



## Fumigation Toxicity of the Essential Oils of *Ferula persica* against *Tribolium castaneum* and *Ephestia kuehniella*

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### ABSTRACT

For the first time, the potential fumigation toxicity of the essential oils (EOs) extracted from the flower and root tissues of *Ferula persica* were evaluated against the adult insects of *Tribolium castaneum* and the fourth instar larvae of *Ephestia kuehniella*. The EOs from the flower and root tissues of *F. persica* were initially prepared using the water distillation method and subsequently utilized for fumigation toxicity assays. To this end, four different series of EO concentrations (each one contained a group of six different concentrations) belonging to both tissues were applied for *T. castaneum* and *E. kuehniella*. The experiment was based on a completely randomized design with three replicates, and the mortality rate after 24 hours was employed as a dependent criterion. According to the four individual ANOVA results, in all cases, EO concentration substantially affected the mortality rate of both storage pests of interest. Furthermore, the median lethal concentrations (LC<sub>50</sub>) of EOs from the flower and root of *F. persica* against *T. castaneum* were calculated as 220.832 and 371.475  $\mu\text{L L}^{-1}$  air, respectively. However, higher LC<sub>50</sub> values of 860.041  $\mu\text{L L}^{-1}$  air and 1268.148  $\mu\text{L L}^{-1}$  air were respectively detected upon exposure of the fourth instar larvae of *E. kuehniella* versus EOs from flower and root tissues. Considering LC<sub>50</sub> values of the current fumigant assay, it seems that the insecticidal activity of EOs from both flower and root tissues of the plant is less toxic against *E. kuehniella* rather than *T. castaneum*. Altogether, the results revealed that these EOs could be possibly nominated as safe botanical pesticides for biocontrol of storage pests, including *E. kuehniella* and particularly *T. castaneum*.

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### 1. Introduction

The genus *Ferula* encompasses ~172 species which are geographically spread from central Asia westward throughout the Mediterranean region to northern Africa (Mabberley, 2017). According to (Asili *et al.*, 2009), ~30 species of the genus *Ferula* spp. have been reported to be grown in different mountainous regions of Iran. The native habitat of *F. tabasensis*, *F. gummosa*, and *F. persica* is assumed to be Iran. Resembling some medicinal plants, the genus *Ferula* spp. benefits from a broad spectrum of naturally occurring products, which are commonly produced in different tissues of the plant, including flower, stem, leaf, and root. Both aerial and underground tissues of the plant species are rich

sources of essential oils (EOs), and could be accordingly utilized as biomaterials with various biological activities, including antibacterial, antifungal and antioxidant activities (Salehi *et al.*, 2019; Zomorodian *et al.*, 2018), followed by insect pest biomanagement/biocontrol, as the new emerging term of “biopesticides”.

Among various stored product pests, the Mediterranean flour moth of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) is globally assumed as one of the most destructive ones of stored grains, predominantly in the flour form (Hill, 2002; Kurtuluş *et al.*, 2020; Özder, 2004; Tarlack *et al.*, 2015). Owing to its substantial destruction on either flour or whole grain,

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the pest is economically considered a key limiting factor to the quality dropping of many grain products worldwide (Javanshir et al., 2022; Kurtulus et al., 2020). Furthermore, among 100 insect species belonging to the family of *Tenebrionidae* (Order: *Coleoptera*) with enough capabilities to cause substantial damage to the quality of stored agricultural products, the stored product pest of *Tribolium castaneum* is considered as the superior one (Upadhyay et al., 2018). As a model insect for fundamental and applied research over history (Campbell et al., 2022), the pest activity can negatively affect the quality of various stored products such as cereals, legumes, spices, oilseeds, etc., remarkable destructive damage with huge economic losses could be accordingly occurred (Abou-Taleb et al., 2016). Due to the economic importance of both previously mentioned stored product pests, numerous attempts have been made to diminish their undesirable damages, including various chemical insecticides. Despite their superiority, the application of such synthetic products is normally accompanied by different unwanted consequences, not only on the environment but also on livestock and human health throughout the world, as reviewed comprehensively by (Rani et al., 2021). Recently many efforts have been made to find bio-based alternative options known as “biopesticides”, normally extracted from plant materials and/or other living organisms to minimize such undesirable consequences (Copping and Menn, 2000; Glare et al., 2012; Kumar et al., 2021; Mnif and Ghribi, 2015). In this sense, the EOs of diverse medicinal plants have been nominated as “green pesticides” and also as one of the most suitable candidates to biologically control of various insect-pests including storage pests (Mossa, 2016; Singh and Pandey, 2018; Upadhyay et al., 2018).

The mechanism of action of plant-based EOs against insect-pest activity could be generally divided into toxic, repellent, ovicidal, larvicidal, antifeedant, attractants, insect growth regulators (IGR), and fumigant activities, as reviewed comprehensively by (Mossa, 2016). The fumigant activity which is mainly due to the volatile nature of aromatic terpenes convey volatility (Upadhyay et al., 2018), has been widely proposed as a solution to control the stored insect-pests including *T. castaneum* (Abouelatta et al., 2020; Baccari et al., 2020; Oviedo-Sarmiento et al., 2021; Papanikolaou et al., 2022; Pavela et al., 2020; Wu et al., 2021) and *E.*

*kuehniella* (Aouadi et al., 2020; Bouzeraa et al., 2019; Chaaban et al., 2019; Ghasemi et al., 2014). Furthermore, regarding the EOs from *Ferula* spp., several investigations have focused on the fumigant activities against *E. kuehniella* (Ghasemi et al., 2014), *T. castaneum* (Baccari et al., 2020; Pavela et al., 2020), *Plodia interpunctella* (Sokuti and Ghasemi, 2018), *Tetranychus urticae* Koch (Fatemikia et al., 2017), and *Aphis gossypii* Glover. (Koorki et al., 2022).

Irrespective of such or similar efforts, there is no relevant information about assessing the fumigation toxicity of the EOs from *F. persica* on the adult insects of *T. castaneum* and the fourth instar larvae of *E. kuehniella*, as a potential biopesticide. Here, as a result, an endeavor was made to scrutinize the potential fumigation toxicity of the EOs extracted from flower and root tissues of *F. persica* against the adult insects of *T. castaneum* and the fourth instar larvae of *E. kuehniella*.

## 2. Materials and methods

### 2.1. Plant materials and preparation of EOs

Plant sampling of *F. persica* was taken place in the mountainous regions of the Sharestanak district of Karaj (Alborz, Iran) in May 2017, with an altitude of 2200 m above sea level (MASL; 35° 58' 10" N and 51° 21' 13" E). The two individual tissues of the harvested plants, including flowers and roots, were carefully separated and air-dried to achieve plant materials with homogeneous dried patterns. Subsequently, the EO isolation was accomplished from ~30 grams of the resultant fine powder of each tissue via a 5-hour hydro-distillation process using a Clevenger-type apparatus. Finally, water elimination of the isolated EOs was performed using anhydrous sodium sulfate, and the resulting EOs of both flower and root tissues were poured into sealed glass containers and kept at 4 °C until further biological assays.

### 2.2. Fumigant toxicity assay

The fumigant toxicity of the EOs derived from the flower and root tissues of *F. persica* was appraised versus the adult stage of *T. castaneum* (< 24-h old) as well as the fourth instar larvae of *E. kuehniella* using the fumigation method (Choi et al., 2004). The experiment was conducted at the Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (2020),

and both pests under study were prepared by the same department. For the fumigant toxicity assay, filter papers (1 × 1 cm) were initially treated with different concentrations of the corresponding EOs by means of micropipettes and pasted immediately on the internal surfaces of the door of each petri dishes (10 cm diameter). Regarding the control group, the filter papers were treated with the same volumes of distilled water (DW). For both pests under study, after putting 20 biological samples into each petri dish, each one was carefully sealed with parafilm. All the control and treated dishes were kept in a growth chamber (25 ± 1 °C, 60 ± 10% RH) for 24 hours, and subsequently mortality rate was recorded. Notably, based on a preliminary logarithmic analysis, four different series of EO concentrations (each one contained a group of six different concentrations) belonging to both tissues were applied for *T. castaneum* and *E. kuehniella*. In this sense, for the first storage pest of *T. castaneum*, six different concentrations of “147.91, 194.98, 257.04, 338.84, and 446.68 μL L<sup>-1</sup> air” were deduced for flower, while the second group of “223.87, 295.12, 389.04, 512.86, and 676.08 μL L<sup>-1</sup> air” was applied for root tissue. Regarding the second storage pest of *E. kuehniella*, the first EO concentration group from flower tissue comprised “0.00 (DW), 501.18, 704.94, 1000, 1412.54, and 1995.26 μL L<sup>-1</sup> air”, whereas for the second tissue of root the last group was decorated as “0.00 (DW), 741.31, 1000, 1348.96, 1819.7, and 2454.71 μL L<sup>-1</sup> air”.

### 2.3. Statistical analysis

The fumigant toxicity assay was analyzed based on a completely randomized design (CRD) with three replicates for each pest of interest. The mortality rate was employed as the dependent criterion, and the resultant data were checked for normality by applying Shapiro-Wilk's test. Subsequently, statistical analysis of the raw data was performed via one-way analysis of variance (ANOVA), and Duncan's multiple range test was employed to measure specific differences between pairs of means ( $p \leq 0.05$ ). The statistical analyses were carried out by SPSS software package (Khattree and Naik, 2000). Furthermore, for the calculation of lethal concentrations (LC10-LC90) for each EO, the mortality rate (%) was corrected using Abbott's formula:

$$\text{Corrected mortality} = (T-C/100-C) \times 100 \quad (1)$$

where T and C indicate the number of dead pests in treatment and control groups, respectively (Abbott, 1925). Then, the corrected mortality data were analyzed using Probit analysis in POLO-Plus 1.0 software (LeOra 1994).

## 3. Results and discussion

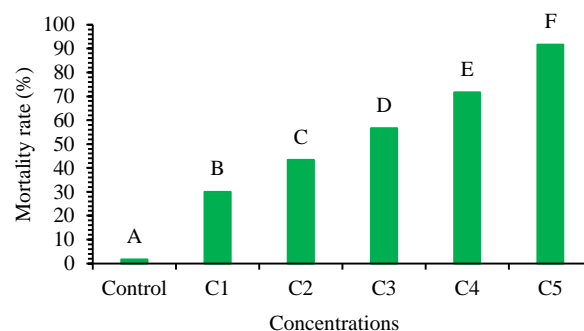
### 3.1. Fumigant toxicity of the EOs from *F. persica* on *T. castaneum*

Based on the Shapiro-Wilk method, the data possessed a normal distribution, so, the possibility of further analysis was provided.

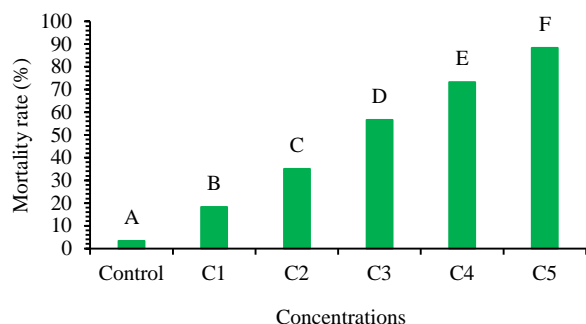
According to the ANOVA results, for both tissues, EO concentration was statistically significant on the mortality rate (%) of *T. castaneum* population ( $p$ -value < 0.0001; Table 1). As could be seen in Fig. 1 and 2, for both tissues, as expected, the average mortality rates of the pest were increased in line with a growth in the applied EO concentrations, which were statistically significant ( $p$ -value < 0.05). Regarding the EOs belonging to the first tissue of the flower, the mean mortality rate of *T. castaneum* population ranged between 1.67 % (control) to 91.67 % (C5; 446.68 μL L<sup>-1</sup> air), while for the second one, this ratio varied from 3.33 % (control) to 83.33 % (C5; 676.08 μL L<sup>-1</sup> air). Furthermore, the median lethal concentrations (LC<sub>50</sub>) of *F. persica* EOs derived from both tissues of flower and root were computed as 220.832 μL L<sup>-1</sup> air and 371.475 μL L<sup>-1</sup> air, respectively (Table 2).

**Table 1. ANOVA results for the application of flower and root EOs concentrations of *F. persica* on the mortality ratios (%) of *T. castaneum* population.**

Source of variation	df	Mean Square (flower)	Mean Square (root)
EO concentration	5	3015.833***	3215.833***
Error	12	23.61111	15.27778



**Figure 1. Mean comparison of insecticide effect (mortality rate; %) of the EO extracted from flower tissue of *F. persica* against *T. castaneum* population. Note: Control and C1-C5 indicate 0.00 (DW), 147.91, 194.98, 257.04, 338.84, and 446.68 μL L<sup>-1</sup> air of EOs.**

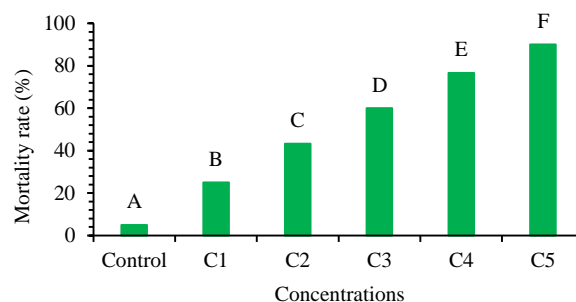


**Figure 2.** Mean comparison of insecticide effect (mortality rate; %) of the EO extracted from root tissue of *F. persica* against *T. castaneum* population. Note: Control and C1-C5 indicate 0.00 (DW), 223.87, 295.12, 389.04, 512.86, and 676.08  $\mu\text{L L}^{-1}$  air of EOs

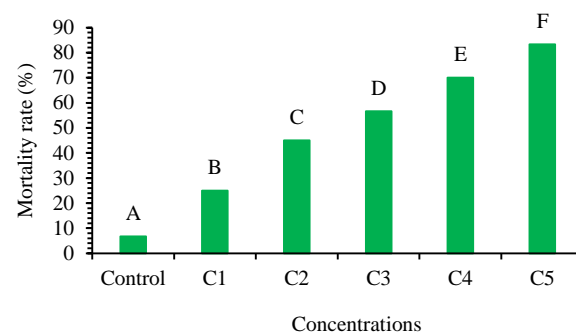
## 2.2. Fumigant toxicity of the EO of *F. persica* on *E. kuehniella*

Similar to the previous section, the effect of “EO concentration” was statistically significant on the mortality rate (%) of the current *E. kuehniella* population ( $p$ -value < 0.0001; Table 3). As Fig. 3 and 4 are shown (i.e., mean comparison results), the average mortality rates were gradually boosted in parallel with an increase in the applied EO concentrations. In this sense, regarding flower EOs, the mean mortality rate of the current *E. kuehniella* population ranged from 5.00 % to 90.00 % for control and the last applied EO treatment of C5 (i.e., 1995.26  $\mu\text{L L}^{-1}$  air), whereas for the second tissue of root, this parameter was quantified from 6.67 % (control) to 83.33 % (C5; 2454.71  $\mu\text{L L}^{-1}$  air). Furthermore, the median lethal concentrations ( $LC_{50}$ ) of *F. persica* EOs

extracted from flower and root tissues were reported as 860.041  $\mu\text{L L}^{-1}$  air and 1268.148  $\mu\text{L L}^{-1}$  air, respectively (Table 4).



**Figure 3.** Mean comparison of insecticide effect (mortality rate; %) of the EO extracted from flower tissue of *F. persica* against *E. kuehniella* population. Note: Control and C1-C5 indicate 0.00 (DW), 501.18, 704.94, 1000, 1412.54, and 1995.26  $\mu\text{L L}^{-1}$  air of EOs.



**Figure 4.** Mean comparison of insecticide effect (mortality rate; %) of the EO extracted from root tissue of *F. persica* against *E. kuehniella* population. Note: Control and C1-C5 indicate 0.00 (DW), 741.31, 1000, 1348.96, 1819.7, and 2454.71  $\mu\text{L L}^{-1}$  air of EOs.

**Table 2.** The estimated  $LC_{50}$  and  $LC_{90}$  values of EOs from *F. persica* were applied against *T. castaneum*.

EOs	df	$LC_{50}$ ( $\mu\text{L/L}$ air; 95% CI) (lower-upper)	$LC_{90}$ ( $\mu\text{L/L}$ air; 95% CI) (lower-upper)	Chi-Square	Heterogeneity
Flower	13	220.832 (195.698-244.355)	490.471 (414.466-643.92)	4.662	0.359
Root	13	371.475 (335.999-406.712)	716.271 (623.198-884.068)	2.365	0.182

**Table 3.** ANOVA results for applying flower and root EOs concentrations of *F. persica* on the mortality ratios (%) of *E. kuehniella* population.

Source of variation	df	Mean Square (flower)	Mean Square (root)
EO concentration	5	3063.333***	2432.22222***
Error	12	27.77778	25

**Table 4.** The estimated  $LC_{50}$  and  $LC_{90}$  values of EOs from *F. persica* were applied against *E. kuehniella*.

EOs	df	$LC_{50}$ ( $\mu\text{L/L}$ air; 95% CI) (lower-upper)	$LC_{90}$ ( $\mu\text{L/L}$ air; 95% CI) (lower-upper)	Chi-Square	Heterogeneity
Flower	13	860.041 (827.127-740.73)	2082.515 (1730.248-2776.335)	3.29	0.253
Root	13	1268.148 (1086.234-1446.735)	3176.939 (2555.356-4615.378)	2.423	0.186

The mean mortality rates of *T. castaneum* population ranged between 1.67 % (control) to 91.67 % (C5; 446.68  $\mu\text{L L}^{-1}$  air) against flower EOs, while for the root EOs, this parameter varied from 3.33 % (control) to 83.33 % (C5; 676.08  $\mu\text{L L}^{-1}$  air). There was also a positive association between six levels of EO concentrations and the average mortality rates of the pest resulting from the fumigant toxicity assay. Furthermore, the  $\text{LC}_{50}$  calculated for the flower and root tissues were detected as 220.832  $\mu\text{L L}^{-1}$  air and 371.475  $\mu\text{L L}^{-1}$  air, demonstrating the higher toxicity levels of the former than the latter against *T. castaneum*. Such a difference could be explainable by the possible variability in the metabolite contents of the flower and root tissues of *F. persica* (Iranshahi et al., 2006; Javidnia et al., 2005).

Considering the average mortality rates for EOs of flower and root against *E. kuehniella*, the superiority of the former (50.00 %) versus the latter (47.78 %) was detected, as the maximum mortality rate for flower and root tissues was acquired as 90.00 % (C5; 1995.26  $\mu\text{L L}^{-1}$  air) and 83.33 % (C5; 2454.71  $\mu\text{L L}^{-1}$  air), respectively. Consistent with this, the  $\text{LC}_{50}$  value recorded for flower (860.041  $\mu\text{L L}^{-1}$  air) was  $\sim 68$  % lower than root tissue (1268.148  $\mu\text{L L}^{-1}$  air), again corroborating greater fumigant toxicity power of the aerial parts than the underground tissue against *E. kuehniella*. Notably, excluding tissue type, it seems that the lethality power of EOs from this plant species is much higher against *T. castaneum* than *E. kuehniella*, which could be probably owing to the less sensitive nature of *E. kuehniella* against chemical compositions that existed in the EOs of *F. persica*.

Based on the fumigant toxicity of the EOs (different concentrations of 0, 16, 32, 64, 128, and 256  $\mu\text{L/L}$  air) from the stems and leaves of *Piper hancei* Maxim applied on the 7-14-day old adults of *T. castaneum* (Wu et al., 2021), obvious fumigant activities were identified against *T. castaneum* adults ( $\text{LC}_{50}=322.52$  (48 h) and 145.82 (72 h) ( $\mu\text{L/L}$  air) (Wu et al., 2021). Insecticidal activity of EOs from *Ferula tunetana* against *T. castaneum* adults was assessed using different doses of 25, 50, 100 and 200  $\mu\text{L/L}$  air based on the fumigation and contact bioassay (Baccari et al., 2020). After 24 h EOs exposure, the mortality rate ranged from 6.6% for the lowest concentration (25  $\mu\text{L/L}$  air) to 73.3% for the maximum level concentration (200  $\mu\text{L/L}$  air). Furthermore, the  $\text{LD}_{50}$  of the flowers-derived EO on the

adult red flour beetle was 161.89  $\mu\text{L/L}$  air after 24 h (Baccari et al., 2020).

As mentioned above, the  $\text{LC}_{50}$  recorded here for the flowers and roots against *T. castaneum* adults were quantified as 220.832 and 371.475  $\mu\text{L/L}$  air, which is nearly lower than that reported by (Baccari et al., 2020), which was 161.89  $\mu\text{L/L}$  air. Such a difference in the current and earlier recorded  $\text{LC}_{50}$  values could be owing to the following two possible explanations. The first one involves the possible variations in the quality and/or quantity of the natural compounds available in the EOs of *F. persica* as compared with the *F. tunetana*. The second one may be rooted in the place of experiment and variability in the population under study. In other words, since both investigations have been conducted independently and in two different geographical regions, such a difference could be attributed to the different “susceptibility thresholds” of the two different populations of *T. castaneum* against EOs of *F. persica* and *F. tunetana*. Meanwhile, comparing our results with (Wu et al., 2021), in which an  $\text{LC}_{50}$  of 322.52 of EOs of *P. hancei* against *Piper hancei* was recorded, it seems that EOs from *F. persica* is presumably more toxic.

On the other hand, both toxicological and haematological impacts of EOs from *Callistemon viminalis* and the Iranian *F. gummosa* were assessed on the mortality rate of the fifth larva instars of *Ephestia kuehniella* via fumigant and topical exposure. Based on the results of fumigant toxicity, the  $\text{LC}_{50}$  values of 76.44 and 24.60  $\mu\text{L/L}$  air were recorded for EOs of *F. gummosa* and *C. viminalis*, respectively (Ghasemi et al., 2014). Looking more carefully, the insecticidal activity of the EOs from *F. gummosa* is apparently much higher than that of recorded here for either flower (860.041  $\mu\text{L/L}$  air) or root (1268.148  $\mu\text{L/L}$  air) tissues of *F. persica*. Again, the two potential reasons explained above could be applied here to describe such differences between the current and earlier  $\text{LC}_{50}$  values.

According to (Pavela et al., 2020), the probable mode of action toward the toxicity perceived from the EOs in the current and earlier similar studies could be related to the following assumptions. The occurrence of sulphur-containing volatiles, which is nearly popular in the genus *Ferula* spp., as they could easily react with the thiol groups of proteins (Soltani et al., 2018), including the detoxicant enzymes of some insects (Pavela et al., 2020). Meanwhile, the monoterpene

compounds available in the EOs of genus *Ferula* spp. may exert toxicity on the nervous system (Pavela et al., 2020), for instance, possible interaction thereof with the GABAergic and octopaminergic systems of insects (Campos et al., 2019; Pavela and Benelli, 2016). Since EOs of the genus *Ferula* spp. possessed nearly (but not essentially) similar major components, such a high observed mortality rate in both stored product pests could be due to the compounds reported earlier, such as  $\alpha$ -pinene (Fatemikia et al., 2017), and probably other volatile compounds in EOs, as the same has also been emphasized by (Baccari et al., 2020).

#### 4. Conclusion

In conclusion, an effort was made here to assess the potential insecticidal (fumigant) activity of EOs extracted from aerial and underground tissues of *F. persica* against the two main stored product pests, including *T. castaneum* (adult insects) and *E. kuehniella* (fourth instar larvae). In this context, the LC<sub>50</sub> of EOs from the flower and root of *F. persica* against *T. castaneum* were respectively calculated as 220.832 and 371.475  $\mu\text{L L}^{-1}$  air, while higher amounts of 860.041  $\mu\text{L L}^{-1}$  air and 1268.148  $\mu\text{L L}^{-1}$  air were respectively detected against the fourth instar larvae of *E. kuehniella*. Considering LC<sub>50</sub> values of the current fumigant assay, it seems that the insecticidal activity of EOs from both flower and root tissues of the plant species is less toxic against *E. kuehniella* rather than *T. castaneum*. In addition, considering LC<sub>50</sub> values, it appears that the lethality power of flower-extracted EOs is higher than root tissue either against *T. castaneum* or *E. kuehniella*. Altogether, the results demonstrated that EOs from *F. persica* could be nominated as a potential biopesticide/natural insecticide for the storage pests like *E. kuehniella* and predominantly *T. castaneum*.

#### Conflict of interests

All authors declare no conflict of interest.

#### Ethics approval and consent to participate

No human or animals were used in the present research.

#### Consent for publications

All authors read and approved the final manuscript for publication.

#### Availability of data and material

All the data are embedded in the manuscript.

#### Authors' contributions

All authors had an equal role in study design, work, statistical analysis and manuscript writing.

#### Informed consent

The authors declare not to use any patients in this research.

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