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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Contact Toxicity of *Hypericum* Extracts against *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae)

Hypericum Ekstraktlarının *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae)'ya Karşı Kontak Toksisitesi

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Abstract

Medicinal and aromatic plants have popular recently to investigate their usability as natural resources in many areas due to their superior biological activity properties. *Hypericum* genus contains important medicinal plant species known worldwide. In this study, the ethanol extracts from different plant parts (flower, leaf and stem) of three *Hypericum perforatum* L., *Hypericum heterophyllum* Vent., *Hypericum scabrum* L. were screened for their toxicity against adults of *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae), an important insect of stored grains in many regions of the world. Insecticidal activity was analyzed at 10 % concentration of ethanol extracts, and measurements were taken at three different exposure times (24, 48 and 72 hours). Considering the factors analyzed on this insect, *Hypericum* species, plant part, exposure time as well as the interaction of *Hypericum* species and plant part displayed a statistically significant effect. The mortality values of extracts from *H. perforatum*, *H. heterophyllum* and *H. scabrum* varied from 44.8 % to 88.9 %, 26.0 to 78.8 %, 26.1 % to 50.3 % for adult of *R. dominca*, respectively, after 72 h. *H. perforatum* showed a stronger effect than other species. Among plant parts, the leaf showed superior mortality on this insect. In addition, the mortality rate increased with increasing exposure time. According to the interaction between *Hypericum* species and the plant part, the leaf of *H. perforatum* (79.4 %) displayed the strongest mortality, followed by the leaf of *H. heterophyllum* (70.6 %). After 72 hours, the highest mortality was recorded in the leaf parts of *H. perforatum*. The current results showed that the extracts, in particular, the leaf extracts of *H. perforatum* and *H. heterophyllum*, may be evaluated as a new natural potential product of plant-derived insecticide because of its high mortality impact against *R. dominica*.

Keywords: *Hypericum perforatum*, *Hypericum heterophyllum*, *Hypericum scabrum*, *Rhyzopertha dominica*, Insecticidal effect

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Öz

Tıbbi ve aromatik bitkiler, üstün biyolojik aktivite özelliklerinden dolayı birçok alanda doğal kaynak olarak kullanılabilirliklerini araştırmak için son zamanlarda popüler hale gelmiştir. *Hypericum* cinsi dünya çapında bilinen önemli tıbbi bitki türlerini içermektedir. Bu çalışmada, üç *Hypericum* türünün (*Hypericum perforatum* L., *Hypericum heterophyllum* Vent., *Hypericum scabrum* L.) çiçek, yaprak ve gövde kısımlarından elde edilen etanol ekstraktları, dünyanın birçok bölgesinde depolanmış tahılların önemli zararlısı olan *Rhyzopertha dominica* (Fab.) (Coleoptera: Bostrichidae) erginlerine karşı toksisiteleri açısından değerlendirilmişlerdir. Böcek öldürücü aktivitesi, %10 etanol ekstrakt konsantrasyonunda analiz edilmiş ve ölçümler, üç farklı zamanda (24, 48 ve 72 saat) alınmıştır. Bu tür üzerine analiz edilen faktörler değerlendirildiğinde *Hypericum* türleri, bitki kısmı, maruz kalma süresi ve *Hypericum* türleri ile bitki kısmı arasındaki etkileşimin istatistiksel olarak önemli bir etkisi olduğu görülmüştür. *H. perforatum, H. heterophyllum* ve *H. scabrum*'dan elde edilen ekstraktların ölüm değerleri, 72 saat sonra *R. dominca* ergini için sırasıyla %44.8 ila %88.9, %26.0 ila %78.8 ve %26.1 ila %50.3 arasında değişmiştir. *H. perforatum* diğer türlere göre daha güçlü etki göstermiştir. Bitki kısımları arasında yaprak, tür üzerinde üstün ölüm oranı göstermiştir. Ek olarak, maruz kalma süresinin artmasıyla ölüm oranı artmıştır. *Hypericum* türleri ile bitki kısmı arasındaki etkileşime göre en yüksek ölüm oranını *H. perforatum* yaprağı (%79.4) gösterirken, onu *H. heterophyllum* yaprağı (%70.6) izlemiştir. 72 saat sonra, en yüksek ölüm *H. perforatum*'un yaprak kısımlarında kaydedilmiştir. Mevcut sonuçlar, ekstraktlardan, özellikle *H. perforatum* ve *H. heterophyllum*'un yaprak ekstraktlarının, *R. dominica*'ya karşı yüksek mortalite etkisi nedeniyle bitki kaynaklı insektisitin yeni bir doğal potansiyel ürünü olarak değerlendirilebilir.

Anahtar Kelimeler: *Hypericum perforatum, Hypericum heterophyllum, Hypericum scabrum, Rhyzopertha dominica,* İnsektisit etki

1. Introduction

One of the important pests among the stored grain insects is *Rhyzopertha dominica* (Coleoptera: Bostrichidae), known as lesser grain borer *R. dominica* is one of the insects that cause significant damage to many stored products. Larvae of this pest damage the product by eating the inside of the grain or living in flour and flour products. Adults are harmful by gnawing the outside of the grains (Dissanayaka et al., 2020). In some studies both in Turkey and other countries, it is reported that the adults cause weight loss in the stored grains and the damage reaches the economic level after 122 days from the contamination (Malagon and Trochez, 1985; Toğantimur and Özder, 2019). During the storage period, especially stored grain insects damage cereal grains both qualitatively and quantitatively, which causes degradation of seed, food or forage. The economic damage of insects that cause economic loss in granaries is 5-10% in developed countries, and this rate is higher in developing countries, which means an important economic loss (Hernandez Nopsa et al., 2015). In addition, these pests cause food contamination and cause serious problems for the food industry and human health (Wu et al., 2019, Sular et al., 2019).

Synthetic chemicals such as pesticides, fumigants and insecticides have been actively used management stored grain pests for a long time (Kostyukovsky and Shaaya, 2013). These chemical products have caused very important problems such as air pollution, change of balance in the ecosystem, harm to living health, emergence of resistant pests and other. So, recently, scientists have focused to search for new and natural products to control against pests for both these reasons and high costs (Damalas and Eleftherohorinos, 2011; Paoli et al., 2015). To control pests, scientists are looking for low-cost, organic products that are residue-free in nature and are less harmful to human health. Therefore, it has become popular to investigate the toxic effects of various plant species against pests (Guru-Pirasanna-Pandi et al., 2018; Zaka et al., 2019; Lampiri et al., 2020). Because, plant-derived materials are natural products, they are less harmful to the environment and living things (Sodaeizadeh et al., 2010).

Hypericum species (Hypericeae), which is in the group of medicinal plants contain the extremely significant phytochemicals such as phenolic acid, flavonoids (hyperin, hyperoside, luteolin), napthodianthrones (hypericin, protohypericin, pseudohypericin), phloroglucinols (hyperforin), xanthones and essential oils (Napoli et al., 2018). *Hypericum perforatum* are rich in napthodianthrones and phloroglucinols which exhibit many biological activities (Barnes et al., 2019; Lazzara et al., 2020). The type and amount of chemical components belonging to plants can vary in different parts (flower, leaf, stem, root etc.) of plants, which causes efficacy differences among plant parts (Sarrou et al., 2018), and even organic solvent varieties (Khan et al., 2017).

As known, *H. perforatum* and *H. scabrum* are economically important species that are actively used in many areas of the pharmaceutic in most countries (Zorzetto et al., 2015). Also, a few studies were reported about insecdisidal effect of both *Hypericum* species (Tozlu et al., 2011; Rouis et al., 2013; Dastagir et al., 2016). In previous studies, there have been reports of the usability of *Hypericum* species to control of insects and their larvae (da Silva et al., 2013; Biniaś et al., 2016; Erdoğan and Yıldırım, 2016; Puthur et al., 2019). Additionally, *Hypericum* extracts from *H. perforatum* and *H. scabrum* had a strong toxicity on insects, especially against many Coleopteran insects such as *Tribolium castaneum*, *Trogoderma granarium*, *Callosobruchus analis*, *Sitophilus oryzae*, *R. dominica* (Tozlu et al., 2011; Dastagir et al., 2016). But, there is still inadequate information regarding their toxic effects against adults of *R. dominica*. Moreover, there are no references of insecticidal effect of extracts with organic solvent from different plant parts of *H. scabrum* and *H. heterophyllum* on the Coleopteran insects in stored grains. In current study, the ethanol extracts from *H. heterophyllum,* an endemic to Turkey**,** was first time used against *R. dominica*. The aim of this study was to analyze the toxic effect on *R. dominica*, important stored product insect, of ethanol extracts from different plant parts (flower, leaf, stem) of *Hypericum* species (*H. perforatum, H. heterophyllum, H. scabrum*).

2. Materials and Methods

2.1. Plant materials

Hypericum perforatum, *H. heterophyllum* and *H. scabrum* were collected aerial parts of each species including more than 30 individuals at 100% flowering period from their natural habitats (Turkey) showed in *Table 1.* Collection of plants was done between 11:00 and 13:00. The flowers, leafs and stem parts of the plants were separated, and dried under shade at 20 ± 2 ºC for analysis. The plant materials were determined by Prof. Dr. Osman Tugay (Department of Pharmaceutical Botany, Faculty of Pharmacy, Selçuk University). Voucher specimens are kept in KNYA Herbarium of the Selçuk University, Faculty of Science, Konya, Turkey (Herbarium numbers: 28281, 28282, and 28283, respectively).

Table 1. Habitat and collection status of the Hypericum species

Species	Location	Collection time	Latitude (N)	Longitude (E)	Altitude (m)
Hypericum perforatum	Çorum, Yeşilçat Village	01.07.2017	40º41'16	34º7'51	1372
Hypericum heterophyllum[1]	Yozgat, Bozok University Campus	04.07.2017	39º46'42	34º47'51	1332
Hypericum scabrum	Yozgat, Gelin Kayası	13.06.2017	39º50'20	34º45'44	1401

[1]It is endemic to Turkey.

2.2. Extraction of plant samples for analysis

The aerial parts (flower, leaf and stem) of the *Hypericum* species were used for the extraction. The aerial parts were dried under shade and mechanically ground with a blender. 4 g (three replicate) of each grounded plant materials were extracted individually in 40 mL of 100% ethanol at 40 °C for 24 h (Yaman, 2020). The resulting solutions were filtered through whatman paper and the solvent was removed on a rotary evaporator at temperature bellow 40°C.

2.3. Insect source

Stock cultures of insects were obtained from the Department of Plant Protection, Yozgat Bozok University. Wheat grains were used for feeding of *R. dominica* adult. The stock cultures of insects were incubated at 27±2 ºC. Adult individuals were taken into these jars and left to feed. These stock cultures were incubated at 27±2 Cº in dark conditions. Within 45 days, a new generation of adults emerged, and randomly selected adult individuals were used in the study (Abay et al., 2012).

2.4. Contact toxicity

The 10% concentration of *Hypericum* extracts which dissolved with acetone, and used for contact effect according to method used by Gokce et al., (2010). The 1 µl amount of the extracts was applied topically to each insect by micro-aplicator. For control, 1 µl insect-1 of acetone solvent was treated. The experiment was plotted in complete randomized design while six replications were set up for each assay after the insects were taken from stock cultures at a random age, ten adults were transferred to each petri dish. At the end of the application, the insects were incubated with the food under the conditions mentioned above, and the mortality was recorded after 24, 48 and 72 h of exposure. The mortality rates were calculated as %.

2.5. Statistical analysis

No mortality was observed in the control applications of all species. The same vials were examined for mortality at the different exposure intervals (24, 48, and 72 hours), so mortality data were analyzed by using one-way analysis of variance (ANOVA) with *Hypericum* specie, plant part and exposure time as the main effects. Percent mortality data were expressed as mean values and standard error (±SE). It was used Levene's test to check homogenity of variances before ANOVA tests. Also, data given in percentages were subjected to arcsine (√X) transformation before statistical analysis. Differences between the means were compared by Duncan's multiple range tests using SPSS 20.0 Statistical software at 0.01 level.

3. Results and Discussion

Non-synthetic natural origin materials such as crude extracts and essential oils with secondary metabolites from plants, and other biomolecules from microorganisms have been suggested as alternatives for controlling *R. dominica* adults. In this work, ethanolic extracts from flower, leaf and stem parts of *Hypericum* species were evaluated against this insect.

Mortality of *R. dominica* adults was significantly affected from all main effects (*Hypericum* species, plant part and exposure time) and *Hypericum* species x Plant part interaction ($P < 0.01$). However, the associated interactions within exposure time were not significant ($P \geq 0.32$). The plant part among all main effects displayed the highest F value (F=66.75) (*Table 2*).

Table 2. Habitat and collection status of the Hypericum species

Source	*df*	*R. dominica*	
		F	P
Hypericum species	2	21.98	<0.01**
Plant part	2	66.75	<0.01**
Exposure time	2	23.90	<0.01**
Hypericum species x Plant part	4	13.61	<0.01**
Hypericum species x Exposure time	4	1.19	0.32
Plant part x Exposure time	4	0.58	0.68
Hypericum species x Plant part x Exposure time	8	0.77	0.63
Error	135		

total *df* =162. *p*<0.01. **

A very high mortality was observed as a result of the factors examined on the control of this insect. The mortality of *R. dominica* varied from 19.3 to 88.9% for all exposure intervals, and was found to increase with increasing exposure time in all extracts (*Table 3*). After 72 h of exposure, leaf extracts of *H. perforatum* (88.9%) and *H. heterophyllum* (78.8%) displayed strong toxic effect, and were statistically in the same group.

When the efficacy of the tested *Hypericum* species on these insects is examined, *H. perforatum* (52.2%) was found to be powerful effective than other *Hypericum* species, but there was no statistical difference between *H. perforatum* and *H. heterophyllum* (48.2%) (*Table 3*). In a recent study, Yaman and Şimşek (2021) investigated the effect of acetone extracts of *Hypericum* species on *R. dominica*, and found the highest mortality in *H. perforatum* as 42.3%. But, in currently study, ethanol extracts of *H. heterphyllum* displayed the high toxic effect on *R. dominica*. To the best of our knowledge, *H. heterphyllum*, an endemic species to Turkey, was analysed first time toxicity of its ethanolic extracts on this pest, had only a few studies about pest management. For example, Hernandez Nopsa et al. (2015) reported that *H. heterophyllum* exhibited the stronger inhibition zone against *Paenibacillus* larvae than other many *Hypericum* species. Yaman and Şimşek (2021) recorded that *H. heterophyllum* displayed high toxic effect on stored grain pests. In fact, many scientists have investigated usability in pest control of some *Hypericum* species. Erdoğan and Yıldırım (2016) indicated that *Hypericum calycinum* L. exhibited about 60% mortality against *Myzus persicae* Sulzer. Puthur et al. (2019) declared that *Hypericum japonicum* Thunb. displayed 85% mortality on *S. oryzae*. Biniaś et al. (2016) notified that aqueous extract of *H. perforatum* inhibited the feeding activity on larvae of *Leptinotarsa decemlineata*. da Silva et al. (2013) recorded that *Hypericum carinatum* Griseb. was high toxicity against *Aedes aegypti*.

Table 3. Mortality effect of ethanol extracts from flower, leaf and stem parts of Hypericum species on Rhizopertha dominica adults after 24, 48 and 72 h of exposure time.

Source	*df*	Exposure time						Average		Average
		24h		48h		72h				
Control		0.0	c	0.0	e	0.0	d	0.0	e	
Hypericum perforatum	Flower	24.1±0.41	b	36.3±0.27	cd	44.8±0.26	c	35.1	cd	
	Leaf	62.0±0.54	a	87.4±0.58	a	88.9±0.54	a	79.4	a	52.2 a
	Stem	25.8±1.46	b	42.2±0.80	c	56.7±0.33	bc	41.6	cd	
Hypericum heterophyllum	Flower	19.3±0.22	b	22.3±0.37	d	26.0±0.26	d	22.5	d	
	Leaf	56.8±0.26	a	76.2±0.46	ab	78.8±0.15	a	70.6	ab	48.2 ab
	Stem	30.4±1.68	b	60.5±0.51	b	63.7±0.21	b	51.5	bc	
Hypericum scabrum	Flower	26.0±0.26	b	38.0±0.26	cd	41.5±0.24	c	35.2	cd	
	Leaf	32.8±0.63	b	41.3±0.54	c	50.3±0.45	bc	41.5	cd	33.3 b
	Stem	20.9±0.27	b	22.5±0.30	d	26.1±0.18	d	23.2	d	
Exposure time average		33.1 b		47.4 ab		53.0 a				

Means followed by the different letter are significantly different (Duncan test at 0.01).

Our results indicated that the efficacy of *Hypericum* extracts varies among the *Hypericum* species tested, and it was extremely related with the plant parts. Similarly, Haouas et al. (2008) reported that the effect of flower and leaf parts against *Tribolium confusum* Jacquelin Du Val. varied by eight *Chrysanthemum* species which had different toxic effects among themselves.

The plant parts among all main effects had a statistically significant effect on *R. dominica*. Moreover, the leaf (63.8 %) displayed the stronger mortality than other plant parts (*Figure 1*). In fact, it was recorded that it is twice as effective as flower (38.8 %) and stem (30.9 %) parts. Looking at previous studies, many scientists have reported that the leaf part of plants has a superior insecticidal activity compared to other plant parts (Batish et al., 2008; Chauhan et. al., 2015; Alvi et al., 2018; Şimşek et al., 2019; Yaman and Şimşek, 2021).

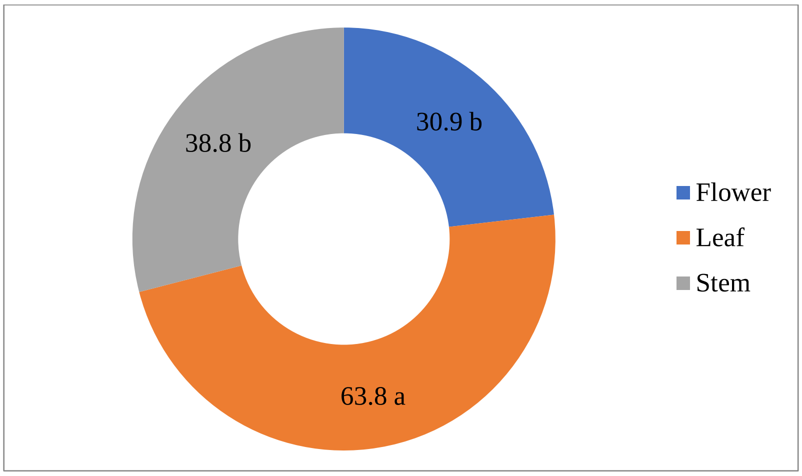

Figure 1. Toxic effect of the flower, leaf and stem parts from Hypericum species against Rhizopertha dominica adults

When examining the interaction between the *Hypericum* species and plant part, the leaf part of *H. perforatum* exhibited the strongest mortality (79.4 %) followed by the leaf part of *H. heterophyllum* (70.6 %). Yaman and Şimşek (2021) were notified that leaf part of *Hypericum* species was more effective against *R. dominica* than their other parts. On the contrary, Dastagir et al. (2016) found that different plant parts of *H. perforatum* had no mortality against *R. dominica*. This was obviously proven in previous studies that different solvent extracts of a species had different toxicologic effects on insects. According to the results of Kısa et al. (2018) notified that methanol extract of *Olea europaea* L. leaf had stronger toxicity on *T. confusum* than its ethanol extract. Boussaada et al. (2008) revealed that some Asteraceae plant extracts showed high mortality on adults of *T. confusum*, and their effect varied with different solvents.

4. Conclusions

In search for the detection of strong toxic botanical plant raw material against stored product insects, safe for botanic use and preparations, the insecticidal effects presented in this study reveals the opportunity to valorize toxicity of plant parts in order to identify superior the *Hypericum* species. Our results indicate that 10% concentration of flower, leaf and stem extracts with ethanol from *H. perforatum*, *H. heterophyllum* and *H. scabrum* from Turkey were screened for insecticidal activity and we identified two extracts with potent toxic activity against *R. dominica*. The results indicated that the leaf extract of *H. perforatum* and *H. heterophyllum* can be useful in controlling adults of *R. dominica*. In the future, *H. perforatum* and *H. heterophyllum* can be evaluated as insecticide because of both its potential effect in controlling *R. dominica*. For the practical application of the extracts from the leaf parts of these *Hypericum* species, including phyto-constituents, further studies are necessary to shed light on the safety and efficacy.

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