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The Acaricidal Activity of Polar and Non-Polar Plant Extract of Artemisia Absinthium and Artemisia Annua on Red Mite

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ABSTRACT

Red mite is one of the most arthropods in layers that has a worldwide distribution. Although there are different synthetic compounds against this mite, because of drug resistance and chemical residuals of these compounds in meat and egg, substitute methods like herbal medicine now are developing. In the following study N-Hexane and Ethanol extract, Artemisia absinthium, and Artemisia annua are used. GC-MS analysis is performed to determine the compounds of these two extracts. Then lethal property on red mite determined by contact toxici-ty. In the field study, ethanolic extract of Artemisia absinthium and Artemisia annua are sprayed on laying hens infected with red mite. This study reveals that the most compound of ethanol and N-hexan extract of Ar-temisia annua was Dimethoxyphenylacetylene and Benzene respectively. thymol and cyclohexane were the major constituents of ethanol and N-hexan extract of Artemisia absinthium respectively. LC50 of ethanol ex-tract of Artemisia absinthium and Artemisia annua was 16 µg/cm³. Field studies show that the ethanolic extract of these two plants is effective on red mite and reduce the population of the red mite. The study shows Ethanol extract of Artemisia absinthium and Artemisia annua could be used as a substitute compound against the red mite.

Keywords: *Red mite, polar and nonpolar extract, Artemisia absinthium, Artemisia annua plant, lethal property*

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1. INTRODUCTION

Dermanyssus gallinae (red mite) is one of the most important arthropods that affect the quality and quantity of egg production (Fletcher et al, 1991; Maurer et al, 1992). Red mite in most parts of the world is important. it causes anemia, bird irritation, and, even in the case of severe contamination, causes the death of the bird. These can transmit some of the pathogens of birds. They cause skin irritation in humans (Kirkwood, 1968: Chauve et al, 1998).

There are several chemical combinations to fight this mite. The widespread use of these compounds increased the resistance to these compounds in these mites (Beugnet et al, 1997; Nordenfors et al, 2001; Guy et al, 2004; Fiddes et al, 2005; Thind et al, 2007; Marangi et al, 2009; George et al, 2009; Tabari et al, 2015; Naqqash et al., 2016). Also, the chemical residues and the harmful effects of these compounds in meat and 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 (Artemisia annua and Artemisia absinthium) to control red mite. The purpose of this study is to investigate the acaricidal activity of polar (Ethanol) and non-polar (N-hexan) extracts of these plants on red mite under in vivo and in vitro conditions.

2. MATERIAL AND METHODS

In vivo experiment

Mites

Red mites are collected from a commercial laying poultry farm in Amol, Iran. Mites are placed in dark containers under 25 °C and humidity of 55% transferred to the laboratory.

Essential oil extraction and GC-MS analysis

The aerial parts of Artemisia annua and Artemisia absinthium are collected in Sari and dried on 25ºC. The extracts are isolated by the Clevenger-type apparatus, essential oil extraction is done according to Negahban et al. (2007). The polar extract was obtained by Ethanol solvent However, the non-polar extract is obtained by the N-hexan solvent. To analyze and identify the constituents of the extract Gas chromatography coupled to mass spectrometry (model Shimadzu-QP5050A) is used (Littlewood et al. 1962). In this study, the gas chromatography Agilent-6890 model equipped with a 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 used detector temperature was 270 ° C. Helium gas is used as a carrier gas and its speed was 0.9 mm / min and Fission ratio of 1 to 43 .The injection rate was 0.1 µl of sample and the source ionization temperature was 230 ° C. The EI ionization mode and the

ionization energy were 70 ev. A series of normal 28C-8C alkanes are also injected under the same conditions to calculate the RI inhibition index. The sample retention index is calculated using a computer program.

Finally, the essential oil components are identified by comparing the mass spectra obtained with the standard mass spectra in the Wiley2000 electronic library in Lab solution GC / MS software and computing the standard inhibition index and comparing them with the standard numbers in the references. (Adams. 2001; Shibamoto. 1987)

Contact toxicity:

Contact toxicity assay is done according to the method described by Tabari et al. (2015). In this study, treatment groups, the control group of solvents, negative control, and standard group are considered. 50 mites are added to all studied groups. The number of mites died at 24, 48, and 72 hours after the study, and then the concentration of LC50 is calculated. Two replicates are carried out for all tested groups of mite.

Treatment groups: In the studied groups, the concentrations of different 0.5, 1, 2, 4, 8, 16, 32, 64, 128 micrograms per cubic centimeter ($\mu g/cm^3$) are prepared. For dilution of polar extract from Ethanol and for the non-polar extract of N- hexane solvent used. Polar extract diluted in 50 µl ethanol and non-polar extract diluted in 50 µl N-hexane, the Wattemn's paper is then embedded with dilutions after 3 minutes, the paper dried The paper is loaded onto the plate, about 50 mites were added to each plate.

The number of red mites that are lost during 24, 48, and 72 hours after treatment are counted. Two replicates are considered for all groups.

Control groups:

Negative control group: without any treatment, we placed 50 red mites on filter paper at the bottom of the plate.

Ethanol solvent control group: The filter paper is smeared with 50 µl of ethanol solvent and after 2 minutes, the filter paper is dried and placed on the bottom of the plate and then added 50 red mites.

N-hexan solvent control group: The filter paper is smeared with 50 µl of N-hexan solvent and after 2 minutes, the filter paper is dried and placed on the bottom of the plate and then added 50 red mites.

Positive control group: 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

For this study, rooms are separate for each treatment is considered. Equipment and cages before washing and disinfection are laying hens. Ventilation and lighting, temperature, and power control based on catalog LSL.

Treatments

In the treatment groups, the ethanolic extracts of Artemisia annua and Artemisia absinthium are sprayed on 20 LSL laying hens at 40 weeks of age.

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) is considered. Two replicates are considered for all study groups.

To create red mite contamination, contaminated fields are collected from laying the farm of Amol city, and then in each group, about 2000 red mites are generated.

According to the LC50 concentration indicated in the in vivo studies, ethanolic extract of Artemisia annua and Artemisia absinthium and cypermethrin toxin are sprayed twice on the bird's body for one week. 24 hours after each spray, the number of dead mites on the floor of each cage is measured and counted using adhesive paper traps.

Statistical analysis:

Data on the toxicity assay were analysis of variance test in SPSS (version 16). Values of $p \leq 0.05$ are considered significant.

3. RESULTS:

GC-MS analysis

The major constituents of the extract as shown in table 1-4. the most compounds of the extract of Ethanol and N-hexan extract of Artemisia annua were Dimethoxyphenylacetylene and Benzene respectively. thymol and cyclohexane were the major constituents of Ethanol and N-hexan extract of Artemisia absinthium respectively.

Table 1. The constituents of Ethanol extract of Artemisia annua

Compound	Retention index	Peak area
Dimethoxyphenylacetylene	45.251	32.61
Nonacosane	48.955	6.97
Benzene	37.752	6.46
Iodophenyl	50.188	5.56
Benzodiazepine	39.027	4.48
Furandione	48.528	3.54
Dimethyltricyclo	47.38	3.16

Table 2. The constituents of N-hexan extract of Artemisia annua

Compound	Retention index	Peak area
Benzene	37.85	8.28
VULGAROL	39.125	6.4
Benzodioxin	39.545	5.23
Oxatricyclo	41	4.92
Clavukerin A	41.56	3.8
Oplopenone	43.53	2.16
Dimethoxyphenylacetylene	45.66	52.69

Table 3. The constituents of Ethanol extract of Artemisia absinthium

Compound	Retention index	Peak area
Thymol	17.4	3.3
palmitic acid	36.6	1.76
Linoleic acid	40.5	2.35
Phenyl	48.7	2

Ergostenol	54	2
Stigmasterol	54.5	2.06
Clionasterol	55.5	1.84

Table 4. The constituents of N-hexan extract of Artemisia absinthium

Compound	Retention index	Peak area
cyclo hexane	27.5	1.83
Palmitic acid	36.8	2
Linoleic acid	40.77	2.44
Tetracosane	49	1.7
Propanediol	49	1.6
Dinostrol	49.5	2.52
Fluoroaniline	49.9	2.1
Phenyl	50.65	2.83
Cinchol	55.57	1.62
Tigmasterol	54.6	1.6
Lycopersin	52.38	3.51
Androstan	51.96	3.3
Indole	50.9	2.36

Contact toxicity

In contact with toxicity assay, Ethanol extracts of these plants were effective on the red mite. LC50 of Ethanol extract of Artemisia absinthium and Artemisia annua were 16 µg/cm^3. The results are shown in Tables 5 to 6.

Table 5. Comparison of the lethal effect of polar and nonpolar extracts of Artemisia absinthium

Treatment	24 hours	48 hours	72 hours	total
N-hexan A. absinthium 128µg/cm^3	5±2.51^{c}	2.33±0.66^{b}	2.66±0.88^{a}	3.33±0.89^{a}
N-hexan A. absinthium 64 µg/cm^3	0.66±0.33^{a,b,c}	0.66±0.33^{a}	0.66±0.33^{a}	0.66±0.16^{a}
N-hexan A. absinthium 32 µg/cm^3	0.66±0.33^{a,b,c}	0.66±0.33^{a}	1.33±0.66^{a}	0.88±0.26^{a}
N-hexan A. absinthium 16 µg/cm^3	0^{a}	0^{a}	0.33±0.33^{a}	0.11±0.11^{a}
N-hexan A. absinthium 8 µg/cm^3	0.33±0.33^{a,b}	0.33±0.33^{a,b}	0.66±0.66^{a}	0.44±0.24^{a}
N-hexan A. absinthium 4 µg/cm^3	0^{a}	0^{a,b}	0.33±0.33^{a}	0.11±0.11^{a}
N-hexan A. absinthium 2 µg/cm^3	0^{a}	0^{a,b}	1^{a}	0.33±0.16^{a}
N-hexan A. absinthium 1 µg/cm^3	1^{a,b,c}	1^{a,b}	0.33±0.33^{a}	0.77±0.14^{a}
N-hexan A. absinthium 0.5 µg/cm^3	0.33±0.33^{a,b}	0.33±0.33^{a,b}	0.33±0.33^{a}	0.33±16^{a}
Ethanol A. absinthium 128µg/cm^3	45.33±0.333^{g}	45.33±0.33^{i}	45.33±0.33^{g}	45.33±0.16^{h}
Ethanol A. absinthium 64 µg/cm^3	38.33±1.66^{f}	40^{h}	40^{f}	39.44±0.55^{g}
Ethanol of A.absinthium 32 µg/cm^3	25±0.57^{e}	36±1^{g}	39.66±0.33^{f}	33.55±2.23^{f}
Ethanol A. absinthium 16 µg/cm^3	21±0.57^{e}	25.57±0.57^{f}	25±0.57^{e}	23.66±0.72^{e}
Ethanol A. absinthium 8 µg/cm^3	15.33±1.45^{d}	21±0.57^{e}	21±0.57^{d}	19.11±1.05^{d}
Ethanol A. absinthium 4 µg/cm^3	12.66±1.45^{d}	15^{d}	15.66±0.33^{c}	14.44±0.62^{c}
Ethanol A. absinthium 2 µg/cm^3	5.33±1.45^{c}	6.33±0.88^{c}	6.33±0.88^{b}	6±0.57^{b}
Ethanol A. absinthium 1 µg/cm^3	0.33±0.33^{a,b}	1.33±0.33^{a}	1.33±0.33a	1±0.23^{a}
Ethanol A. absinthium 0.5 µg/cm^3	0.33±0.33^{a,b}	0.66±0.33^{a}	0.66±0.33a	0.55±0.17^{a}
Standard	45.33±1.45^{g}	46.33±0.33^{i}	46.66±0.33^{g}	46.11±0.26^{d}
n-hexan control	1±0.57^{a,b,c}	2^{a,b}	2^{a}	1.66±0.23^{a}
Ethanol control	0.33±0.33^{a,b}	2^{a,b}	2^{a}	1.44±0.29^{a}
Negative control	0.33±0.33^{a,b}	1.66±0.33^{a,b}	2^{a}	1.12±0.28^{a}

Non-anonymous Latin letters at the top of each group indicate significant difference and P≤0.05

Table 6. Comparison of the lethal effect of polar and nonpolar extracts of Artemisia annua

Treatment	24 hours	48 hours	72 hours	total
n-hexan A.annua 128 µg/cm^3	1.33±0.33^{a}	2±0.57^{a}	2±0.57^{a,b}	1.77±0.27^{a}
n-hexan A. annua 64 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 A. annua 32 µg/cm^3	0^{a}	0.33±0.33^{a}	0.33±0.33^{a}	0.22±0.14^{a}
n-hexan A. annua 16 µg/cm^3	0^{a}	0.33±0.33^{a}	0.33±0.33^{a}	0.22±0.14^{a}
n-hexan A. annua 8 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 A. annua 4 µg/cm^3	0^{a}	0.33±0.33^{a}	0.33±0.33^{a}	0.22±0.14^{a}
n-hexan A. annua 2 µg/cm^3	0^{a}	0^{a}	0^{a}	0^{a}
n-hexan A. annua 1 µg/cm^3	0^{a}	0^{a}	0^{a}	0^{a}
n-hexan A. annua 0.5 µg/cm^3	0.33±0.33^{a}	0.33±0.33^{a}	0.33±0.33^{a}	0.33±0.16^{a}

Ethanol A.annua 128 µg/cm^3	46±1^f	46.66±0.66^g	46.33±0.88^g	46.33±0.44^g
Ethanol A. annua 64 µg/cm^3	38.66±0.66^e	39.66±0.33^f	40.66±0.33^f	39.66±0.37^f
Ethanol A. annua 32 µg/cm^3	24.66±0.88^d	35±2.88^e	37.66±1.45^f	33.44±2.2^e
Ethanol A. annua 16 µg/cm^3	21.66±1.66^d	24.66±1.2^d	25.33±1.45^e	23.88±0.91d
Ethanol A. annua 8 µg/cm^3	11.66±1.66^c	20c	20.66±0.66^d	17.44±1.53^c
Ethanol A. annua 4 µg/cm^3	6.66±1.66^b	6.66±1.66^b	6.66±1.66^c	6.66±0.83^b
Ethanol of A. annua 2 µg/cm^3	2^a	2^a	5^c	3±0.5^a
EthanolA. annua1 µg/cm^3	1±0.57^a	1±0.57^a	2.333±0.33^a	1.44±0.33^a
Ethanol A. annua 0.5 µg/cm^3	0.33±0.33^a	0^a	1±0.57^a	0.44±0.24^a
Standard	45.33±0.33^f	46.33±0.33^g	46.66±0.33^g	46.11±0.26^g
n-hexan control	1±0.57^a	2^a	2^{a,b}	1.66±0.33^a
Etanol control	0.33±0.33^a	2^a	2^{a,b}	1.44±0.29^a
Negative control	0.33±0.33^a	1.66±0.33^a	1.66±0.33^a	1.33±0.28^a

Non-anonymous Latin letters at the top of each group indicate significant difference and P≤0.05

The ethanolic extract of these plants was effective on red mite under field conditions and the differences between groups were significant. (Table 7)

Table 7. Comparison of Lethality of Ethanolic Extract of Artemisia absinthium and Artemisia annua on Red mite under Field Conditions

Treatment	Number of dead red mite after the first spray	Number of dead red mite after the second spray
Ethanol A. absinthium	15±0.57^b	25±1.73^c
Ethanol A. annua	15±0.57^b	20±0.57^c
Standard	60±3.46^d	50±1.15^e
Negative control	0.33±0.33^a	0.66±0.66^a
positive control	5±1.15^a	7±0.57^b

Non-anonymous Latin letters at the top of each group indicate significant difference and P≤0.05

4. DISCUSSION:

The chemical combinations are used to conventional pesticides that were progressively arthropod resistance (Naggash et al., 2016). Alternative products for example selected plant extracts, isolated metabolites and essential oil meet criteria of minimum risk pesticides (Kim et al., 2004; Abdel-Ghaffar et al., 2008, 2009; Semmler et al., 2009; Schmahl et al.,2009; Benelli and Mehlhorn., 2016). Acaricidal effects of essential oils and plant extracts are associated with bioactive constituents. However, limited information is available about plant extract. Locher et al. (2010) described a neem-based product, mite-stop, as an effective acaricide for the control of poultry red mite while George et al (2009) showed that essential oil of Thyme, manuka, and pennyroyal effective on the red mite. Kim et al. (2004) reported that acaricidal activity of 56 essential oil that in contact bioassay, 100% mortality testing 0.07 mg/cm^2 was observed for the bay, cade, cinnamon, clove bud, coriander, horseradish, lime, mustard, pennyroyal, pimento, spearmint, red thyme and while thyme oil (Kim et al. 2004). Tabari et al. (2017) reported α-thujone-rich essential oil of Artemisia sieberi is toxic and repellent potential. Magdas et al. (2010) revealed that oils of sweet basil, coriander, peppermint, and Summer savory are effective on the red mite. However, limited information is available about Artemisia absinthium, Artemisia annua.

The study shows the polar extract of Artemisia absinthium, Artemisia annua are effective on red mite and LC 50 has observed in 16 µg/cm^3. Limited studies are about the effect of the plant extraction method and it affects the lethal property of red mite however our studies show polar extract was more effective than nonpolar extract. Acaricidal and insecticidal effects of essential oils are largely associated with the presence of bioactive constituents. Many botanical oils and their extracts are composed of more than one bioactive compound that can exert different modes of action against ectoparasites (Showler, 2017). Sparagano et al. (2013) showed that terpenes, like eugenol, geraniol, and citral, were effective against the poultry red mite. Archana et al. (2011) observed that thymol is a monoterpene phenol which found in essential oils of thyme, Thymus vulgaris, or Thymus zygis. Our study also shows that most combination of ethanol extract of Artemisia absinthium was thymol that could be one of the causes of the lethal effect against the red mite. Di methoxy phenylacetylene was the most combination in Ethanol extract of Artemisia annua that may have a lethal property against the red mite.

There are no in vitro studies on the effect of Artemisia absinthium and Artemisia annua extract on red mite, However, experimental studies of the effect of other plant extracts on red mite were performed. Faghihzade et al. (2014) Showed twice the spray of garlic extract With one week to the 96% decrease in the population of red mites. Locher et al. (2010) reported that Neem seed extract was effective on all stages of red mite

and in this study it was found that the best effect of this plant extract was 1.33 twice dilution treatment with 5 to 7 days interval on the red mite. our study shows that Ethanol extract of Artemisia annua and Artemisia absinthium are effective on red mite in field conditions.

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