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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Possible Efficacy of Some Plant Crude Extracts Against *Orosanga japonica* (Hemiptera: Ricaniidae) Nymphs

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Abstract: In this study, twenty species from twelve plant families were tested for potential effectiveness against *Orosanga japonica* control on nymph. Methanol extracts mortality rates were generally found to be higher than ethyl acetate extracts. Most effective plant crude extracts were *Salvia verticillate* (Lamiaceae) and *Daucus carota* (Apiaceae) respectively. Consequently, we reported the high or moderate potential insecticidal activity against *Orosanga* nymphs from eleven different plant families extract. That is why, we recommend extensive and more detailed study for the establish biological based control methods against invasive *Orosanga japonica* in Black Sea countries.

Keywords: Biological control, insecticidal activity, mortality, *Orosanga japonica*, plant extract, Ricaniidae, Turkey.

Bazı Bitki Ham Özütlerinin *Orosanga japonica* (Hemiptera: Ricanidae) Nimflerine Karşı Olası Etkinlikleri

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Öz: Bu çalışmada, on iki bitki familyasından yirmi türün *Orosanga japonica* nimf kontrolüne karşı potansiyel etkinlikleri edilmiştir. Metanol ekstraktları ölüm oranlarının genellikle etil asetat ekstraktlarından daha yüksek olduğu bulunmuştur. En etkili bitki ham özütleri sırasıyla *Salvia verticillate* (Lamiaceae) ve *Daucus carota* (Apiaceae) olarak görülmüştür. Sonuç olarak, on bir farklı bitki familyası türlerinden alınan özütlerin *Orosanga* nimflerine karşı yüksek veya orta derecede potansiyel insektisidal aktivite rapor edilmiştir. Bu nedenle Karadeniz ülkelerinde istilacı *Orosanga japonica*'ya karşı biyolojik bazlı kontrol yöntemlerinin oluşturulması için kapsamlı ve daha ayrıntılı bir çalışma yapılması gerekmektedir.

Anahtar kelimeler: Biyolojik kontrol, insektisidal aktivite, ölüm oranı, *Orosanga japonica*, bitki özütü, Ricaniidae, Türkiye.

INTRODUCTION

Ricaniidae species have an important group in the Hemipteran pests that include invasive species. This family is represented in the world with 46 genera and 450 species. (Chou et al., 1985; Shcherbakov, 2006; Gnezdilov, 2009; Bu et al. 2010). Only species belonging to the *Ricania* genus of this family are distributed in Palaearctic Region (Demir, 2009). However, some Ricaniidae species have spread to beyond to the original habitats. *Orosanga japonica* was firstly detected in Russia (Krasnodar), Ukraine (Crimea) in West Palearctic. It was transported from native habitats to Russia in the 1900s via seedling or plants transportation for the Botanical garden. In 1950s, the species was recorded in Georgia (Nast, 1987; Gnezdilov & Sugonyaev, 2009; EPPO, 2016; Bourgoin, 2017). For the last two decades, it has been reported in the Eastern Black Sea area and began to spread rapidly to the west (Demir, 2009; Cebir, 2016; Demir, 2018; Akıner et al., 2019). It was also recorded in the Western Black Sea region by Öztemiz, (2018) and İstanbul by Arslangündoğdu & Hizal, (2019). Adult and nymph stages of this species are fed with plant sap like beans, corn, cabbage, pepper and eggplant, especially in all agricultural plants, shrubs and shoots (Gokturk & Aksu, 2014). Furthermore, they caused indirect by damage on weaken the plant. This situation makes the plant vulnerable to attack by some important plant pathogenic fungi (*Cylindrocarpon sp.*, *Fusarium sp.* and *Pestalotiopsis quepinii*) (Eken et al., 2013). *O. japonica* is an important plant pest, generally in tropical regions but recently some authors reported the different degree of damage situation about chestnut in Abhazia (related to vector situation of the fungus pathogen), orchards in around Caspian Sea in Iran, tea and kiwifruit in Turkey (Lukmazova & Selikhovkin, 2013; Ak et al., 2015; Mozaffarian, 2018). In recent years, the population of *O. japonica* has increased step by step in the Eastern Black Sea region (Akıner et al., 2019).

Eastern Black Sea is a region characterized by its topographic characteristics and precipitation regime, and it differs from other regions of Turkey. Agricultural production is limited due to the limited cultivable areas and the sloping structure of the land. The most important agricultural products of the region are tea (*Camelia sinensis*), hazelnut (*Corylus avellana*) and kiwi (*Actinidia deliciosa*), respectively. The increase in *O. japonica* population level and its rapid spread are arised problems in the agriculture of these commercial products and cause production losses. Pest has an important detrimental effect on different plants grown such as *Vitis vinifera, Ficus carica, Phaseolus vulgaris* and *Cucumis sativus*. According to the decision of Turkey's General Directorate of Tea Enterprises, step by step, also including fertilizers are applied zero synthetic chemicals in the region. Therefore, chemical control agent (insecticides) usage is very limited in the Eastern Black Sea area though. In this mean, the area is the only region in the world where tea plant grows in naturally without any input except limited amount of chemical fertilizer.

The long term control of Ricanidae species with insecticides has yet been developed in any country (Jeon et. al., 2017). Synthetic insecticide based control methods are most influential method for pest control in many areas (human, animal and plant health). But, the use of synthetic chemicals is known to bring serious problems such as environmental contamination and insecticide resistance (Nicoletti e al., 2010; Liu et al., 2012). Additionally, toxic and detrimental effects on non target organisms are very big problem in many areas (Dinesh et al., 2015).There is a need to develop selective and naturally-based control methods in the control of the Ricaniidae species due to the harmful effects of chemical based insecticides and above mentioned reasons. Botanical based materials are safer for non target organisms and environmentally friendly agents for pest control operations (Lee & Lee, 2016; Lee et al., 2016). Although different kinds of biological based control agents and mechanical control option studies were conducted against adult stage of *O. japonica* (Güçlü et al., 2010; Ak et al., 2013; Eken et al., 2013; Gokturk & Mıhlı, 2015; Gokturk et al., 2017), there is a need more study of subject.

All of the selected plants are belonging to some plant families identified as to have a significant insecticidal and larvicidal activity against different kind of insects. Around 110 plant families with these properties have been identified worldwide and Lamiaceae, Fabaceae, Asteraceae, Apiaceae and, Solanaceae are the most important families in this group (Boulogne et al., 2012). Similar studies for different insect are usually about obtaining essential oils by hydrodistillation method and investigating their insecticidal properties. In this study, examined the possible efficacy of different plant crude extracts against nymph stage of *O. japonica*. Therefore, firstly, our aim in this study is to investigate the contact toxicity of crude extracts derived from a plant twenty naturally grown in Turkey.

MATERIAL AND METHOD

Plant material: Selected twenty plant species were collected from different localities in Turkey. The specimen identification was performed by Prof. Vagif Atamov from the Faculty of Science and Arts, Recep Tayyip Erdogan University in Rize, Turkey. A sample of each plant was deposited in the Herbarium of Biology Department. Information about these plants are given in Table 1.

Preparation of plant extracts: During the extraction of plants, it is important to avoid contamination of the extract as a result of extraction conditions or solvent impurities. The aerial parts of fresh plants were cleaned and washed with distilled water and ethyl alcohol mixture against fungus like contaminations (1:1, v/v). Cleaned plant materials were dried for one week at room temperature with reduced humidity (25%). Subsequently, all materials were powdered using an electric blender and made as in the literatıre by ethyl acetate and methanol (Dmitrienko et al., 2012; Mokrani & Madani, 2016). The crude extracts were filtered, evaporated to dryness and lyophilized under pressure to completely remove the solvent used in the extraction. 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 a solvent of studied plant materials are given in Table 1.

Table 1. Crude oil yields obtained from extractions with a solvent of studied plant materials

Family	Plant species	Date	Origin	Used part	Yield (%)	
					MeOH	**EtOAc**
Lamiacae	*Salvia vertisillate*	May 2018	Antalya	Aerial part	7.80	2.93
	Phlomis lychitis	May 2018	Konya	Aerial part	9.71	2.46
	Salvia tomentosa	May 2018	Antalya	Aerial part	8.07	6.43
Apiaceae	*Foeniculum vulgare*	July 2018	Denizli	Aerial part	5.30	1.62
	Daucus carota	Aug. 2018	Rize	Aerial part	5.98	3.12
Asteraceae	*Matricaria chamomilla*	May 2018	Denizli	Aerial part	2.34	6.71
	Laucantheum vulgare	April 2018	Rize	Aerial part	3.67	3.68
	Tripleurospermum vulgare	May 2018	Rize	Aerial part	6.97	4.03
	Erigeron annuus	June 2018	Rize	Aerial part	4.55	2.23
	Inula vulgaris	Sept. 2018	Artvin	Aerial part	2.78	3.33
Primulaceae	*Primula vulgaris*	April 2019	Artvin	Flower	10.99	3.53
	Primula vulgaris	April 2019	Artvin	Leaf	12.69	2.64
Ericaceae	*Rhododendron luteum*	Aug. 2018	Rize	Flower	2.27	0.86
	Rhododendron ponticum	June 2018	Rize	Leaf	1.16	1.26
Rutaceae	*Citrus sinensis*	Febr. 2018	Rize	Fruit peel	1.36	1.16
Lauraceae	*Laurus nobilis*	April 2018	Denizli	Leaf	5.59	3.66
Solonaceae	*Nicotiana tabacum*	Aug. 2018	Denizli	Leaf	5.23	5.73
Polygonaceae	*Polygonum persicaria*	Sept.2018	Rize	Aerial part	2.91	1.03
Papaveraceae	*Papaver somniferum*	May 2018	Konya	Seed	6.68	12.57
Lythraceae	*Lythrum salicaria*	Sept. 2018	Rize	Aerial part	7.05	3.71
Boraginaceae	*Heliotropium europaeum*	July 2018	Antalya	Aerial part	5.84	3.82

MeOH: Methyl alcohol; EtOAc: Ethyl acetate

Herbarium numbers: *S. vertisillate* (RTEUB:4305); *P. lychitis* (RTEUB:4307); *S. tomentosa* (RTEUB:4406); *F. vulgare* (RTEUB:4435); *D. carota* (RTEUB:4408); *M. chamomilla* (RTEUB:4436); *L. vulgare* (RTEUB:4503); *T. vulgare* (RTEUB:4503); *E. annuus* (RTEUB:4505); *I. vulgaris* (RTEUB:4509); *P. vulgaris* (RTEUB:4511); *R. luteum* (RTEUB:4506); *R. ponticum* (RTEUB:4507); *C. sinensis* (RTEUB:4513); *L. nobilis* (RTEUB:4537); *N. tabacum* (RTEUB:4538); *P. persicaria* (RTEUB:4508); *P. somniferum* (RTEUB:4514); *L. salicaria* (RTEUB:4515); *H. europaeum* (RTEUB:4509).

Test organisms

The nymphs of *O. japonica* were collected with electric aspirator in and around Rize city (41.025461 Lat 40.482803 Lon Alipasa, 41.036468 Lat 40.493461 Lon Campus). About 1000 *Orosanga* nymphs collected from region were placed into the cages (20 x 20 x 20 cm, maximum of 50 nymphs) and transferred to the laboratory. The samples brought to the insectarium were taken into cages of 50 x 50 x 50 cm for easy to feed (maximum of 100 nymphs in each cage) during acclimatisation period. The cages to be used were previously sterilised in order to avoid any contamination. Samples were held at 26 ± 2 °C, 65 ± 10 % relative humidity and a photoperiod regime of 12:12 h (L:D) in the insectarium. Fresh blackberry branches were put into the cages for feeding. Samples were kept for 24 hours in the insectarium for to acclimate to the environment before the tests.

Insecticidal Activity

250 mL glass jars and filter paper were used for evaluating the contact toxicity of crude extracts. The each of stock solutions (500 ppm) prepared by using DMSO was impregnated to filter papers (10x20 cm) prepared separately. DMSO impregnated paper was used as a control. Filter papers were placed in the jars after impregnation. Glass jar was cleaned and autoclaved before experiment. Collected and acclimatised *O. japonica* nymphs for 24 hours were distributed to 250 mL glass jars. 20 individuals were used for every test chamber for avoiding uncontrolled death rate related to the density effect. Fresh blackberry branch was put into every jar for feeding.

24 hours and 48 hours death rate were counted. The per cent mortality was calculated according to the death rate and corrected *Abbott formula* (Abbott, 1925). All tests were done in two replications.

RESULTS AND DISCUSSION

In this study, 20 species from eleven plant families were tested for potential efficacy against nymph stage of *O. japonica* was evaluated by contact toxicity assay by using impregnated paper (Table 2). The effect rate (death rate) of two kinds of crude extracts obtained by using different polarity solvents such as ethyl acetate and methyl alcohol varies between 2.5 and 100 per cent.

Table 2. The effectiveness of different plants crude extracts against nymph stage of *O. japonica*

Family	Plant species	% mortality			
		EtOAc		MeOH	
		24 h	48 h	24 h	48 h
Lamiacae	*Salvia vertisillate*	80	100	87.50	100
	Phlomis lychitis	17.50	56.25	16.25	71.25
	Salvia tomentosa	33.75	55	35	81.25
Apiaceae	*Foeniculum vulgare*	33.75	52.5	27.50	53.75
	Daucus carota	87.75	100	78.75	100
Asteraceae	*Matricaria chamomilla*	8.75	87.5	2.50	2.50
	Leucanthemum vulgare	18.75	47.5	25	75
	Tripleurospermum caucasicum	20	43.75	71.25	98.75
	Erigeron annuus	65	82.5	73.75	100
	Inula vulgaris	22.50	48.75	18.75	66.25
Primulaceae	*Primula vulgaris* (flower)	52.5	90.25	56.25	96.25
	Primula vulgaris (leaf)	32.5	86.25	61.25	98.75
Ericaceae	*Rhododendron luteum*	31.25	76.25	30	76.25
	Rhododendron ponticum	51.25	82.5	52.5	80
Rutaceae	*Citrus sinensis*	61.25	90	72.5	98.75
Lauraceae	*Laurus nobilis*	70	95	53.75	91.25
Solonaceae	*Nicotiana tabacum*	22.50	61.25	27.50	72.5
Polygonaceae	*Polygonum persicaria*	23.75	60	36.25	77.5
Papaveraceae	*Papaver somniferum*	47.75	80	30	60
Lythraceae	*Lythrum salicaria*	57.5	96.25	67.5	93.75
Boraginaceae	*Heliotropium europaeum*	38.75	93.75	66.25	98.75

h: hour; MeOH: Methyl alcohol; EtOAc: Ethyl acetate

Among the tested species in the Lamiaceae family, *Salvia vertisillate* caused the highest mortality rates after 24 and 48 h periods for two tested extract types. *Salvia tomentosa* and *Phlomis lychitis* caused high mortality rates after 48 h test period for two tested extract types. EtOAc extract gave 52.5-55% mortality values for this two plant species after 48 h. For Apiaceae family, *Daucus carota* caused high mortality for two extract types after 24 and 48 h and mortality values ranged between 78.75% (MeOH 24 h) and 100% (EtOAc and MeOH 48 h). In contrast this result *Foeniculum vulgare* caused low mortality for all tested periods and extract types. Asteraceae family plant species test results ranged between 2.5% (*Matricaria camomilla* MeOH extract 24 h) and 100% (*Erigeron annuus* MeOH extract 48 h). *E. annuus* caused highest mortaliy after two tested periods and extract types among the tested Asteracea family species. Only one species tested from the Primulaceae family member, but two different plant parts tested (flower and leaf). Whole tested design caused moderate and high mortality after 24 and 48 h except EtOAc leaf extract 24 h tested period (32.5%) for *Primula vulgaris*. *Rhododendron ponticum* caused moderate and high mortality rates for two tested periods and extracts. *Rhododendron luteum* caused high mortality for two tested extracts 48 h after the test. Alhough two Ericaceae species gave moderate and high mortality, it did not exceed 82.5% (EtOAc extract 48 h). *Citrus sinensis* (Rutaceae), *Laurus nobilis* (Lauraceae), *Lythrum salicaria* (Lythraceae) and *Heliothropium europium* (Boraginaceae) species extracts caused moderate and high mortality rates after both extract types and tested periods among the other tested plant family members.

In general, methyl alcohol extracts mortality rates were generally found higher than ethyl acetate extracts. It was determined that the most effective plant extracts against *O. japonica* nymphs were *S. verticillate* (Lamiaceae) and *D. carota* (Apiaceae) for two extracts and two intervals after experiment (24-48 h). Duration times increased the mortality rates except *M. camomilla* (Asteraceae) methanol extract. Although *M. camomilla* methyl alcohol extract mortality rates were the same, the highest increase of mortality rates was found in ethyl acetate extract for this plant.

Some authors reported the effective activity range for *Salvia* species against vector mosquito species larvae, biting deterrent activity (Ali et al., 2014; Gün et al., 2011) and plant pest species (Kara et al., 2014; Kostic et al., 2007). Our results showed moderate or high mortality rates for two different *Salvia* species, but *S. tomentosa* activity was lower than the *S. verticillate*. *Salvia* species include high rate of different terpenoid and sesquiterpenoid components according to the some studies about the chemical composition (Sefidkon & Khajavi, 1999; Tabanca et al., 2017). Chemical composition of the plant species related to the insecticidal activity refers to terpenoids, alkoloids and phenolic compounds (Boulogne & Petit, 2012). Furthermore, methyl alcohol and ethyl acetate are the best solvents for the phenolic, terpenoid and flavonoid constitutients respectively (Dimitrienko et al., 2012; Mokrani & Madani, 2016). The last Lamiaceae member of this study (*P. lychitis*) caused low and moderate mortality during 24 h and 48 h. Although chemical composition of the *Phlomis* species include different degree of sesquiterpene and terpenoid, this result may be explained by lower ratio of terpenoid than *Salvia* species

(Lopez et al., 2010; Sarıkaya & Fakir, 2017). Some studies refer to *D. carota* extracts for effectiveness against different plant pest species and mosquito groups (El Idrisii, 2016; Hrudova et al., 2006; Muturi et al., 2019). Muturi et al., (2019) indicated that three chemical constitutents (terpinolene, para cymene, and γ-terpinene) have more toxic than the other six tested chemical compositions. Our results gave high efficiency against nympheal stages of the *O. japonia*. Although *D. carota* extracts gave high mortality rates, other Apiaceae species (*F. vulgare*) caused low and moderate mortality after 24 h and 48 h duration. Some studies reported the high constituent of tepenoid compound and antifungal acitivity for *F. vulgare*, but our results did not indicate high insecticidal activity (Diao et al., 2014; Dukc et al., 2003). Five Asteraceae species caused moderate or high mortaliy rates during 48 h duration except *M. camomilla* methyl alcohol extract. Asteraceae family reported as the third family that showed insecticidal activity (Boulogne & Petit, 2012). Our results indicated that methyl alcohol extracts for Asteraceae species are more effective than ethyl acetate extracts. *P. vulgaris* leaf and flower extracts showed moderate and high activity against nymph. Death rate of 48 h gave up to 86% and high potential of the insecticidal activity. Methyl alcohol extract mortality rates were higher than the ethyl acetate extract. It was reported that the high phenolic, monoterpene and terpene like compounds *P. vulgaris* and other *Primula* species (Yaylı et al., 2016; Baczek et al., 2017). Therefore, high insecticidal activity may be related to this compounds in crude extracts. Two different Ericaceae species were tested in this study and results showed that the moderate or high mortality after 48 h. Chemical constituents of *R. luteum* and *R. ponticum* include different degree of terpenoid and phenolic compounds (Tasdemir et al., 2003; Usta et al., 2012). This constituent may be related to the higher toxicity of the nymphs, but does not completely explain these results. Giatropoulos et al., (2012) reported the larvicidal effects against the mosquito *Aedes albopictus* and relation to the limonene and pinene in the essential oils. Furthermore, essential oil of fruit peels and seeds of *C. sinensis* killed the larvae and adults of *Triboluim castaneum* by contact action (Salem et al., 2013). In our study, *C. sinensis* gave mortality up to 61% for 24 and 48 h duration. Our results revealed that *C. sinensis* was the high potential of the control. *L. nobilis* is one of the very well known plant for the tropical and subtropical region. It is known that the bioactive compunds are related to many usage areas such as antibacterial, antifungal, antioxidant and insecticidal (Charal et al., 2017). Our results indicated high insecticidal properties against nymphs. *Nicotiana tabacum* gave low (24 h) or moderate (48 h) toxicity against nymphs. This plant based biological activities against different kind of plant pest caused high mortality *Grapholita molesta* (Lepidoptera: Tortricidae) (Sarker **&** Lim, 2018) and also other *Nicatiana* species extracts caused 100% mortality against key insect pests Brassicas (Amoebeng et al., 2018). But our results did not exceed 72.5% (methyl alcohol 48 h). *Polygonum persicaria* results showed a similar pattern with *N. tabacum* and gave moderate mortality after 48 h for two different extraction types. Although terpenoid components of *Polygonum* species were reported to be high (Saeidnia et al., 2014), insecticidal activities were not consistent with this situation in our result. *Papaver somniferum* seed extract caused low or moderate mortality after 48 h duration period. *Papaver* chemical component is generally related to the drug industry and pharmaceutical benzylisoquinoline alkaloids and terpenoid constituent is not working well (Hao et al., 2015). Therefore constituent of alcohol based extraction may provide moderate toxicity after 48 h. *L. salicaria* results referred to the high inseciticidal potential. Mortality results were found to be up to 57.5 % for 24 h and 48 h results were up to 93.75%. High phenolic compounds to this species (Rouha et al., 2001) were reported and it maybe related to the high potential of the insecticidal activity for this group. *H. europaeum* gave similar results like *L. salicaria*. It was reported the high alcoholic and different degree of flavonoid and triterpenoid constituent *Heliothropium* species. *H. europeaum* is also used for folk medicide as antipyretic, antihelmintic, cholagogue, emmenagogue, cardiotonic (Saeedi & Morteza-Semnani, 2009; Goyal & Sharma, 2014). Here we evaluated high potential of insecticidal activity.

CONCLUSION

Here, we reported that a total of 20 different plants distributed into 12 families have high or moderate potential insecticidal activities against *O. japonica* nymphs. Seven family groups are also well known insecticidal properties (Boulogne & Petit, 2012). Five other families such as Primulaceae, Ericaceae, Papaveraceae, Lythraceae and Boraginaceae were included in the study to see the comparison in terms of their insecticidal activity. It was determined that the plants in this family also show activity against *Orosanga* nymphs in varying proportions. There is a need for an extensive study about the insecticidal activity and detailed chemical composition related to the this data in near future to establish biological based control methods for this group. Nevertheless, it is thought that the observed activity values will contribute to the elimination of deficiencies in the literature and to create different thoughts on the subject.

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