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Effects of *Oliveria decumbens* and *Ferula assa-foetida* essential oils and their nanoemulsions on Mediterranean flour moth (*Ephestia kuehniella*)

Faezeh Bagheri[a*], **Mohammad Ramezani**[a], **Fatemeh Raouf Fard**[b]

[a] Department of Plant Protection, School of Agriculture, Shiraz University, Shiraz, I. R. Iran.
[b] Department of Horticultural Sciences, School of Agriculture, Shiraz University, Shiraz, I. R. Iran.

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ABSTRACT- In this study, effects of essential oils and their nanoemulsions extracted from *Oliveria decumbens* flowers and *Ferula assa-foetida* oleo-gum-resin were evaluated on late-instar larvae of the Mediterranean flour moth (*Ephestia kuehniella*). The selected concentrations of the essential oils and their nanoemulsions were as follows: *O. decumbens* essential oils (250, 300, 370, 410, and 490 µL/L), *F. assafoetida* essential oils (100, 120, 150, 210, and 270 µL/L), *O. decumbens* essential oil nanoemulsion (170, 230, 300, 350, and 410 µL/L), and *F. assa-foetida* essential oil nanoemulsion (30, 60, 90, 110, and 130 µL/L). The results showed that the nanoemulsion of *F. assa-foetida* essential oil was more effective than that of *O. decumbens*, with a 50% lethal dose (LD_{50}) of 79.249 µL/L being more effective than a 50% lethal dose (LD_{50}) of 284.991 µL/L. In addition, the toxicity levels of *F. assa-foetida* were considerably higher than those of *O. decumbens* in both its nanoemulsion and essential oil forms, with lethal dose ratios recorded at 0.278 and 0.456, respectively. These findings suggest that essential oils could serve as an alternative to synthetic pesticides for controlling Mediterranean flour moths.

INTRODUCTION

Insect pests are estimated to damage 10–25% of the world's annually stored crops. In the absence of improved storage technologies, these losses can rise to as much as 40%. Systemic insecticides are often used to protect stored products, but their application can accelerate the development of pesticide resistance. Among the most destructive pests, the Mediterranean flour moth (*Ephestia kuehniella*) not only causes direct damage to the stored grains but also reduces quality through contamination and obstructs machinery with its excrement and webbing (Upadhyay & Ahmad, 2011). The adverse consequences of conventional pesticides, including environmental harm, negative impacts on non-target organisms, high costs, and the development of resistance, have intensified interest in eco-friendly alternatives such as plant essential oils. These oils interfere with the metabolic, physiological, and behavioral processes of pests; degrade quickly in the environment; are generally harmless to non-target organisms; and act as toxicants, repellents, and antifeedants against a variety of storage pests (Isman, 2020; Peixoto et al., 2015; Regnault-Roger, 2013). Their insecticidal action is partly explained by their hydrophobic nature, which disrupts cuticular waxes and blocks insect spiracles, leading to death through asphyxia or dehydration. The effectiveness of essential oils is typically attributed to their major constituents, although synergistic compounds can enhance their activity by facilitating cellular accumulation or adsorption (Isman, 2020; Regnault-Roger, 2013). Some essential oil components, including acetylcholinesterase inhibitors, have been shown to exert neurotoxic effects by disrupting cholinergic, octopaminergic, and GABAergic systems (López & Pascual-Villalobos, 2010). Despite these advantages, essential oils face limitations when applied in natural storage environments. Their low water solubility, chemical instability, and rapid evaporation restrict their persistence, while factors such as oxidation, ultraviolet exposure, and heat further reduce their efficacy. Consequently, research has turned to methods that stabilize plant essential oils in environmentally friendly ways. One promising approach is the development of nanoemulsions with droplet sizes of 100 nm or smaller. These formulations provide controlled release, protection against evaporation and degradation, and ease of application in natural settings. Moreover, they enhance insecticidal efficacy compared to conventional formulations by offering greater surface area, improved solubility, increased mobility, and enhanced cellular adsorption. Unlike many synthetic pesticides, nanoemulsions do not rely on organic solvents, making them less harmful to the environment while still being soluble in both water and organic media (Ikawati et al., 2021; Menossi et al., 2021). The potential of nanoparticles against storage pests has been well documented. For instance, nano-alumina has shown efficacy against *Rhyzopertha dominica* and *Sitophilus*

*Corresponding Author: Assistant professor, Department of Plant Protection, School of Agriculture, Shiraz University, I. R. Iran
E-mail address: f.bagheri@shirazu.ac.ir
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oryzae, while aluminum oxide and silica nanoparticles have demonstrated effectiveness against *S. oryzae* (Abduz Zahir et al., 2012; Debnath et al., 2011; Goswami et al., 2010).

This study aimed to evaluate the insecticidal potential of essential oils and nanoemulsions derived from *Oliveria decumbens* and *Ferula assa-foetida* as safe, plant-based alternatives to synthetic pesticides. In addition, it sought to compare the efficacy of these nanoemulsions with that of conventional emulsions against the Mediterranean flour moth (*Ephestia kuehniella*).

MATERIALS AND METHODS

Insect rearing

To establish a Mediterranean flour moth colony, eggs of uniform age were obtained from the Fars Province insectarium. The insects were reared in plastic containers measuring 20 × 40 × 30 cm^3, each filled with a mixture of 200 g of wheat flour and wheat bran at a 3:1 ratio, supplemented with 0.1 g of pest eggs (Safa et al., 2014). Prior to use, both the containers and the diet were sterilized in a hot-air oven at 50 °C for 24 hours and then cooled to ambient temperature. Each container was covered with double-layered netting (Karalius & Bûda, 1995) and placed in a growth chamber maintained at 25 ± 5 °C, 60 ± 5% relative humidity, and complete darkness (Lima Filho et al., 2001). After approximately 30 days, late-instar larvae were transferred to the laboratory for accurate identification. They were carefully collected with a soft brush and placed in ventilated plastic vials partially filled with the same rearing medium to reduce handling stress. Transfers were performed in the morning to minimize exposure to temperature fluctuations. The vials were then promptly transported in an insulated container to the laboratory within one hour, where the larvae were examined under a stereomicroscope for precise identification.

Plant collection

The oleo-gum resin of *Ferula assa-foetida* and flowers of *Oliveria decumbens* were gathered from Alamarvdasht and Nurabad-e-Mamasani regions, respectively, located in Fars province, Iran. Plant voucher specimens were verified and deposited at Shiraz University Herbarium (HSHU), situated in Shiraz, Iran.

Essential oil extraction

Powdered blossoms of *O. decumbens* and oleo-gum resin of *F. assa-foetida* were separately combined with 1000 mL of distilled water and subjected to hydrodistillation for 3.5 and 4 h, respectively. Hydrodistillation took place in a Clevenger-type apparatus (Haghshenas et al., 2023; Sereshti et al., 2011). The resultant essential oils were dehydrated using anhydrous sodium sulfate and stored at 4 °C until further use.

Nanoemulsion preparation

To prepare nanoemulsions of *F. assa-foetida* and *O. decumbens* essential oils at a concentration of 1200 µL/L, the method of Hazrati et al. (2017) was followed with slight modifications. First, 24 µL of Tween 80 was added to 20 mL of distilled water in a 100 mL beaker and stirred at 850 rpm for 30 min. Subsequently, 24 µL of each essential oil was added dropwise while stirring was continued for an additional 45 min, resulting in a uniform solution (Hazrati et al., 2017). The emulsion was then filtered through 0.22 µm mesh filters to remove large droplets. Particle size of the nanoemulsion was determined at 25.1 °C in the Central Research Laboratory of Shiraz University using dynamic light scattering (DLS; Horiba SZ-100 model).

Determining the concentration of filtered nanoemulsion

First, the maximum photon absorption of both unfiltered and filtered nanoemulsions was measured. Since the photon absorption processes were identical, a series of concentrations was prepared: 100, 200, 300, 400, and 500 µL/L of the unfiltered *O. decumbens* nanoemulsion, and 30, 60, 90, 110, and 150 µL/L of the unfiltered *F. assa-foetida* nanoemulsion. The maximum absorption wavelength (λmax) was then determined for each concentration. The resulting data were entered into Microsoft Office Excel to generate absorption–concentration calibration curves and obtain linear regression equations. Using these equations, the concentration of the filtered nanoemulsion was calculated by substituting its absorption data, after which additional concentrations were determined based on this calculated value.

Bioassay screening of F. assa-foetida and O. decumbens essential oils and nanoemulsions

To evaluate the most effective doses of essential oils and their effects on Mediterranean flour moths, preliminary trials were conducted on twenty 30-day-old last-instar larvae of uniform age, with two replicates per treatment group. The median lethal dose (LD_{50}) for last-instar larvae was determined for the essential oils of *F. assa-foetida* and *O. decumbens*. Essential oil solutions were applied topically using a microapplicator (Burkard Manufacturing Co. Ltd., Hertfordshire, UK), with droplets of a defined volume placed on the dorsal thoracic region of each larva. After absorption, larvae were individually transferred into Petri dishes of uniform size, whose edges were sealed with Parafilm to maintain a controlled environment and prevent contamination. Initial experiments were performed to determine the optimal nanoemulsion doses. For *F. assa-foetida* and *O. decumbens* essential oils, concentrations that resulted in 75% mortality were 270 µL/L and 490 µL/L, respectively, while concentrations causing 25% mortality were 100 µL/L and 250 µL/L, respectively. In the case of nanoemulsions, 75% mortality was achieved at 130 µL/L for *F. assa-foetida* and 410 µL/L for *O. decumbens*, whereas 25% mortality occurred at 30 µL/L and 170 µL/L, respectively. For each essential oil, three additional concentrations were selected between these two mortality thresholds, with the zero dose (water and Tween 80) serving as the control. All bioassays were conducted with four replicates per concentration, each replicate comprising 20 larvae. In the final experiments, five treatment groups were tested, again using 20 thirty-day-old larvae of uniform age per replicate, with four replicates per group. Following treatment, larvae were incubated in a germinator under controlled conditions (25 ± 5 °C, 60 ± 5% relative humidity, and complete darkness), and mortality was recorded after 24 hours. In total, 4,820 larvae were used in the general bioassay, corresponding to 2,410 larvae tested per plant essential oil.

Mortality was assessed 24 hours post-treatment. Larvae were classified as dead if they failed to respond to gentle stimulation with a fine hair brush. Each trial was independently reviewed by two trained observers to verify the mortality data and minimize observer bias. Mortality in the control group was also recorded and adjusted using Abbott's formula when necessary.

Statistical analysis

Probit analysis of bioassay data was conducted using PoloPlus version 2.0. Mortality data were corrected relative to the control group using Abbott's formula (Abbott, 1925). Additional statistical calculations were performed in Microsoft Office Excel 2016, and graphical representations were generated using SigmaPlot 12.0.

RESULTS

Effect of essential oils on larval mortality

The mortality of late-instar larvae of the Mediterranean flour moth increased with rising concentrations of *F. assa-foetida* essential oil. The highest mortality rate (73.33%) was recorded at 270 µL/L. Similarly, exposure to *O. decumbens* essential oil showed a dose-dependent increase in larval mortality, with the highest rate (75.32%) observed at 490 µL/L.

Characterization of nanoemulsions

To improve the efficacy of the essential oils, nanoemulsions were formulated and analyzed using DLS. The mean particle sizes of *O. decumbens* and *F. assa-foetida* nanoemulsions were 53.8 nm and 109.9 nm, respectively, with corresponding polydispersity indices (PDI) of 0.439 and 0.339 (Fig. 1 and Fig. 2).

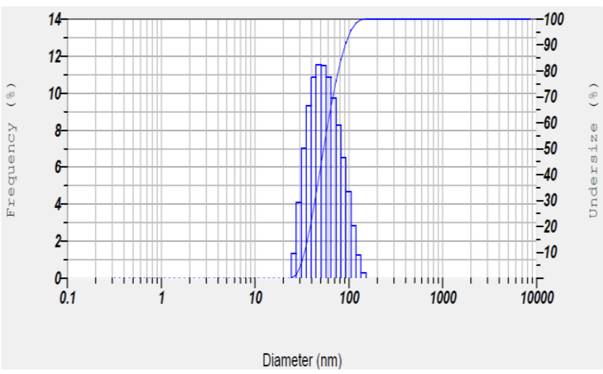

Fig. 1. The average particle size of the nanoemulsion of *Oliveria decumbens* essential oil.

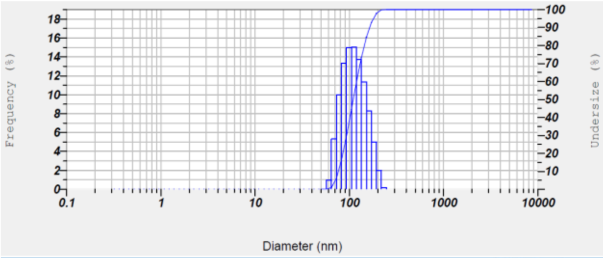

Fig. 2. The average particle size of the nanoemulsion of *Ferula assa-foetida* essential oil.

Effect of nanoemulsions on larval mortality

Analysis of the average mortality rates of late-instar larvae of the Mediterranean flour moth exposed to different concentrations of essential oil-based nanoemulsions showed a clear dose-dependent trend. The highest mortality rate (61.64%) was recorded for the *F. assa-foetida* nanoemulsion at 130 µL/L, while the *O. decumbens* nanoemulsion produced the highest mortality rate (75.75%) at 410 µL/L.

Determination of LD_{50} values

The LD_{50} values (i.e., the lethal dose required to cause 50% mortality in the pest population) were calculated from mortality data of 30-day-old larvae treated with selected doses of essential oils and their nanoemulsions. For the nanoemulsions, the LD_{50} values of *O. decumbens* and *F. assa-foetida* were 284.991 µL/L and 79.249 µL/L, respectively (Table 1). For the crude essential oils, the LD_{50} values were higher, at 351.414 µL/L for *O. decumbens* and 160.156 µL/L for *F. assa-foetida* (Table 2).

Table 1. Effects of essential oil nanoemulsions of *Oliveria decumbens* and *Ferula assa-foetida* on the Mediterranean flour moth

Nanoemulsion	LD_{50} (upper limit-lower limit)	Slope ± SE	Df*	Chi-square
Oliveria decumbens	284.991 (249.457-317.232)	3.95 ± 0.67	18	3.451
Ferula assa-foetida	79.249 (63.506-94.85)	2.20 ± 0.39	18	5.158

* Degrees of freedom

Table 2. Effects of essential oils of *Oliveria decumbens* and *Ferula assa-foetida* on the Mediterranean flour moth

Essential oil	LD_{50} (upper limit-lower limit)	Slope ± SE	Df *	Chi-square
Oliveria decumbens	351.414 (316.324-384.220)	4.32 ± 0.75	18	4.35
Ferula assa-foetida	160.156 (138.887-182.113)	3.0 ± 106.48	18	4.979

* Degrees of freedom

Comparison of essential oils and nanoemulsions

A comparison of the toxic effects of nanoemulsions and essential oils derived from *O. decumbens* and *F. assa-foetida* on late-instar larvae of the Mediterranean flour moth is presented in Table 3. Statistical comparisons of LD_{50} values were conducted using lethal dose ratio

(LDR) analysis, with significance determined by whether the 95% confidence interval (CI) included the value 1 (Robertson et al., 2007). For the nanoemulsions, the LDR between *O. decumbens* and *F. assa-foetida* was 0.278, indicating a statistically significant difference since the CI did not include 1. The *F. assa-foetida* nanoemulsion was therefore more toxic, as evidenced by its lower LD_{50} value. Similarly, for the crude essential oils, the LDR was 0.456, again indicating a significant difference. In this case too, we infer *F. assa-foetida* essential oil was more toxic than *O. decumbens*, owing to its lower LD_{50} value.

Table 3. Comparison of *Oliveria decumbens* and *Ferula assa-foetida* nanoemulsion lethal concentrations for 50% mortality of late-instar larvae of the Mediterranean flour moth

Variable	Lethal dose ratio is 50%	5% confidence interval	Significant
Lethal Dose 50 of *O. decumbens* and *F. assa-foetida* essential oils	0.456	0.388-0.535	+
Lethal Dose 50 of *O. decumbens* and *F. assa-foetida* nanoemulsions	0.278	0.223-0.347	+

A statistically significant difference was found between the LD_{50} values of *O. decumbens* and *F. assa-foetida* essential oils (Table 1). The essential oil of *F. assa-foetida* demonstrated greater toxicity against *E. kuehniella* larvae, which may be attributed to its unique chemical composition, particularly its higher content of sulfur-containing compounds known for their insecticidal properties (Bamoniri et al., 2019). These compounds not only account for the oil's characteristic pungent odor but have also been associated with enhanced larval mortality. Similarly, a comparison of the LD_{50} values of the nanoemulsions showed a significant difference between the toxicities of the two formulations (Table 2). The *F. assa-foetida* nanoemulsion was approximately 3.6 times more toxic than that of *O. decumbens*. This increased efficacy is likely due to the retention of bioactive sulfur compounds in the nanonized formulation (Bamoniri et al., 2019). In line with this, Abdalla and Mühling (2019) also reported the insecticidal potential of sulfur compounds in plant essential oils for pest control.

DISCUSSION

Insecticidal activity of essential oils: Evidence from previous studies

In recent years, plant essential oils have attracted considerable interest as natural and environmentally safe alternatives to synthetic insecticides. Numerous studies have reported their insecticidal activities. For instance, Aslan et al. (2004) showed that essential oils extracted from *Ocimum basilicum*, *Satureja hortensis*, and *Thymus vulgaris* exerted strong toxic effects on the respiratory systems of *Tetranychus urticae* and *Bemisia tabaci*, with mortality rates increasing proportionally with concentration. Our findings are consistent with these observations, although the LD_{50} values obtained in this study were considerably lower, likely due to the specific plant species used and interspecific differences in insect susceptibility. Similarly, Heydarzade et al. (2011) reported that increasing concentrations of essential oils from *Teucrium polium* and *Satureja hortensis* enhanced the mortality of *Callosobruchus maculatus*. In addition, Tapondjou et al. (2005) demonstrated that essential oils derived from *Cupressus sempervirens* and *Eucalyptus saligna* exhibited both toxic and repellent effects against *Sitophilus zeamais* and *Tribolium confusum*.

Nanoemulsions as enhanced insecticidal agents

In recent years, the development of nano-formulations of essential oils has emerged as a promising strategy for pest control, largely due to their unique physicochemical properties. Among these, nanoemulsions are particularly notable for their small particle size, high surface area-to-volume ratio, and enhanced stability, which collectively contribute to the improved penetration and prolonged efficacy. Anjali et al. (2012) demonstrated that nanoemulsions of *Azadirachta indica* essential oil effectively eliminated *Culex quinquefasciatus* larvae, with efficacy increasing over longer exposure periods. Similarly, Ghosh et al. (2013) reported that nanoemulsions of *Ocimum basilicum* essential oil achieved complete mortality of *Aedes aegypti* larvae across a range of concentrations and exposure times. In another study, Nenaah (2014) found that nanoemulsions prepared from three Asteraceae species exhibited toxicity levels up to five times higher than those of the corresponding pure essential oils. Consistent with these reports, our findings showed that the LD_{50} values of nanoemulsions were approximately 1.5–2 times lower than those of the respective essential oils.

Mechanisms underlying the superior toxicity of nanoemulsions

The insecticidal activity of essential oils is primarily attributed to their ability to penetrate the insect respiratory system or cuticle. Non-polar or weakly polar components typically enter through these pathways, leading to physiological disruptions (Veal, 1996; Sfara et al., 2009). Nanoemulsions, with their reduced particle size and enhanced surface adhesion, facilitate deeper and more sustained penetration. Hashem et al. (2018) reported that nano-formulations caused more severe histological damage in insects than their bulk counterparts. Similarly, Margulis-Goshen and Magdassi (2013) emphasized that the superior adhesion of nanoemulsions to the insect cuticle increases contact, thereby amplifying their toxic effects. Werdin Gonzalez et al. (2015) further demonstrated that encapsulating essential oils from *Pelargonium graveolens* and *Citrus bergamia* in polyethylene glycol nanoparticles enhanced contact toxicity and induced marked physiological alterations in the digestive tissues of target insects. In addition, Gonzalez et al. (2016) showed that the controlled release of terpenes from such nano-formulations played a crucial role in maintaining insecticidal efficacy over extended periods.

Comparative analysis and contribution of the present study

Our findings corroborate previous research on the insecticidal activity of essential oils and their nanoemulsified forms. Consistent with the results of Aouadi et al. (2020) and Zallaghi & Ahmadi (2021), we observed a positive correlation between essential oil concentration and insect mortality. The magnitude of LD_{50} reduction, however, varies across studies. For example, Nenaah (2014) reported a fivefold decrease, whereas our results showed a 1.5–2-fold decrease. Such discrepancies may stem from differences in oil composition, nanoemulsion preparation methods, or the varying susceptibilities of insect species. A key limitation of essential oils is the instability and rapid degradation of their monoterpene constituents, which leads to short-lived effects. As noted by Sabbour & Abd-El-Aziz (2019) and Isman (2020), maintaining insecticidal activity with pure essential oils often necessitates frequent reapplication. In contrast, nanoemulsions address this drawback by enabling controlled and sustained release, thereby prolonging their effectiveness.

Implications and future perspectives

The present study demonstrated that nanoemulsions derived from *O. decumbens* and *F. assa-foetida* hold considerable promise as natural alternatives to synthetic pesticides. These nano-formulations exhibit superior penetration, increased toxicity, and greater stability, making them well-suited for use in stored-product pest management. However, further research is needed to evaluate their long-term stability, field efficacy, and potential environmental effects on non-target organisms. In addition, exploring their interactions with insect physiological systems may yield valuable insights into mechanisms of action and the potential for resistance development. The essential oil of *F. assa-foetida* is particularly notable for its strong toxicity, primarily attributed to its distinctive chemical profile, especially its high levels of sulfur-containing compounds. These compounds, including thiols and sulfides, are well-known for their insecticidal properties, as they disrupt metabolic enzymes and impair insect respiratory systems (Bamoniri et al., 2019). Bamoniri et al. (2019) further emphasized that sulfur compounds play a central role in the toxicity of *F. assa-foetida* oil, noting their ability to damage both the insect cuticle and respiratory tract, thereby increasing mortality rates.

CONCLUSION

This study highlighted the insecticidal potential of essential oils from *O. decumbens* and *F. assa-foetida*, particularly when formulated as nanoemulsions, against the Mediterranean flour moth (*Ephestia kuehniella*). Compared to the oils alone, the nanoemulsions exhibited markedly greater efficacy, likely due to their enhanced stability and increased surface area, positioning them as strong candidates for natural pest management. Given rising concerns over pesticide resistance and the ecological risks associated with synthetic chemicals, such plant-based nano-formulations represent a safer and more environmentally sustainable alternative. To advance their practical use, future studies should focus on identifying active constituents, optimizing formulation techniques, and addressing key challenges related to large-scale production, standardization, regulatory approval, and end-user acceptance. Incorporating these natural pesticides into integrated pest management programs could significantly reduce dependence on conventional insecticides.

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CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Faezeh Bagheri and Mohammad Ramezani; Methodology: Faezeh Bagheri and Fatemeh Raouf Fard; Software: Faezeh Bagheri and Mohammad Ramezani; Validation: Faezeh Bagheri and Fatemeh Raouf Fard; Formal analysis: Faezeh Bagheri and Mohammad Ramezani; Investigation: Mohammad Ramezani; Resources: Faezeh Bagheri and Mohammad Ramezani; Data curation: Mohammad Ramezani; Writing—original draft preparation: Faezeh Bagheri; Writing—review and editing: Faezeh Bagheri and Fatemeh Raouf Fard; Visualization: Faezeh Bagheri; Supervision: Faezeh Bagheri; Project administration: Faezeh Bagheri; Funding acquisition: Faezeh Bagheri.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

None.

DATA AVAILABILITY

The data can be obtained from the corresponding author upon reasonable request.

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