

***Annona muricata* Bitki Ekstraktının *Ephestia kuehniella* (Lep: Pyralidae) ve Yumurta Parazitoiti *Trichogramma evanescens* (Hym: Trichogrammatidae)'e Karşı Böcek Öldürücü Aktivitesinin Belirlenmesi**

Determination of insecticidal activity of *Annona muricata* plant extract against *Ephestia kuehniella* (Lep: Pyralidae) and its egg parasitoid *Trichogramma evanescens* (Hym: Trichogrammatidae)

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Abstract

In the study, the insecticidal effect of *Annona muricata* plant extract on stored product pest, *Ephestia kuehniella* and biological control agent *Trichogramma evanescens* was investigated. The plant used in the study was commercially available in capsule form. 0.5 gr. *A. muricata* extract was applied to the adults of *E. kuehniella* and mortality was determined after 24, 48 and 72 h. For *T. evanescens* adults, 0.005 g/ml *A. muricata* was given to the adults by dropping onto egg cards and the mortality rates were determined after 24, 48 and 72 h. It has been observed that death increased with the time increasing for both stored pest and natural enemy. It was concluded that plant extract was toxic to both species.

Keywords: *Annona muricata*, *Ephestia kuehniella*, *Trichogramma evanescens*, biological control agent, insecticide

Özet

Bu çalışmada, *Annona muricata* bitki ekstraktının depolanmış ürün zararlısı, *Ephestia kuehniella* ve biyolojik mücadele ajanı, *Trichogramma evanescens* üzerine böcek öldürücü etkisi araştırılmıştır. Çalışmada kullanılan bitki, kapsül formunda ticari olarak temin edilmiştir. *A. muricata*'nın 0.5 gr. ekstraktı *E. kuehniella* erginlerine uygulanmış ve 24, 48 ve 72 saat sonra ölüm oranları belirlenmiştir. *T. evanescens* erginleri için 0.005 g/ml *A. muricata*

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yumurta kartlarının üzerine damlatılarak erginlere verilmiş ve 24, 48 ve 72 saat sonra ölüm oranları tespit edilmiştir. Hem depo zararlısı hem doğal düşman için süre artışı ile ölümün arttığı gözlemlenmiştir. Bitki ekstraktının her iki tür için de toksik olduğu sonucuna varılmıştır.

Anahtar sözcükler: *Annona muricata*, *Ephestia kuehniella*, *Trichogramma evanescens*, biyolojik mücadele ajanı, böcek öldürücü

Introduction

Ephestia kuehniella, known as Mediterranean flour moth or mill moth, is found in temperate climates around the world and severely damages stored food products such as grains (Lynn and Ferkovich, 2004). This insect significantly reduces food quality and is controlled using fumigants such as phosphine and methyl bromide. However, methyl bromide has a toxic effect on humans and animals and is prohibited by the United Nations Montreal Protocol (Ercan *et al.*, 2013). Therefore, a natural enemy such as *Trichogramma evanescens*, which is an egg parasitoid, is frequently used in biological control. In addition to natural enemies, tropical plants such as *Annona muricata* and their extracts are a good alternative in comparison with fumigant pesticides.

The *A. muricata* plant, which is a member of the Annonaceae family with about 130 **genera** and 2300 species, is found in tropical and subtropical regions of the world, including South and North America, India, Malaysia, and Nigeria (Adewole and Caxton, 2006). This plant, known as a cinnamon apple in our country, is named in different names in every country such as soursop, graviola, guanabana, claw-claw, and sirsak (Leboeuf *et al.*, 1980) (Mishra *et al.*, 2013). As a result of phytochemical evaluations on the seeds, leaves, roots, and fruits of this plant the presence of many compounds has been identified. These components found in *A. muricata*; alkaloids (Yang *et al.*, 2015) (Leboeuf *et al.*, 1980), megastigmanes (Matsushige *et al.*, 2012), flavonol triglycerides (Nawwar *et al.*, 2012), phenolics (Jiménez *et al.*, 2014), cyclopeptides and essential oils (Pellissier *et al.*, 1994) (Kossouh *et al.*, 2007). In addition, it is a rich source of acetogenic compounds and plays a role in many important biological activities such as antiarthritic activity, antiparasitic activity, insecticidal activity, and anticancer activity (Soheil *et al.*, 2015). Especially in the last decade, *A. muricata*'s insecticidal properties and its potential to be biopesticides have been investigated. In a study on different *Annona* species; The toxic effect of *A. muricata* seeds have been tested on *Trichoplusia* larvae (Ribeiro *et al.*, 2014). In another study, *A. muricata* seeds have been

tested against stored product pest *Sitophilus zeamais* and 70% - 100% deaths have been determined (Djamin and Idris, 2012). Additionally, *A. muricata* seeds were applied against *Aedes albopictus*, *Culex quinquefasciatus* larvae (Raveloson et al., 2014) and *Anastrepha ludens* larvae (González et al., 2012). It has been observed that it exhibited promising insecticidal activity.

No attempt has been made to investigate the insecticidal action of *A. muricata* plant extract against *E. kuehniella* (Lep: Pyralidae) and *T. evanescens* (Hym: Trichogrammatidae). Therefore, the aim of this study was to examine the insecticidal activity of *A. muricata* plant extract on *E. kuehniella* and *T. evanescens*.

Materials and methods

2.1. Rearing of *Ephestia kuehniella* Zeller (Lep: Pyralidae)

E. kuehniella Zeller was obtained from Erciyes University, Faculty of Science, Department of Biology. Adults were reared in approximately 27°C temperature and 70% relative humidity and under a light regime of 14 h light followed by 10 h darkness. During the cultivation, 1 kg of wheat flour, 5% yeast and 30 g of wheat germ were supplemented with a mixture (Tunçbilek et al. 2009). Large numbers of 1-day-old *E. kuehniella* adults were collected from stock cultures and placed in plastic jars with screen bottoms. At the end of a day, the eggs were collected and adults and eggs were transferred to clean petri dishes.

2.2. Rearing of *Trichogramma evanescens* Hartig (Hymenoptera: Trichogrammatidae)

T. evanescens adults were obtained from Erciyes University, Faculty of Science, Department of Biology and were reared on *E. kuehniella* eggs in glass rearing vials (2–7.5 cm). During cultivation, *T. evanescens* were incubated at about 25°C temperature and 70% relative humidity for 14 hours under a light light regime followed by 10 hours in a dark environment (Ercan et al., 2013).

2.3. Preparation of *Annona muricata* plant extract

A. muricata was purchased commercially in capsule form (Best Naturals®, USA). *A. muricata* capsules consist of 100% pure *A. muricata* leaf. For *E. kuehniella*; 0.5 grams the *A. muricata* capsule was weighed on a precision scale. Then, 0.5 grams of *E. kuehniella* medium was weighed and homogeneously mixed. For *T. evanescens*; 0.005 grams *A. muricata* capsule

was weighed on a precision scale and was dissolved 0.1% DMSO. Incubated for 1 hour using an orbital shaker. Then, the supernatant was filtered off by centrifugation (Mohamad *et al.*, 2015).

2.4. *Annona muricata* plant extract effects on different stages of the insects

E. kuehniella adults were placed in 1000-mL glass jars. 0.5 gram *A. muricata* plant capsule were mixed with *E. kuehniella*'s food for 24, 48 and 72 h. To determine the mortalities, adults were removed from the jar and checked with a fine brush. If they did not move, they were evaluated to be dead. The similar procedure was used for *T. evanescens* adults. *T. evanescens* adults were placed in 2-7.5 cm glass rearing vials. 0.005 g/ml *A. muricata* plant extract was dropped onto egg cards. Mortality were recorded after 24, 48 and 72 h. for *T. evanescens*.

Results

The number of adult mortalities of the two insects after using a single concentration of *A. muricata* plant extract for 24 h, 48 h, 72 h are shown in Table 1. The adult mortality rates increased at different time intervals. With the time change, *A. muricata* plant extract is both toxic to *E. kuehniella* and *T. evanescens*. The results of this study showed that the toxicity of *A. muricata* plant extract changed according to different time stages of the insects.

Table 1: The number of adult mortalities of *Ephestia kuehniella* and *Trichogramma evanescens*

Life Cycle	Number of Individuals	Applied Dose	Time (h)	Live	Dead
<i>Ephestia kuehniella</i>					
Adult	10	0.5 g.g ⁻¹	24	8	2
			48	5	5
			72	1	9
<i>Trichogramma evanescens</i>					
Adult	10	0.005 g.ml ⁻¹	24	6	4
			48	3	7
			72	1	9

Discussion

Turkey has an important role in flour production and exports in the world. According to FAO's (Food and Agriculture Organization of the United Nations) data; Turkey, that exporters of about 2 million tons of flour in every year is in the leadership position in the world flour exports with this amount. However, grain products must be protected from insect pests during the storage period. Especially, chemical insecticides used to protect cereals are very dangerous for human health. Therefore, studies on natural and biological insecticides have increased in physiological and pharmacological areas in recent years. There are many studies that explained the insecticidal activity of *A. muricata*, *Sitophilus zeamais*, *Aedes albopictus*, *Culex quinquefasciatus*, *Anastrepha ludens*, and *Trichoplusia* larvae and many insect species have been tested (Soheil *et al.*, 2015).

With this study, we investigated the potential of this plant as a likely biopesticide and as a result of the study, we have observed that this plant may have insecticidal activity against *E. kuehniella* and *T. evanescens*. Therefore, plant extract effected the egg parasitoid adult stage, so combined usage of both plant extract and egg parasitoid against stored product pest in any biological control program will not be applicable. As a result, it seems more appropriate to use of natural enemies and plant extracts consecutively.

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