

INFLUENCE OF SELECTED ESSENTIAL OILS AS A NATURAL REPELLENT OF POULTRY RED MITES (*IN VITRO* STUDY)

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Abstract: High economic impact of the poultry red mite (*Dermanyssus gallinae*), the lack of natural acaricides and the resistance of present chemicals have all led to the need to find new ways to control these ectoparasites. One promising alternative method of control focuses on employing repellent effects of selected essential oils against poultry red mite. This experiment was conducted in order to investigate the repellent effects of thyme (*Thymus vulgaris* L.), lavender (*Lavandula angustifolia* L.) and oregano (*Origanum vulgare* L.) on poultry red mites. The best results were observed for lavender essential oil (96% mortality after 72 h) and thyme (82% mortality after 72 h) at a dose of 0.15 mg/cm². In addition, thyme and lavender essential oils showed significant ($p < 0.05$) persistent toxic effects 15 and 30 days post application to filter papers. Thyme was the most effective (100% mortality at 72 h), followed by lavender (78% mortality after 72 h). Results have showed that the thyme and lavender essential oils exhibited promising results when tested *in vitro* for toxic and repellent effects against poultry red mite. Based on the obtained results we suggest that future experiments should focus on *in vivo* experiments using these oils in laying hens farm conditions.

Key words: mites; essential oils; repellent; acaricides; ectoparasites

ВЛИЈАНИЕ НА ИЗБРАНИ ЕТЕРИЧНИ МАСЛА КАКО ПРИРОДНИ РЕПЕЛЕНТИ ПРОТИВ ЦРВЕНИТЕ ПАЈАЦИ КАЈ ЖИВИНАТА (ИСТРАЖУВАЊЕ *IN VITRO*)

Апстракт: Големото влијание на црвениот пајак (*Dermanyssus gallinae*) кај живината врз економичноста на производството, недостигот на природни акарициди и резистенцијата на сегашните хемикалии доведоа до потреба да се најдат нови начини на контрола на овие ектопаразити. Еден ветувачки алтернативен метод на контрола се фокусира на употребата на репелентни ефекти на избраните есенцијални масла против црвениот пајак кај живината. Овој експеримент беше спроведен со цел да се испитаат репелентните влијанија на мајчината душичка (*Thymus vulgaris* L.), лавандата (*Lavandula angustifolia* L.) и ориганото (*Origanum vulgare* L.) врз црвените пајаци кај живината. Најдобри резултати беа забележани кај етеричното масло од лаванда (96% смртност по 72 часа) и мајчината душичка (82% смртност по 72 часа) во доза од 0,15 mg/cm². Освен тоа, етеричните масла од мајчина душичка и лаванда покажаа значајни ($p < 0,05$) перзистентни токсични влијанија 15 и 30 дена по апликацијата на филтер-хартијата. Мајчината душичка беше најефикасна (100% смртност по 72 часа), по неа следеше лавандата (78% смртност по 72 часа). Етеричните масла од мајчина душичка и лаванда дадоа ветувачки резултати кога беа тестирани *in vitro* за токсични и репелентни ефекти против црвените пајаци кај живината. Врз основа на добиените резултати укажуваме дека идните истражувања треба да се фокусираат на експерименти *in vivo* со користење на овие масла во услови на фармско чување на живината.

Клучни зборови: пајаци; етерични масла; репелент; акарициди; ектопаразити

INTRODUCTION

Dermanyssus gallinae (De Geer, 1778), also known as the “poultry red mite”, is considered one of the most important causer of welfare and health problems for poultry, particularly laying hens [4, 5, 9], and causes large economic losses worldwide [3]. This hematophagous mite parasitizes hens, residing on its host only during blood meals, then retreating into the hiding places in the farm buildings [12]. Although not entirely species specific, poultry red mites are seen most frequently in systems for laying hens, largely due to the lengthy turnover of approximately 72 weeks which allows time to establish large populations. The mite progresses through 5 life stages: egg, larva, protonymph, deutonymph and adult [12]. Under favourable conditions this life cycle can be completed within seven days, so populations can grow rapidly [13]. A characteristic of the red mite infestation is that once they are present in a flock, it is almost impossible to eradicate them [5]. The control of the poultry red mite is mainly based on the use of acaricides, such as organophosphates, carbamates, pyrethroids and formamidines. The lack of new acaricides, resistance to present acaricides, and increasingly stringent requirements for chemicals used on food animals greatly limit the options for controlling this pest [1, 13]. Therefore, alternative methods for the *D. gallinae* control are urgently needed [10, 2]. One alternative method focuses on employing the repellent or toxic effects of selected plant derived products, particularly essential oils, for the management of numerous pests in-

cluding *D. gallinae*. *In vitro* tests have shown promising activities of plant derived products against many foodborne pathogens and spoilage microorganisms. More recently, scientists have [7, 8, 6, 12] investigated the influence of the “time since last blood meal” on the toxicity of essential oils to the poultry red mite, the repellence of plant essential oils to *D. gallinae*, and their toxicity *in vitro*, as well as [12] *in vivo* acaricidal activity and residual toxicity against the poultry red mite.

The aim of this study was to investigate the effects of thyme (*Thymus vulgaris* L.), lavender (*Lavandula angustifolia* L.) and oregano (*Origanum vulgare* L.) essential oils for the repellent effects on poultry red mites.

MATERIAL AND METHODS

Mites. Selection of plant essential oils. Bioassays

For experimental purposes poultry red mites were collected from a laying hen facility in the northern Serbia region (Vojvodina). In the laboratory, the mites were transferred into an open jar surrounded by two successive barriers of water and subsequently left for 10 to 15 days before the start of experiment to stabilize the population and to acclimatize the parasites to the laboratory conditions.

All plant essential oils used in this study were produced by and purchased from Eskage-Eskamint Schierholz GmbH (Bad Oldesloe, Germany) (Table 1).

Table 1

*Essential oil parameters quality**

Treatments	Essential oil quality parameters			
	Essential oil	Specific density at 20°C	Refractive index at 20°C	Optical rotation at 20°C
T1	Control treatment	0.0	0.0	0.0
T2	<i>Thymus vulgaris</i> L.	0.910 – 0.930	1.493 – 1.513	-6.0° – +4.0°
T3	<i>Lavandula angustifolia</i> L.	0.878 – 0.892	1.455 – 1.466	-12.5° – -6.0°
T4	<i>Origanum vulgare</i> L.	0.915 – 0.975	1.504 – 1.524	-0.6° – +4.0°

*Provided by supplier of essential oils.

Filter paper contact bioassays were used to evaluate the toxicity and persistence of the toxic and repellent effects of the essential oils towards *D. gallinae*. For the toxicity bioassay, a 20% dilution of each essential oil in distilled water was made with

0.15 mg/cm³ of the oil. Control filter papers (Whatman No. 2) without essential oils addition. After drying in a fume hood at room temperature, each filter paper (length: 8 cm; width: 1 cm) was folded lengthwise and placed into a Pasteur pipette, allow-

ing mites to walk on the impregnated paper or on the glass wall of the pipettes. Batches of 30 adult *D. gallinae* were introduced into the pipettes using a vacuum pump. For each experiment, three replicates were performed for each essential oil in addition to negative control. All pipettes were maintained under the same conditions as those used for batch maintenance. The mites were examined after 24, 48 and 72 h of exposure to the essential oils to determine mite mortality using a binocular microscope. The results were expressed as the percentage of mortality. The persistence was tested after 15 and 30 days using the same procedure as described above with filter papers introduced in to Pasteur pipettes and left under a hood at room temperature for 15 or 30 days before the experiments.

Statistical analyses

Statistical analyses were conducted using the Statistica for Windows statistical package program, (version 13) to determine if variables differed between groups. Significant effects were explored using analysis of variance (ANOVA), least squares mean (LSM) and Fisher's LSD post-hoc multiple range test to ascertain differences among treatment means. A significance level of $p < 0.05$ was used.

RESULTS AND DISCUSSION

Toxicity and persistence of essential oils

The toxic effect of 20% dilutions of three essential oils at an application rate of 0.15 mg/cm^2 ,

varied according to the plant species. At this dose, the best results were observed for lavender (more than 96% mortality after 48 and 72 h) and thyme (more than 80% mortality after 48 and 72 h) at 0 day of the experiment (Table 2). On the other hand, oregano oil resulted in a mortality rate close to 46% after 72 h. In addition, the persistence of the toxic effect of these oils after 15 and 30 days from application on filter paper varied according to the plant species.

As shown in Table 2 and Figures 1, 2 and 3 lavender, oregano and thyme essential oils showed significant activity 30 days after application to the filter papers. Lavender essential oil was being effective nearly 82% mortality after 72 h at the end of 15th day of experiment, while the thyme was the most effective with 100% mortality rate of poultry red mite at 72 h at the end of 30th day of the experiment. Oregano essential oil was the least effective, with more than 40% mite mortality after 72 h at the end of the experiment.

One of the alternative methods for the control of different culture breeding pests is the use of plant essential oils, which have been recognized for their insecticidal, antibacterial, antifungal, and antiviral effects and are widely used in medicine and the food industry. In comparison to other studies [8, 11], lavender and thyme were most effective at a dose of approximately 0.15 mg/cm^2 of the oil, corresponding to a 20% dilution and over 80% mortality of poultry red mites. A problem with the use of essential oils against different pests is that oils can have different chemical profiles and therefore, varying in acaricidal activity, even if the oils are from the same plant species.

Table 2

The acaricidal activity of selected essential oils

Treatments	Poultry red mite mortality during experiment (%)								
	0 day			15 day			30 day		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
T1 LSM	0.0 ^c	0.0 ^c	0.0 ^c	4.0 ^c	4.0 ^c	6.0 ^c	2.0 ^b	4.0 ^d	8.0 ^d
T2 LSM	70.0 ^a	80.0 ^a	82.0 ^a	18.0 ^b	38.0 ^b	26.0 ^b	72.0 ^a	98.0 ^a	100.0 ^a
T3 LSM	90.0 ^a	96.0 ^a	97.0 ^a	70.0 ^a	84.0 ^a	82.0 ^a	80.0 ^a	68.0 ^b	78.0 ^b
T4 LSM	24.0 ^b	40.0 ^b	46.0 ^b	18.0 ^b	36.0 ^b	28.0 ^b	6.0 ^b	38.0 ^c	42.0 ^c

Treatments with different letter indexes in the same column are statistically significantly different ($p < 0.05$)

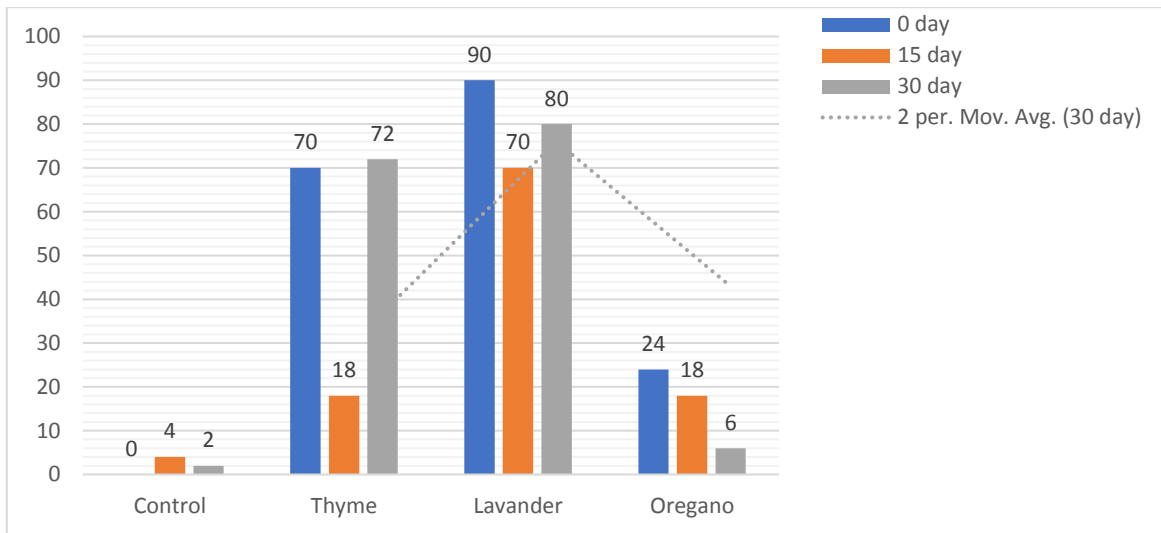


Fig. 1. Poultry red mite mortality during 24 h of experiment, %

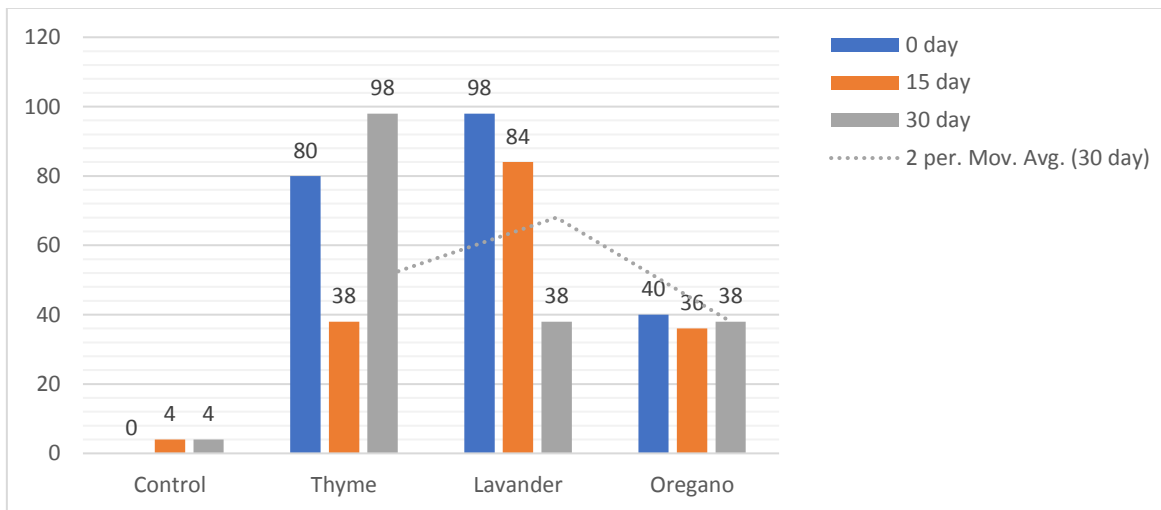


Fig. 2. Poultry red mite mortality during 48 h of experiment, %

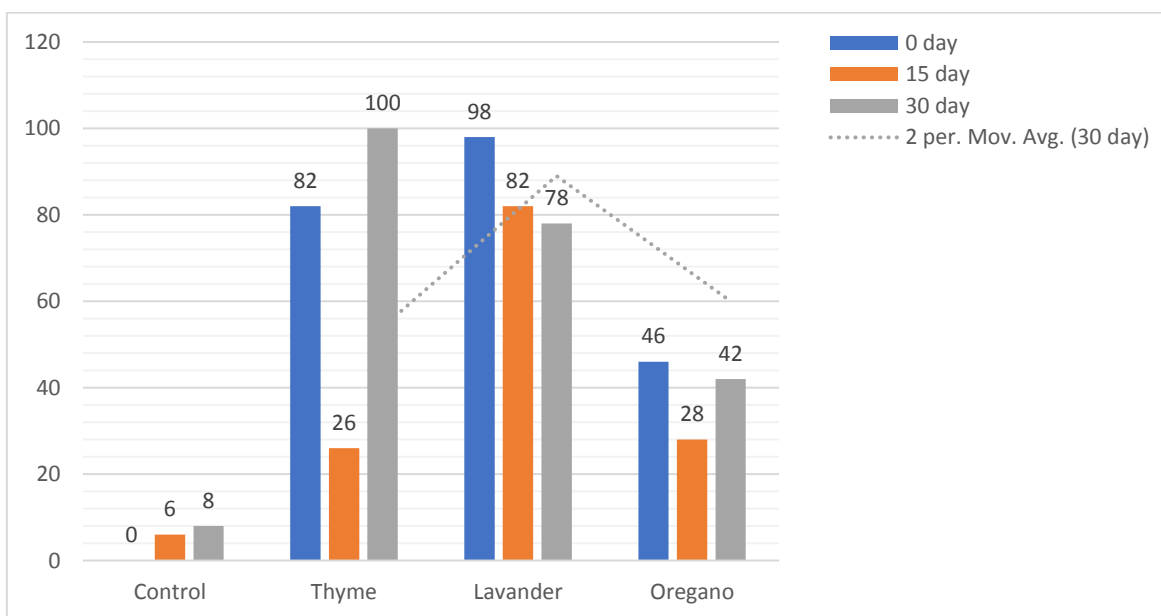


Fig. 3. Poultry red mite mortality during 72 h of experiment, %

CONCLUSIONS

Based on the obtained results this study showed that thyme and lavender essential oils exhibited promising results when tested *in vitro* for toxic and repellent effects against *D. gallinae*, and further experiments should focus on *in vivo* tests of these essential oils in laying hens farms to confirm gained *in vitro* results.

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