

Fumigant Toxicitiy of Essential Oils from *Thymus argaeus* Boissier & Balansa and *Thymus sipyleus* Boissier(Lamiaceae) Againts *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)

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Abstract

In the present study, insecticidal activity of essential oils of *Thymus argaeus* Boissier & Balansa and *Thymus sipyleus* Boissier were evaluated by fumigation method against different developmental stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Essential oils were isolated by Clevenger apparatus. Both essential oils were highly effective against egg and adult stages of pest. On the other hand larvae of *E. kuehniella* were more resistant than other stages. The mortality values reached 100% when the adults were exposed to 5 and 1,5 µl/L air concentrations of *T. argaeus* and *T. sipyleus* essential oil, respectively. In all stages, *T. sipyleus* essential oil was found to be more effective than *T. argaeus* essential oil. Results showed that these essential oils have potential as fumigants against *E. kuehniella* under storage conditions.

Keywords: *Thymus argaeus*, *Thymus sipyleus*, *Ephestia kuehniella*, essential oil, insecticidal activity.

1. Introduction

In stored cereals, insects may cause serious damages (Negahban et al.2007) . Control of these insects is based on the usage of synthetic insecticides and fumigants that have some problems, such as pest resistance to these insecticides, adverse effects on non-target organisms, especially natural enemies and toxic effects on users (Jembere et al.1995). Among the stored product insects, the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is one of the major pest of amylaceous products and the larvae of moth cause a reduction on product quality by webbing, directly feeding and fecal matter (Rees D. 2003, Johnson et al.1997). The disadvantages of synthetic fumigants like phosphine and methyl bromide, has led the researchers to develop new methods.

Alternative pest control strategies include environmentally friendlier and more effective methods than synthetic insecticides and fumigants. Among these alternative methods, natural insecticides in other words essential oil-based pest control is most promising method. Essential oils are good alternatives against fumigant insecticides because of their environmental safety characteristics. Recent studies showed that essential oils may be successfully used for supression the stored product insects (Erler F.2005, Negahban et al.2007, Ayvaz et al.2009). So that essential oils have complex mixture of terpenes, sesquiterpenes, their oxygenated derivatives and other aromatic compounds (Ogendo et al.2008). These natural substances may have volatile compounds and these compounds may have fumigant activity, so this property can be used against pest insects. Therewithal, these compounds have harmful effects against target organism and effect longevity and reproduction of different developmental stages of insects.

Aromatic plants are widely distributed in Turkey and they are used in many areas such as production of cosmetics and detergents, in pharmacology and food flavouring (Aslan et al.2004). Essential oils which were produced from these plants have been used in many studies against stored-product insects (El Nahal et al.1989, Haung et al. 1997, Tuncbilek et al.2009, Ayvaz et al.2010, Ercan et al.2013). Usage of fumigants is an economical tool for controlling these stored-product pests[5]. Essential oils are a possible alternatives to chemical fumigants that used against pests (Negahban et al.2007).

The genus *Thymus* (Lamiaceae) represented by 40 species and two subspecies, of which 17 are endemic to Turkey (Yıldız B. 2012). This genus members are herbaceous perennials and subshrubs, native to Southern Europe and Asia (Wasson RJ.1999) and used as herbal tea and condiments in Turkey. Antioxidant and antimicrobial activities of the essential oils and extracts of several *Thymus* species have formerly been reported (Cosentino et al.1999, Bounatirou et al.2007, Bentes et al.2009, Ouariachi et al.2011). Therewithal, the essential oil compositions of *Thymus argaeus* and *Thymus sipyleus* have been reported before (Vural et al.2008, Ozcan et al.2008). However, insecticidal effects of these plants essential oils seem not to be reported before.

This study was undertaken to evaluate the insecticidal activity of the two abovementioned thyme oils against different developmental stages of *E. kuehniella*. The mortality rate of different developmental stages after treatments were determined.

2. Materials And Methods

2.1 Rearing of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae)

Ephestia kuehniella Zeller, was obtained from the Adana Plant Protection Research Institute. Insects were reared on a diet consisting of a mixture of 1 kg wheat flour, 30 g wheat germ and 5% yeast (Tuncbilek et al.2009). Mouth of the containers was covered with fine mesh cloth for ventilation as well as to prevent escape of the insects. Cultures were maintained at 27±1 °C and 70±5% relative humidity and 14 h light-10h darkness in growth chamber. Adult insects, 1-2 days old, were used for toxicity tests. Adults were collected from the cultures and they placed in plastic jars. Eggs were collected daily and placed in petri dishes. They were used for the experiments and rearing new cultures. All experimental procedures were carried out under the same environmental conditions as the cultures.

2.2 Plant Materials

Thymus argaeus Boissier & Balansa and *Thymus sipyleus* Boissier were collected from the Mount Erciyes in Turkey (Table 1). The plant samples were dried via herbarium techniques, determined by The Flora of Turkey (Davis PH. 1972).

Table 1. Origins of *Thymus argaeus*Boissier & Balansa and *Thymus sipyleus*Boissier used in the study

Species	Locality/Origin	Year collected
*Thymus argaeus*Boissier & Balansa	Çaybağları Elevation: 1932m.	2000
*Thymus sipyleus*Boissier	Beğendikbağları Elevation: 1130m.	1997

2.3 Isolation of the Essential Oil

200 g of plants were dissolved in 3 L distilled water for 3 h using a Clevenger type apparatus. At the end of the time period concentrates of essential oil samples were collected. The obtained essential oil was

stored at +4 °C until used.In this study we isolated essential oils of herbarium materials of *T. argaeus* and *T. sipyleus* by the same method of (Vural et al.2008, Ozcan et al.2008).

2.4 Bioassays
The essential oil of *T. argaeus* and *T. sipyleus* were tested for their insecticidal effects against different stages of *E. kuehniella*. Insect adults and last larval instars were placed in 1 L glass jars. Six repetitions were done for each concentration and each replicate consisted of 10 adults and larvae. The essential oil of plants was applied on a filter papers (25×25 mm). Then the filter paper was attached to the under surface of glass jar's cap. The adults and larvae of *E. kuehniella* were exposed to essential oil vapour with different concentrations (0-5,0 µl/L air for *T. argaeus* and 0-1,50 µl/L air for *T. sipyleus*; and 0-350 µl/L air for *T. argaeus* and 0-250 µl/L air for *T. sipyleus*, respectively) for 24 h. At each concentration, to specify the mortalities, insects were removed from the jar and checked that if they were alive or dead. The insects were considered as dead when they didn't move, fly or response to gentle touch. The values of LC_{50} and LC_{99} were determined by Probit analyses (lethal concentration to kill 50% and 99% of the population, respectively) and given in Table 2 and Table 3. The control insects were exposured to the same conditions but no material was applied in control jars. Mortality was recorded daily. For egg treatment; equal number of eggs (100±5) were spreaded on egg cards and glued for determining the effect of essential oil on egg stage of *E. kuehniella*. These cards were placed to the bottom of 1 L glass jars. Essential oils were applied to glass jars (0-9,0 µl/L air for *T. argaeus* and 0-5,0 µl/L air for *T. sipyleus*) for defining the fumigant toxicity. After 24 h treatments, egg cards were placed in petri dishes and the number of hatched eggs was scored. Egg hatchability was compared with control and then recorded.

Table 2. LC_{50} and LC_{99} values of *T. argaeus* essential oils against different life stages of *E. kuehniella*.

Time (24 h)	**N**	**LC_{50}, µl/L air**	**LC_{99}, µl/L air**	**DF**	**c^2**
Egg	100	6,17484	10,52516	7	33,781[a]
Larvae	10	159,84144	346,65140	7	5,015
Adult	10	3,67708	6,49184	7	53,734[a]

N: Number of the tested stages. a: Since the goodness-of-fit chi-square was significant (P <0.05), a heterogeneity factor was used in the calculation of confidence limits.

Table 3. LC_{50} and LC_{99} values of *T. sipyleus* essential oils against different life stages of *E. kuehniella*.

Time (24 h)	**N**	**LC_{50}, µl/L air**	**LC_{99}, µl/L air**	**DF**	**c^2**
Egg	100	2,60739	5,25770	6	9,018
Larvae	10	101,12928	254,93381	7	39,976[a]
Adult	10	1,03840	1,78732	7	33,607[a]

N: Number of the tested stages. a: Since the goodness-of-fit chi-square was significant (P <0.05), a heterogeneity factor was used in the calculation of confidence limits.

3. Data Analysis
In order to calculate significant differences between concentrations in different life stages of the insects, one-way analysis of variance (ANOVA) followed by Tukey multiple comparision were used in SPSS 11.5 ($P < 0.05$). Probit analysis was used to estimate LC_{50} and LC_{99} values (Abbott W.1925).

4. Results And Discussion
In this study, insecticidal activity of essential oils of *Thymus argaeus* and *Thymus sipyleus* were evaluated against different developmental stages of *Ephestia kuehniella*. Essential oils can be used as an alternative method for pest management. Fumigant toxicity of essential oils contents like terpenoids and phenols, show insecticidal activity and may be important for controlling stored product insects (Ayvaz et al.2009).

Moreover, some components of essential oils can be shown neurotoxic effects on pests and monoterpenes can be act competitive inhibitors of acetylcholinesterase, cholinesterase, and octopaminergic system of insects (Kostyukovsky et al.2002).

The major components of *T. argaeus* and *T. sipyleus* were found to be as nerol (16,328%) and carvacrol (35,327%), respectively by Vural et al. (2008) and Özcan et al. (2008). Also linalool (10.53%) and thymol (13.22%) were detected in the essential oil of *T. argaeus* and *T. sipyleus* in same studies and these monoterpenoids were known to have insecticidal activity. Therefore, the toxicity of *T. argaeus* and *T. sipyleus's* essential oils may be resulted from their chemical contents. These chemicals were reported to have an insecticidal activity on some important pests (Lee et al.1997). These data support our findings which were observed in this study.

The fumigant toxicity of several plant essential oils against different developmental stages of *E. kuehniella* were determined in previous studies (Ayvaz et al.2009, Ercan et al.2013, Emamjomeh et al.2017). Our results indicated that the adult stage of *E. kuehniella* was more sensitive to both essential oils than other stages. Mortality of adults was decreased with increasing doses (Fig. 3). Larvae of *E. kuehniella* was found to be more tolerant than egg stage (Fig. 2) like previous studies (Erler R.2005, Ercan et al.2013).

The results show quite clearly that essential oils of both plants were toxic to all stages of *E. kuehniella*. But the most susceptible stage was adult to both essential oils. We obtained 100% mortality by vapours of essential oil of *T. argaeus* in 9. 0 µl/L air and *T. sipyleus* in 5.0 µl/L air for 24 h againstegg stage of *E. kuehniella* (Fig. 1). Essential oil vapours can be effect insect species eggs in different ways (Isıkber et al.2009). In current study, the eggs of *E. kuehniella* were more tolerant to the essential oils than adult stage (LC_{99}:10.52516 µl/L air for *T. argaeus* and LC_{99}:5,25770 µl/L air for *T. sipyleus*). Larvae of *E. kuehniella* were tolerant to fumigant toxicity ofboth essential oils compared to adults and 100% mortality was obtained in 350 and 250 µl/L air for *T. argaeus* and *T. sipyleus*, respectively (Table 2-3, Fig. 2).

Gözek (2007) demonstrated that adult and larval stages of *Tribolium confusum* were the most tolerant stages to treatments of garlic essential oils. On the other hand, Ercan et al. (2013) found that adult stage of *E. kuehniella* was the most sensitive stage to exposure of essential oil of *Prangos ferulaceae*. So, effects of different plants' essential oils may be varied against different stored product pests.

Ayvaz et al. (2010) obtained 100% mortality after 24 h at 9 µl/L air essential oils of oregano and savory application against *E. kuehniella* adults (Ayvaz et al. 2010). In our study, 100% mortality of *E. kuehniella* adult was obtained at 5 and 1.5 µl/L air essential oils of *T. argaeus* and *T. sipyleus*, respectively. Saraç & Tunç (1995) showed that last larval instars of *E. kuehniella* were less susceptible than the egg stage towards anise essential oil. The present work reported the same results that larval stage of *E. kuehniella* was more tolerant than egg stage after exposure to essential oils.

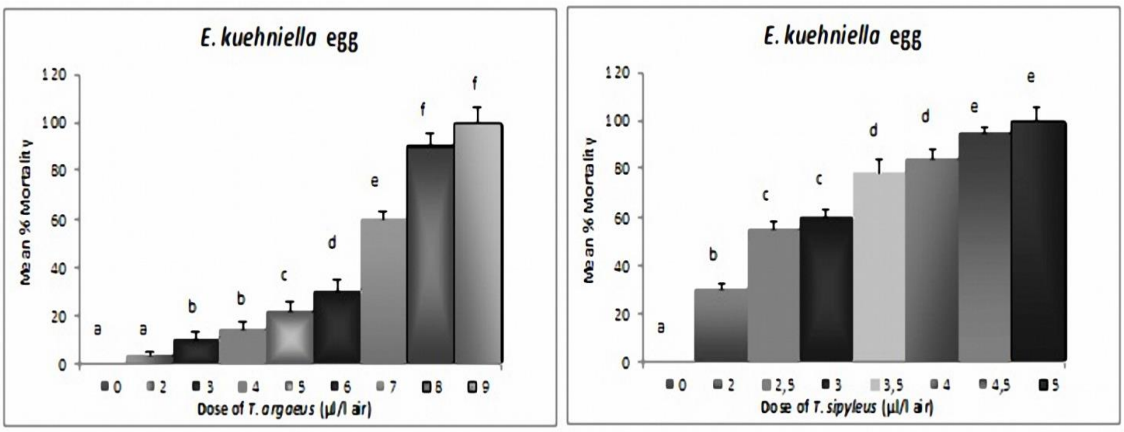

Figure 1. The percentage of mortalities of *E. kuehniella* egg stages after exposure to *T. argaeus* and *T. sipyleus* essential oils for 24 h. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means.

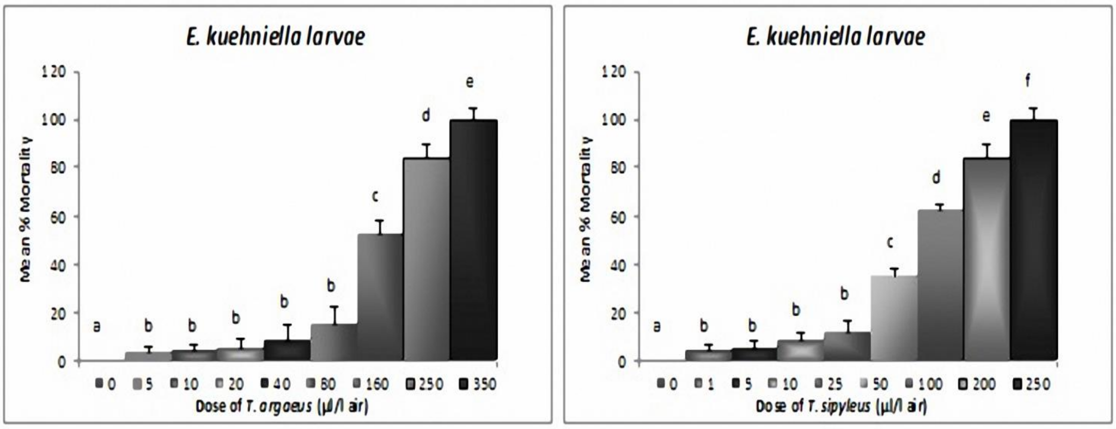

Figure 2. The percentage of mortalities of *E. kuehniella* larval stages after exposure to *T. argaeus* and *T. sipyleus* essential oils for 24 h. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means.

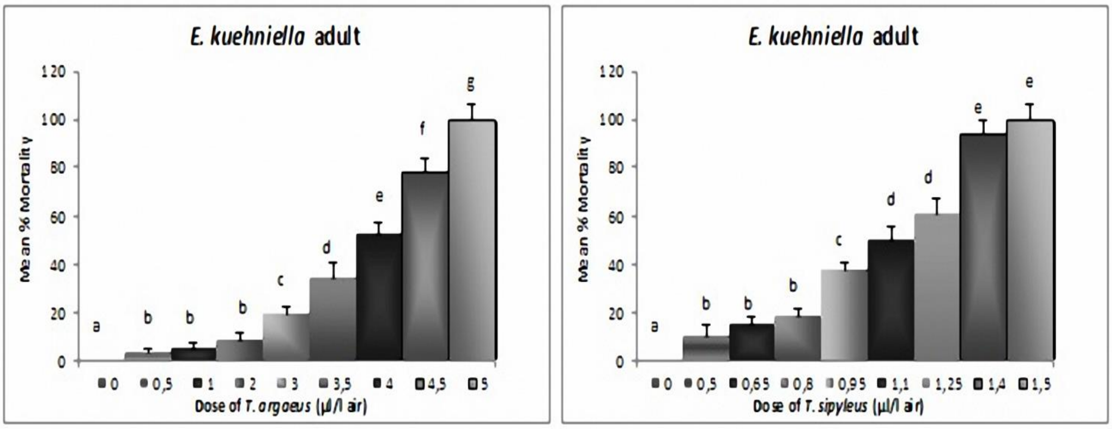

Figure 3. The percentage of mortalities of *E. kuehniella* adults after exposure to *T. argaeus* and *T. sipyleus* essential oils for 24 h. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means.

This is the first study on the insecticidal effects of essential oils of *T. argaeus* and *T. sipyleus* against different life stages of *E. kuehniella*. In summary tested doses of essential oils of *T. argaeus* and *T. Sipyleus* caused 100% mortality against egg, larvae and adult stages of *E. kuehniella* in our study. Our results clearly indicated that the fumigant toxicity against especially adults of this important stored product pest as well as cause 100% mortality at relatively low doses. These findings suggest that the essential oils of *T. argaeus* and *T. sipyleus* have potential to be developed as a natural fumigant for the control of stored-product insects.

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