

## Stomach poison activity of some plant extracts on Colorado potato beetle (Coleoptera: Chrysomelidae)

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### ABSTRACT

#### Bazı bitki ekstraktlarının Patates böceği (Coleoptera: Chrysomelidae) üzerindeki mide zehiri aktivitesi

Discovery of new eco-friendly methods for management of insect-pests is very important in integrated pest management. Toxicities of six different plant extracts (*Acanthus dioscoridis* L., *Achillea millefolium* L., *Bifora radians* Bieb., *Heracleum platytaenium* Boiss, *Humulus lupulus* L. and *Phlomis tuberosa* (L.) Moench) were evaluated against Colorado potato beetle (CPB) larvae under laboratory conditions. Methanol extracts prepared from vegetative parts of plants were initially tested against 3<sup>rd</sup> instar larvae via feeding method. Maximum mortality was observed in the case of *H. platytaenium* and *H. lupulus* extracts after 48 hours when tested against 3<sup>rd</sup> stage larvae. So these extracts bearing the maximum biological activity were further evaluated using 2<sup>nd</sup> and 4<sup>th</sup> instar larvae. Second instar larvae were more susceptible to *H. platytaenium* and *H. lupulus* than 3<sup>rd</sup> instar larvae. However, 4<sup>th</sup> stage larvae were the most resistant to these extracts' activities. These results indicated that *H. platytaenium* could be potentially used in the management of CPB, especially as a stomach poison.

**Keywords:** *Leptinotarsa decemlineata*, bio-pesticides, stomach poison, *Heracleum platytaenium*, *Humulus lupulus*, plant extract, CPB

### ÖZ

Entegre zararlı yönetiminde zararlı böceklerin yönetimi için yeni, çevre dostu metodların geliştirilmesi çok önemlidir. Altı farklı bitki ekstraktının (*Acanthus dioscoridis* L., *Achillea millefolium* L., *Bifora radians* Bieb., *Heracleum platytaenium* Boiss, *Humulus lupulus* L.

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ve *Phlomoides tuberosa* (L.) Moench) toksisiteleri Patates böceğine karşı laboratuvar koşullarında değerlendirilmiştir. Bitkilerin vejetatif aksamlarından hazırlanan metanol ekstraktları beslenme yoluyla 3.dönem larvalara karşı test edilmiştir. Maksimum ölüm oranı *H. platytaenium* ve *H. lupulus* ekstraktında 48 saat sonra gözlemlenmiştir. Bu yüzden bu ekstraktlar ile ileri biyolojik aktivite testleri 2. ve 4. dönem larvalar ile de yürütülmüştür. *H. platytaenium* ve *H. lupulus* ekstraktları için 2. dönem larvalar 3. dönem larvalardan daha hassas olmuşlardır. Test edilen dönemlerden 4. dönem larva bu ekstraktların aktiviteleri bakımından en direçli dönem olmuştur. Bu sonuçlar *H. platytaenium*'un Patates böceğinin yönetiminde özellikle mide zehiri olarak potansiyel olarak kullanılabileceğini ortaya koymuştur.

**Anahtar kelimeler:** *Leptinotarsa decemlineta*, biopestisit, mide zehiri, *Heracleum platytaenium*, *Humulus lupulus*, bitki ekstraktı

## INTRODUCTION

Worldwide, potato is the fifth highest crop by acreage (Anonymous 2015a). The tubers are rich source of proteins, carbohydrates, minerals (K, Mn, Mg, Fe, Cu and P) and vitamins (C, B1, B3, B6, K, folate, pantothenic acid) (Alisdair et al. 2001). Turkey is among the important potato producing countries of the world. Area under potato cultivation was around 130000 hectares and total potato production was nearly 5 million tones in 2014 (Anonymous 2015b).

However per acre production of potato in Turkey is less as compared to many countries primarily due to attack of insect-pests. Around 270 insects and 17 mite species are reported on potato under field and storage conditions worldwide. In case of severe insect attack, yield losses may reach to 100% (Chalfant 1990). The CPB is considered as one of the most serious insect pest of potato crop (Weber and Ferro 1994) and is also a vector of bacterial ring rot disease (Christie et al. 1991). It is a multivoltine insect and uncontrolled populations can completely defoliate potato plants/crop during the growing season (Hsiao and Fraenkel 1968). Severe attack during the plant growth cycle may lead to 100% yield loss (Hare 1980). Chemical control is most commonly used strategy for management of insect-pests (Jaleel et al. 2014, Alkan et al. 2015). The CPB has an important role in creating the modern pesticide industry, with hundreds of chemicals tested against it (Alyokhin 2009).

Insecticides are heavily used to control insects by killing them or preventing them from eating in undesirable behaviors (Afzal et al. 2015). The frequent and uncheck use of insecticides can lead to phytotoxicity and resistance problem (Stewart et al. 1997, Mota-Sanchez et al. 2000). Ability to adapt to toxic substances together with high selection pressure eventually resulted in a large number of insecticide-resistant CPB populations. It has developed resistance to 52 different compounds belonging to all major classes of insecticides (Whalon et al. 2015). Resistance levels vary greatly in different populations and beetle life stages, but in some cases can be very high (up to 2,000-fold) (Alyokhin 2009). The frequent use of

insecticides can also affect the physiological make-up of the target pests by causing changes in reproduction parameters, growth and development (Afzal et al. 2015).

Since 1990, the bio-pesticides have been used commercially especially when the problems associated with the intensive use of pesticides against various pest species started (Nitao 1987, Pascual-Villalobos and Robledo 1999, Chiasson et al. 2004, Thacker 2002). Biopesticides (plant based) are generally made from the secondary metabolites that are synthesized after pest attacks or unfavorable conditions by the plants (Bourgau et al. 2001). They are natural repellents, antifeedants and have toxic properties against many insect pest species (Mordue (Luntz) and Nisbet 2000). Although there are many reports regarding insecticidal properties of plant extracts, the practical application of them is still limited (Hassan and Gökçe 2014).

An alternative control strategy is needed against CPB to delay the resistance development against insecticides and also to manage the negative impact of insecticides on the environment and human health. As pointed out above, the plant extracts have various modes of action against insect pests and they might have important roles in eco-friendly approaches to manage the pests. In this study, the residual toxicity of six different plants extracts were tested on various development stages of CPB. Additionally, dose-response bioassay was carried out with *H. platytaenium* and *H. lupulus* extracts.

## MATERIALS AND METHODS

The experiment was conducted at Laboratory of Entomology, Department of Plant Protection, Gaziosmanpasa University, Tokat, Turkey.

### Collection of plant materials

Collected plants species, their material used in experiment and their location are listed in Table 1. Samples were collected in summer and spring during the year 2009 (Gökçe et al. 2005). Plant materials (seeds, leaves, stem and flowers) were washed with distilled water to remove dust particles and dried at room temperature for two weeks in the laboratory. Samples were ground to get fine powder using grinder (M-20 IKA Universal Mill, IKA Group, Wilmington, NC, USA) and then preserved in glass jar (25 cmL×12cmW) at 15±5°C for further usage.

Table 1. Name of selected plants species, their analyzed part and location of collection

Botanical Name	Family	Analyzed part	Location
<i>Humulus lupulus</i>	Cannabaceae	Cone	Tokat, Turkey
<i>Heracleum platytaenium</i>	Apiaceae	Leaf, Stem	Trabzon, Turkey
<i>Achillea millefolium</i>	Asteraceae	Leaf, Stem	Tokat, Turkey
<i>Acanthus dioscoridis</i>	Acanthaceae	Leaf, Stem	Erzincan, Turkey
<i>Phlomoïdes tuberosa</i>	Lamiaceae	Leaf, Stem	Erzincan, Turkey
<i>Bifora radians</i>	Apiaceae	Leaf, Stem	Tokat, Turkey

### **Preparation of plant extracts**

Plant extracts were prepared by using maceration method (Alkan and Gökçe 2012). The hexane, ethyl acetate and methanol were added according to their polarities into 200g plant sample using a glass jar. The samples were treated with hexane till 48 hours and suspension was filtered from plant material using filter paper. Later on, plant material was treated with ethyl acetate till 48 hours and suspension obtained from ethyl acetate extract was again filtered using filter paper. Finally, methanol was added to the plant materials, and same procedure was adopted for methanol extraction. The suspension was placed in an evaporator (RV 05 Basic 1-B, IKA® Werke GmbH & Co. KG, Germany) and excess solvent was evaporated. Methanol extracts obtained from *H. lupulus*, *B. radians* and *A. millefolium* were transferred into glass tubes and stored at 4°C in the refrigerator for further use.

### **Rearing of Colorado potato beetle**

The CPB larvae were reared at Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection (Gökçe et al. 2006). The CPB colony was continuously reared on potato plants (*Solanum tuberosum* L. cultivar Granola) which were planted at Gaziosmanpasa University Research Station in Tasliciftlik, Tokat, Turkey. Field was designated for organic potato production and there were no pesticide application for 3 years prior to the initiation of this project. Granola cultivar was planted in a 0.2 ha potato field. When the potato plants reached 3-5 leaves stage *L. decemlineata* adults were released into the field and all required stages for the studies were collected from that field.

### **Single dose efficacy trails**

The extracts were diluted with acetone to obtain the concentration of 10.00% plant extract in acetone (w/v). Each side of potato leaves were sprayed with 20 µl of plant extracts suspension using a hand spray. Spinosad (Laser®, Dow Agro Sciences write E.C or percent formulation) was used as negative control at recommended dose of 0.1 ml/L for *L. decemlineata*. Spinosad suspension (20 µl) was applied to each side of potato leaves. Simple acetone (20 µl) was applied to potato leaves in control. During the first phase of the study, all plant extracts were screened against 3<sup>rd</sup> instar larvae of the CPB. In the second phase, *H. platytenium*, *H. lupulus* extracts and spinosad were also tested on 2<sup>nd</sup> and 4<sup>th</sup> instar larvae. In each replicate, 15 *L. decemlineata* larvae were used in three different groups as 5 larvae were released on each treated potato leaf. The mortality was recorded after 24, 48 and 72 hours of treatment. The experimental trials were performed using completely randomized design (CRD) with eight treatments and three replications.

### **Dose-response bioassay**

Based on the single dose screening test, further dose-response bioassays were carried out with *H. platytenium* and *H. lupulus* extracts. Four different concentrations of these plants extracts i.e. 1%, 3%, 5% and 7.5% (w/v) were tested

on 3<sup>rd</sup> instar larvae. The treatment was carried out as described above. The larval mortality was recorded after 72 hours. Completely randomized design (CRD) was used for the experiment set up and each treatment was replicated thrice. Each replicate contained three groups of 5 larvae and total 45 larvae were used at each dose.

### Statistical analysis

Percent mortality results were subjected to arcsine transformation. Data were subjected to analysis of variance and differences among treatment were analyzed by Tukey Multiple Comparison Test ( $\alpha = 0.05$ ). All statistical analysis were done using MINITAB<sup>®</sup> version 16 software package (Minitab Inc. US2010).

## RESULTS AND DISCUSSION

### Single dose bio-assays

In single-dose screening test, methanol extracts of 6 plants were tested on 3<sup>rd</sup> instar larvae of CPB. All plant extracts, except *A. dioscoridis* and *P. tuberosa*, caused mortality of CPB larvae after 48 hours (Table 2). Highest percentage mortality (43.20%) of CPB larvae was recorded in *H. platytaenium* treatment. This was followed by *H. lupulus* extract with 27.20% mortality. Negative control (spinosad) was very toxic to 3<sup>rd</sup> instar larvae and caused 98.30% mortality at 48 h. The mortality rates recorded from these two extracts and spinosad were statistically different to a great extent from other treatments ( $F=58.79$ ,  $df=7.15$ ,  $P<0.05$ ). These results are in conformity with the work of Çam et al. (2012) who tested different plant extracts against various developmental stages of CPB and reported that the plant extract, *H. lupulus* was effective against all the stages of CPB except adult stage. However, our results are different from some other studies (Ermel et al. 1983, Pavela et al. 2008, Sajfirtova et al. 2008), which could be result of difference in collection place and time of the plant species and difference in extraction methods that were used in the studies. Collecting time and place of plant species affect the specific amount of chemical molecules present in leaves and also the extraction methods are directly related to the presence of chemical compounds in the extracts e.g.  $\alpha$ - Thujame,  $\alpha$  - Pinene, Sabinene, 1,8- Cineole, camphor and  $\rho$  - Cyr man -8-ol. These factors play important roles for inducing the insecticidal properties of extracts (Karuppusamy and Muthuraj 2011). Promising results of *Heracleum* spp. extracts against insect-pests like *Sitophilus zeamais* and *Tribolium castaneum* adults are known (Chu et al. 2012). *Heracleum* spp. essential oils contain aliphatic esters, terpenes and these components are known to have antibacterial, antifungal, anti-dermatophytic activities (Torbati et al. 2013). The stomach poison activity of *H. platytaenium* observed in this study could be related with the presence of above chemical compounds in leaves of *H. platytaenium* selected for extraction.

Table 2. Stomach poison effects of plant extracts and spinosad to 3<sup>rd</sup> stage larvae of CPB after 48 hours

Treatment	Mortality% $\pm$ SD*
Control	0.00 $\pm$ 0.00 e
<i>Humulus lupulus</i>	27.19 $\pm$ 1.77bc
<i>Heracleum platytaenium</i>	43.19 $\pm$ 2.05 b
<i>Achillea millefolium</i>	11.61 $\pm$ 0.14 cd
<i>Acanthus dioscoridis</i>	0.00 $\pm$ 0.00 e
<i>Bifora radians</i>	6.67 $\pm$ 0.00 de
<i>Phlomis tuberosa</i>	0.00 $\pm$ 0.00 e
Spinosad	98.30 $\pm$ 3.37 a

The mean follow-up of the column that different lowercase letters, shows that the average differs significantly (ANOVA  $P < 0.05$ , Tukey test).

\* SD: Standard Deviation

*H. platytaenium* and *H. lupulus* extracts alongside with spinosad as a chemical standard 1 were tested against the 2<sup>nd</sup> instar larval stage of CPB (Table 3). Plant extract of *H. lupulus* caused about 98.30% mortality that was statistically similar to the chemical standard, spinosad treatment. These two treatments were statistically significantly different from both *H. platytaenium* (46.70% mortality) and control (0.00% mortality) ( $F=146.29$ ,  $df=3.9$ ,  $P < 0.05$ ). First three stages of CPB are more susceptible to the plants extracts than bigger stage larval instars (Scott et al. 2003, 2004; Çam et al. 2012).

Table 3. The stomach poison effect of plant extracts and spinosad to 2<sup>nd</sup> stage larvae of CPB after 48 hours

Treatment	Mortality% $\pm$ SD*
Control	0.00 $\pm$ 0.00 c
<i>Heracleum platytaenium</i>	46.65 $\pm$ 0.90 b
<i>Humulus lupulus</i>	98.30 $\pm$ 3.37 a
Spinosad	98.30 $\pm$ 3.33 a

The mean follow-up of the column that different lowercase letters, shows that the average differs significantly (ANOVA  $P < 0.05$ , Tukey test).

\* SD: Standard Deviation

The plants extracts were also tested on the 4<sup>th</sup> instar larvae of CPB. There was a significant differences among the mortality rates of 4<sup>th</sup> instar larvae of CPB when they were fed on *H. lupulus*, *H. platytaenium* and spinosad treated potato leaves ( $F=44.80$ ;  $df=3.11$ ,  $P < 0.05$ ). Interestingly, *H. platytaenium* extracts produced 13.10% mortality after 48 hours and it was less than those were seen on 2<sup>nd</sup> and 3<sup>rd</sup> stage. *H. lupulus* extracts stomach poison activity fluctuated depending on the tested stage of CPB and it caused 46.50% mortality after 48 hours. Spinosad activity remained at the similar level and 93.30% mortality was observed in the insecticide treated replicates (Table 4). Similar results were also reported by other researchers that the plants extracts are effective on the immature stages of the CPB (Scott et al. 2004).

Table 4. Stomach poison effects of single concentration of plant extracts and spinosad to 4<sup>th</sup> stage larvae of CPB after 48 hours

Treatment	Mortality %± SD*
Control	0.00±0.00 d
<i>Heracleum platytaenium</i>	13.05±3.64 c
<i>Humulus lupulus</i>	46.54±3.62 b
Spinosad	93.33±0.00 a

The mean follow-up of the column that different lowercase letters, shows that the average statistical significantly different (ANOVA P <0.05, Tukey test).

\* SD: Standard Deviation

### Dose-response bioassay

Dose-response bioassay with the most promising plant extracts (*H. platytaenium* and *H. lupulus*) revealed that toxicity of plant extract was not directly proportional to tested dose of plant extracts in this study. Population treated with *H. platytaenium* extract showed 100% mortality at 5.00% and 7.50% tested concentrations. Whereas 95.80% mortality was recorded at 3.00% concentration followed by 92.20% mortality at 1.00% tested concentration (Table 5). These results suggested that 1.00% concentration of *H. platytaenium* could be the best option for testing stomach poison effect of this plant. This could be due to the larval preference of feeding on leaflets that were treated with low concentration of plant extract. As a result, the more active ingredient entered the insect's body and leading to the death of the CPB larvae. Similar results were reported by Wawrzyniak and Lamparski (2006) who tested different plant extracts effects on CBP feeding and development. Maximum mortality i.e. 58.91% was obtained at 3.00% concentration followed by 53.35% mortality at 7.50% concentration of *H. lupulus* extracts. Around 51.12% mortality was observed in case of leaves treated with 5.00% concentration. In control treatments mortality wasn't recorded. This low percent mortality observed in *H. lupulus* treatments comparing with *H. platytaenium* could be due to repellent effects of the plants extracts (Gökçe et al. 2012).

Table 5. Dose-response results of 3<sup>rd</sup> stage *Leptinotarsa decemlineata* larvae treated with various concentration *Heracleum platytaenium* and *Humulus lupulus* after 72 hours

Treatment	Tested Concentrations	Mortality%± SD*
<i>Heracleum platytaenium</i>	1.00%	92.24±7.46 b
	3.00%	95.81±5.85 ab
	5.00%	100.00±0.00 a
	7.50%	100.00±0.00 a
	Control	0.00±0.00c
<i>Humulus lupulus</i>	1.00%	0.00±0.00 b
	3.00%	58.91±8.72 a
	5.00%	51.12±3.42 a
	7.50%	53.35±1.01 a
	Control	0.00±0.00 b

The mean follow-up of the column that different lowercase letters, shows that the average statistical significantly different (ANOVA P <0.05, Tukey test).

\* SD: Standard Deviation

In this study, the stomach poisoning effects of different plant extracts were tested against various larval stages of CPB. Greatest stomach poison activity was found in case of plant extracts of *H. platytaenium* and *H. lupulus*. Dose efficacy studies indicate that even 1.00% concentration of *H. platytaenium* could be effective in controlling the beetle larvae. However, further field studies are needed to evaluate the original potential of this plant extracts. Meantime, isolation and characterization of active compound(s) from this plant extract should be studied for development of this plant extract as a plant based bio-pesticide for controlling CPB.

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