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Original article (Orijinal araştırma)

Toxicological and behavioral effects of some plant extract on Colorado potato beetle, *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae)[1]

Bazı bitki ekstraktlarının Patates böceği [*Leptinotarsa decemlineata* Say,1824 (Coleoptera: Chrysomelidae)]'ne karşı toksikolojik ve davranışsal etkileri

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Summary

Repellent, ovicidal and oviposition deterrent effects of six plant extracts [*Heracleum platytaenium* Boiss (Apiaceae), *Humulus lupulus* L. (Cannabaceae), *Achillea millefoliu*m L. (Asteraceae), *Acanthus dioscoridis* L. (Acanthaceae), *Phlomoides tuberosa* (L.) Moench (Lamiaceae), *Bifora radians* Bieb. (Apiaceae)] were tested on *Leptinotarsa decemlineata* Say, 1824 (Coleoptera: Chrysomelidae) under laboratory conditions. Methanol extracts prepared from plant vegetative components were tested on *L. decemlineata*. *Heracleum platytaenium* extract was significantly more toxic against the egg stage than all other extracts, except for *A. millefolium* 5 d after treatment. It was followed by *A. millefolium* extract reducing the egg hatch rate to 15%. Significant mortality was not observed in the case of other plant extracts. In the second series of experiments, different dose-response bioassays with *H. platytaenium* against *L. decemlineata* eggs were conducted. The lowest egg hatch rate of 1% was observed at 7.5% [w/v (plant extract/acetone]. The greatest oviposition deterrent effect was seen with the *H. platytaenium* extract treatment, which resulted in no egg laying. Plant extracts showed a high level of repellent activity to *L. decemlineata* and their activity increased with extended incubation time. The greatest repellency was observed with the *A. millefolium* extract treatment, which gave 0.01% repellency in the first 15 min. These results show that *H. platytaenium* extract could be a useful toll in the control of *L. decemlineata*.

Keywords: Biopesticide, Colorado potato beetle, ovicidal effect, plant extract, repellency

Özet

Altı bitki ekstraktının [*Heracleum platytaenium* Boiss (Apiaceae), *Humulus lupulus* L. (Cannabaceae), *Achillea millefoliu*m L. (Asteraceae), *Acanthus dioscoridis* L. (Acanthaceae), *Phlomoides tuberosa* (L.) Moench (Lamiaceae), *Bifora radians* Bieb. (Apiaceae)], *Leptinotarsa decemlineata* Say 1824 (Coleoptera: Chrysomelidae)'ye karşı repellent, ovisidal ve yumurta bırakmayı engelleyici etkileri test edilmiştir. Bitkilerin vejetatif aksamlarından hazırlanan methanol ekstraktları *L. decemlineata* üzerinde test edilmiştir. En yüksek ovisidal etki beşinci gün itibari ile *H. platytaenium* uygulamasında gözlemlenmiş ve bunu *A. millefolium* ekstraktının etkisi izlemiştir. *Heracleum platytaenium* ekstraktının etkinliğini %15 yumurta açılım oranı ile *A. millefolium* ekstraktının etkinliği takip etmiştir. Diğer bitki ekstraktlarında önemli ölüm gözlenmemiştir. İkinci seri denemelerde Patates böceğinin yumurtalarına karşı *H. platytaenium*'un doz-etki çalışmaları yürütülmüştür. Önemli derecede düşük yumurta açılım oranı sadece %7.5 [w/v (bitki ekstraktı/aseton)] konsantrasyonda %1 etki ile gözlemlenmiştir. En yüksek yumurta bırakmayı engelleyici etki *H. platytaenium* ekstraktında görülmüş ve bu muamelede hiç yumurta bırakılmamıştır. Bitki ekstraktları yüksek oranda repellent aktivite göstermiş ve bu aktivite inkübasyon süresine bağlı olarak artmıştır. Test edilen bitki ekstraktları arasında en yüksek derecede uzaklaştırıcı etki %0.01'lik oransal değer ile ilk 15 dakikada *A. millefolium* ekstraktında saptanmıştır. Bu sonuçlar *H. platytaenium* ekstraktının *L. decemlineata* kontrolünde önemli bir potansiyele sahip olduğunu ortaya koymuştur.

Anahtar sözcükler: Biyopestisit, Patates böceği, yumurta bırakmayı engelleyici etki, bitki ekstraktı, repellent

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Introduction

Leptinotarsa decemlineata Say, 1826 (Coleoptera: Chrysomelidae), Colarado potato beetle is a polyphagous pest, and occurs over an area of 12 million km^2 in North America, Asia and Europe (Alyokhin, 2009). It causes serious damage to various crops from the Solanaceae family including potato, tomato and eggplant (Hsiao, 1978; Hare, 1990) and in the absence of pest control losses may reach 100% (Christie et al., 1991). Additionally, it is also a vector of certain plant viruses (Borror & De Long, 1966; Kısmalı, 1973; Jolivet et al., 1988; Booth et al., 1990).

Control of *L. decemlineata* almost totally relies on insecticides. Heavy insecticide usage to control *L. decemlineata* has led to many environmental problems (Ioannidis et al., 1991; Stewart et al., 1997; Mota-Sanchez et al., 2000). Insecticide resistance is the most serious problem in managing *L. decemlineata,* since it has become resistant to 54 compounds (Whalon et al., 2013). Alternative control methods for *L. decemlineata* are urgently needed for insect resistance management programs. Use of biopesticides in the control of pests has become common since 1990 following outbreaks of resistance of many insect species to synthetic insecticides. Biopesticides including microorganisms, including fungi, nematodes, bacteria and plant secondary metabolites, are important tools for management of resistant pest species. Plant secondary metabolites, e.g., terpenes, nitrogen containing compounds and phenolic compounds, are mainly produced when plants are stressed, e.g., by drought or pest attacks. These metabolites have been tested against many insect pest species and promising results for control of *L. decemlineata* have been reported (Hough-Goldstein, 1990; Scott et al., 2003, 2004; Gökçe et al., 2005, 2006, 2011; Alkan et al., 2015; Tampe et al., 2015).

Solvent systems used for extraction of plant secondary metabolites have effects on both yield and composition. Especially plant waxes, that cause low viscosity in the plant extracts suspension, are removed from the plant residue using non-polar solvent, e.g., hexane is used to obtain a more uniform plant residue (Hassan & Gökçe, 2014). Additionally, removing of plant waxes from the extracts may also increase bioactive compound concentrations in the plant extract suspension. In the present study, secondary metabolites from plants having various activities to different insect species (Gökçe et al., 2005, 2011), were extracted with different solvent systems and tested against the important potato to pest, *L. decemlineata*. The objectives of the study were to evaluate the selected plant extracts behavioral effects, including repellence, oviposition deterrence and ovicidal effects and to determine effective dose for specific activity. To achieve these objectives, the plant extracts were tested against *L. decemlineata* adults and dose-response bioassays were also conducted with promising extracts.

Material and Methods

Collection of plant materials

Details of the plant material used are presented in Table 1. The plants were identified to species at Istanbul University with reference to herbarium samples. The plants were collected in the spring and summer of 2009 as described by Gökçe et al. (2006). Plant materials were passed through preliminary purification process in that the plants parts to be used were separated from other parts. The plants parts, cone, leaf and stem, were placed on blotting papers in a dark room and were left to dry at room temperature (25°C) for 2 weeks. The dry plant materials were ground into small pieces by using a mill (M20 IKA Universal Mill, IKA Group, Wilmington, NC, USA) and transferred to 5-L glass jars and placed in a dark room at 15±5°C until used.

Table 1. Plants, analyzed parts and place of collection in Turkey

Botanical Name	Family	Analyzed part	Sample location
Humulus lupulus L.	Cannabaceae	Cone	Tokat
Heracleum platytaenium Boiss	Apiaceae	Leaf, Stem	Trabzon
Achillea millefolium L.	Asteraceae	Leaf, Stem, Flower	Tokat
Acanthus dioscoridis L.	Acanthaceae	Leaf, Stem, Flower	Erzincan
Phlomoides tuberosa (L.) Moench	Lamiaceae	Leaf, Stem, Flower	Erzincan
Bifora radians Bieb.	Apiaceae	Leaf, Stem	Tokat

Preparation of plant extracts

Plant extracts were obtained by maceration method as described by Alkan & Gökçe (2012). *Acanthus dioscoridis* L., *Heracleum platytaenium* Boiss and *Phlomoides tuberosa* (L.) Moench extracts were processed using three different solvents (hexane, ethyl acetate and methanol) to remove the plant waxes that prevent homogenous plant extract suspension being obtained. Two hundred g of dried plant material for each species were put into separate glass jars and then treated with solvents (hexane, ethyl acetate and methanol) according to their polarity range. The plant materials were first treated with hexane for 48 h, then the plant suspension was separated from plant materials using Whatman No. 4 filter paper. After that, the separated plant materials were treated with ethyl acetate further 48 h at room temperature. The plant suspensions were again filtered through the filter paper to separate the plant parts. Finally, methanol was added on the plant materials and then incubated 48 h under the same conditions. Similarly, the methanol soluble plant extracts were separated from the plant material using the filter paper. Excess hexane, ethyl acetate and methanol were evaporated from the plant suspension using a rotary evaporator (RV 05 Basic 1-B, IKA-Werke GmbH & Co. KG, Staufen, Germany). *Humulus lupulus* L., *Bifora radians* Bieb. and *Achillea millefolium* L. were only extracted with methanol because these plant species contain limited amount of the plant waxes. The total yield of plant extracts was about 10% of their dry weight. The plant residues were transferred into glass tubes, and stored at 4ºC. The methanol extracts were used in all the experiments described below.

Rearing of *Leptinotarsa decemlineata*

Leptinotarsa decemlineata larvae were reared at Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection as described by Gökçe et al. (2006). *Leptinotarsa decemlineata* colony was continuously reared on potato plants (*Solanum tuberosum* L. cv. Granola) which were planted at Gaziosmanpasa University Research Station (Taşliçiftlik, Tokat, Turkey) in a 0.2-ha field used for organic potato production with no pesticide application in the previous 3 years. The potato tubers were planted in the first week of April each year. When the potato plants reached to three- to five-leaf stage*, L. decemlineata* adults from a lab colony were released into the field and all stages used in for this study collected when needed.

Single dose ovicidal effects

The ovicidal effects of the six plant extracts were tested on *L. decemlineata* egg masses 1 to 3 d old. Plant extracts were diluted with 70% acetone to give the concentration of 10% (w/v) plant extract/acetone suspension. Twenty µl of each plant extract suspension were applied to each egg mass (n ≈ 20 eggs) using a hand spray. In the control group, each mass was treated with 20 µl of 70% acetone. The egg masses on potato leaflets were then transferred into Petri dishes and the petiole of each leaflet was covered with a distilled water soaked cotton wool to prevent the leaflets withering. Egg hatch was recorded up to 7 d. Bioassays were set up in the randomized block design. The experiment was repeated on three different days (blocks) and each treatment in a block contained three subset groups of egg mass (n ≈ 60). Total around 180 eggs were used for each treatment.

Dose-response study with *Heracleum platytaenium* extract

Based on the single dose screening ovicidal test, a dose-response bioassay was carried out with *H. platytaenium* extract, given it gave the greatest ovicidal effect. The plant extract was diluted with 70% acetone to give 3, 5 and 7.5% (w/v) plant extract in acetone. The application of plant extract and incubation of egg masses were conducted as described above. Randomized block design was used and all treatments were replicated three times with three replicates of each dose and the control groups.

Oviposition deterrent effects

Oviposition deterrent effects of *H. platytaenium* and *H. lupulus* extracts, the most bioactive extracts in the preliminary test, were tested against *L. decemlineata* adults. Plant extract suspensions were prepared as described in the single dose ovicidal effect tests. A 3-5-leaf stage potato plant was sprayed with one of the extracts until run off using a hand spray. Control plants were treated with 70% acetone. The treated potato plants were left to dry at room temperature for 30 min. A choice-test was used for this experiment. One treated plant and one control plant were placed inside a curtain net cage, 60 x 60 x 60 cm, and two *L. decemlineata* adults (one female and one male) were released inside the cage and these were then incubated at 27ºC and a 16:8 h L:D photoperiod for 7 d. Paired treatments were replicated nine times. The number of eggs laid on the plants were recorded.

Repellent effects

In the repellent effect tests, the six plant extracts were screened against *L. decemlineata* adults. The plant extracts were prepared at 5% (w/v) in 70% acetone. Similar sized potato leaves, consisted of six to eight leaflets, were sprayed with a hand spray until run off. After application of the extracts, the petioles were wrapped with cotton wool moistened with distilled water to prevent the leaves wilting and the leaves left to dry under a fume hood for 30 min. A choice-test was used in the experiment. Two potato leaves (one treated and one control) were placed in a 150 x 300 x 50 mm plastic container. Ten mixed-sexed *L. decemlineata* adults were released into each container. To test both repellence and desensitizing of the adult beetles, the number of adults on each leaf was recorded after 15 min and 1, 12 and 24 h after release. The trial was repeated nine times for each plant extract-control paired combination.

Statistical analysis

The ovicidal test results were converted into percentages and arcsine transformed. The transformed data was analyzed by analysis of variance (ANOVA) and the means compared by Tukey's multiple comparison test. The number of egg laid in the oviposition deterrent test were compared with the paired t-test ($P < 0.05$). The results obtained in the repellence tests were converted to percentages and arcsine transformed. The converted data were analyzed with the paired-t test ($P < 0.05$).

Results and discussion

Heracleum platytaenium and *A. millefolium* extracts had the greatest ovicidal effect when compared with other extracts 5 d after treatment (DAT) ($F = 53.3$; $df = 6,14$; $P < 0.05$). The other extracts caused no significant reduction in hatching rate with 84.7% egg hatch rates for *H. lupulus*, 99.7% for *P. tuberose,* and 100% for both *B. radians* and *A. dioscoridis* 5 DAT (Table 2). *Heracleum platytaenium* and *A. millefolium* extracts produced similar ovicidal effects 6 DAT and these extracts were significantly different from the other treatments ($F = 276$, $df = 6,14$; $P = 0.05$). The hatch rates were nearly 100% for extracts 6 DAT. At 7 DAT, *H. platytaenium* and *A. millefolium* extracts remained significantly different from other treatments ($F = 276$; $df = 6,14$; $P < 0.05$) with 3.7% and 18.6 % hatching rates, respectively (Table 2).

Table 2. Ovicidal effect of plant extracts on *Leptinotarsa decemlineata* eggs over time

Extracts	Egg hatch rate ± SEM* (%)		
	5 DAT**	6 DAT	7 DAT
Control	99.4±0.47 ab***	99.4±0.47 a	99.4±1.40 a
Heracleum platytaenium	2.1±1.05 c	3.7±0.84 c	3.7±0.84 c
Humulus lupulus	84.7±15.18 b	100.0±0.00 a	100.0±0.00 a
Achillea millefolium	15.1±0.46 c	18.7±0.52 b	18.7±0.52 b
Bifora radians	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
Acanthus dioscoridis	100.0±0.00 a	100.0±0.00 a	100.0±0.00 a
Phlomoides tuberosa	99.7±0.37 ab	99.7±0.37 a	99.7±0.37 a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the same letter are not statistical significantly different (ANOVA P < 0.05, Tukey's test).

The testing of different doses of *H. platytaenium* extract on *L. decemlineata* eggs showed that the ovicidal effect of different concentrations were statistically different from each other and the control, except at 3%. The data indicated that the increasing concentration of *H. platytaenium* significantly decreased the egg hatch of *L. decemlineata* over time (5 DAT, F = 37.7; 6 DAT, F = 39.5; 7 DAT, F = 39.5; df = 3,9; P < 0.05) (Table 3). The greatest ovicidal effect was seen at 7.5% concentration, and it was only 1% at this concentration during the incubation period. This was followed by the 5% concentration, for which relatively low rate of egg hatch (<44%) was observed compared to the other concentrations and the control.

Table 3. Ovicidal effect of *Heracleum platytaenium* concentrations on *Leptinotarsa decemlineata* eggs

Extract concentration (%)	Egg Hatch Rate ± SEM* (%)		
	5 DAT**	6 DAT	7 DAT
7.5	1.0±0.59 c***	1.0±0.59 c	1.0±0.59 c
5	26.9±1.53 b	43.4±3.01 b	43.3±3.01 b
3	82.6±9.56 a	88.5±7.40 a	88.5±7.40 a
Control	99.4±0.44 a	99.7±0.44 a	99.4±0.44 a

* SEM: Standard error of the mean;
** DAT: Days after treatment;
*** Means in a column followed by the letter are not statistical significantly different (ANOVA, P < 0.05, Tukey's test).

The *H. platytaenium* extract had the greatest ovicidal effect in the single dose ovicidal test. A previous study on chemical components of *H. platytaenium* essential oil revealed that its essential oil contains isopropyl butyrate, octanal, heptyl acetate, hexyl butyrate, octyl acetate, (Z)-4-hexenyl butyrate, decanal, octanol, hexyl hexanoate and octyl butyrate (Iscan et al., 2004). Among these components, octyl acetate was the most abundant (Kürkçüoğlu et al., 1995) and is well known for being detrimental to insects (Carroll & Berenbaum, 2002). The *H. platytaenium* extract caused a significant reduction in egg hatch of *L. decemlineata*. The ovicidal activity of *A. millefolium* extract was not as high as that of the *H. platytaenium* extract, and it was the second most toxic extract among those tested. The other plant extracts did not show any ovicidal effects against *L. decemlineata* eggs. This difference in ovicidal activity of the tested extracts is likely to be related to their chemical composition. As mentioned above, octyl acetate is one of the main chemical component of *H. platytaenium* essential oil and this compound is known to have detrimental effects on insects. The ovicidal activity of *H. platytaenium* extract could be

attributable to this compound but further studies are needed to characterize the actual active compound(s). Notably, *H. lupulus* extract did not have any ovicidal effect on *L. decemlineata* eggs even though this extract is known to have high contact toxicities against the larvae and adults of this pest (Gökçe et al., 2006). This could be due to the chemical differences between the cuticle of egg and larvae. Solvent systems used for extraction of plant species have effects on both yield and composition. This leads to differences in insecticidal activities of the extracts. Arivoli & Tennyson (2013) showed that the solvent used for the preparation of plant extracts also effect the ovicidal activity of the extracts. Additionally, the ovicidal activity of the same plant ranged from 6 to 66% depending on the extraction solvent used (Jeyasankar et al., 2013).

The data presented in Table 4 shows that extracts of both *H. platytaenium* and *H. lupulus* had an oviposition deterrent effect on the *L. decemlineata* females. The greatest oviposition deterrence was seen with *H. platytaenium* extract and this was statistically different from the control ($t = 4.66$; $P < 0.05$). At the end of the incubation period, there were no eggs deposited on plants treated with *H. platytaenium* extract. An average of 8.8 eggs were laid on the plants treated with *H. lupulus* extract, which was significantly different from the control ($t = 4.29$; $P < 0.05$) (Table 4). Although oviposition deterrent effects of different plant extracts have been documented, there is no reports on the activity of *H. platytaenium* extracts against any insect species (Rajkumar & Jebanesan, 2005; Rehman et al., 2009; Tomasek & Dvorak, 2009; Sezer & Özalp, 2011; Singh et al., 2014; Dehghani & Ahmadi, 2013). High oviposition deterrent activity of *H. platytaenium* can be due to presence of larger concentrations of octyl acetate and octyl butyrate (Carroll & Berenbaum, 2002). Various oviposition deterrent effect of *H. lupulus* against different insect species *L. decemlineata* was reported in previous studies (e.g., Gökçe et al., 2005) While *H. lupulus* extract did not display oviposition deterrent activity against *Choristoneura rosaceana* Harris, 1841 (Lepidoptera: Tortricidae) and *Argyrotaenia velutinana* Walker, 1863 (Lepidoptera: Tortricidae), it showed a strong effect against *L. decemlineata* (Gökçe et al., 2005). These differences in oviposition deterrent activity could be related to differences in the taxonomic group of the test insect or due to behavioral differences between insect species. For example, *C. rosaceana* and *A. velutinana* larvae feed on various fruit and forest trees as well as vegetables, so that their tolerance to any kind of odor may be higher than that of *L. decemlineata*.

Table 4. Oviposition deterrent effects of *Heracleum platytaenium* and *Humulus lupulus* extracts against *Leptinotarsa decemlineata*

Extracts	Number of laid eggs	
	Control	Extract
Heracleum platytaenium	86.3 a[1] (38.8-133.8)	0.0 b (0.0-0.00)
Humulus lupulus	59.2 a (34.1-84.3)	8.8 b (4.6-22.2)

[1]Means in a row followed by the same letter are not statistical significantly different (paired t-test, $P < 0.05$).

The plant extracts tested also showed significant repellence of *L. decemlineata* adults. Repellent effects of different plant extracts against insect pest species has been tested in many studies with some promising results (Schearer, 1984; Wyrostkiewicz, 1987; Chiasson et al., 1994; Mateeva, 1998; Przybylski, 2002; Pavela, 2004; Sarbu et al., 2004; Wawrzyniak & Lamparski, 2008). Among the plant extracts tested, the greatest repellency was observed with *A. millefolium* extract with 0.01% proportional repellency in the first 15 min. It was significantly different from the control ($t = 5.99$; $P = 0.00$). This was followed by *H. platytaenium* with 0.03% proportional repellency with most of the released adults moving to the control potato leaflets. Notably, neither *B. radians* nor *H. lupulus* extracts showed any significant repellency in the first 15 min. Desensitizing of insect species is one of the important factors affecting their successful use in pest control. However, in the present study the repellent activity of plant extracts was consistent over the entire incubation period (Figure 1). After 12 and 24 h, *A. millefolium*, *B. radians* and *A. dioscoridis* did not show any significant repellency when compared to the control. Notably, *H. lupulus* repellency increased as the incubation time was extended and the number of insect moving to the plant extract treated leaflets dropped to 0.03% proportional repellency after 24 h (Figure 1). A similar increase in activity of plant extracts with increasing incubation time was also reported by Alkan & Gökçe (2012),

who tested the contact toxicity and behavioral effects of *Tanacetum abrotanifolium* (L.) Druce, 1914 (Asteraceae) stem and flower extracts against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and reported that the plant extract repellency activity increased in parallel to the incubation time.

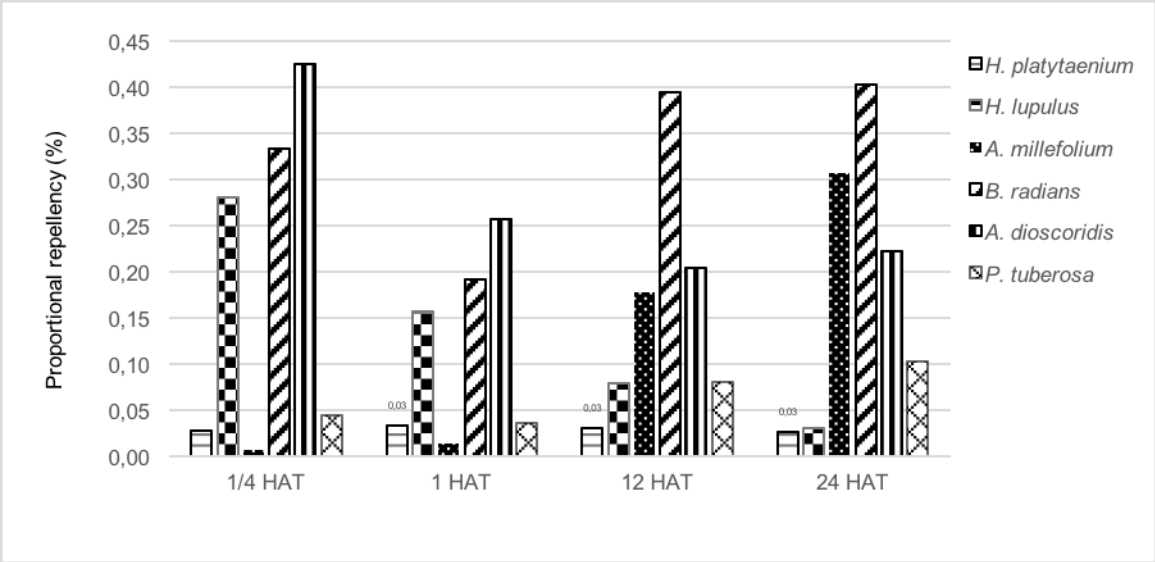

Figure 1. Proportional repellency of plant extracts against *Leptinotarsa decemlineata* at four time periods after treatment (HAT, Hours after treatment).

In the current study, ovicidal activity, oviposition deterrence and repellency of the extracts were tested against the *L. decemlineata*. Of the plant extracts tested, *H. platytaenium* extract showed the greatest ovicidal activity and repellency, and indicated that this extract has potential for development as biopesticide in a Colorado potato beetle control program. However, further studies are needed to elucidate the active compound(s) in this extract. Following the elucidation of active compound(s), further laboratory and field studies should be undertaken to examine the full potential of this plant extract in the control of *L. decemlineata*.

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