast to these results, high doses of methanol extract gived moderate mortality for two test duration and high doses of ethyl acetate extracts gived high mortality for two test duration. In a similar study, He et al. (2014) described larvicidal activity of another *Inula* species (*I. racemosa*) root ethanol extract against *Ae albopictus* larvae. They showed that 25.23 μg/mL LC_{50} value of ethanol extract.

There are numerous studies on plant extracts against mosquito larvae. Insects and especially mosquitoes control becomes more diffucult day by day related to the insecticide resistance. Literature survey has shown that there is no report on larvicidal activity of *P. lychitis, L. vulgare, T. caucasicum, E. annuus* unlike other tested species. Botanical orginated insecticides may have a chance for safe control option beyond the harmfull effects of synthetic insecticides and also may help to solve insecticide resistance obstacle for mosquito control. This study indicates that the methanol and ethyl acetate extract of *Salvia* and some daisy species naturally found in our country have a potential to be used for the control of *Ae albopictus*. Therefore, more detailed studies are needed for exact and effective candidate of invasive *Aedes* species recently distrubited in Turkey.

Table 2. Mortality rate of *Aedes albopictus* by different concentrations of some plant extracts belonging to Asteraceae and Lamiaceae families.

Consantration (ppm)	% mortality			
	Ethyl acetate		Methyl alcohol	
	24 hour	48 hour	24 hour	48 hour
Salvia vertisillate L.				
50	0	7.5	2.5	5
500	90	100	85	90
Salvia tomentosa Mill.				
50	0	0	0	2.5
500	2.5	15	7.5	15
Phlomis lychitis L.				
50	0	0	2.5	5
500	10	17.5	2.5	2.5
Matricaria chamomilla L.				
50	12.5	57.5	0	25
500	90	95	30	75
Leucanthemum vulgare (vall.) Lam.				
50	50	62.5	7.5	10
500	100	100	45	50
T. caucasicum (Wild.) Haya				
50	17.5	20	2.5	20
500	30	75	10	10
Erigeron annuus (L.) Pers				
50	10	30	15	17.5
500	35	67.5	17.5	22.5
Inula vulgaris (Lam.) Trevisan				
50	10	30	5	27.5
500	75	95	57.5	62.5

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