

Lethal effect of thiophene and phenol rich leaf extract from *Ferula asafoetida* on *Dacus ciliatus* (Loew)

Seyed Hasan Zamani

University of Zabol

Abbas Khani

abbkhani@yahoo.com

University of Zabol <https://orcid.org/0000-0003-1751-6233>

Kazem Mohammadpour

Iranian Research Institute of Plant Protection

Ali Mirshekar

University of Zabol

Research Article

Keywords: Bioassay, Chemical composition, *Dacus ciliatus*, Extract, Insecticide

Posted Date: July 30th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-3602057/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Studies were conducted to evaluate the toxicity of methanol (MeOH) extract from *Ferula asafoetida* Linn. (Umbelliferae) leaves on female adults and larvae of the lesser pumpkin fly, *Dacus ciliatus* (Diptera: Tephritidae). Direct-dip bioassays for larvae and topical bioassays for adults of fly were used to assess mortalities. Mortality increased significantly with rising concentrations. The intermediate lethal concentrations (LC₅₀), calculated 7.3 mg/ml (7300 ppm) for larvae. The female adult LC₅₀ was 409 µg/insect. The chemical composition of the isolated MeOH extract from *Ferula asafoetida* leaves was examined by gas chromatography-mass spectrometry. The major compounds were 2, 3, 4, 5-Tetramethyl thiophene (22.1%), Phenol, 3-pentadecyl- (21.2%) and Mono (2-ethylhexyl) phthalate (15.98%). The results suggested that the extract from the tested plant could be used as a potential control agent for this pest.

Introduction

Dacus ciliatus Loew (Dip.: Tephritidae) is an important agricultural pest worldwide that mainly attacks cucurbit crops. In many areas such as Africa, Atlantic Islands, and oriental Asia, this fly is responsible for high infestation rates in cucurbit crops and induces important damages (White and Elson–Harris 1992). Pumpkin flies usually lay their eggs in the young fruit; Larvae eat surface, which renders it unsuitable for marketing. The published reviews, such as the one by White and Elson-Harris (1992), are frequent and provide a simple background to suggest integrated pest management methods for this pest. In addition, most of the knowledge is based on laboratory studies or field experiments using either male pheromone or food–baited traps or collecting of infected fruits (Dhillon et al. 2005).

Today, there is increasing interest in the use of natural products from plants to control pests. Secondary metabolites consist of compounds the concentration of which may be only few percent or less of the dry weight. The function or importance of these compounds to plant development is not always clear, although many of them are involved in defense mechanisms or act as signaling substances in plants (Luckner 1990).

Limited natural products have reached the marketplace, partially because their efficiency is still lower than that of presently used synthetic compounds. However, there is a new attention in plant products as sources of novel insect controlling agents, because they may be decomposable to harmless compounds, thus reducing the accumulation of adverse residues, leading them to be more ecologically friendly compared to synthetic mixtures. The search for environmentally safe pesticides has fostered research on the use of plant materials for the protection of crops against pests, both on the field and in storage. Secondary plant metabolites are a large reservoir of chemicals with biological activities. Phytochemicals such as rotenone, nicotine and pyrethrin were all used as pesticides before the advent of synthetic insecticides. Numerous species of Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae families are known to have different chemical compounds which act as antifeedants, repellents or development inhibitors to many insect pest species (Srivastava et al. 2001).

Ferula assafoetida is a monoecious, herbaceous, constant plant. It is giant plant 2–3 m high, with a circular mass of 30–40 cm. Leaves are compound, 2–4 pinnate very huge and bipinnate, pubic. It is native to central Asia, eastern Iran to Afghanistan.

The chemistry of *Ferula* genus has been studied by many and is well documented as a good source of biologically active compounds such as sesquiterpene derivatives (Kogure et al. 2004; Motai et al. 2004) and sulfur containing compounds (Iranshahi et al. 2008).

Literature on biological activity and insecticide evaluation on *F. asafoetida* essential oil were reported in various studies (Sonigra and Meena 2021; Estekhdami et al. 2020; Bahrami et al. 2016; Koorki et al. 2022).

Essential oil characterized by readily evaporation when exposed to air at normal room temperature. Their toxicities, arresting and repellent effects to stored-product insects and greenhouse pests have been of special interest. Although some commercial products based on repellent odours have been developed recently (for example, Isman 2000), owing to the high volatility of some plant essential oils, their application in open fields may not control insect pests satisfyingly. So, MeOH extract from *Ferula asafoetida* leaves studied in this research. On the other hand, to our knowledge, there is no literature referring to the use of *Ferula assafoetida* extract against *Dacus ciliatus*.

The objective of the present study was to determine chemical composition and its insecticidal potential of MeOH extract from *Ferula asafoetida* leaves against the lesser pumpkin fly, *Dacus ciliatus* (Dip.: Tephritidae).

Materials and methods

Plants and Solvent Extraction

Aerial parts (foliage) of *F. assafoetida* were collected from Takhteh-ye Jan villages, located in Darmian County, South Khorasan Province, Iran (33° 13' 32" N, 59° 59' 39" E, 1900 m a.s.l.). The plant materials were dried naturally on laboratory benches at room temperature (23–27°C) until crisp.

For the extraction of secondary metabolites, the leaves of *F. assafoetida* were dried under shade and then powdered with a mechanical grinder. The powder was fermented with MeOH alcohol 95% (ratio 1:4 w/v), the containers were covered with Para film and the mixture was shaken (300 rpm) for 24 h to ensure proper soaking of the plant product. After the completion of extraction, the liquid extract was filtered through Whatman No.1 filter paper fitted in a Buchner funnel using suction. The extracted plant substances were kept in container cover with aluminum foils at 4°C.

To determine the dry weight of pure extract at one milliliter of solution, the small parts of solution (5 ml) were collected in three replicate, separately and allowed to dry completely at 60°C in oven. The methanol extraction of leaves of *F. asafoetida* gave a crude extract in 1.5% (w/v) density.

Larvae Bioassay

Larval-dip bioassays were conducted to determine the contact toxicity of the MeOH extract of *F. asafoetida* on *Dacus ciliatus* larvae. On the basis of preliminary testes, six concentrations of MeOH extract were used in the test. The randomly selected last instars larvae were separately immersed in a prepared concentration of extract solution for 3 s as described by Watkinson et al. (1984). To absorb excess moisture, the treated larvae placed on paper towel and then ten randomly selected larvae transferred to a plastic petri dish (9cm diameter), the bottom of which was lined with a filter paper (Whatman no. 1, 9 cm) and held at 25 ± 1 °C. Control larvae were dipped in MeOH for the same period of time.

The treatments were replicated four times with ten larvae per each treatment, and larval mortality was recorded 24 h after treatment. Larvae were considered dead if they did not move when lightly prodded with forceps.

Adult Bioassay

A direct contact bioassay was conducted to compare the response of newly emerged (aged two days) adult cucurbit fly. For bioassay test, various volumes of the MeOH extracts of *Ferula asafoetida* leaves (250–3000 μ l) were completely dried at room temperature under mild fanning and the residue was weighted and re-dissolved in the 0.5 ml water as solvent.

The bioassays were carried out by topical application of a 10 μ l (5 μ l droplet in two stages with 10 minutes interval times) of each concentration of the MeOH extract (in distilled water) to the thorax of chilled (5°C for 5 min), anaesthetized female flies (1- to 2-day-old adults) by using a an Eppendorf micropipette (0.5–10.0 μ l, Brinkmann Instruments, Westbury, NY). Total of 25 flies were treated for each concentration of extract with five replicates for each treatment. The treated flies were transferred to the breeding room (25 ± 2 °C) and were kept in a 120 ml net-coated plastic cup (5 adults / cup), the bottom of which was lined with a filter paper, and fed with fifty percent honey solution. Control insects were treated with methanol dried and water re-dissolved, alone. Mortality was observed 24 h after exposure and LC₅₀ (median lethal dosage) was calculated. No mortality was recorded in the controls.

Gas chromatography–mass spectroscopy of extract

The MeOH extract was analyzed on a gas chromatograph mass spectrometer (GC-mass) (Shimadzu-17A-QP5050, Japan). The GC column was DB-5 (30 m \times 0.25 mm i. d, 0.25 μ m film thickness). The column oven temperature was set at 60°C for 3 min, and then increased to 260°C at a rate of 5°C/min. Injector and detector temperatures were 230 and 245°C, respectively. The GC mass analysis was carried out with the same characteristics as used in GC. The ionization energy was 70 ev with a scan time of 1s and mass range of 40–500 amu. Unknown essential oil was identified by comparing its GC retention time to that of known compounds and by comparison of its mass spectra, either with known compounds or published spectra.

Statistical analysis

Mortality data from bioassays were analyzed using probit analysis and analysis of variance (ANOVA) with SPSS₂₄. LC₅₀ values were considered significantly different if 95% confidence limit did not overlap.

Results

Contact Toxicity

Probit analysis of the concentration-mortality response of *Dacus ciliatus* indicated that the larvae and adults were susceptible to *F. asafaetida* extract. There were significant differences between tested concentrations after 24 h. The mortality increased significantly with rising concentrations (Fig. 1, 2) ($F_{5,23} = 34.07$; $P < 0.001$ for larvae and $F_{6,34} = 22.43$; $P < 0.001$ for adults).

Table 1 shows the parameters of probit regression line of *Dacus ciliatus* adults and larval susceptibility to MeOH extract of *F. asafaetida* at different concentration.

After examination of different concentration on last instar larvae, crude extract in concentration of 21 mg/ml showed 95% mortality. By testing other concentration, LC₅₀ calculated 7.3 mg/ml (7300 ppm) for larvae. Concentration of 900 µg/female showed 84% mortality in tested adults. The adult LC₅₀ was determined 409 µg /female (Fig. 2).

Chemical composition of MeOH extract

The chemical composition of the MeOH extract from *F. asafaetida* leaves is presented in Table 2. The major compounds were 2, 3, 4, 5-Tetramethyl thiophene (22.09%), Phenol, 3-pentadecyl- (21.18%), Mono (2-ethylhexyl) phthalate (15.98%), 2, 3, 4, 5, 6, 7, 9,10-octamethyl- syntricyclo [4.2.2.0(2, 5)] deca-3, 7, 9-triene-1, 8- dicarboxyanhydride (5.79%), Phytol (5.18%), Sulfonium, dimethyl (5.02%), Octadecatrienoic acid methyl ester (4.33%), Neophytadiene (3.93%), Benzylcarbamate (2.65%), 1-Deuteronaphthalene (2.09%) and Photonerol B (2.04%) in the MeOH extract from *F. asafaetida* leaves.

Discussion

Due to the development of insecticide resistance and risks to human health and the environment of synthetic compounds, the search for alternative pesticides is encouraged. The use of plant essential oils and extract in insect pest control is a suitable alternative method for reduction of the side effects of chemical pesticides on the environment (George et al. 2014).

The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities. In the present study, the plant extract exhibited good insecticidal properties. According to the results, insecticidal activity of the extract of *F. asafaetida* showed that the MeOH

extract with $LC_{50} = 7.3$ mg / ml for larvae and LC_{50} equal to 409 μ g /female for adults had high toxicity effect.

Based on our knowledge, although, there are some literatures on cytotoxic, pesticide and insecticidal activity of *F. asafoetida*, no study has been reported concerning the toxicity of *Ferula asafoetida* extract against *Dacus ciliatus*. It has been reported that dried root latex powder of *F. asafoetida* is an important source of botanical molluscicides which possess time- and concentration dependent toxicity (Kumar and Singh 2006) and this toxicity seems to be mainly due to the water-soluble components, particularly ferulic acid and umbelliferone (Saleem et al. 2001; Hirota et al. 2003). The methanol extracts of oleo-gum-resin of *F. asafoetida* exhibited dose-dependent cytotoxic effect on *Artemia salina* larvae, a simple zoological organism (marine invertebrate), with LC_{50} value of 28 μ g/mL (Bagheri et al. 2010).

Ferula species essential oil has been described by other research groups as insecticidal and some of its active derivatives were identified. Lethal concentrations (LC_{50}) of the essential oil of *F. asafoetida* were examined after 12 (9.04 μ L/L air) and 24 (4.64 μ L/L air) hours whereas at a 10 μ L/ml concentration the essential oil showed repellent activity against *Aphis gossypii* Glover (Koorki et al. 2022).

In other research, the poisonous effects of the essential oil obtained from the oleo-gum-resin of *F. asafoetida* was studied on two species of stored-product pests, larger grain borer, *Prostephanus truncatus* (Coleoptera: Bostrichidae) and *Trogoderma granarium* Everts (Coleoptera: Dermestidae), an agricultural insect pest, cotton leaf worm, *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae), and two public health relevance, *Culex quinquefasciatus* Say (Diptera: Culicidae) and *Musca domestica* Linnaeus (Diptera: Muscidae), and according to the results, the essential oil were active against important insect pests and vectors (Pavela et al. 2020).

Effects of *Ferula asafoetida* L. ethanolic extracts on lethality and some physiological features of Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae, were studied and the results showed that the *F. asafoetida* extract considerably reduced the activity of chymotrypsin, β -glucosidase, lipase and trypsin enzymes compared to the control insects (Ajamhassani, 2021).

Analyzing the MeOH extract from *F. asafoetida* leaves by GC/MS showing that twenty compounds representing 97% of the total detected compounds, in which 2, 3, 4, 5-Tetramethyl thiophene (22.09%), Phenol, 3-pentadecyl- (21.18%), Mono (2-ethylhexyl) phthalate (15.98%) were reported as the chief compounds. The present result was similar to the other literature reported earlier. The most distinctive feature that can be applied for the classification of *Ferula* genus is the presence of sulfur containing compounds. In common, sec-butyl part of sulphur-containing compounds is considered as the chemotaxonomic indicator of the family Apiaceae. sec-Butyl derivatives of sulphur containing compounds have formerly been reported from *Ferula* species including *F. asafoetida* (Iranshahi and Iranshahi 2011).

Relative richness of sulfur-containing compounds in the seed and oleogum resin essential oil of *F. asafoetida* were 50 and 80 percent, respectively (Sahebkar and Iranshahi 2010). A sulfur-containing

hydrocarbon (E)-sec-butyl propenyl disulfide, was the chief element (62.7%) in the essential oil of this plant (Kavoosi et al. 2013).

Some studies showed that *F. assafoetida* possesses a highly strong, persistent and nasty alliaceous aroma. The main constituents of its essential oil which cause its odor are sulfur compounds. The study of chemical profiles of nine samples revealed that (E)-propenyl sec-butyl disulfide (37–54%) was the abundant compound with (Z)-propenyl sec-butyl disulfide (12–23%) in the essential oils (Hassanabadi et al. 2019). Also, (E)-sec-butyl propenyl disulfide (Z) and (E)- β -ocimene, β and α -pinene were the major chemical components of *Ferula assa-foetida* (Koorki et al. 2018).

Similar to our results, sulfur-containing metabolites such as 2,5-diethylthiophene, trimethylthiophene and phenol 2-methyl-5-(1-methylethyl) was found in the essential oil of *Ferula assafoetida* (Kavoosi and Purfard 2013) and diethyl phthalate (13.09%) was observed in essential oil of *Ferula galbaniflua* Boiss. & Buhse (Andrade et al. 2016). Also, 2-isopropyl-5-methylphenol were recognized as primary chemical constituents in methanolic extracts and essential oil of *Ferula microcolea* (Boiss.) (Amiri 2014)

The results showed the presence of bioactive compounds in the MeOH extract from *F. asafoetida* leaves extracts. The toxicity of the extract could be caused by the bioactive compounds present in the extract. Some of these compounds and their derivatives have been previously reported for their insecticidal properties against other insect pests.

Existence of the sulphur-containing compounds in MeOH extract of *Ferula assa-foetida* leaves, are of pharmacological interest because they could have insecticidal effect and so contribute to the plant's overall defense mechanism (Breteler and Ketel 1993; Huang et al. 2000; Nwachukwu et al. 2012). Sefidkon et al. (1998) showing that essential oil, (E)-1-propenyl sec-butyl disulfide is the major component (58.9%) in Iranian *Ferula assafoetida*. Also, polysulfides were found in the volatile oil. It has been concluded that these compounds might be responsible for the oil desirable properties such as insecticidal activity (Huang et al. 2000).

The thiophene derivatives symbolize one of the most thoroughly examined groups of phototoxic phytochemicals (Downum 1992; Breteler and Ketel 1993). The sulfur containing compounds such as thiophenes detected from *Tagetes* owning high insecticidal (Perich et al. 1995) and nematocidal activity (Breteler and Ketel 1993; Kyo et al. 1990). Three new insecticidal thiophene derivatives, xanthopappins A-C, were isolated from *Xanthopappus subacaulis*, that exhibited significant photoactivated insecticidal action against the fourth-instar larvae of the Asian tiger mosquito, *Aedes albopictus* Skuse (Diptera: Culicidae) (Tian et al. 2006).

The effect of phenolic compounds on larvae of the mosquito, *Stegomyia aegypti* (= *Aedes aegypti*), the vector of dengue, has been also reported (Marston et al. 1993; Chowdhury 2008). Aluminium chloride obtained from alder leaf, known for its phenolic complexing activity, is reported to have the larvicidal activity against *Stegomyia aegypti* (David et al. 2000).

This study suggests that *F. assafoetida* leaf extract may be a potential protectant due to its contact toxicity activity against *Dacus ciliatus*. The results of this study highlight the potential benefit of further research into suitable formulations, as well as cheaper and more potent synthetic analogs.

Conclusion

In conclusion, the results obtained in this study clearly indicate that *F. assafoetida* has a sufficient potential for use as a source for management and control of insect pests. However, the exact mechanism of this effect remains to be clarified and further investigations including chronic toxicity studies and activity-guided fractionation must be performed in order to assess the real toxicological profile and the active compounds of the extract.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgment

This study is a part of MSC program in the University of Zabol, Iran. The corresponding author gratefully acknowledges the financial support from the University of Zabol (Grant No 0084).

References

1. Ajamhassani M (2021) Effects of *Ferula assa-foetida* L. and *Aloysia citriodora* Palau ethanolic extracts on mortality and some physiological characteristics of *Ephestia kuehniella* (Lepidoptera: Pyralidae) larvae. *Iran J Med Arom Plants* 37(2):300–314
2. Amiri H (2014) Chemical composition and antioxidant activity of essential oil and methanolic extracts of *Ferula microcolea* Boiss (Apiaceae). *Int J Food Prop* 17(4):722–730
3. Andrade MA, Azevedo C, Motta FN, Santos ML, Silva CL, Santana JM, Bastos IMD (2016) Essential oils: *In vitro* activity against *Leishmania amazonensis*, cytotoxicity and chemical composition. *BMC Complement Altern Med* 16(1):444
4. Bagheri SM, Sahebkar A, Gohari AR, Saeidnia S, Malmir M, Iranshahi M (2010) Evaluation of cytotoxicity and anticonvulsant activity of some Iranian medicinal *Ferula* species. *Pharm Biol* 48(3):242–246
5. Bahrami R, Kocheili F, Ziaee M (2016) Fumigant toxicity and persistence of essential oils from asafetida, geranium, and walnut on adults of *Rhyzopertha dominica* (Col.: bostrichidae). *Toxin Rev* 35(3–4):63–68

6. Breteler H, Ketel DH (1993) *Tagetes spp.* (Marigolds): In vitro culture and the production of thiophenes. In: Bajaj YPS (ed) *Biotechnology in Agriculture and Forestry, Volume 21, Medicinal and Aromatic Plants IV*, Springer-Verlag Berlin-Heidelberg, pp 387–412.
7. Chowdhury N, Ghosh A, Chandra G (2008) Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. *BMC Complement Altern Med* 8:10
8. David JP, Rey D, Meyran JC, Marigo G (2000) Involvement of lignin like compounds in toxicity of dietary alder leaf litter against mosquito larvae. *J Chem Ecol* 27:161–174
9. Dhillon MK, Singh R, Naresh JS, Sharma HC (2005) The melon fruit fly, *Bactrocera cucurbitae*: A review of its biology and management. *J Insect Sci* 5(40):16pp
10. Downum KR (1992) Tansley review No. 43 Light-activated plant defense. *New Phytol* 122:401–420
11. Perich MJ, Wells C, Bertsch W, Tredway KE (1995) Isolation of the insecticidal components of *Tagetes minuta* (Compositae) against mosquito larvae and adults. *J Am Mosq Control Assoc* 11(3):307–310
12. Estekhdami P, Dehsorkhi AN, Kalvandi R (2020) Insecticidal efficacy of essential oils from *Cinnamomum zeylanicum*, *Thymus vulgaris*, *Ferula assafoetida* L on *Callosobruchus maculatus* F. *Asian J Agric Res* 13(2):52–62
13. George DR, Finn RD, Graham KM, Sparagano OA (2014) Present and future potential of plant-derived products to control arthropods of veterinary and medical significance. *Parasit Vectors* 7:28
14. Hassanabadi M, Ebrahimi M, Farajpour M, Dejahang A (2019) Variation in essential oil components among Iranian *Ferula assa-foetida* L. accessions. *Ind Crops Prod* 140:111598
15. Hirotaka F, Shuhei Z, Yumiko I, Hiroshi M, Toyokazu Y, Toru N (2003) Ferulic acid production from clove oil by *Pseudomonas fluorescens* E118. *J Biosci Bioeng* 96:404–405
16. Huang Y, Chen SX, Ho SH (2000) Bioactivities of methyl allyl disulfide and diallyl trisulfide from essential oil of garlic to two species of stored-product pests, *Sitophilus zeamais* (Coleoptera: Curculionidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). *J Eco Entomol* 93(2):537–543
17. Iranshahi M, Hassanzadeh-Khayat M, Fazly Bazzaz BS, Sabeti Z, Enayati F (2008) High content of polysulphides in the volatile oil of *Ferula latisecta* Rech. fruits and antimicrobial activity of the oil. *J Essent Oil Res* 20:183–185
18. Iranshahi M, Ghiadi M, Sahebkar A, Rahimi A, Bassarello C, Piacente S, Pizza C (2009) Badrakemonin, a new eremophilane-type sesquiterpene from the roots of *Ferula badrakema*. *Iran J Pharm Res* 8:275–279
19. Iranshahi M, Iranshahi M (2011) Traditional uses, phytochemistry and pharmacology of asafoetida (*Ferula assa-foetida* oleo-gum-resin)-a review. *J Ethnopharmacol* 134(1):1–10
20. Isman MB (2000) Plant essential oils for pest and disease management. *Crop Prot* 19:603–608
21. Kavooosi G, Purfard AM (2013) Scolicidal effectiveness of essential oil from *Zataria multiflora* and *Ferula assafoetida*: disparity between phenolic monoterpenes and disulphide compounds. *Comp Clin Path* 22(5):999–1005

22. Kavoosi G, Tafsiroy A, Ebdam AA, Rowshan V (2013) Evaluation of antioxidant and antimicrobial activities of essential oils from *Carum copticum* seed and *Ferula assafoetida* latex. *J Food Sci* 78(2):46
23. Kogure K, Yamauchi I, Tokumura A, Kondou K, Tanaka N, Takaishi Y, Fukuzawa K (2004) Novel antioxidants isolated from plants of the genera *Ferula*, *Inula*, *Prangos* and *Rheum* collected in Uzbekistan. *Phytomedicine* 11:645–651.
24. Koorki Z, Shahidi-Noghabi S, Mahdian K, Pirmaoradi M (2018) Chemical composition and insecticidal properties of several plant essential oils on the melon aphid, *Aphis gossypii* Glover (Hemiptera: aphididae). *J Essent Oil-Bear Plants* 21(2):420–429
25. Koorki Z, Shahidi-Noghabi S, Smagghe G, Mahdian K (2022) Insecticidal activity of the essential oils from yarrow (*Achillea wilhelmsii* L.) and sweet asafetida (*Ferula assa-foetida* L.) against *Aphis gossypii* Glover. (Hemiptera: Aphididae) under controlled laboratory conditions. *Int J Trop Insect Sci* 42(4):2827–2833
26. Kumar P, Singh DK (2006) Molluscicidal activity of *Ferula asafoetida*, *Syzygium aromaticum* and *Carum carvi* and their active components against the snail *Lymnaea acuminata*. *Chemosphere* 63(9):1568–1574
27. Kyo M, Miyauchi Y, Fujimoto T, Mayama S (1990) Production of nematocidal compounds by hairy root cultures of *Tagetes patula* L. *Plant Cell Rep* 9:393–397
28. Luckner M (1990) *Secondary Metabolism in Microorganisms, Plants and Animals*, Springer, Heidelberg.
29. Marston A, Maillard M, Hostettmann K (1993) Search for antifungal, molluscicidal and larvicidal compounds from African medicinal plants. *J Ethnopharmacol* 38:215–223
30. Motai T, Daikonya A, Kitanaka S (2004) Sesquiterpene Coumarins from *Ferula fukanensis* and Nitric Oxide Production Inhibitory Effects. *J Nat Prod* 67:432–436
31. Nwachukwu ID, Slusarenko AJ, Gruhlke MC (2012) Sulfur and sulfur compounds in plant defense. *Nat Prod Commun* 7(3):395-400
32. Pavela R, Morshedloo MR, Lupidi G, Carolla G, Barboni L, Quassinti L, Bramucci M, Vitali LA, Petrelli D, Kavallieratos NG, Boukouvala MC, Ntalli N, Kontodimas DC, Maggi F, Canale A, Benelli G (2020) The volatile oils from the oleo-gum-resins of *Ferula assa-foetida* and *Ferula gummosa*: a comprehensive investigation of their insecticidal activity and eco-toxicological effects. *Food Chem Toxicol* 140:111312
33. Sahebkar A, Iranshahi M (2010) Biological activities of essential oils from the genus *Ferula* (Apiaceae). *Asian Biomed* 4(6):835-847
34. Saleem M, Alam A, Sultana S (2001) Assafoetida inhibits early events of carcinogenesis – A chemopreventive study. *Life Sci* 68:1913–1921.
35. Sefidkon F, Askari F, Mirza M. 1998. Essential oil composition of *Ferula assafoetida* L. from Iran. *J Essent Oil Res* 10:687–689

36. Sonigra P, Meena M (2021) Metabolic Profile, Bioactivities, and Variations in the Chemical Constituents of Essential Oils of the *Ferula* Genus (Apiaceae). *Front Pharmacol* 12(11):608649
37. Srivastava S, Gupta MM, Prajapati V, Tripathi AK, Sushil K (2001) Insecticidal activity of myristicin from *Piper mullesua*. *Pharm Biol* 39:226–229
38. Tian Y, Wei X, Xu H (2006) Photoactivated insecticidal thiophene derivatives from *Xanthopappus subacaulis*. *J Nat. Prod* 69(8):1241–1244
39. White IM, Elson-Harris MM (1992) Fruit flies of economic significance: their identification and bionomics, C.A.B. International, Wallingford, UK. 601 pp.

Tables

Table 1. Efficiency of methanol extract from *Ferula asafoetida* leaves against *Dacus ciliatus* larvae and female adults.

Insect stage	No.	χ^2	Probability	Slope \pm SE	LC ₅₀	95% Confidence Interval	
						Lower limits	Upper limits
Larvae	280	3.97 (4)	0.41	2.58 \pm 0.37	7.29	6.10	8.51
Adults	175	0.76 (4)	0.94	2.57 \pm 0.45	408.9	335.3	510.0

Ten individuals per replicate, four replicates per concentration, six concentrations per assay for larvae. Five individuals per replicate, five replicates per concentration, six concentrations per assay for adults.

Lc: lethal concentration (mg/ml solvent for larvae and μ g/insect for female adults)

Table 2. Chemical composition of methanol extract from *Ferula asafoetida* leaves

Compounds	Concentration (%)	Retention time (min.)
Chlorophenylacetic acid	0.77	3.82
2,3,4,5-Tetramethyl thiophene	22.09	4.34
1-Deuteronaphthalene	2.09	6.26
(+)-5,6-dehydrocamphor	0.45	11.23
2-propyldecan-1-ol	0.97	12.08
Di-isodecyl phthalate	0.38	13.50
Sulfonium, dimethyl	5.02	14.90
Photonerol B	2.04	15.50
Neophytadiene	3.93	19.54
Citronellyl propionate	0.61	20.43
Octadecatrienoic acid, methyl ester	4.33	21.13
Phytol	5.18	24.80
2-chloro-1,3-dimethyl-1,3,- diazaphosphacyclopentane	0.41	25.01
3,3,4,4-Tetramethyl-2-pentanone	1.35	26.10
Phenol, 3-pentadecyl-	21.18	26.92
2,3,4,5,6,7,9,10-octamethyl-Syntricyclo[4.2.2.0(2,5)]deca-3,7,9-triene-1,8-Dicarboxyanhydride	5.79	27.45
Phenol, 3-methyl-	0.96	29.55
Benzylcarbamate	2.65	29.74
1-(2-Hydroxyethoxy) tridecane	0.76	29.85
Mono(2-ethylhexyl) phthalate	15.98	29.95
Other compounds	3.05	

Figures

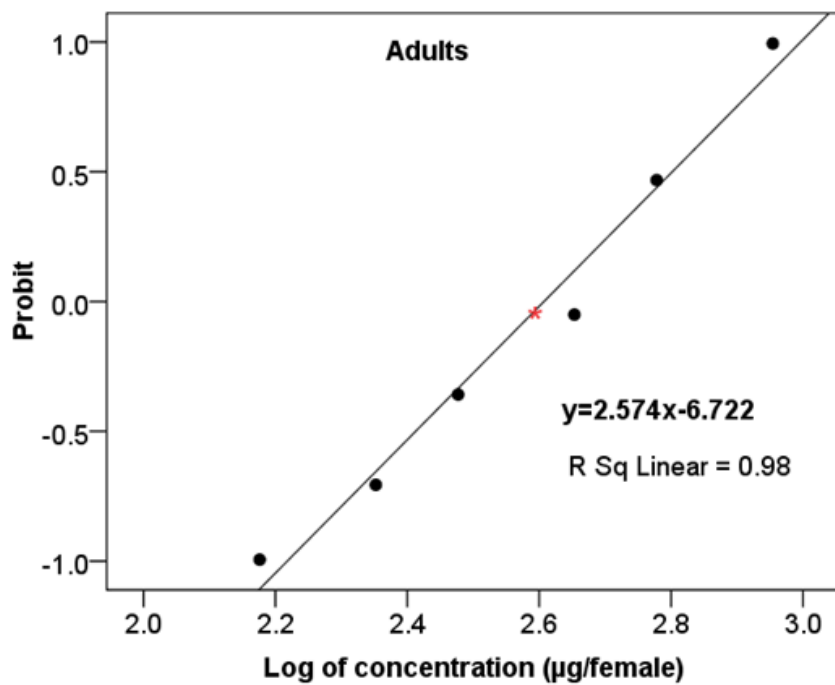
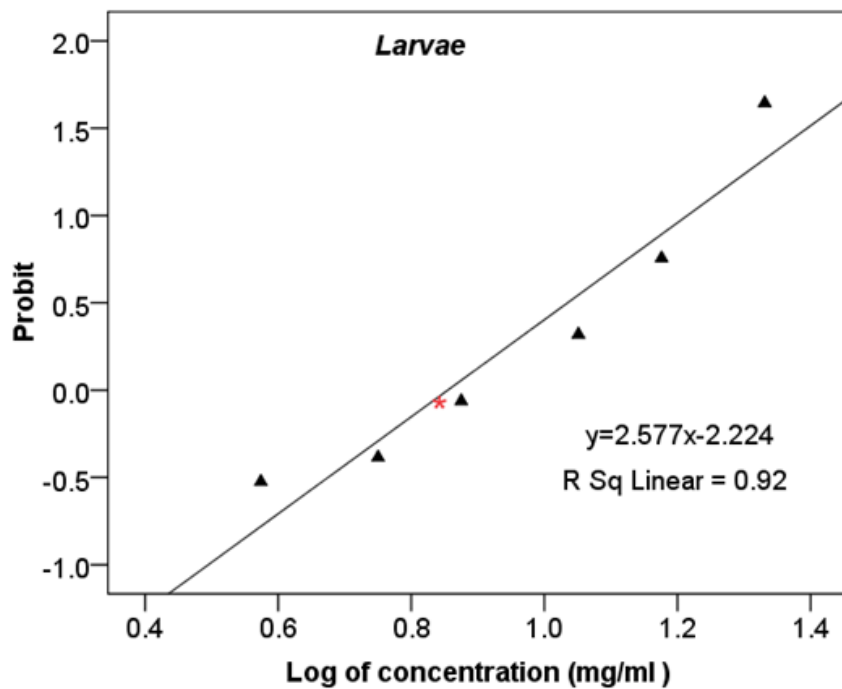


Figure 1

Mortality probit regression line for female adults and larvae of *Dacus ciliatus* after 24 h exposure to methanol extract from *Ferula asafoetida* leaves. LC₅₀ point is shown by the red marker.

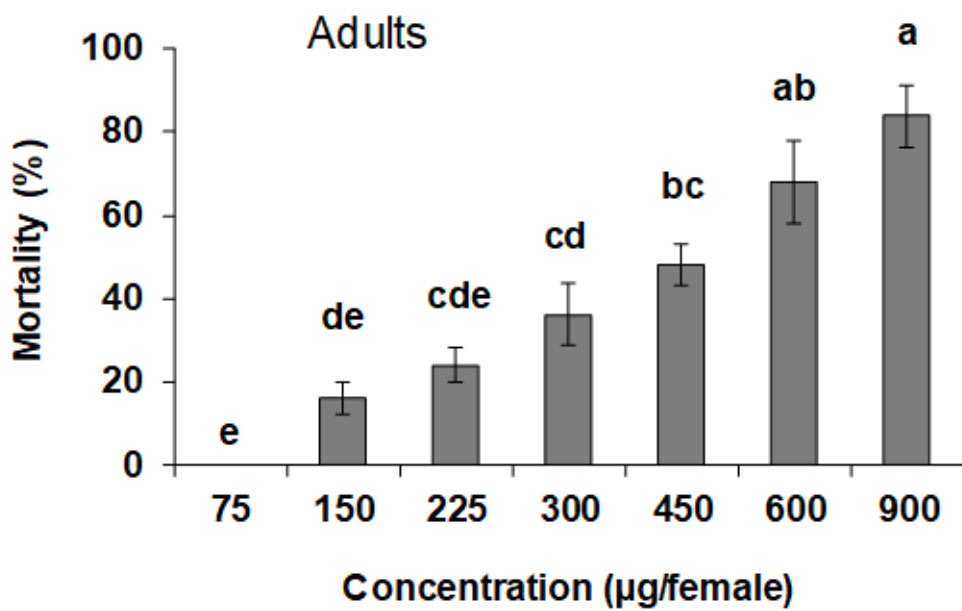
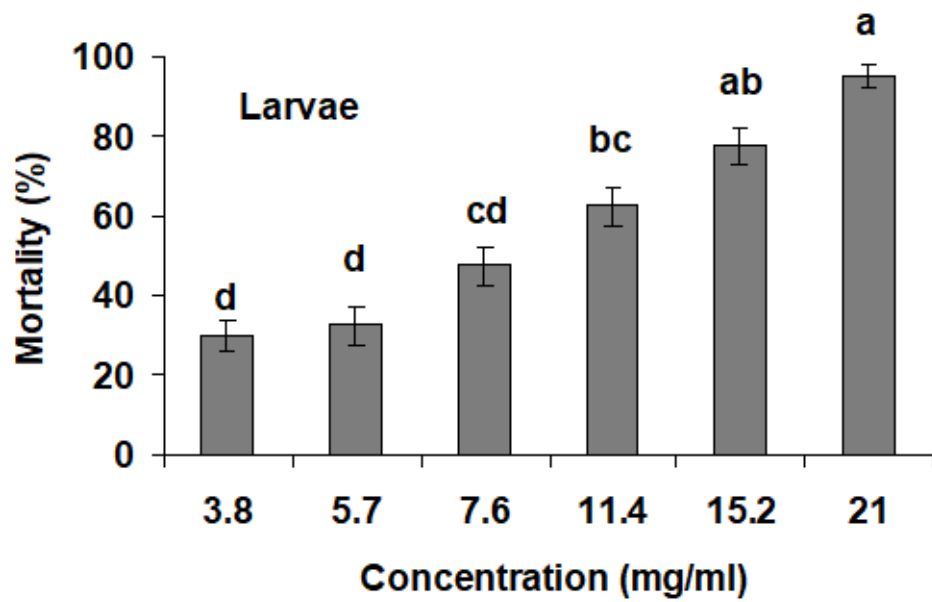


Figure 2

Lethal effect of methanol extract from *Ferula asafoetida* leaves on female adults and larvae of *Dacus ciliatus* after 24 h. Means with the different letters are significantly different ($p < 0.05$) (Tukey post-hoc test after analysis of variance)