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Effect of Combined Plant Essential Oils on Dermanyssus gallinae: *In vitro* and *in vivo* study

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ABSTRACT

The present study was carried out to evaluate the effect of plant essential oils on *Dermanyssus gallinae (D. gallinae). In vitro* six groups of red mites, 20 mites in each group were exposed to direct spray of combined plant essential oils (Alisal) in rate of 0.25% on mites. activity and changes under stereomicroscope showed that sprayed mites completely stopped movements at both 1- and 2- h after treatment with completely stretched legs and white bead-like spots of oils accumulation on legs and bodies at 1- and 2- h. while, the non-treated mites were active with pale light brown colour. *In vivo* effect of Allisal to control red mite infestation in laying hens was investigated. In case of drinking water method mite count reduction rate on the bird was 60 %, 10%, and 0% as well as 0%, 0% and 10% in their traps at 4,7, and 12 days respectively, from the start of treatment in drinking water. While, in spray method mite reduction was 40%, 20%, and 10% on birds and 0%, 0% and 30% in the traps. On the other hand, water intake, feed intake, general health condition, skin health, and feather condition scores were improved at 4th day post treatment (DPT). Total lesion score at 12 DPT was improved. The present study concluded that in vitro combined plant essential oils have rapid and strong acaricide effect in contact sprays. *In vivo*, there was obvious improvement in groups treated with plant oils than non-treated group. Drinking water treated birds showed good results than spray treated group. Therefore, it is recommended to use combined plant essential oils in *D. gallinae* control strategies in poultry.

Key words: Acaricides, Chickens mite, *Dermanyssus gallinae*, Plant essential oils, Red mite.

INTRODUCTION

Dermanyssus gallinae (D. gallinae) infestation has significant impacts on poultry industry and productivity. Significant economic losses are due to reduction in weight gain and egg production, moreover their role as a disease vector (Flochlay et al., 2017). Also, Blood feeding mite can cause losses due to anemia where layers lose more than 3% of its blood volume every night. In severe cases hens may die from severe anemia (Van Emous, 2005). The veterinary and human medical impact needs adequate therapeutic measures to control parasites in poultry (Meyer-Kuhling et al., 2007; Roy et al., 2009; Sparagano et al., 2009). Where, *D. gallinae* is involved in transmission of many pathogenic agents responsible for severe outbreaks in both animals and humans (Chirico and Tauson, 2002; Moro et al., 2009). In a study it's concluded that northern fowl mite infestation (NFM) has negative impact on interior egg quality and hen integument (Vezzoli et al., 2016). *D. gallinae* life cycle possessed five stages (egg, larva, protonymph, deutonymph, adult) Infestation can triple its numbers in only 10 days where nymphs need a blood meal for metamorphosis and adult females need blood meals for egg maturation. The mites are small and grey in color but may appear red if they have filled with blood after feeding on the bird (Pritchard et al., 2015). Various synthetic contact acaricides such as permethrin, carbaryl, diazinon, dichlorvos are the most used for control *D. gallinae* infestation lead to drug chemical pollution and the development of resistance (Flochlay et al., 2017). In addition, drug and chemical residues in eggs and meat are an important problem for human health (Cernea, 2006; Kim et al., 2007). So that, there is an urgent need for using of herbal organic pharmaceuticals products as an alternative control measures to avoid drawbacks and maintain a good animal health (Lee et al., 1997; Abbas et al., 2014).

Plant essential oils are rich sources of bioactive components to control of mite infestations (Kim et al., 2004). The main chemical constituent of intact garlic is the amino acid allicin, an alkyl derivative of cysteine alkyl sulfoxide, which may vary from 0.2 to 2.0% fresh weight (Michahelles, 1974; Lutomski, 1987). Garlic contains at least 100 sulfur-containing compounds basic to medicinal uses. Allicin represents 70-80 % of the total thiosulfinates (Lawson and Hughes, 1992; Lawson, 1993; Srivastava et al., 1995). Valuable phytochemicals such as phenolic compounds (2554 µg/g), carotenoids (2.92 µg/g), and ascorbic acid (1798 µg/g) are contained in the rosehip seed. Also, it was rich in polyunsaturated fatty acids, linoleic acid (54.05%), linolenic acid (19.37%), and phytosterols, mainly β-sitosterol (82.1%) (Ilyasoğlu, 2014). Rapeseed oil is plant-based oil extracted from the seeds of the rapeseed plants contained

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higher levels of total tocopherols and carotenoids. So that, it widely used in animal nutrition (Marčić et al., 2009).

Acaricide/insecticide activity of some plant extracts, oils and active components were examined and used as alternative to the chemical acaricides to control chicken's mite infestation (Kim et al., 2004; Magdaş et al., 2010; George et al. 2010 and 2014).The activity of numerous essential oils were evaluated against adult *D. gallinae* which collected from poultry using direct contact and fumigation methods, the results showed great effect due to action in the volatile oils (vapour phase) (Kim et al., 2004). *In vitro* the effect of eleven essential oils using direct contact method was evaluated and the results revealed that the most effective oils were sweet basil, coriander, peppermint and summer savory (Kim et al., 2004; Magdaş et al., 2010). Acaricidal activity of plant bioactive components was evaluated against *D. gallinae* by contact toxicity to carvacrol and thymol, the author concluded that two components were found to be toxic to *D. gallinae* with LD50 values of 1 and 3.15 µg/cm, respectively (Tabari et al., 2015). Carvacrol-thymol combination in ratio 4:1 at 2 % concentration displayed good residual toxicity and was effective against *D. gallinae* till 14 days post spraying (Masoumi et al., 2016). Garlic essential oil was toxic to *T. molitor larva*, followed by pupa and adult. Diallyl disulfide was the most toxic induced symptoms of intoxication and necrosis in larva, pupa, and adult of *T. molitor* between 20–40 h after exposure, therefore, garlic essential oil and their compounds have the potential for pest control (Plata-Rueda et al., 2017). The rosehip-seed oil plant oils were efficient natural phytocompounds against the treated larvae of cotton leaf-worm and combination of oils has synergistic action against the 4th larval in star of *S. littoralis* (Mesbah et al., 2006). Rapeseed oil used in spray treatment against spider mites, green peach aphid, pear psylla, summer population of *P. ulmi* and *T. urticae* resulted in efficacy rate 97.4% -84.1 % at 7-11 DPT (Marčić et al., 2009). Antimicrobial activity of the synergistic action of essential oils mixture was also reported (Mesbah et al., 2006; Bassolé and Juliani, 2012; Hyldgaard et al., 2012; Masoumi et al., 2016). Susceptibility of *D. gallinae* to combined essential oil was carried out by direct contact fumigation or spray to fulfill contact toxicity (Kim et al., 2004; Magdaş et al., 2010; Faghihzadeh Gorji et al., 2014; Rahimian and Sparagano, 2017).

The present study was aimed to evaluate the acaricidal potential of combined plant essential oils (includes garlic, rosehip, rapeseed and polysorbate) in vitro using direct contact method and *in vivo* through drinking water and direct spray on *D. gallinae* (red mite) of chickens.

MATERIAL AND METHODS

Red mite collection

Five hundred and twenty red mites (*D. gallinae)* of different stages were collected from naturally infested 5 layer poultry farms. The mites were collected with the aid of a brush in plastic jars and were used for tests within 2 days of collection. Until experiment duration, the mites were kept at 24 C° under a photoperiod of 16:8 h light/dark (Magdas et al, 2010). The collected mites were used for testing of efficacy of mixture of vegetable oil extracts (Allisal®) *in vitro* and *in vivo* (Faghihzadeh Gorji et al., 2014; Rahimian and Sparagano, 2017).

Essential oils

Allisal® is a liquid supplement product of ENVISAL EUROPE BV Vlambloem 85B 3068 JG Rotterdam, Netherlands. It was an aqua suspension of natural plant oils contained garlic oil, rosehip oil, rapeseed oil and polysorbate at concentration of 2.5%, 4.2%, 4.8% and 14.0% respectively. The product was diluted to 0.25% in water and used for in vivo and in vitro study.

In vitro study

Grouping and treatment for using direct contact with the mixture of plant oils. Six groups, 20 mites each were transferred to separate glass petri dishes (3 replicates) and exposed to the following treatments: Group 1 kept as non-treated control (sprayed with only water). Group 2 was sprayed with 0.25% plant oils mixture in water. All treated mite groups were observed under stereomicroscope at 1 and 2 h after direct spray, non-moving mites considered dead, the time for parasite death was recorded and photos taken for comparative evaluation of the acaricidal effect.

In vivo evaluation of plant oil mixture effect on red mite

Sixty mites infested 45 weeks-old layer hens having marked signs were selected and transferred to our laboratory. Birds were randomly divided into 3 equal groups (1 to 3); 20 hens each. Two hundred mites for each treated group were released for 4 days before start of treatment the average mite count from the first date will be set as 100% and the following counts will be compared to the first count. Each group was kept in separate cage on cross straw litter, feed on commercial layer ration under natural day light dark time. Hen groups were treated as group 1 kept as control non-treated. Groups 2 and 3 were treated with 0.25% plant oils mixture for 4 days, followed by 3 days stop of treatment and

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return other 3 days treatment via drinking water and spray, respectively. Four traps were laid in each cage corner and were examined with counting of mite for calculation of counts % at 4,7 and 12 days from start of treatment and release it again. All groups were subjected to daily observation with recording of comb color, skin color, scape in skin and feather condition for calculation of lesion score. Also, water intake, feed intake and laid egg were considered in observation.

Scoring

The feather cover on six different body areas (head/neck, breast, vent, wings, back, and tail) was scored using a 0 to 2 scale, with 0 indicating that all body parts were completely feathered, 1 indicating that one or more body areas had damaged feathers or featherless spots < 5 cm in diameter, and 2 indicating that there were one or more body areas with featherless spots ≥ 5 cm (Nicol et al., 2009; Welfare Quality, 2009; Vezzoli et al., 2016). The normal layer hens have no lesion (Score 0). The layers with lesions known as one lesion (score 1), two lesions (score 2), three lesions (score 3), four lesions (score 4). The all counted lesions were observed on feather, skin color, scabs and comb color.

Statistical analysis

The groups were compared at each time point by one-way analysis of variance (ANOVA) in SPSS (Statistics software, 2017) (Allen, 2017). The level of significance of the formal tests was set at 5%.

RESULTS

***In vitro* results of anti-red mites**

Mite's description

Mites were directly subjected to treatment and examined under stereomicroscope until complete stop movements and their features were recorded at 1 and 2 hours after treatments (Figure 1). The non-treated mites showed active movement under stereomicroscope with pale light brown color at 1 and 2 hours after treatment (Figures 1A and 1B). The Allisal essential oil sprayed mites with 0.25% solution were completely stopped movements which considered dead at 1 and 2 hours after treatment with completely stretched legs and white bead like spots of oils accumulation on legs and bodies (Figures 1 C and 1 D). Results of essential oils contact on *D. gallinae* at different dose were listed in table (1). For the 0.25% Allisalessential oils after 1 and 2 h of contact a strong acaricide effect of 100% death of red mites at 1 and 2 h was seen.

***In vivo* results of anti-red mites**

In vivo effect of Allisal oil to control red mite infestation in laying hens (Table 2). First treatment showed that at the 3rd day post- administration in drinking water mite was reduced to 60% on bird and 0% in traps, while in sprays; the mite population was reduced to 40% on bird and 0% in trap. At the 7th day (1st day of starting 2nd treatment) mite count was reduced to 10% and 0% on bird and in trap; respectively, while in spray it was reduced to 20% and 0% on birds and in trap; respectively. By the 12th day (2nd day after full treatment course) birds treated via drinking water showed no mite on hens and 10% in traps, while those spray treated showed 10% on birds and 30% in traps. Infested chickens showed dirty dull feather, red spots in pale skin and area free from feather at 0 time, these signs started to improve in treated via drinking water and spray at the 4th day (figure 2) while feathers and skin returned to normal at the 12th day as compared with non-treated. Lesion score (table 3) drinking water treated group 2 at the 4th day of treatment showed lower feather, skin color, scabs , comb color and total score (0.70, 0.6, 0.70, 0.50 and 2.50) than those of infected non-treated group 1(0.75, 0.7, 0.85, 0.65and 2.90), respectively. The spray treatment (group 3) showed slight improvement (0.70, 0.5, 0.70, 0.50 and 2.40). The improvement in feather, skin color, scabs, comb color and total score were the highest at 12 days post-treatment in water (0.30, 0.4, 0.30, 0.30and 1.30 followed by spray (0.70±, 0.6, 0.60,0.50and 2.30) compared with non-treated (0.80, 0.8, 1.00, 0.80 and 3.45), respectively. At 4th day there is marked improvement in water and feed intake, bird's activity and egg production (quantity and quality) were started to improve in water treated groups than spray and non-treated groups from the 4th day till the 12th. In general drinking water treated birds showed suitable general health condition and lower lesion scores than spray treated as compared with non-treated control group.

Table 1. Acaricidal effect of Allisal essential oils against *D. gallinae* collected from layers farms

Treatment	Dose	Mites Mortality (%)	
		1 h.	2hs
Non treated	Water	0	0
Allisal®Oils	0.25%	100	100

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Table 2. Effect of Allisal 0.25%in drinking water and direct spray on red mites count in infested treated hens

Parameter	Observation/ days							
	Drinking water				Spray			
	0	4D	7	12	0	4	7	12
Red mites count on birds (%)	100	60*	10	0	100	40	20	10
Red mites count in traps (%)	100	0	0	10	100	0	0	30

*All numbers are rounded to the nearest ten.

Table 3. Lesion score of Allisal 0.25%treatment in drinking water and direct spray on red mites in infested laying hens

Treatment	Time/ days	Lesion				Total
		Feather	Skin colour	Scabs	Comb colour	
		Mean* ± SD**	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Non-treated	0	0.65±0.49	0.6±0.53	0.75±0.44	0.55±0.51	2.50±0.69
	4	0.75±0.45	0.7±0.49	0.85±0.37	0.65±0.49	2.90±0.79
	7	0.80±0.42	0.75±0.45	0.90±0.31	0.80±0.41	3.25±0.64
	12	0.80±0.41	0.8±0.419	1.00±0.0	0.80±0.41	3.45±0.51
Drinking water	4	0.70±0.48	0.6±0.52	0.70±0.48	0.50±0.53	2.50±0.53
	7	0.60±0.52	0.5±0.523	0.80±0.42	0.4±0.52	2.30±0.82
	12	0.30±0.48	0.4±0.51	0.30±0.48	0.30±0.48	1.30±1.16
Spray	4	0.70±0.48	0.5±0.53	0.70±0.48	0.50±0.53	2.40±0.69
	7	0.60±0.52	0.5±0.53	0.60±0.52	0.60±0.52	2.40±0.84
	12	0.70±0.48	0.6± 0.52	0.60±0.51	0.50±0.53	2.30±1.05

*Means: Mean number of birds with lesion/ total birds per group. ** SD: Standard deviation.

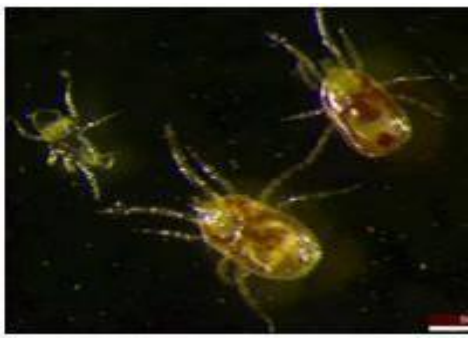
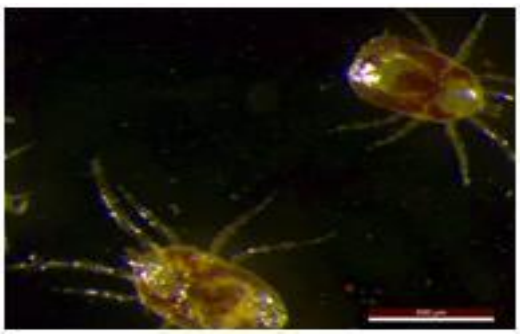
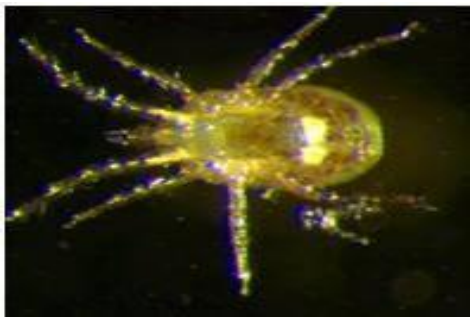
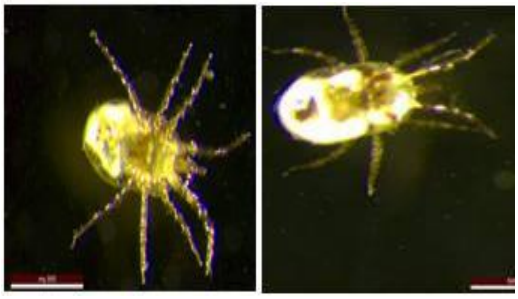
Group and treatment	Time after treatment	
	1 hour	2 hours
1. Control non-treated	 A	 B
2- Oils treated	 C	 D

Figure 1. Acaricidal effect of essential oils against *D. gallinae* collected from layers farms

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DPT	Control non-treated	Drinking water	Direct spray
0			
4			
7			
12			

Figure 2. Effect of Allisal essential oil at 0.25% concentration in drinking water and water direct spray in red mites natural infested laying hens. DPT: Day post treatment. 0: Time before start treatment. 4-, 7- and 12- Days post- treatment

DISCUSSION

Recently, the susceptibility of field collected *D. gallinae* from naturally infested commercial chicken houses to essential oil mixture and/or prepared nanoparticles as acaricide was evaluated. The susceptibility of collected *D. gallinae* to essential oil as acaricide was carried out by direct spray to fulfill contact toxicity (Faghihzadeh Gorji et al., 2014; Rahimian and Sparagano, 2017).

In the present study, we used essential oils mixture (Allisal) which contains garlic oil, rosehip oil and rapeseed oil to evaluate their effect on the chicken's mites in vitro and vivo. As shown in (figure 1 and table 1) results of in vitro essential oils contact on *D. gallinae* at different doses of the 0.25% Allisal after 1 and 2 h of contact were revealed that the non-treated mites showed active movement under stereomicroscope with at 1 and 2 h after treatment. The Allisal essential oil sprayed mites with 0.25% solution were completely stopped movements which considered dead at 1 and 2 h after treatment with completely stretched legs and white bead like spots of oils accumulation on legs and bodies A strong acaricide effect of 100% death of red mites at 1 and 2 h was seen. This in accordance with Kim et al. (2004 and 2007)

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and George et al. (2010a, b, 2014) the authors concluded that plant-derived essential oils are shown to have a lethal characteristics, where garlic and thyme oils were the most effective. In the other hand, the synergistic action of essential oil for cuticular penetration was also reported (Tong and Bloomquist, 2013; Tak and Isman, 2015). Where the outer part of the mite exoskeleton, known as the epicuticle, consists of a layer of wax, which further limits water loss, and a cement layer, which protects the cuticle from external abrasion (Pritchard et al., 2015; Flochlay et al., 2017). The hydrophobic nature of the oils can cause mechanical effects on the parasite by disrupting the cuticular waxes and blocking the spiracles, leading to death by water stress or suffocation (Burgess, 2009). On the other hand, some of essential oils can be toxic effect on the insect nervous system (Mills et al., 2004; Lopez and Pascual-Villalobos, 2010) and maybe they have similar effects in other parasites.

The synergistic acaricidal action of essential oil was recorded, and the treated mites showed stop movement and detached legs and abnormal keratin surface of dark brown color at 2 h (Amagase et al., 2001). High concentration extract of Garlic (*Allium sativum*) juice and Chrysanthemum (*Chrysanthemum cinerariae folium*) were found to be effective, against *D. gallinae* (Maurer et al., 2009).

Strong acaricide effect with 100% deaths at 1.2 and 3 hours was previously reported by many researchers. Carvacrol (essential oil of *Origanum vulgare*), garlic extract, cinnamon, eucalyptus and mint extract reduction in mite control with 92%, 96%, 66.97, 80.85 and 90.19%; respectively (Rahimian and Sparagano, 2017). The acaricidal activity of essential oils was tested on movable *D. gallinae*; regardless of the stage of their development, garlic extract was 96% effective after two successive sprays (Faghihzadeh Gorji et al., 2014).

Results of in vivo effect of Allisal oil to control red mite infestation in laying hens showed in **(**table 2 and figure 2**)** and revealed that in drinking water treated group mites reduction rate on the bird was 60 %, 10%, and 0% as well as 0%, 0% and 10% in their traps at 4,7, and 12 days respectively, from the start of treatment in drinking water. While, in spray method mite reduction rate was 40%, 20%, and 10% on birds and 0%, 0% and 30% in the traps. On the other hand, water intake, feed intake, general health condition, skin health, and feather condition scores were improved at 4th (DPT). Total lesion score at 12 (DPT) was improved. Where feathers and skin returned to normal at the 12th day as compared with non-treated group (Table 3). Drinking water treated group showed suitable general health condition and lower lesion scores as well as egg production improvement more than spray treated group.

The improvement in all parameters proved safety of used essential oil mixture in red mite control in poultry. These results may be attributed to efficacy of used oil as antihistaminic, anti-inflammatory, high vitamin E and antioxidant activity. These findings are consistent with the result recorded by (Rabinkov et al., 1998; Miron et al., 2000; Xu et al., 2011; Pillaiyar et al., 2017; Qing, 2017). Moreover, essential oil had been approved as food additives and fall in the category of generally recognized as safe by the US FDA (Bilsland and Strong, 1990). For example, Rapeseed oil is plant-based oil contained higher levels of total tocopherols and carotenoids. So that, it widely used in animal nutrition (Marčić et al., 2009).

CONCLUSION

From the obtained results its concluded that the treatment with special and formulated essential oil mixture is highly effective for *D. gallinae* when used in drinking water than direct spray (0.25ml/liter) as acaricidal. It can be considered as safe recommended alternative to chemical compound used in control strategies of *D. gallinae* (red mites) in poultry industry.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

Author`s contributions

Aziza M. Amer and Mohamed M. Amer designed, planned the study, Hoda M. Mekky and Hanaa S. Fedawy collecting samples. All authors shared performed experimental work, manuscript writing, drafted, revised the manuscript, and approved the final manuscript.

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