



Effect of Polar and non Polar Extract of *Ferula assafoetida* *Dermanyssus gallinae* in vivo and in vitro Conditions

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ABSTRACT

Dermanyssus gallinae is one of the most common arthropods in layers that affects the quality and quantity of egg production. Although there are different synthetic compounds against this mite, but despite these compounds, drug resistance and the presence of these compounds and synthetic compounds in meat and eggs makes that the use of alternative methods, as well as increased use of herbal extracts and essential oils. In the present study, the N-Hexane and Ethanol extracts of *Ferula assafoetida* were used. GC-MS analysis revealed the constituents of the two extracts. The lethal properties of the extracts were determined by contact toxicity. In this field study, ethanolic extract of *Ferula assafoetida* was sprayed on laying hens that infected with red mite. The most available compounds of the Ethanol extract and N-hexane extract of *Ferula assafoetida* were Diethylpyridine and Aurapten respectively in this study. The LC50 of Ethanol extract of *Ferula assafoetida* was 16 µg/cm³ and in vitro study determined that ethanolic extract of *Ferula assafoetida* has been able to reduce the red mite population. This study indicated Ethanol extract of *Ferula assafoetida* could use as a substitute compound against red mite.

Key words: Polar and nonpolar extract, *Ferula assafoetida*, Red mite

INTRODUCTION

Dermanyssus gallinae (red mite) is one of the most important arthropods, especially in laying hens all over the world (Sparagano et al., 2013; Kim et al., 2016; Tabari et al., 2017; Kim et al., 2018) that affects the quality and quantity of egg production, irritation, anemia and, even in the case of severe contamination, causes the death of the bird (George et al., 2009; Spargano et al., 2013). *D. gallinae* plays an important role in the transmission of rickettsial, viral and bacterial pathogens and occasionally causes skin irritation in humans (George et al., 2009; Na et al., 2011; Spargano et al., 2013).

There are various chemical combinations including organophosphates, pyrethroids and carbamates to counteract this mite. The continuous applications of these compounds have increased the resistance to these compounds in this mite (Marangi et al., 2009; George et al., 2009; Tabari et al., 2015). In addition, the chemical residues of these compounds in meat, eggs and the environment are among the limitations of the use of these compounds (Dalton et al., 2001). Because of this, it

increases the importance of using alternative methods such as extracts and essential oils of plants of *Ferula assafoetida* to control red mite. The acaricidal properties of many plant extracts and essential oil have been reported against *D.gallinae* (Kim et al., 2004, 2007; George et al., 2009; Tabari et al., 2015; Nechita et al., 2015; Masoumi et al., 2016; Kim et al., 2016, 2018)

The genus of *Ferula* belong to the family of Apiaceae that distributed the Mediterranean area and central Asia including Iran and Afghanistan (Bagheri et al., 2010). *F. assafoetida* is traditionally use for the treatment disease including parasitic disease (Iranshahi et al., 2011). The compounds of this plant are 40-46% resin, 25% gum, 10-17 % volatile oil and 1.5 10% ash. The resin consist of ferulic acid esters, free ferulic acid, umbelliferone and coumarine (Iranshahi et al., 2011). The sesquiterpenes and sesquiterpene coumarins are the most compound of the genus *Ferula* (Pimenov et al., 1982). The essential oils of *F. assafoetida* are strong ovicides and larvicides of mosquitoes (Muturi et al., 2018). anthelmintic (Kakar et al., 2013; Upadhyay et al., 2017) antiprotozoal activity (El Deeb et al., 2012; Bafghi et al., 2014; Barati et al., 2014)

are a characteristic of this plant. The acaricidal activity of *F. assafoetida* has not been investigated against *D. gallinae*. The purpose of this study was to investigate the acaricidal activity of polar (Ethanol) and nonpolar (N-hexane) extracts of *F. assafoetida* on red mite under *in vivo* and *in vitro* conditions.

MATERIALS AND METHODS

Mites source

Between October 2018 and May 2019 *D. gallinae* samples were collected from a laying poultry farm in Amol, Iran. The mites were placed in dark containers under 25 °C and humidity of 55% transferred to the laboratory in Science and Technology Park of Sari, Iran.

Essential oil extraction and GC-MS analysis

The aerial parts of *F. assafoetida* were collected in Mashhad city, Iran and dried at 25°C. The aerial parts of *F. assafoetida* were ground mechanically using a commercial electric mill. To provide extract, mill plant was macerated with ethanol and N-hexane in Soxhlet apparatus and subsequently, the extract was filtered and solvent was evaporated by using a rotary evaporator and the acquired extract was dried in desiccator (Sonar *et al.*, 2016). The polar extract was obtained by ethanol solvent. However, the non-polar extract was obtained by N-hexane solvent. To analyze and identify the constituents of the extract, Gas chromatography coupled to mass spectrometry (model Shimadzu-QP5050A, Japan) was used. In this study, gas chromatography Agilent-6890 model equipped with DB-5 column with a length of 40 m, an inner diameter of 0.18 mm to 0.25 mm thick layer of stationary phase are used. The column heat program was adjusted from 60 to 210 °C with a gradient of 5 °C/min. The injection chamber temperature was 280 °C and the detector temperature was 270 °C. Helium gas was used as carrier gas and its speed was 0.9 mm/min and split ratio of 1 to 43. The injection rate was 0.1 µl of sample and the source ionization temperature was 230 °C. The electron ionization mode and the ionization energy were 70 eV. A series of normal alkanes were also injected under the same conditions to calculate the retention index inhibition index. The sample retention index was calculated using a computer program.

Finally, the essential oil components were identified by comparing the mass spectra obtained with the standard mass spectra in the Wiley 2000 electronic library in Labsolution GC/MS software and computing the standard inhibition index and comparing them with the standard

numbers in the references (Shibamoto, 1987; Adams, 2001).

Contact toxicity

Contact toxicity assay was done according to the method described by Tabari *et al.* (2015). In this study, treatment groups, control group of solvents, negative control and standard group were considered. 50 mites were added to all studied groups. The number of dead mites were recorded during 24, 48 and 72 hours after spraying the extract, and then the concentration of Lethal Concentration 50 (LC50) were calculated. Two replicates were carried out for all tested groups of mite.

Experimental groups

In the studied groups, the different concentrations were prepared (0.5, 1, 2, 4, 8, 16, 32, 64, 128 µg/cm³). For dilution of polar extract from ethanol and for nonpolar extract of N-hexane solvent used. Polar extract diluted in 50 µl ethanol and non-polar extract diluted in 50 µl N-hexane, the Whatman's paper was then embedded with dilutions after three minutes, the paper dried and the paper was loaded on to the plate, about 50 mites were added to each plate. The number of red mites that were lost during 24, 48, and 72 hours after treatment were counted. Negative control group without any treatment was placed with 50 red mites on filter papers at the bottom of the plate. In ethanol solvent control group, the filter paper was smeared with 50 µl of ethanol solvent and after two minutes, the filter paper was dried and placed on the bottom of the plate and then 50 red mites added to the plate. In N-hexane solvent control group, the filter paper was smeared with 50 µl of N-hexane solvent and after 2 minutes, the filter paper was dried and placed on the bottom of the plate and then 50 red mites added to the plate. In standard group, the filter paper impregnated with 50 µl of diluted cypermethrin solution and then dried at the bottom of the plate, about 50 red mites were added to the plate.

In vitro experiment

Preparation nest

The research was conducted in one of the laying farms of Amol city in May 2019. Each study group contained 20 laying hens (at 40 weeks of age) that reared in cage system (5 laying hens in each cage). Ventilation, lighting and temperature controlled on the basis of breed recommendations (LSL catalog, 2018).

Treatments

In the treatment groups, the ethanolic extracts of *F. assafoetida* was sprayed on 20 LSL laying hens at 40 weeks of age based on the LC50 concentration obtained *in vivo* studies. The negative control group (without conflict with the red mite and untreated), positive control group (involved with the red mite untreated), standard (involved with red mite and treatment with Cypermethrin Mahan Chemical Company) was considered. Two replicates were considered for all study groups. In order to create red mite contamination, contaminated fields were collected from laying farm of Amol city, Iran and then in each group about 2000 experimental red mite were generated. According to the LC50 concentration indicated in the *in vivo* studies, Ethanolic extract of *F. assafoetida* and cypermethrin toxin were sprayed on the bird's body and repeated for one week more. 24 hours after each spray, the number of dead mites on the floor of each cage was measured and counted using adhesive paper traps.

Statistical analysis

The mortality rates of mites were analyzed using a one-way ANOVA in SPSS software (version 16). Values of $P \leq 0.05$ were considered significant.

RESULTS

GC-MS analysis

The major constituents of the extract are shown in tables 1 and 2. The most available compounds in Ethanol extract were Diethylpyridine (23.54%), Aurapten (15.58%), Coumarin (5.11%) respectively and N-hexan extract of *Ferula assafoetida* were Aurapten (16.39%), Lutidine (7.36%) and Ergosten (4.87) respectively.

Table 1. The constituents of Ethanolic extract of *Ferula assafoetida*

| Compound | Retention index | Peak area |
|-----------------|-----------------|-----------|
| Coumarin | 41.65 | 5.11 |
| Quinolium | 48.37 | 1.68 |
| Benzene | 48.76 | 1.96 |
| Ethylene | 48.98 | 2.1 |
| Methoxyindole | 49.82 | 7.19 |
| Phenol | 50.11 | 2.37 |
| Naphthalenone | 50.33 | 3.1 |
| Benzopyran | 50.62 | 2.93 |
| Aurapten | 51.16 | 15.58 |
| Diethylpyridine | 51.63 | 23.54 |
| Costol | 52.48 | 2.63 |
| Lavandulol | 53.92 | 3.72 |

Table 2. The constituents of N-hexan extract of *Ferula assafoetida*

| Compound | Retention index | Peak area |
|--------------------------|-----------------|-----------|
| Geranyl | 33.467 | 3.62 |
| a.-Selinene | 33.99 | 3.78 |
| Farnesal | 34.81 | 1.07 |
| Myristoleate | 36.9 | 1.2 |
| Oleic acid | 37.03 | 1.85 |
| Decanone | 39.4 | 1.56 |
| Farnesol | 41.89 | 1.17 |
| Phenyl ethanone | 47.45 | 1.89 |
| Oelsauere | 48.15 | 2.06 |
| Dehydrogingerdione | 48.33 | 1.84 |
| Formamide | 48.83 | 1.27 |
| Isothiocyanate | 49 | 3.69 |
| Trienoic acid | 49.54 | 1.97 |
| Dimethoxyindole | 49.76 | 3.04 |
| Benzenedicarboxylic acid | 50.12 | 2.7 |
| Xanthene | 50.42 | 2.9 |
| Aurapten | 51.35 | 16.39 |
| Lutidine | 51.7 | 7.36 |
| Ergosten | 52.89 | 4.87 |
| Quinoline | 53.21 | 3.91 |
| Squalene | 55.91 | 1.32 |

Contact toxicity

Generally in all studied timings (After 24 hours, 48 hours and 72 hours), the results indicated that the responses to treatments were the extraction method and dose dependent in contact toxicity assay, Ethanol extracts of *Ferula assafoetida* was effective than N-hexan extract on red mite ($P \leq 0.05$). The LC50 was $16 \mu\text{g}/\text{cm}^3$ for Ethanol extracts (Table 3).

In vitro study

The ethanolic extract of *F. assafoetida* was effective on red mite under field conditions and the differences between groups were significant ($P \leq 0.05$). After first spray, the number of dead red mite was not significant between positive and negative control ($P > 0.05$) but mean mortality rates of mites in treatment group with *F. assafoetida* extract and standard groups significantly higher than the control groups ($P \leq 0.05$). After the second spray, the number of dead red mites in treatment group with *F. assafoetida* and standard group were significantly higher than the control groups ($P \leq 0.05$, Table 4).

Table 3. Comparison of lethal effect polar and nonpolar extracts of *Ferula assafoetida* on red mite at different times

| Treatment | After 24 hours | After 48 hours | After 72 hours | Total |
|---|-------------------------|-------------------------|-------------------------|-------------------------|
| N-hexan <i>F. assafoetida</i> 128 µg/cm ³ | 1 ^a | 1.33±0.33 ^a | 1.33±0.33 ^a | 1.22±0.14 ^a |
| N-hexan <i>F. assafoetida</i> 64 µg/cm ³ | 0 ^a | 0.33±0.33 ^a | 0.33±0.33 ^a | 0.22±0.14 ^a |
| N-hexan <i>F. assafoetida</i> 32 µg/cm ³ | 0.33±0.33 ^a | 0.33±0.33 ^a | 0.33±0.33 ^a | 0.33±0.16 ^a |
| N-hexan <i>F. assafoetida</i> 16 µg/cm ³ | 0 ^a | 0.33±0.33 ^a | 0.33±0.33 ^a | 0.22±0.14 ^a |
| N-hexan <i>F. assafoetida</i> 8 µg/cm ³ | 1±0.57 ^a | 1±0.57 ^a | 1±0.57 ^a | 1±0.28 ^a |
| N-hexan <i>F. assafoetida</i> 4 µg/cm ³ | 1 ^a | 1 ^a | 1 ^a | 1 ^a |
| N-hexan <i>F. assafoetida</i> 2 µg/cm ³ | 0 ^a | 0 ^a | 0 ^a | 0 ^a |
| N-hexan <i>F. assafoetida</i> 1 µg/cm ³ | 0.66±0.33 ^a | 0.66±0.33 ^a | 0.66±0.33 ^a | 0.66±0.16 ^a |
| N-hexan <i>F. assafoetida</i> 0.5 µg/cm ³ | 0 ^a | 0 ^a | 0 ^a | 0 ^a |
| Ethanol <i>F. assafoetida</i> 128µg/cm ³ | 41±0.57 ^f | 41.33±0.66 ^f | 41.33±0.66 ^f | 41.22±0.32 ^g |
| Ethanol <i>F. assafoetida</i> 64µg/cm ³ | 35 ^e | 41±0.57 ^f | 41±0.57 ^f | 39±1 ^g |
| Ethanol <i>F. assafoetida</i> 32µg/cm ³ | 25±0.57 ^d | 33.33±1.6 ^e | 33.33±1.6 ^e | 30.55±1.55 ^f |
| Ethanol <i>F. assafoetida</i> 16µg/cm ³ | 16.66±1.66 ^c | 24.66±0.88 ^d | 24.66±0.88 ^d | 22±1.46 ^d |
| Ethanol <i>F. assafoetida</i> 8µg/cm ³ | 8.33±1.66 ^b | 17.66±1.45 ^c | 17.66±1.45 ^c | 14.55±1.73 ^c |
| Ethanol <i>F. assafoetida</i> 4µg/cm ³ | 3.33±1.66 ^a | 10 ^b | 10.33±0.33 ^b | 7.88±1.24 ^b |
| Ethanol <i>F. assafoetida</i> 2µg/cm ³ | 0.33±0.33 ^a | 0.33±0.33 ^a | 0.66±0.33 ^a | 0.44±0.24 ^a |
| Ethanol <i>F. assafoetida</i> 1µg/cm ³ | 0 ^a | 1 ^a | 1 ^a | 0.66±0.16 ^a |
| Ethanol <i>F. assafoetida</i> 0.5µg/cm ³ | 0 ^a | 0.33±0.33 ^a | 0.33±0.33 ^a | 0.22±0.14 ^a |
| Standard | 45.33±0.33 ^g | 46.33±0.33 ^a | 46.33±0.33 ^a | 46.11±0.26 ^h |
| N-hexan control | 1±0.57 ^a | 2 ^a | 2 ^a | 1.66±0.23 ^a |
| Ethanol control | 0.33±0.33 ^a | 2 ^a | 2 ^a | 1.44±0.29 ^a |
| Positive control | 0.33±0.33 ^a | 1.66±0.33 ^a | 2 ^a | 1.33±0.28 ^a |

Non-anonymous latin letters in each column indicate significant difference ($P \leq 0.05$).

Table 4. Comparison of lethality of ethanolic extract of *Ferula assafoetida* on red mite under field conditions

| Treatment | Number of dead red mite after first spray | Number of dead red mite after second spray |
|--|---|--|
| Ethanolic Extract of <i>F. assafoetida</i> | 30±2.3 ^b | 40±1.52 ^c |
| Standard | 60±3.46 ^c | 50±1.15 ^d |
| Negative control | 0 ^a | 0 ^a |
| Positive control | 5±1.15 ^a | 7±0.57 ^b |

Non-anonymous Latin letters in each column indicates significant difference ($P \leq 0.05$)

DISCUSSION

F. assafoetida is a well-known traditional plant with anti-parasitic properties. Anti-parasitic activity of this plant against leishmania (Bafghi et al. 2014, Gholami et al. 2013), cestode (Farhadi et al. 2016) culex (Muturi et al. 2018) Giardia (Nazer et al., 2019) and mosquito (Evergetis et al., 2012) has been demonstrated. Despite there are extensive reports concerning excellent insecticidal activity of *F. assafoetida*, there was no study to evaluate its acaricidal activity on *D. gallinae*. The present study is one the first *in vitro* and *in vivo* acaricidal studies of *F. assafoetida* against *D. gallinae*. In present study it was obtained that ethanolic extract was effective on *D. gallinae in vitro* and *in vivo* but N-Hexan extract had no significant effect on *D. gallinae*. This could be due to such active compounds in the ethanolic extract of *F. assafoetidae*.

The GC-MS analyses indicated that Ethanolic extract of *F. assafoetidae* was Diethylpyridine (23.54%) but N-Hexan extract of *F. assafoetidae* was Aurapten (16.39%). The anti parasitic effect of Diethylpyridine has been demonstrated against leshmania (Abdala et al., 2002). Generally, the major components of plant materials play the main role to determine the biological properties, but this point should not be ignored that the potential of the major compositions may be regulated by other minor components and the biological activities of plant materials are on account of synergistic/antagonistic interactions of all constituents (Szcepanik et al., 2012).

El-Razek et al. (2001) and Pimenov et al. (1982) and Iranshahi et al. (2012) have reported that the most constituent compounds of *F. assafoetida* are sesquiterpenes and sesquiterpene coumarins. Numerous factors like geographic origin, seasonality, method of oil extraction, year of harvest and even storage conditions could affect the composition of essential oils, so the results from different toxicity studies might not always be adequate (Chalchat et al., 2007).

According to the present study ethanolic extract of *F. assafoetidae* was effective on red mite and LC50 was obtained 16 µg/cm³. However N-Hexan extract of *F. assafoetidae* was not effective on red mite. In a similar study the aquatic and ethanolic extracts of *Conocarpus erectus*, regarding relative toxic and repellency properties, were used as botanical, safe acaricide and repellent agent for control of *D. gallinae* in avicultures (Rajabpour et al., 2018). The similar results were reported about Ethanol extract of *Syzygium cumini* and indicated the most efficient acaricidal activity against *Tetranychus urticae*

followed by hexane extract, ether and ethyl acetate extracts (Afify et al., 2011). The similar studies reported Ethanolic extracts of *Veratrum album* and *Tanacetum parthenium* could be useful to control *Tetranychus urticae* populations on vegetable plants grown through Integrated Pest Management and organic systems of agriculture (Yildirim et al., 2012).

Present results shows the ethanol extract of *F. assafoetidae* was effective on red mite *in vitro* condition. Some studies reported that the neem seed extract acaricide has positive against red mite *in vitro* condition (Abdelghafar et al. 2008; Locher et al., 2010). Similar findings were reported on garlic (*Allium sativum*) extract for controlling red mite infestation in a layer farm in Babol, North of Iran (Faghihzade et al., 2014).

CONCLUSION

Present findings indicated that the ethanolic extract of *Ferula assafoetidae* was effective on red mite and reduced the red mite population *in vivo* and *in vitro* condition but N-Hexan extract of *Ferula assafoetidae* was not effective on red mite, due to active components like Diethylpyridine and LC50 of ethanolic extract that was indicated 16 µg/cm³.

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