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# Fumigant toxicity of three essential oils of *Rosa hybrida* L., *Citrus aurantium* L. and *Ixora chinensis* L., on second, third and fourth instars larvae of *Ephestia kuehniella* Zeller (Lep.: Pyralidae) under laboratory conditions

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**Introduction**: Stored-grain insect pests have been damaging food grains in granaries and store houses and accounts for 10-40% loss worldwide (Mossa, 2016). The Mediterranean flour moth, *Ephestia kuehniella* (Zeller), is a cosmopolitan pest of cereal (Nagan *et al.*, 2003). It is one of the most important pests of stored products which causes a lot of damages (Nielsen, 1998). Infestations of stored product insects are typically controlled by the use of synthetic insecticides and fumigants. The insecticides are the most effective applications for the protection of flour mills, grocery shops, warehouses, and other agricultural commodities from stored insect infestation. However, they have a number of associated disadvantages, such as environmental pollution, development of insect resistance and potential toxicity to non-target organisms. Therefore, today there is a need to develop alternatives that is capable of reducing the large-scale utilization of synthetic pesticides for crop protection (Mossa, 2016). The use of plant essential oils is one of the suitable methods for controlling stored product pests, which has been considered in recent years. Plant essential oils in general have been recognized as an important natural source of pesticides.

**Materials & Methods**: The primary colony of The Mediterranean flour moth was prepared from the storage pest breeding room of the entomology department of the plant protection of Urmia University. The plants *Ixora chinensis* L. from the family Rubiaceae, *Citrus aurantium* L. from the family Rutaceae and *Rosa hybrida* L. from the family Rosaceae were collected from the Urmia Medicinal Plants Research Institute. In this study the lethality effect of essential oils of these plants were studied on the second, third and fourth instars larvae of *E. kuehniella* at laboratory conditions. The experiments were conducted at 27±2 °C, and 75±5% relative humidity under dark conditions. All the experiments were carried out under the same environmental conditions. In preliminary experiments, the minimum and maximum concentrations that caused 10% and 90% mortality, respectively, were determined (Pourmirza, 2005). Then, between these two concentrations, four concentrations were determined by the method of logarithmic intervals. Finally, six concentrations were used along with the control treatment (distilled water) for each larval stage. Bioassay tests

were performed according to the method of Negahban and Moharramipour (2007). Plastic containers with a volume of 100 cc and a lid with a diameter of 2 cm were used for the experiments. Certain concentrations of each essential oil along with 3% tween were poured using a micro sampler on a flat paper that was placed inside the lid of the test containers. Then 10 larvae of the same stage were placed inside the jars along with food. The number of dead insects in treated and control containers was counted and recorded after 24, 48 and 72 hours. This experiment was performed on second, third and fourth instar larvae. Different larval ages were separated based on the width of the head capsule (Yazdanian *et al.,* 2005). The data obtained from the mortality of larvae of different ages were analyzed by Probit analysis method in SPSS23 software after correction with Abbott formula (1925) and $LC_{25}$ and $LC_{50}$ values were calculated in Probit program.

Results & discussion: $LC_{50}$ values of *Rosa hybrida* essential oil on the second, third and fourth instars larvae after 24, 48 and 72 hours were «640.60, 409.22 and 308.76», «1030.61, 812.26 and 446.26» and «2019.84, 1841.97 and 1120.32» $\mu l.L^{-1}$ air, respectively. $LC_{50}$ values of *Citrus aurantium* essential oil on the second, third and fourth instars larvae at the three times were «1078.70, 949.06 and 658.72», «1170.25, 1098.25 and 804.58» and «2311.99, 2200.46 and 2072.13» $\mu l.L^{-1}$ air, respectively. $LC_{50}$ values of *Ixora chinensis* essential oils on the second, third and fourth instars larvae after 24, 48 and 72 hours were «1273.18, 951.35 and 636.88», «1254.98, 2073.28 and 1183.33» and «3288.35, 2604.56 and 1761.68» $\mu l.L^{-1}$ air, respectively. Based on the results of calculating the toxicity index, rose essential oil with a toxicity index of 100% in all three time periods of 24, 48 and 72 hours had the highest lethality on the second, third and fourth larval instars. By increasing the dose of plant essential oils, the mortality rate increased in all three larval stages. Also, the respiratory toxicity of all three essential oils on the second, third and fourth instar larvae increased with the increase in the exposure time of the larvae to the toxic vapors of the essential oils.

In a research, the effect of ethanolic extract of *Eugenia aromatica* (L.) on the rate of egg hatching, the emergence of adult insects and the mortality of larvae and adult insects of *Ephestia cautella* (Walker) after 24, 48, 72 and 96 hours of treatment was investigated and the results the research determined that the effect of *E. aromatica* essential oil on this pest was dependent on the concentration and duration of exposure to the extract, so that with the increase in the concentration of the essential oil and the duration of exposure, the effect of the essential oil on the investigated indicators increased and the mortality of larvae and adult insects increased significantly (Akinneye *et al*., 2019), which is consistent with the results of the present research. In another study, the insecticidal effect of *Agastache foeniculum* essential oil against *Plodia interpunctella* was studied and it was found that their insecticidal rate increased with increasing concentration and duration of treatment (Ebadollahi *et al.,* 2010), which is similar to the results of the present study. Other researchers obtained similar results and found that with the increase in the duration of the effect of essential oils, the number of losses caused by essential oils increases (Tandorost & Karimpour, 2012; Mehany, 2014; Zarei *et al*., 2015; Pandir & Bas, 2016; Sokuti & Ghassemi, 2018). In another study, by investigating the insecticidal effect of *Laurus nobilis* L. and *Myrtus commonis* L. essential oils against the second, third and fourth instar larvae and adults of The Mediterranean flour moth, it was found that with the passage of time, the respiratory toxicity of the essential oils decreased (Salehi *et al.,* 2010), which is not consistent with the results of the present research. The difference in the conditions of the two researches and the type of the investigated population can be the reason for the difference.

Conclusion*:* The results of the present research showed that the two essential oils of *Rosa hybrida* and *Citrus aurantium* can be used in order to reduce the harmful effects and risks caused by the use of chemical insecticides to control the Mediterranean flour moth pest in the form of integrated management in warehouses. Although the practical use of plant essential oils as storage preservatives requires extensive research in the field of standardization, identification and isolation of active compounds and obtaining suitable and cost-effective formulation methods for use in the storage environment. It is hoped that the current research will open a clear horizon for the practical use of some plant essential oils as a suitable alternative to common chemical poisons in the control of warehouse pests at large levels.

***Keywords: Citrus aurantium, Ephestia kuehniella, Ixora chinensis, plant essential oils, Rosa hybrid***

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*Statement on ethics:* All ethical matters have been observed.