

THE EFFECTS OF SOME NATURAL SUBSTANCES ON ACANTHOSCELIDES OBTECTUS SAY (COLEOPTERA: BRUCHIDAE)

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Abstract

To date, numerous studies have documented the effectiveness of plant extracts, but the number of studies with *Acanthoscelides obtectus* (Col.: Bruchidae) is very low. This study was conducted to determine the effects of *Nicotiana tabacum* L., *Urginea maritima* (L.), *Euphorbia aleppica* L., *Nerium oleander* L. extracts and chitinase enzyme on *A. obtectus*, one of the main pests of stored legume grains. Neem Azal, a plant extract, and Pyriproxyfen, an insect growth regulator, were used as positive controls. It has been demonstrated for the first time that the effects of 4% dose of tobacco extract is exhibited as potential candidate on *A. obtectus* control with the effect of 68,25%. Additionally, it has been demonstrated with the results of this study that the mortality rates of some extracts (2% and 4% doses of spurge extracts, 2% doses of oleander extract, 4% doses of chitinase enzyme and 2 l/ha application dose of neem azal preparation) are higher than 50%.

Key words: *acanthoscelides obtectus*, bean weevil, bio-pesticides, plant extracts, stored products

1. INTRODUCTION

Phaseolus vulgaris L, the green bean, is a legume that originated from America. The bean seeds are a very rich source of vitamin A, B, D and contain %22 protein, %61 carbohydrate and % 1-2 oil (Şehirali, 1988). Especially in developing countries, dried bean is one of the most important nutrient source in the human diet. Bean plants can increase the amount of nitrogen fixation (64 kg/ha) in the soil through biological nitrogen fixation (Kün et al., 2005). Beans constitute an important group of plants in terms of crop rotation and meet the nitrogen needs of subsequent generations. Due to such reasons they are indispensable for vegetable gardens (Adams et al., 1985). Moreover, with its high protein content, bean straw is an important source of fodder for the TR71 region, one of livestock centers in Turkey (Ergül, 1988).

According to the data of 2012, dry beans with 28,780,377 hectares of cultivation area and 23,140,276 tons of production, rank first in the world production of edible legumes (Anonymous, 2014a). But with 93.174 hectares of cultivation area and 200.000 tons of production (Anonymous, 2014b) it ranks third in Turkey after the chickpeas and lentils (Önder et al., 2012).

The bean weevil (*Acanthoscelides obtectus* Say.) (Col: Bruchidae) which is quite common in Turkey, is one of the most important pest of beans (Anonymous, 2012). The body of adult bean weevil is oval and slightly flat. Female bean weevils are small beetles, ranging in size from 3,8-4,8 mm. Adult males, ranging in size from 3,1-4,2 mm, are smaller than females. The color is light or dark brown. The bottom of the body is reddish yellow. The legs are red and there is a bulge on the back end of the femur in the form of two small and one large spines. The range in egg diameter is 0,63 to 0,77 mm. Larvae, 0,6-0,8 mm in length, are white and pupa, 2,9-4,6 mm in length, are light brown.

It spends the winter as adults on stored crops or in naturally sheltered places. With the warm weather in the spring, after the first week of May, bean weevil becomes widespread in the fields. After the second week of July, females lay 4-20 eggs as individually or in clusters on drying legume capsules through inserting ovipositor into a crack which is on the fruit peel. A female *Acanthoscelides obtectus* may lay 41-108 eggs in her lifetime. The emerged larvae are spread over the fruit peel and after finding the grains, fruit shell is pierced and larvae enter the seed. Then the inlet opening is closed but a small point remains as a trace on the grain.

Larvae eat the interior part of the seed and this period takes a few weeks. Old larvae enter the pupal stage in a section near the peel of the seed. The pupal period lasts 9-12 days. The adult that results

from pupation emerges from the bean through a valve. More than one larva can enter the same seed and more than one adult emergence can be seen. Thus, the adults maintain their generations through passing other beans in the area.

They usually give 5-6 generations annually (Atak, 1975). Larvae fed on seeds constitute grooves, reduce the nutritional value of the seeds and pollute the seeds with excrement and body parts. In addition, they cause the loss of some properties such as grain quality, germination power and weight and market value (Anonymous, 2012).

Several groups of insecticides, especially organophosphorus compound, pyrethroids and fumigants, are widely used for controlling pests of stored grains. Excessive use of insecticides causes the development of resistance on the pest populations and leads some negative impacts on human and animal healths (Lee and Lees, 2001). These negative effects such as resistance problem and insecticide residues in products, constitute a need to develop alternative methods and their applications in control strategies against pests of stored grains. Researches on plant-based insecticides, as alternative pest control methods, has gained pace in recent years (Varma and Dubey, 2001; Lee et al., 2001; 2004; Karakoç et al., 2006; Negahban et al., 2007; Rajendran and Sriranjini, 2008; Chu et al., 2010). Plant extracts have a high potential for the development of agents for controlling of pests (Wink, 1993; Rahman and Schmidt, 1999). These agents may inhibit pest populations in different ways such as disruption of metabolic pathways, rapid death, attractive and deterrent factors, prevention of nutrition and ovulation and other negative effects on the insect development. Today over 2000 species of plants are known that possess some insecticidal activity. Some products derived from various plant species has been proven as toxic and repellent against pests of stored grains (Raja et al., 2001; Papachristos and Stamopoulos, 2002; Tapondjou et al., 2002; Isman, 2006).

Recently, plant-derived products have become more popular in our country and around the world (Civelek and Weintraub, 2004; Yanar and Düzdemir, 2012). In this study, the pesticidal effects of chitinase enzyme and the effects of some plants extracts such as *Nicotiana tabacum* L., *Urginea maritima* (L.), *Euphorbia aleppica* L., *Nerium oleander* L. on *A. obtectus*, bean weevil, were determined under the laboratory conditions.

2. MATERIALS AND METHODS

This research was carried out under laboratory conditions in Muğla Sıtkı Koçman University, Science Faculty, Biology Department, Entomology laboratory between April and July 2014. The main material of this study consists of *A. obtectus* and some natural compounds.

2.1. Care and Maintenance of Insect Culture

In this study we used *A. obtectus* laboratory stock, which is being maintained since 2010, on the dry bean seeds under laboratory conditions (28 ± 2 °C temperature and 65 ± 5 % relative humidity). Newly emerged adult *A. obtectus* were transferred from stock culture to the new culture containers and oviposition was provided for 48 hours. After this time, adult individuals were removed from the culture and new adult emergence were provided and these individuals were selected for the experiment.

2.2. Obtaining Plant Extracts

Plants and their body parts used in the study are presented in Table 1. The plants used for extraction were collected from Ortaca district of Mugla province between 2012 and 2013 when the plants are in their flowering period. Collected plants were transferred to the laboratory and dried in the drought and shade place. The dried plants were crushed and pulverized and then were packed with the filter paper to be 5 grams each and placed on the column of soxhlet apparatus. 250 ml water was used as a solvent. The transition of plant material into the water were provided through thermal treatment and evaporation (Civelek and Weintraub, 2004). Chitinase enzyme obtained from the Konsey Tarım (Antalya) and commercial preparations are sold by the manufacturer.

Table 1. The plants and body parts used in the study.

English Name	Scientific Name	Family	Extracted Body Part
Tobacco	<i>Nicotiana tabacum</i> L.	Solanaceae	Leaf
Spurge	<i>Euphorbia aleppica</i> L.	Euphorbiaceae	Leaf
Mountain Onion	<i>Urginea maritima</i> (L.)	Liliaceae	Leaf
Oleander	<i>Nerium oleander</i> L.	Apocynaceae	Leaf

Obtained plant extracts and chitinase were used at the doses of 1%, 2%, and 4%. At this point, in order to ensure a better homogenization of chitinase enzyme in the water, the mixture was stirred in the magnetic stirrer for a while until it became blurred white. Neem Azal (Verim Construction-Tourism, İstanbul), a plant extract, and Pyriproxyfen (Admiral 10 EC (Sumi Agro), an insect growth regulator, were used as positive control with the doses of 200 ml/ha and 50 ml/ha respectively. Although these preparations are not licensed formulations on bean seed beetle, their recommended doses for control of other pests were preferred in our study.

2.3. Applications and Counting

Three replicates were performed for each application and 20 bean seeds were used for each replicate. Plant extracts, chitinase and witnessed preparations were sprayed with 500 ml hand sprayers on bean seeds. The soaked bean grains were placed in different places to prevent the contact each other and were dried for 24 hours. Each container was labeled to avoid mixing of cultures. A total of 15 *A. obtectus* adults (randomly selected males and females) were transferred to labeled culture dishes and culture containers were covered with tulle in order to prevent the escape of individuals and to provide air. Cultures were regularly checked every day and surviving individuals were counted. The dead individuals were counted and removed from the culture with a soft forceps (Karakoç et al., 2006). Counting process was performed every 24 hours and recorded daily during seven days.

2.4. Statistical Analysis

The effects of extracts, chitinase and witness preparation were determined by applying the Henderson-Tilton formula to the obtained results. According to the % effects of the preparations used in the experiment, one-way ANOVA and Duncan test was conducted ($p=0,05$) through SPSS (15.0) Software package program.

3. RESULTS AND DISCUSSION

The average effects and Duncan test results obtained from this study, which was carried out under the laboratory conditions to investigate the effects of certain natural substances as an alternative to synthetic pesticides against *A. obtectus*, are shown in the Table 2. According to Table 2, the highest mortality rate and the effect (%) was determined at the highest dose of *N. tabacum* extract and the rate of death was determined to be parallel with the increase in application dose. According to statistical analysis, the effects of the highest concentration (4%) of tobacco extract was determined to be significant ($p<0,05$). The highest concentration (4%) of *E. aleppica* was also determined to be the most effective dose. Although the rate of death was determined to be parallel with the increase in application dose, all application doses (1, 2 and 4%) of *Euphorbia aleppica* were determined to be in the same statistical group. The lowest concentration (1%) of *U. maritima* was determined to be the most effective dose. According to statistical analyses, no significant variation was established among the application doses of *U. maritima*. The second application dose (%2) of *N. Oleander* was established to be the most effective dose and analyses, no significant variation was established among the application doses of *N. oleander*. The most effective application dose of chitinase enzyme was the third concentration (%4). The second and third concentrations of chitinase enzyme were determined to be in the same statistical group.

Table 2. The effects of different doses of natural substances applied by spraying on *A. obtectus* adults (Mean± Standard deviation).

Plant extract	Dose	Mortality Rate (%)	Effect (%)
<i>Nicotiana tabacum</i>	1 l/100 l	44,44	32,60±13,07ab
	2 l/100 l	48,25	30,27±16,47ab
	4 l/100 l	68,25	57,55±14,00a
<i>Euphorbia aleppica</i>	1 l/100 l	36,19	19,83±12,84ab
	2 l/100 l	55,56	45,42±3,11ab
	4 l/100 l	56,19	46,64±5,72ab
<i>Urginia maritima</i>	1 l/100 l	46,67	26,54±10,00ab
	2 l/100 l	46,35	25,13±5,02cab
	4 l/100 l	40,63	20,36±13,50ab
<i>Nerium oleander</i>	1 l/100 l	44,76	26,21±13,51ab
	2 l/100 l	61,27	48,62±8,52ab
	4 l/100 l	47,30	28,89±8,55ab
Chitinase	1 l/100 l	23,49	5,65±4,73b
	2 l/100 l	35,24	31,26±25,53ab
	4 l/100 l	53,33	41,40±23,65ab
Neem Azal®-T/S	2 l/ha	58,73	51,22±14,24ab
Admiral 10 EC	50 ml/ha	46,93	31,83±9,90ab
Control	-	22,06	-

* The small letters in the same column indicates that the averages are statistically significant (Anova $p<0,05$, Duncan test).

According to the results of the study, among the preparations used in the experiment the highest mortality rate was obtained with 4% doses of tobacco extract. Neem Azal showed the second highest effect (%) with the dose of 200 ml/da and it was determined to statistically close to the 4% dose of tobacco extract. The results obtained from the application dose of Admiral 10 EC were found to be close to the average results obtained from the study. The lowest mortality rate (22,06%) were seen in the control group during the trial.

To date, numerous studies have documented the effectiveness of plant extracts but the number of studies with *A. obtectus* is very low. According to the results of a study about the fumigant toxicity of some monoterpenoids on *A. obtectus* adults, LC_{50} value has been identified for 24 and 48 hours application time and linalool was found to be the most effective compound while estragole was the lowest toxic compound (Regnault-Roger and Hamraoui, 1995).

In another study, the contact effect of a volatile oil of Chrysanthemum on *Bemisia tabaci* (Genn.), *Tribolium castaneum* (Herbst), *A. obtectus* (Say) and *Ephestia kuehniella* (Zell.) has been investigated through in vitro bioassay methods. Rapid and devastating effects has been observed on *T. castaneum* and *E. kuehniella* larvae and on *A. obtectus* adults (Perez and Pascual-Villalobos, 1999).

The effects of some volatile oils extracted from lavender, eucalyptus and rosemary on *A. obtectus* larvae has been investigated and all extracts has been determined to be toxic (0.6-76 ppb LC_{50} values)

depending on the insect life stage and the structure of compound. An increase on larval tolerance with the growth of larvae has also been reported (Papachristos and Stamopoulos, 2002). Aslan et al., (2004), reported the toxic effects of some compounds of *Sideritis trojana* (sarıkız tea) such as entkaurene diterpenoids, 7-epicandicandiol and 18-acetylsideroxol on *A. obtectus*. Papachristos and Stamopoulos (2004), reported the fumigant activity of some essential oils, extracted from lavender, rosemary and eucalyptus, against *A. obtectus* eggs and LC₅₀ values were determined to be 1,3-35,1 ppb.

In another study, the fumigant effects of acetone on *A. obtectus* adults has been investigated and mortality rate has been determined to be connected with the exposure time to acetone vapour (Çetin et al., 2009). Düzdemir and Yanar (2012), evaluated the effects of some plant extracts, used against *A. obtectus*, on bean grain yield under field conditions. Some oil extracts such as azadiractin, cypermethrin and eucalyptus were used in the research. This study, however, was not found satisfactory considering the usage of herbal preparations against *A. obtectus* instead of using synthetic chemical pesticides.

As a result of the literature surveys, we did not encounter any research related to pesticidal effects of some preparations on the *A. obtectus*. According to the results of the study, the effects of tobacco extract (4%) is exhibited as potential candidate on the *A. obtectus* control studies with the effect of 68,25%. In addition, it has been demonstrated with the results of this study that the mortality rates of some extracts (2% and 4% doses of spurge extracts and 2% doses of oleander extract), 2 l/ha application dose of neem azal preparation and 4% doses of chitinase enzyme) are higher than 50%.

REFERENCES

- Adams, M.V, Coyne, D.P., Davis, J.H.C., Grahaw, P.H., Francia, C.A. (1985). *Grain Legumes Crops*. Collins, London, 478.
- Anonymous, (2012). *Baklagil tohum böcekleri Bruchus Spp. (Col., Bruchidae) zirai mücadele teknik talimatı*. (http://www.gkgm.gov.tr/birim/bitki_karantina/faaliyet/teknik_talimat/yemeklik_baklagiller/baklagil_tohum_bocekleri.pdf), (August, 2012).
- Anonymous, (2014a). *Food and agriculture organization statistical database*, (<http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>), (Ağustos, 2014).
- Anonymous, (2014b). Republic of Turkey Prime Ministry-Turkish Statistical Institute. *Crop production statistics*. (<http://www.tuik.gov.tr/itkiselapp/bitkisel.zul>), (September, 2014).
- Aslan, I., Kılıç, T., Gören, A. C., Topçu, G. (2004). *Toxicity of acetone extract of Sideritis trojana and 7-epicandicandiol, 7-epicandicandiol diacetate and 18- acetylsideroxol against stored pests Acanthoscelides obtectus (Say), Sitophilus granarius (L.) and Ephestia kuehniella (Zell.)*. Atatürk University, Faculty of Agriculture, Plant Protection Department, Erzurum, Balıkesir University, Faculty of Arts and Science, Department of Chemistry, Balıkesir, TÜBİTAK, UME, P.O. Gebze-Kocaeli, Istanbul University, Faculty of Pharmacy, Beyazıt-Istanbul, TURKEY Volume 23, Issue 2, Pages 171–176.
- Atak, E. D. (1975). *Fasülye Tohum Böceği (Acanthoscelides obtectus Say)'nin Biyokolojisi ve Mücadelesi Üzerinde Araştırmalar*. Ministry of Agriculture and Forestry of the Republic of Turkey, Plant Protection Research Institute, Research Works Series. No:7, İstanbul, 67 s.
- Civelek, H. S., Weintraub, G. P. (2004). *Effects of Two Plant Extracts on Larval Leafminer Liriomyza trifolii (Diptera: Agromyzidae) in Tomatoes*. J. Econ. Entomol, 97(5), 1581–1586.
- Chu, S. S., Liu, Q. R. Liu, Z. L. (2010). *Insecticidal activity and chemical composition of the essential oil of Artemisia vestita from China against Sitophilus zeamais*. Biochemical Systematics and Ecology, 38 (4): 489-492.

- Çetin, H., Uysal, M., Alaoğlu, Ö., Şahmaz, A. (2009). *Fumigant effect of acetone to bean weevil [Acanthoscelides obtectus Say (Coleoptera: Bruchidae)] adults*. Türkiye Entomoloji Dergisi, 33(1):23-90.
- Düzdemir, O., Yanar, D. (2012). *Effects of using some plant extracts and plant originated pesticides against bean weevil (Acanthoscelides obtectus (Say.)) on bean yield in field conditions*. Tarım Bilimleri Araştırma Dergisi, 5 (1): 51-54.
- Ergül, M. (1988). *Yemler Bilgisi ve Teknolojisi*. Ege University, Faculty of Agriculture Publications, No.487, Bornova-İzmir.
- Isman, M.B. (2006). *Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world*. Annual Review of Entomology, 51: 45-66.
- Karakoç, Ö. C., Gökçe A., Telci İ. (2006). *Fumigant activity of some plant essential oils against Sitophilus oryzae L., Sitophilus granarius L. (Col.: Curculionidae) and Acanthoscelides obtectus Say. (Col.: Bruchidae)*. Türkiye Entomoloji Dergisi, 30 (2): 123-135.
- Kün, E., Çiftçi, G.Y., Birsin, M., Ülger, A.C., Karahan, S., Zencici, N., Öktem, A., Güler, M., Yılmaz, N., Atak, M., (2005). *Tahıl ve yemelik dane baklagil üretimi: Yemelik dane baklagiller*. Türkiye Ziraat Mühendisliği V. Teknik Kongresi, 3-7 Ocak 2005. Ankara, 396-407.
- Lee, S. E., Lees, E. M. (2001). *Biochemical mechanisms of resistance in strains of Oryzaephilus surinamensis (Coleoptera: Silvanidae) resistant to malathion and chlorpyrifos-methyl*. Journal of Economic Entomology, 94 (3): 706-713.
- Lee, B. H., Choi, W. S., Lee, S. E., Park, B. S. (2001a.) *Fumigant toxicity of essential oils and their constituent compounds towards the Rice weevil, Sitophilus oryzae (L.)*. Crop Protection, 20 (4): 317-320.
- Lee, B. H., Annis, P. C., Tumaalii F., Choi, W.S. (2004). *Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineole against 3 major stored-grain insects*. Journal of Stored Products Research, 40 (5): 553-564.
- Negahban, M., Moharramipour, S., Sefidkon, F. (2007). *Fumigant toxicity of essential oil from Artemisia sieberi Besser against three stored-product insects*. Journal of Stored Products Research, 43 (2): 123-128.
- Önder, M., Ateş, M.K., Kahraman, A., Ceyhan, E. (2012). *Konya ilinde fasulye tarımında karşılaşılan problemler ve çözüm önerileri*. Tarım Bilimleri Araştırma Dergisi, 5: 143-148.
- Papachristos, D.P., Stamopoulos D.C. (2002). *Toxicity of vapours of three essential oils to the immature stages of Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae)*. Journal of Stored Products Research, 38:365-373.
- Papachristos, D.P., Stamopoulos D.C. (2004). *Fumigant toxicity of three essential oils on the eggs of Acanthoscelides obtectus (Say) Coleoptera:Bruchidae*. Journal of Stored Products Research, 40:517-525.
- Perez, M.P., Pascual-Villalobos, M.J.. (1999). *Effect of the essential oil of flower Chrysanthemum coronarium L. on white fly and stored product pests*. Estacion Sericicola, Consejeria de Medio Ambiente, Agrilutura y Agua, CIDA, La Alberca, Spain 14 (1-2) : 249-258.
- Rahman, M. M., Schmidt, G. H. (1999). *Effect of Acorus calamus (L.) (Araceae) essential oil vapours from various origins on Callosobruchus phaseoli (Gyllenhal) (Coleoptra: Bruchidae)*. J.Stored Prod. Res., 35: 285-295.
- Raja, N., Albert, S., Ignasmuthu, S., Dorn, S. (2001). *Effect of plant volatile oils in protecting stored cowpea Vigna unguiculata (L.) against Callosobruchus maculatus (F.) (Coleoptra: Bruchidae) infestation*. J. Stored Prod. Res., 37:127-132.

- Rajendran, S., Sriranjini, V. (2008). *Plant products as fumigants for stored-product insect control*. Journal of Stored Products Research, 44 (2): 126-135.
- Regnault-Roger, C., Hamraoui, A. (1995). *Fumigant toxic activity and reproductive inhibition induced by monoterpenes on Acanthoscelides obtectus (Say) (Coleoptera), a bruchid of kidney bean (Phaseolus vulgaris L.)*. Journal of Stored Products Research, 31:291-299.
- Şehirali, S. (1988). *Yemeklik dane baklagiller*. Faculty of Agriculture Publications, Ankara University:1089, Textbook:314, Ankara, 435 p.
- Tapondjou, L., Adler, A., Bouda, C., Fontem, D.A. (2002). *Efficacy of powder and essential oil from Chenopodium ambrosioides leaves as post-harvest grain protectants against six-stored product beetles*. J. Stored Prod. Res., 38(4): 395-402.
- Varma, J., Dubey, N. K. (2001). *Efficacy of essential oils of Caesulia axillaris and Mentha arvensis against some storage pests causing biodeterioration of food commodities*. International Journal of Food Microbiology, 68 (3): 207-210.
- Wink, M., (1993). *Production and application of phytochemicals from an agricultural perspective*. Van Beek, T.A. and H. Breteler, (eds.). Vol.34, Claredon, Oxford, UK, pp. 171-213.
- Yanar, D., Düzdemir, O. (2012). *Effects of Some Plant Extracts and Plant Originated Pesticides to Bean Weevil (Acanthoscelides obtectus (Say.))*. Tarım Bilimleri Araştırma Dergisi, 6 (1): 36-40.