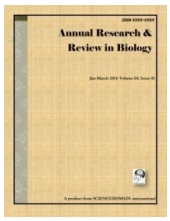

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Acaricide and Fungicide Effects of the *Artemisia vulgaris* Essential Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author LBS managed the literature searches and produced the initial draft. Authors ECT and EG anchored the acaricidal and fungicidal studies, gathered the initial data and performed preliminary data analysis. Author RCF collaborated in the GC/MS analysis. Author JDF designed the study, wrote the protocol and interpreted the all data, led the searches and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study aimed to determine the chemical constitution of the essential oil from *Artemisia vulgaris* leaves and also evaluated the toxicity of the essential oil against *Dermanyssus gallinae* (poultry red mite) and *Aspergillus flavus*.
Methodology: The essential oil was obtained from *A. vulgaris* leaves by hydrodistillation and analyzed by GC-MS. *D. gallinae* mortality was observed over a 24 h period at concentrations of 0.10; 0.19; 0.39; 0.59, and 0.78 µL /cm^2 of essential oil, respectively. The fungal growth was evaluated by disk diffusion assay.
Results: The main chemical constituents were monoterpens α–thujone (48.46%), β– thujone (7.95%), caryophyllene (6.29%), and camphor (3.36%). Both concentrations (0.59 and 0.78 µL/cm^2) were toxic to *D. gallinae*, with mortality of 62.0 and 97.8 %, respectively. The LC_{50} and LC_{90} values were 0.522 and 0.738 µL/cm^2, respectively. *A. flavus* growth was inhibited from 41 to 88.58%.

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Conclusion: These results indicate that *A. vulgaris* will be a promising alternative for the control of *D. gallinae* and *A. flavus*, contributing to pest management of importance in agribusiness.

Keywords: Dermanyssus gallinae; Aspergillus flavus; α-thujone; essential oil; Asteraceae.

1. INTRODUCTION

The excessive use of pesticides, fungicides and acaricides has been at present a major topic in debates about the agro-ecosystems, due to their residues in food and environment that can cause serious risk to human health. In addition, the indiscriminate use of them has led to some organisms to develop resistance to most widely used synthetic agrochemicals.

Natural substances are a suitable alternative to synthetic pesticides as a mean to reduce negative impacts on human health and the environment [1]. The search for alternative control methods of pests without the use of synthetic substances has been a worldwide trend in both agriculture area and veterinary area, seeking better quality foods, without toxic wastes, therefore enabling a better life quality for people. Like this, the evaluation of acaricide and antifungal activity of essential oils from plants is a way used to find alternatives to synthetic substance [2].

Currently, *Dermanyssus gallinae* (De Geer) is one of the main mites that parasitize laying hens raised in confinement systems, and control represents a major challenge to the producer by the fact that this species shows resistance to most chemicals used in its control. Its control by conventional means (i.e., synthetic acaricides) has become increasingly problematic [3]. This mite is currently the most economically deleterious ectoparasite of laying hens. Infestations by *D. gallinae* can result in significant stress to hens with subsequent declines in growth, quality and production of the eggs. In extreme cases, mite population levels may be so high and cause anemia, and even death of hens [3,4].

Thus, a possible alternative to control the *D. gallinae* is the essential oils. Several plant essential oils have already been identified as being toxic to these mites: for example, *Artemisia absinthium, Ocimum basilicum; Cymbopogon citratus; Origanum vulgare; Thymus vulgaris; Eucalyptus citriodora* [5].

The exploitation of natural substances with bioactivity against fungi has been the target of interest in the search for ecologically safe products [6]. The genus *Aspergillus* is widely found in the environment. It relates to the process of biodegradation in industrial and food stored in the processes of food processing and production of antibiotic and acid. *A. flavus* and *A. parasiticus* are the main producers of aflatoxin, a toxic substance. It may cause damage to health, such as toxic hepatitis, hemorrhage, edema, immunosuppression, hepatic carcinoma and death when ingested by man or animals [7,8]. One of the strategies to reduce the adverse effects of aflatoxin problems is prevention of mold growth on the substrate [9].

Several papers report fungicidal, insecticidal, acaricidal, effects of essential oils and extracts from some species of Asteraceae family [10]. The genus *Artemisia* (commonly wormwood or sagebrush) is one of the largest and most widely distributed genera of the family Asteraceae. It consists of around 400 species of herbs and shrubs well-known for their volatile oil that is extensively used in food and pharmaceutical industry [11]. *Artemisia* species are interesting in terms of phytochemical, biological and chemical diversity. They have been used to control free radicals, fungi, insects, bacteria [12,13] and may possess insecticidal, repellent or antifeedant properties [14,15].

Essential oils derived from *Artemisia* species (*A. sieberi*; *A. vulgaris*, *A. annua*, *A. vestita*) have been evaluated as fumigant, repellent and toxic against Coleoptera species as *Sitophilus zeamais* Motschulsky (*Coleoptera*: *Curculionidae*), *Sitophilus granarius* Mots. (*Coleoptera*: *Curculionidae*) and *Tribolium castaneum* Herbst. (*Coleoptera*: *Tenebrionidae*). *S. zeamais* is a pest of stored grains worldwide, responsible for significant losses [16]. *A. scoparia* Waldst and Kit showed fumigant activity against several stored-product pests [14].

Essential oils and extracts of several *Artemisia* species have shown inhibitory effect on growth of pathogenic fungal species in agriculture. There are several reports about antifungal activity of *Artemisia* species against *Aspergillus* spp.,

Fusarium spp., *Penicillium* spp., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* Lib., among others [14,17-20].

A. vulgaris aromatic plant is used in traditional medicine for treatment of diabetes, insomnia, stress, parasites, amenorrhea, dysmenorrhea and colic. It also is used as anthelminthic, antiseptic, antispasmodic and tonic for vital organs and for various disorders including hepatosis. In previous studies, *A. vulgaris* showed antibacterial, antitumor activity [21]. The active components of *A. vulgaris* identified include flavonoids, coumarins, sesquiterpene lactones, volatile oils, insulin and traces of alkaloids. The main compounds of volatile oils include camphor, camphene, α-thujone, germacrene D, 1,8-cineole, and β – caryophyllene [21,22].

This paper reports the main constituents of the essential oil from *A. vulgaris* leaves and its effects on *D. gallinae* (De Geer) and *A. flavus*.

2. MATERIALS AND METHODS

2.1 Plant Materials

Leaves of *A. vulgaris* were collected in the garden of the Instituto Biológico, São Paulo city, São Paulo State, Brazil, in April/2010. A voucher specimen (No. 13445 PMSP) was deposited in the herbarium of the City Hall of São Paulo City.

2.2 Oil Extraction and Analysis

The fresh leaves were cut into small pieces and placed in a distillation Clevenger apparatus for 2 hours. The hydrolyte was extracted with hexane and evaporated at room temperature, and the resulting oil was stored in dark glass bottles in a freezer until it was used by test in *A. flavus* and *D. gallinae* and GC/MS analysis.

GC-MS analyses of the main components of essential oil and its fraction were done in a Shimadzu QP-5000 equipped with an OV-5 (30 m x 0.25 mm x 0.25 µm, Ohio Valley Specialty Chemical, Inc) capillary column. Operating conditions were undertaken at oven temperature from 60°C to 240°C at 3°C/min, injector and detector temperatures of 240°C and 230°C, respectively; system operated by electron impact ionization (70 eV), helium as a carrier gas at a constant flow of 1.7 mL/min, split 1/20. The oil components were identified using retention indices with those of authentic compounds or with literature data [23,24].

2.3 Acaricidal Assay

Essential oil of *A. vulgaris* was applied on filter papers (Whatman No. 2, 4x8 cm) at known concentration (0.10; 0.19; 0.39; 0.59, and 0.78 µL /cm^2); volumes of the essential oil were dissolved in soybean oil. Filter papers which had received 125 mL of soybean oil or 125 ml water were used as control. The filter papers impregnated with the essential oil, soybean oil and water were placed into glass petri dishes with 20 adult females of *D. gallinae* deprived of the opportunity to take a blood meal for 24 hours. Mite mortality was assessed after 24 h. All treatments consisted of five replicates. Percentage mortality between concentrations of the essential oils and treatments control was then assessed by ANOVA and Tukey tests.

2.4 Antifungal Assay

2.4.1 Culture conditions

A. flavus strain producer of aflatoxin B_1 was isolated from peanut and provided by the Instituto de Biociências /USP, São Paulo, Brazil. The fungi were plated onto potato dextrose agar (PDA) and incubated for 10 days at 25ºC. The spore suspension used as inoculum was prepared washing cultures with sterile 0.01% Tween 80 solution.

2.4.2 Disk diffusion assay

Filter paper disks (6 mm diameter) containing 2.5, 5.0, 7.5, 10.0, and 15.0 µL of the crude essential oil of *A. vulgaris* were applied on the dextrose agar in petri dishes previously inoculated with the fungal inoculum on the surface. The inoculated plates were incubated at 25°C for 5 days. At the end of the period, antifungal activity was evaluated by measuring the zone of inhibition (cm) against the test fungus [25]. The commercial fungicide was used as the positive control. All treatments consisted of three replicates and repeated three times, and the averages of the experimental results were determined. Antifungal experiments were performed in triplicate and data analyzed are mean subjected to one way ANOVA.

3. RESULTS AND DISCUSSION

The hydrodistillation of leaves of *A. vulgaris* yielded pale yellow colored oil (yield: 0.014%, v/w). The yield of essential oil can vary

considerably depending upon the source plant, location, time and period of collection.

The chemical composition of *A. vulgaris* essential oils used, determined by GC-MS analysis, emphasized the presence of different major compounds (Table 1). The essential oil obtained contains 24 constituents eluted between 6 and 35 min. Of these, 21 constituents accounting for 94.77% of the essential oil were identified; monoterpens represent 81.36% and sesquiterpens 13.41% (Table 1). The main constituents of *A. vulgaris* essential oil were α-thujone (48.46%), β-thujone (7.95%), 1,8-cineole (7.37%) and *trans*-caryophyllene (6.29%). Of the 21 substances identified, six had been not previously reported as constituents of the essential oil of the plant (*p*-menthene, *p*-cymenene, fenchocanfene, dehidrosabieno ketone, *cis*-limonene oxide, *cis*-calamenene). Five of these substances have been reported as constituents of the genus *Artemisia* [21,22,26].

Table 1. Chemical analysis of *A. vulgaris* essential oil

KI*	Constituent	%
972	*p*-menthene	1.93
1026	1,8-cineole	7.31
1094	*p*-cymenene	0.66
1100	α-thujone	48.46
1106	fenchocampherone	0.78
1110	β-thujone	7.95
1115	dehydrosabinocetone	1.40
1118	crysanthophenone	0.81
1129	*cis*-limonene oxide	1.06
1134	pinocarveol trans	1.45
1136	camphor	3.36
1159	isoborneol	0.97
1171	terpin-4-ol	2.08
1181	thuj-3-en-10-al	0.90
1212	cymen-8-ol (*para*)	1.10
1220	cumin aldehyde	1.14
1415	caryophyllene (*E*)	6.29
1448	humulene (alpha)	1.44
1476	calamenene (*cis*)	2.22
1558	nerolidol (*E*)	1.04
1575	caryophyllene oxide	2.42
	Monoterpene hydrocarbons	2.59
	Oxigenated monoterpenes	78.77
	Sesquiterpene hydrocarbons	9.95
	Oxigenated sesquiterpenes	3.46

(*) Kovats índices experimental

The α-thujone is the main constituent of the essential oil of *A. vulgaris* L. collected in São Paulo. This substance has also been reported as a major constituent of *A. vulgaris* essential oil by Govindaraj et al. [26]. 1,8-cineole is a component identified in most essential oils of *Artemisia* species, and , in some cases, it is among the main constituents [27-29]. Camphor is also the main constituent of the essential oil of *A. vulgaris* collected in India and Italy [26]. In a study on chemical constituent of *Artemisia* spp. essential oils performed by Baeda and Delian (2014) [20], germacrene D (27.78%) was the major constituent in the *A. vulgaris* essential oil. The author attributes the significant variation in the composition of the essential oil to the ecological niche occupied by the plant. Such differences in chemical constitution of *A. vulgaris* essential oil occur due to different geographical locations, environmental conditions and abiotic factors to which plants were exposed [20,21,26].

The percentage mortality of the mites was 97.85 and 62% for concentrations 0.78 and 0.59 μL/cm^2, respectively. These results were significant differences when compared to negative controls ($p < 0.001$ and $p < 0.01$) (Table 2). The results obtained for concentrations 0.78 and 0.59 μL/cm^2 were compared with each other , and it was observed a significant difference ($p < 0.05$), showing that the first concentration is more active than the second. The other concentrations tested were not significant when compared to control $p > 0.05$.

The acaricidal activity of 56 plant essential oils at 0.07 mg/cm^2 concentration against poultry house-collected adult *D. gallinae* was examined by Kim et al. [5]. Mortality of 100% was observed in *Pimenta racemosa*, *Juniperus oxycedrus*, *Eugenia caryophyllata*, *Coriandrum sativum*, *Cocholearia armoracia*, *Citrus aurantifolia*, *Brassica juncia*, *Mentha pulegium*, *Pimenta officinalis*, *Mentha spicata* and *Thymus vulgaris* oils.

The acaricide action of the essential oil of *Artemisia* species against species of the genus *Tetranychus* has been reported [30]. On the other hand *A. absinthium* essential oil showed activity against *D. gallinae* [5], but for the extract of *A. princeps* var. orientalis no activity was observed on *D. gallinae* [31]. This is the first report of acaricidal activity of *A. vulgaris* L. essential oil against *D. gallinae*.

The curve of concentration of essential oil versus percentage of mortality obtained showed the IC_{50} and IC_{90} values at 0.522 and 0.738 μL/cm^2, respectively (Fig. 1), suggesting that the

essential oil may become an effective acaricide against this mite. The results are quite interesting since only 52.2 mL of *A. vulgaris* essential oil are necessary to control 50% of *D. gallinae* infestation in an area of 1000 m^2.

Another important finding was reported by Kim et al. [31] that evaluated toxic effects of 41 plant extracts on adult *D. gallinae* at dose of 0.35 mg/cm^2; 10 plants gave 100% mortality against adult mites. Among them, *Foeniculum vulgare* fruit, *Glycyrrhiza glabra* root, *Paeonia suffruticos*a root bark and *Schizonepeta tenuifolia* whole plant extracts (LD_{50}, 0.11– 0.15 mg/cm^2) were comparable to that of chlorpyrifos-methyl (0.15 mg /cm^2), but more effective than that of diazinon (0.25 mg/cm^2). These data showed a possible alternative of the *A. vulgaris* essential oil as potential acaricide for the control of *D. gallinae* populations.

The fungal growth inhibition assessed by the disk diffusion test has been used in the evaluation of plant extracts and essential oils [32,33]. The influence of the essential oil on the inhibitory zone against *Aspergillus flavus* was measured at 0.90, 1.18, 1.29, 1.40, and 1.94 cm for the volumes of 2.5, 5.0, 7.5, 10.0, and 15 µL, respectively. The commercial fungicide (control) was measured at 2.16 cm. The largest percentage of the fungal growth inhibition was 88.58% (Table 3). All volumes of *A. vulgaris* L. essential oil tested showed growth inhibitory effect of *A. flavus*. Inhibition of fungal growth was dependent on the volume of essential oil used (Table 3), and, when compared to control, all volumes used were statically significant ($p < 0.001$).

Several authors have reported the inhibition of fungal growth of *A. flavus* and aflatoxin biosynthesis by essential oils [10,34,35]. Nogueira et al. [10] related that *Ageratum conyzoides* essential oil at 30, 20, 10, 5.0, 1.0, 0.40, 0.20, 0.10, 0.04, 0.02, 0.01 µg/mL inhibited the fungal growth on average 55%.

The results of fungicidal activity obtained are quite interesting since there is no previous report of the fungicidal activity of the essential oil of *A. vulgaris* against *A. flavus*. Thus, the results obtained in this work demonstrate that essential oils are a great promise for the control of fungi, especially *A. flavus*, showing a path to sustainable agriculture.

Table 2. Concentration used in square centimeter, number of mites used, number of dead mites, and mortality % representing the acaricidal activity of *A. vulgaris* essential oil against *D. gallinae*

***A. vulgaris* essential oil (%)**	**Concentration (µL/cm^2)**	**N**	**Number of dead mites**	**Mortality (%)**
20	0.78	90	87	97.85
15	0.59	84	63	62.00
10	0.39	104	26	24.13
5.0	0.19	88	8	12.50
2.5	0.10	107	5	4.87
soybean oil	3.91	100	3	1.96
distilled water	3.91	100	2	3.0

N - Number of mites used in the trial

Table 3. Essential oil volumes, size of inhibition zone, and *A. flavus* growth inhibition

Volumes (µL)	**size of inhibition zone (cm)**	***A. flavus* growth inhibition (%)**
Control	2.19±0.115	100
2.5	0.90±0.051	41.10
5.0	1.18±0.121	53.88
7.5	1.29±0.073	58.90
10	1.40±0.083	63.93
15	1.94±0.174	88.58

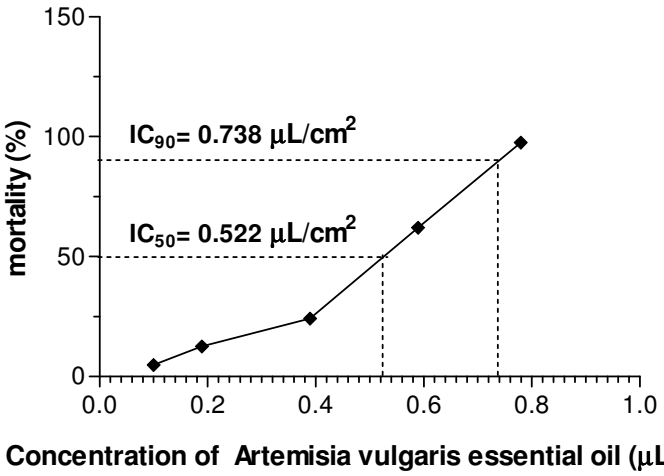

Fig. 1. Evaluation of *A. vulgaris* essential oil against *D. gallinae*

4. CONCLUSION

The results show that the *A. vulgaris* essential oil is an inhibitor of growth of *A. flavus* and toxic against *D. gallinae*, glimpsing the possibility that this plant species could be used in control of these two pests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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