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Insecticidal activity of essential oil of *Prangos ferulacea* (Umbelliferae) against *Ephestia kuehniella* (Lepidoptera: Pyralidae) and *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae)

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Abstract: Essential oil vapors obtained by the hydrodistillation of *Prangos ferulacea* (Umbelliferae) were tested on the different stages of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and egg parasitoid *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae). Extracts of the volatile fractions from *P. ferulacea* were analyzed by capillary gas chromatography-mass spectrometry. The major compound of the essential oil was detected as 2,3,6-trimethyl benzaldehyde (66.59%) and the minor compound was heneicosane (0.02%). The third instar larvae of *E. kuehniella* (LC_{50}: 379.662 µL L^{-1} air and LC_{99}: 538.755 µL L^{-1} air) and the pupal stage of *T. embryophagum* (LC_{50}: 5.947 µL L^{-1} air and LC_{99}: 19.568 µL L^{-1} air) were found to be the most tolerant stages. The essential oil was toxic to the adult stages of both the pest and its parasitoid with 100% mortality obtained after 24 h at 1.0 and 0.25 µL L^{-1} air, respectively. The LC_{50} and LC_{99} values of the essential oil against the egg stages of *E. kuehniella* and *T. embryophagum* were 320.372–486.839 µL L^{-1} air and 2.121–5.662 µL L^{-1} air, respectively. In general, the mortality rate increased with the increasing concentration of essential oil. The results of the study indicated that essential oil of *P. ferulacea* should be used as a control agent against *E. kuehniella* for an integrated pest management program.

Key words: *Ephestia kuehniella*, essential oil, insecticidal activity, *Prangos ferulacea*, *Trichogramma embryophagum*

1. Introduction

The Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), is a serious and common pest found in stored food products, such as cereal (Lynn and Ferkovich 2004). *E. kuehniella* larvae reduce the quality of products because of webbing and they also cause direct damage by feeding (Johnson et al. 1997; Ayvaz et al. 2010). A reduction in the quality of food makes *E. kuehniella* an important target pest for control methods. Controlling the pest is possible with the use of fumigants such as phosphine and methyl bromide. Methyl bromide destroys the ozone layer, and it is also very toxic to humans and animals. For this reason, methyl bromide was banned until 2015 by the United Nations Montreal Protocol. Therefore, the use of alternative control methods has become a necessity. One of these methods is biological control through integrated pest management (IPM) strategies. In biological control, egg parasitoids, especially of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), have been used much more often than other natural enemies (Goebel et al. 2001). In addition to natural enemies, aromatic plants and their powders, extracts, and essential oils are being researched for their insecticidal properties as biopesticides (Lee et al. 2004). Essential oils are good alternatives against fumigant insecticides because of their environmental safety characteristics. Fumigation is an effective method to decrease pests in stored products. Accordingly, the insecticidal activity of essential oils against different stored product pests was investigated by many researchers (Lamiri et al. 2001; Lee et al. 2004; Çalmaşur et al. 2006; Ayvaz et al. 2009; Ebadollahi 2010; Karabörklü et al. 2011; Jemaa et al. 2012) and the results of their studies suggest that essential oils have potential as control agents against stored-product pests.

The genus *Prangos* (Umbelliferae) consists of 12 taxa in the flora of Turkey and the East Aegean islands, and some species have been used in folk medicine for abortifacient effects and as antibacterial agents (Davis 1972; Davis et al. 1988; Kazerooni et al. 2006; Massumi et al. 2007; Özgen et al. 2012). The distribution of *Prangos ferulacea* ranges from East Europe to the Middle East and Central Asia. It is a perennial herb that can measure up to 150 cm in

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length. A full description of *P. ferulacea* is given in *The Flora of Turkey and the East Aegean Islands* (Davis 1972). In this study, the medicinal plant *P. ferulacea* was collected from eastern Anatolia and the chemical composition of the essential oil of this plant was determined by gas chromatography-mass spectrometry (GC-MS).

There have been no attempts to study the effect of the essential oils of *P. ferulacea* on economically important pests in stored products. Thus, the objective of this study was to examine the potential of the essential oil of *P. ferulacea* to control *E. kuehniella* and its effects on the nontarget egg parasitoid, *Trichogramma embryophagum* Hartig.

2. Materials and methods

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

The Mediterranean flour moth, *Ephestia kuehniella* Zeller, was obtained from the Adana Plant Protection Research Institute and was reared on a mixture consisting of 1 kg wheat flour, 5% yeast, and 30 g wheat germ (Tunçbilek et al. 2009). Throughout the rearing, cultures were kept in a rearing room at 27 ± 1 °C and 70 ± 5% relative humidity and under a light regime of 14 h light followed by 10 h darkness. Large numbers of 1-day-old *E. kuehniella* adults were collected from stock cultures and placed in plastic jars with screen bottoms. Eggs that fell through the screen were collected the following days and placed in petri dishes. Eggs were collected daily. These eggs were used for both the experiments and new cultures.

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

The *Trichogramma embryophagum* Hartig strain used in this study was obtained from the Adana Plant Protection Research Institute. In the laboratory, *T. embryophagum* was mass-reared on *E. kuehniella* eggs as a host for several generations. Throughout the rearing, cultures were kept in the rearing room at 24 ± 1 °C and 70 ± 5% relative humidity, and under a light regime 14 h light followed by 10 h darkness. Parasitoid cultures were started from a single female on *E. kuehniella* eggs and maintained in glass rearing vials (2–7.5 cm).

2.3. Plant material

Aerial parts of *Prangos ferulacea* (Umbelliferae) were collected at the flowering and fruit stages from the area between Tunceli and Pulumur at an altitude of 1.210 m in July 2011 in Turkey. They were enumerated as M.Koç 1334. Samples were pressed and dried according to herbarium techniques, identified by *The Flora of Turkey* (Davis 1972), and kept at the herbarium of the Bozok University Department of Biology (Yozgat, Turkey).

2.4. Isolation and analysis of the essential oil

The air-dried and ground fruit parts of the plant were exposed for 4 h to water distillation using a Clevenger-type apparatus. The obtained essential oil was stored at 4 °C until used. The chemical composition of the essential oil was determined by GC-MS technique (Table 1) using an Agilent Technologies 6890 N Network GC System 5973 MSD, ionization energy of 70 eV; 19091 N - 136 HP-Innowax column of 60 m × 0.25 mm i.d.; helium at 1 mL/min.

The GC was programmed at 60 °C for 4 min and then 5 °C min^{-1} to 250 °C, with injector and transfer line temperatures of 250 and 280 °C, respectively. Compounds were tentatively identified by GC-MS, and identifications were confirmed by comparison of the retention times and mass spectra with those of authentic samples. The relative amount of each compound was determined from the area under GC peaks. The machine was connected to an e-computer system that manages ADAMS, NISTO5, and WILEY mass spectrum libraries.

2.5. Bioassays

The essential oil of *Prangos ferulacea* was tested for its insecticidal effect against different stages of the Mediterranean flour moth, *Ephestia kuehniella*, and its natural enemy, *Trichogramma embryophagum*.

2.6. Essential oil effects on different stages of the insects

Ephestia kuehniella adults were placed in 1000-mL glass jars. Each replicate consisted of 10 adults and 6 replicates that were used for each concentration. Essential oil was applied on a filter paper strip (2.5 × 2.5 cm) attached to the bottom of the jar's cover. The same procedure was used for *T. embryophagum* adults. *E. kuehniella* and *T. embryophagum* adults were exposed to essential oil vapor with different concentrations (0–1.0 and 0–0.250 μL L^{-1} air, respectively) for 24 h. To determine the mortalities at each concentration, adults were removed from the jar and checked with a fine brush. If they did not move, they were evaluated to be dead. The concentrations that killed 50% and 99% of the exposed insects (LC_{50} and LC_{99}, respectively) were determined (Table 2). The control groups contained the same conditions but without essential oil. The larval-stage experiment of *E. kuehniella* was conducted in the same manner as the adult-stage experiment. Different concentrations were applied to larvae (0–500 μL L^{-1} air) for 24 h.

One-day-old *E. kuehniella* eggs were placed in petri dishes. An equal number of eggs (100 ± 5) for each concentration were strewn on egg cards that were glued with gum arabic. These egg cards were placed on the bottom of 1000-mL glass jars. The egg stages of *E. kuehniella* were exposed to essential oil vapor (0–450 μL L^{-1} air) for 24 h. After 24 h, egg cards were placed in petri dishes and the number of hatched eggs was scored. Egg hatchability was compared with the control and recorded.

Mated, individual females of *T. embryophagum* (24 h old) were prepared for the experiments by isolating them

Table 1. Percentages of compounds detected by GC-MS in *Prangos ferulacea* fruit essential oil.

Group	Chemical compounds	*P. ferulacea* %
Alcanes	Cyclopentane	0.29
	Heneicosane	0.02
Alcenes	α-Pinene	2.69
	Sabinene	0.21
	β-Myrcene	1.40
	Limonene	0.13
	β-Ocimene	3.76
	γ-Terpinene	1.68
	Cymene	0.51
	Mesitylene	0.23
	Verbenene	0.63
Aldehydes	Bicyclohept-2-ene-2-carboxaldehyde	0.21
	Benzaldehyde	0.07
	2,3,6-Trimethyl benzaldehyde	66.59
	3,3,4-Trimethylcyclohex-1-ene-carbaldehyde	0.09
Acetates	Chrysanthenyl acetate	15.06
Acids	2-Butenoic acid	0.08
	n-Hexadecanoic acid	0.63
Esters	Benzyl benzoate	0.13
Alcohol	Bicycloheptan-2-ol	0.88
	p-Mentha-1,5-dien-8-ol	3.57

in glass tubes for laying. *E. kuehniella* eggs were used as a host. Host eggs were sprinkled on egg cards and placed in tubes along with single females of *T. embryophagum*. After 24 h, parasitized eggs were isolated from the tubes and used for egg stage tests of *T. embryophagum*. After the eighth day of parasitism, the parasitized eggs were exposed to essential oil for testing the essential oil effect on the pupal stage of *T. embryophagum*. The egg and pupal stages of *T. embryophagum* were exposed to essential oil vapor (0–5 and 0–20 μL L^{-1} air, respectively) for 24 h. Each replicate consisted of 50 eggs and pupae, and 6 replicates were done for each concentration.

2.7. Data analysis

In order to calculate significant differences in toxicity between concentrations in different life stages of the insects, a one-way analysis of variance (ANOVA) in SPSS 10.0 at the $P < 0.05$ level was used (SPSS 2001). Probit analysis was used to forecast LC_{50} and LC_{99} values (Abbott 1925).

3. Results

3.1. Fumigant toxicity of essential oil against different stages of the insects

The percentage of adult mortalities of the 2 insects (*E. kuehniella* and *T. embryophagum*) after using different concentrations (0–1.0 μL L^{-1} air and 0–0.250 μL L^{-1} air, respectively) of essential oil for 24 h are shown in Figure 1. The differences among essential oil concentrations on adult mortality rates were statistically significant. The percentages of adult mortality of the pests were high when the concentrations of essential oil increased (*E. kuehniella* adult stage: $F = 377.404$, $DF = 8$, $P < 0.05$; *T. embryophagum* adult stage: $F = 287.667$, $DF = 6$, $P < 0.05$). According to the probit analysis, LC_{50} and LC_{99} values of the essential

Table 2. LC_{50} and LC_{99} values of *P. ferulacea* fruit essential oil against different life stages of *E. kuehniella* and *T. embryophagum*.

Time (24 h)	N	LC_{50}, µL L^{-1} air	LC_{99}, µL L^{-1} air	DF	c^2
E. kuehniella					
Egg	100	320.372	486.839	5	16.226[a]
Larvae	10	379.662	538.755	7	1.390
Adult	10	0.570	1.099	7	1.699
T. embryophagum					
Egg	50	2.121	5.662	7	14.798[a]
Pupae	50	5.947	19.568	5	50.998[a]
Adult	10	0.143	0.283	5	2.224

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.

oil were 0.570–1.099 and 0.143–0.283 µL L^{-1} air against the adult stages of *E. kuehniella* and *T. embryophagum*, respectively.

The fumigant toxicities of essential oil against *E. kuehniella* and *T. embryophagum* egg stages are shown in Figure 2. Mortality increased significantly by increasing the concentrations of essential oil (*E. kuehniella* egg stage: $F = 1331.658$, $DF = 6$, $P < 0.05$; *T. embryophagum* egg stage: $F = 2196.740$, $DF = 8$, $P < 0.05$). A probit analysis demonstrated that LC_{50} and LC_{99} values of the essential oil against egg stages of *E. kuehniella* and *T. embryophagum* were 320.372–486.839 and 2.121–5.662 µL L^{-1} air, respectively.

The essential oil of *P. ferulacea* was toxic for both the larval stage of *E. kuehniella* and the pupal stage of *T. embryophagum* (Figure 3). LC_{50} and LC_{99} values of larvae of *E. kuehniella* were 379.662 and 538.755 µL L^{-1} air, and for pupae of *T. embryophagum* they were 5.947 and 19.568 µL L^{-1} air (Table 2).

The fumigant effect was more significant on the adult stage than the egg and larval stages of *E. kuehniella*. The most resistant stage of *T. embryophagum* was the pupal stage. The results of this study showed that the fumigant toxicity of *P. ferulacea*'s fruit essential oil changed according to different developmental stages of the insects.

4. Discussion

Chemical insecticides used in agriculture are very dangerous to human health. Therefore, studies on natural and biological insecticides have increased in physiological and pharmacological areas in recent years. The different parts of many plants are utilized for insecticidal and medicinal purposes because of their biological features (Razavi 2012). In previous studies, the antibacterial effects, antioxidant properties, and allelopathic aspects of *P. ferulacea* were reported (Çoruh et al. 2007; Massumi et al. 2007; Razavi 2012). In the current study, the insecticidal effect of *P. ferulacea* was studied against *E. kuehniella* and

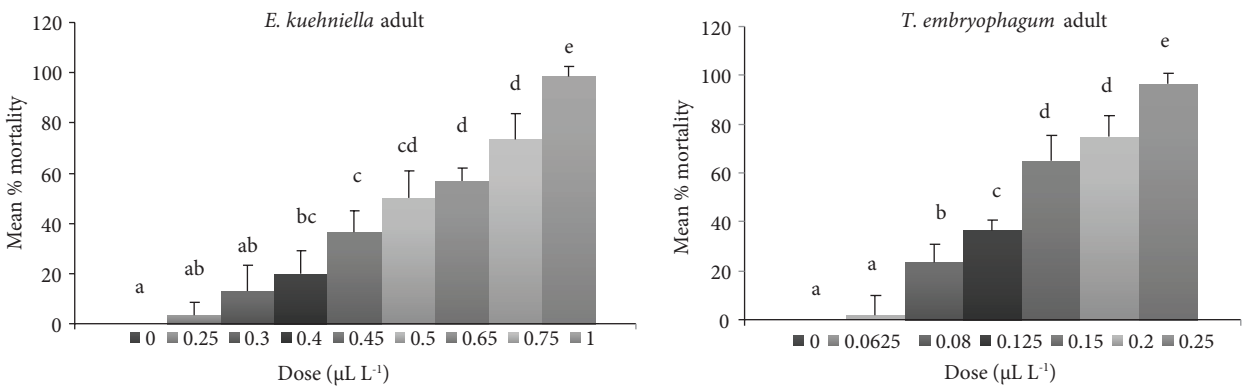

Figure 1. The percentage of mortalities of *E. kuehniella* and *T. embryophagum* adults after exposure to *P. ferulacea* essential oil for 24 h. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means.

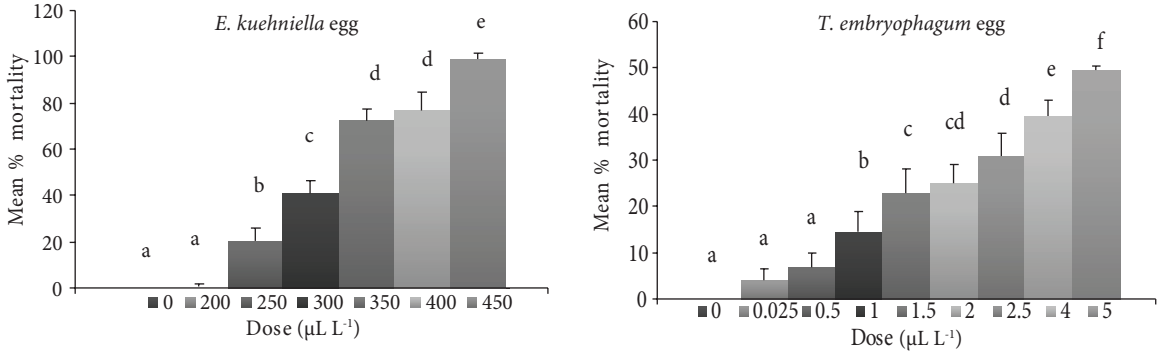

Figure 2. The percentage of mortalities of *E. kuehniella* and *T. embryophagum* egg stages after exposure to *P. ferulacea* essential oil for 24 h. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate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

its egg parasitoid, *T. embryophagum*, for the first time in Turkey.

The genus *Prangos* consists of about 30 species, and some of them have been used in folk medicine as emollient, carminative, tonic, antiflatulent, anthelminthic, and antifungal agents (Ceylan 1987). Many researchers have revealed the chemical constituents of essential oil of *P. ferulacea* from various countries (Massumi et al. 2007; Akhgar et al. 2011; Razavi 2012). Akhgar et al. (2011) and Razavi (2012) found that the main percentage component of *P. ferulacea* fruit oil was α-pinene (25.4% and 57%, respectively). On the other hand, Massumi et al. (2007) identified that chrysanthenyl acetate (26.53%) was the main component of the essential oil of *P. ferulacea* fruit oil. The results of this study showed that the main compounds found in the *P. ferulacea* were 2,3,6-trimethyl benzaldehyde (66.59%), chrysanthemyl acetate (15.06%), β-ocimene (3.76%), p-mentha-1,5-dien-8-ol (3.57%), and α-pinene (2.69%). In the analyses of fruit oil of *P. ferulacea*, 2,3,6-trimethyl benzaldehyde (66.59%) was determined at the highest level and heneicosane (0.02%) was determined at the lowest in all samples (Table 1). Comparing the main components of fruit oil of *P. ferulacea* indicated that the differences originated from different collection regions.

Many chemicals, such as α-pinene, β-myrcene, limonene, β-ocimene, γ-terpinene, and cymene, were detected in *P. ferulacea* (Razavi 2012). These chemicals have phytotoxic and fungitoxic effects in lettuce and against *Sclerotinia sclerotiorum*, respectively. The major components of chrysantenyl acetate (26.53%), limonene (19.59%), and α-pinene (19.50%) were detected by Masummi et al. (2007). The essential oil of *P. ferulacea* showed activity against *Staphylococcus aureus*, *S. epidermis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. We detected the same compounds in *P. ferulacea* fruit by GC-MS analysis in this study. Sajjadi et al. (2009) also detected 47 constituents, of which 2,3,6-trimethyl benzaldehyde (18.4%) was the main constituent, of the essential oil of the aerial parts of the *P. ferulacea*. In this study, trimethyl benzaldehyde (66.59%) was detected as the main constituent of the essential oil of the *P. ferulacea*. This compound may have an insecticidal activity against different stages of *E. kuehniella*.

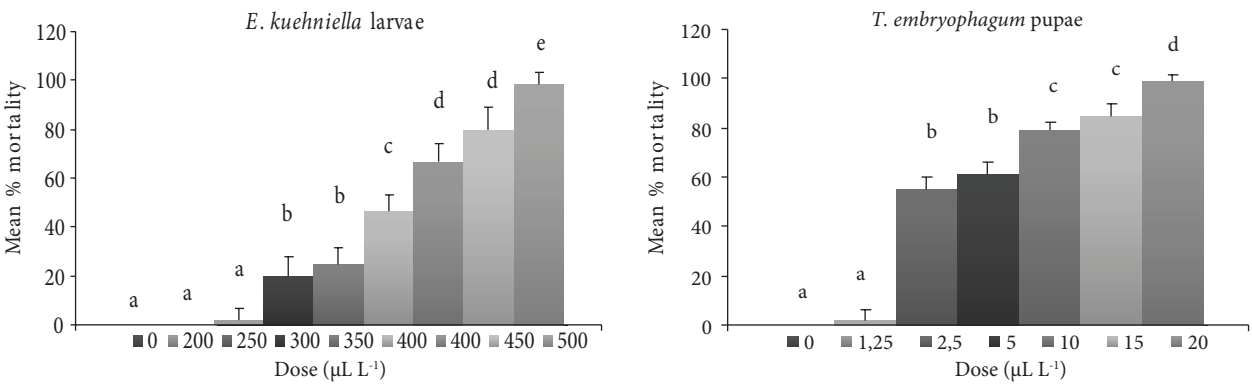

Figure 3. The percentage of mortalities of larval stage of *E. kuehniella* and pupal stage of *T. embryophagum* after exposure to *P. ferulacea* essential oil for 24 h. Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means.

Some previous studies have reported that many of the plant's essential oils have an insecticidal effect on stored-product pests (Negahban et al. 2007; Rajendran and Sriranjini 2008; Ayvaz et al. 2009, 2010; Bachrouch et al. 2010; Karabörklü et al. 2010, 2011). Ayvaz et al. (2010) showed that the main compound of essential oils of oregano and savory were carvacrol and that they caused 100% mortality after 24 h against *Plodia interpunctella* (Hubner) and *E. kuehniella*. Moharramipour et al. (2009) determined the repellency and insecticidal effectiveness of the essential oil residues of a medicinal plant, *Prangos acaulis* (Dc.) Bornm, against *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), and *Callosobruchus maculatus* (F.).

In the current study, the essential oil of *P. ferulacea* effectively killed the adult stages of *E. kuehniella* and *T. embryophagum*. The insecticidal activity changed due to increasing concentrations of essential oil. *T. embryophagum* was more sensitive than *E. kuehniella* to each concentration. Thus, the required concentration for the killing of *T. embryophagum* was much lower than that for *E. kuehniella*. A 100% mortality rate was obtained by the vapors of the essential oil of *P. ferulacea* in 1.0 and 0.25 µL L^{-1} air for 24 h against *E. kuehniella* and *T. embryophagum*, respectively.

Essential oil vapors have different effects on eggs of different insect species (Papachristos and Stamopoulos 2004; Işıkber et al. 2009). These differences in the eggs may originate from different chorion structures such as the thickness, shape, and permeability of the vitellin membrane (Işıkber et al. 2009). In the current study, the eggs of *E. kuehniella* were tolerant of the essential oils (LC_{99}: 486.839 µL L^{-1} air). The *T. embryophagum* egg stage was more sensitive than the pupal stage (LC_{99}: 5.662 µL L^{-1} air and 19.568 µL L^{-1} air, respectively).

A previous study showed that the essential oils of different plant species, *Micromeria fruticosa*, *Nepata racemosa*, and *Origanum vulgare*, killed the larvae of *E. kuehniella* at different concentrations (Aslan et al. 2005). In this study, the larvae of *E. kuehniella* were tolerant to the fumigant toxicity of *P. ferulacea* fruit essential oils compared to the adults (LC_{99}: 538.755 µL L^{-1} air and 1.099 µL L^{-1} air, respectively).

In biological control programs, the parasitoid species *Trichogramma* is used widely against the eggs of Lepidoptera (Silva et al. 2006) in particular. Egg parasitoids of this genus are more sensitive against chemical insecticides. At the same time, Asteraceae extracts were found to have more of a selectively insecticidal effect against *Trichogramma pretiosum* Riley (Taveres et al. 2009). This study found that the essential oil of *P. ferulacea* reduced the development of *T. embryophagum* in the egg stage. On the other hand, the pupal stage was more tolerant than the eggs.

This is the first study on the insecticidal effects of fruit essential oils of *P. ferulacea* against *E. kuehniella* and *T. embryophagum*. The most effective essential oils can affect the different life stages of the pest. The essential oil of *P. ferulacea* caused 100% mortality against the egg, larvae, and adult stages of *E. kuehniella* in tested concentrations in this study. It also affected the life stages of natural enemy *T. embryophagum*, especially in adults. These findings suggest that the essential oil of *P. ferulacea* has potential to be developed as a natural fumigant for the control of stored-product insects. Therefore, the combined use of essential oil and the adult stage of the parasitoid in any biological control program will not be applicable. On the other hand, these results may aid in the development of an IPM program to control *E. kuehniella*. For instance, egg parasitoid *T. embryophagum* adults can be released when *E. kuehniella* adults are observed in a stored-product region. After 24 h of the parasitoid release, the essential oil of *P. ferulacea* can be applied to the region. Thus, the sequential application of parasitoid and essential oil will suppress the population density of the pest.

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