In vitro acaricidal effects of Thyme essential oil, Tobacco extract and Carbaryl against Dermanyssus gallinae (Acari: Dermanyssidae)

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Abstract. Massive economic losses imposed by Dermanyssus gallinae De Geer, 1778 and limitation in using chemical compounds against them, indicates that searching for new alternative miticide compounds is an important necessity. The aim of this study was to investigate acaricidal effects of thyme essential oil and tobacco extract on D. gallinae in comparison to Carbaryl 85%WP in laboratory conditions. For this purpose a filter paper contact test was used and 30 live adult female mites were exposed to different concentrations of each examined compound for 2, 4, 6 and 24h. The mortality rate of mites at each concentration and time was recorded and each treatment was performed in triplicates. Three examined compounds showed acaricidal activities and effects of exposure time and concentration on mortality rate were significant (P<0.05). The highest mortality was achieved at 24h and tobacco extract showed more acaricidal activity than thyme essential oil. The results of this study suggest that tobacco has a promising acaricidal activity and is a potent green pesticide. More research is needed to investigate its side-effects and safety concerns for mammalian and bird hosts.

Keywords: Dermanyssus gallinae; Acaricidal activity; Carbaryl; Thyme essential oil; Tobacco extract.
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Introduction

The poultry red mite, Dermanyssus gallinae parasitizes laying hens and wild birds. These mites can cause anemia, weakness, weight loss and decrease in egg production. Heavy infestation may lead to death in affected birds (Valiente et al., 2007; Marangi et al., 2009). They not only can adversely affect egg production, but also act as vectors for some pathogenic agents such as Salmonella, Fowl pox virus and Newcastle virus (Valiente et al., 2005).

Chemical acaricides have been conventionally used for controlling of this mite in infested aviary systems. Despite the high efficacy, low price and accessibility of chemical compounds, usage of them has been faced with crucial challenges. Mite resistance, chemical residues in human’s food, stricter legislation and increase in consumers demand for organic...
products are only a part of these challenges (George et al., 2009; 2010).

Hence, finding alternatives for chemical pesticides are pursued by some researchers. Spinosad that derived from bacterial species Saccharopolyspora spinosa has showed 97% efficacy and approved for use against D. gallinae in some European countries (Sparagano et al., 2014). Other alternatives such as predatory mites, Bacillus thuringiensis, feeding deterrence and insect growth regulators ought to be developed but none is ready for field use (Chave, 1998; Sparagano et al., 2014). In recent years, a lot of interests and attentions have been drawn to the herbal medicine. Plant essential oils of Thyme, Manuka, Cade and Pennyroyal have showed different acaricidal effects on D. gallinae (Kim et al., 2004; George et al., 2009). Acaricidal properties of some plant extracts such as neem seed oil and Tobacco extract have been revealed (Lundh et al., 2005; Sindhu et al., 2012). Garlic-based products have been recommended for use against D. gallinae (Sparagano et al., 2014). The aim of this study was to investigate acaricidal effects of thyme essential oil, tobacco extract and Carbaryl 85%WP against D. gallinae in laboratory conditions.

Materials and methods

D. gallinae mites were collected from three naturally infested caged laying poultry farms. They were transferred to clean jar and brought to laboratory and kept at 22°C (16:8 L: D cycle).

Essential oil of thyme (Thymus vulgaris) was purchased from Johare Taem Shargh Company, Iran. Dried leaves of tobacco (Nicotiana tabacum) and Carbaryl 85%WP (Ghazal Shimi Company, Iran) were obtained from local market. For preparation of tobacco extract, dried leaves were ground into fine powder by an electric grinder. This powder was soaked in ethanol 70% and put on a shaker at a low speed shake for 36 h. After 36 h soaked materials were filtered through filter paper using a Buchner funnel. For elimination of solvent, the extract was placed in evaporator. Finally dried residue was collected and preserved in airtight container.

Whatman No. 2 filter papers (3*3cm) were impregnated with 500 µl of thyme essential at concentrations: 0.2, 1.12, 2.25, 5.50, 11, 22, 110 and 220 mg/ml. Similarly 500 µl of tobacco extract at concentrations of 0.052, 0.52, 5.2 and 52 mg/ml were applied to filter papers. Concentrations of 1, 2, 3, 4 and 5 mg/ml were prepared from Carbaryl and applied to filter papers. Distilled water was used as solvent in all mentioned substances. Untreated control filter papers were impregnated with 500 µl distilled water. Treated and untreated filter papers were dried in a fume cupboard prior to use.

The treated and untreated papers were placed into 26 ml glass vials with screw caps and a batch of 30 adult mites was transferred to each vial. Then, vials were sealed with a layer of Parafilm and after adding screw caps, kept at 22°C. Mite mortality was assessed after 2, 4, 6 and 24 h under magnification. Each treatment was performed in triplicates.

Statistical analysis

The analyses of mortality rates induced by different concentrations of thyme essential oil, tobacco extract and Carbaryl were performed using one way ANOVA followed by Tuckey-Kramer post-hoc test, while changes in mortality rates among different time points were analyzed using Repeated measures ANOVA. Lethal concentration 50 (LC50) value was calculated by probit test. P value less than 0.05 was considered as significant.

Results

Two plant-derived products and one chemical compound were tested at different concentrations to evaluate their toxic effects at 2, 4, 6 and 24 h against adult D. gallinae. The effect of exposure time was significant (P<0.05) and there was a direct relationship between mortality rate and exposure time for all examined compounds. The highest relationship was achieved at 24 h of exposure. Mortality rate had a direct relation with concentration and this relationship was statistically significant (P<0.05).
Acaricidal effects of thyme essential oil

The paper contact test revealed that mites had a substantial sensitivity to thyme essential oil (figure 1). Indeed, the acaricidal effects of thyme essential oil increased in a time dependent manner in all study groups (P<0.05). Within each time point, the increase in thyme essential oil concentration leaded to enhance mortality of D. gallinae reaching the highest (100%) mortality in concentration of 110 mg/ml (P<0.05).

Acaricidal effects of tobacco extract

The mortality rate of mites depended on the concentration of tobacco and the exposure time. The highest mortality was achieved at concentration of 52 mg/ml. The effect of time was also significant and in each concentration mortality rate increased with the exposure length. As we showed in figure 2 the best acaricidal effects achieved at 24 h.

Acaricidal effects of Carbaryl

Carbaryl at concentrations 1, 2 and 3 mg/ml did not show significant toxicity but at concentration 4 mg/ml mortality rate reached 100%. Like other tested compounds in this study, acaricidal effect of Carbaryl was time and dose-dependent and the highest mortality at each concentration achieved at 24 h exposure (figure 3).

Lethal concentration 50 (LC50) values

For each examined compound LC50 was calculated at 2, 4, 6 and 24h. The LC50 of thyme compared to tobacco and Carbaryl was higher. Tobacco and Carbaryl had very close LC50 values. The LC50 of thyme, tobacco and Carbaryl at 24h were 46.6, 1.71 and 1.60 mg/ml, respectively (figure 4).

Discussion

Huge economic losses imposed by D. gallinae and limitation in using chemical compounds against them, indicates that searching for new alternative miticide compounds is an important necessity. Plant-derived miticides have been investigated in recent years. Among those plants that have been investigated, neem oil yielded from neem tree (Azadarchta indica) has shown great pesticide activity and has been produced commercially as a plant-derived miticide against D. gallinae (Locher et al., 2010; Sparagano et al., 2014). Despite the acaricidal potencies of the neem seed oil against D. gallinae this plant does not raise in Iran. One of the most important factors for selecting a botanical alternative for synthetic pesticides, in addition to its acaricidal effects, is its availability in the region. Both tobacco and thyme are cultivated in extend areas of Iran. The results of this study showed tobacco has more miticidal effects on D. gallinae than thyme and it demonstrated promising acaricidal activity. The pesticidal effect of tobacco has been known for centuries by Iranians and they used its aqueous extract for curing animal’s mange. Nicotine is the main compound of tobacco plant. This compound binds to cholinergic acetylcholine nicotinic receptors in the nerves of arthropods and other animals and continuously stimulates post neurons. Its LC50 to rats is 50-60 mg/kg (Copping and Menn, 2000). Tobacco has showed its efficacy in controlling ectoparasites in poultry and domestic animals when its decoction or crude water extract was applied on the test group as a spray, powder, dip and sponge bath application (Rosa, 2001). Fajimi et al. (2003) found tobacco aqueous extract had better therapeutic effects than Ivermectin to cure pediculosis when applied topically on Linognathus sp. infested dwarf goats. They found the eggs of lice on tobacco test group were looking dried and dead, unlike the Ivermectin test group that parasite eggs were looking shiny and hatchable. The acaricidal activities of tobacco against Boophilus microplus and two-spotted spider mite (Tetranychus urticae) have been reported (Sindhu et al., 2012; Potenza et al., 1999). Furthermore, Lans and Turner (2011) reported tobacco is using for control of poultry external parasites including D. gallinae in organic poultry farms in British Colombia, Canada. No one of these researchers reported any side-effect in animals and birds after using Tobacco in liquid or powder forms.
Figure 1. Acaricidal effects of thyme essential oil on *D. gallinae* increased in a time and concentration dependent manner in laboratory conditions.

Figure 2. Acaricidal effects of tobacco extract against *D. gallinae* and its relationship with time and concentration in laboratory conditions.
In this study thyme essential oil showed low acaricidal activity in comparison to tobacco extract. The acaricidal effect of this plant was investigated by Kim et al. (2004) and George et al. (2010) and results of both studies showed thyme essential oil is a good candidate for *D. gallinae* control as a biopesticide. Despite using the same methodology (filter paper contact bioassay) in these studies, our results revealed thyme essential oil had weak acaricidal activity and for achieving effective mite control high concentrations are needed that it is not economically justifiable. This disagreement can be attributed to chemical profile of examined plant in these studies. According to Koul et al. (2008) chemical profile of plant species can vary naturally depending on genetic,
geographic, climatic, seasonal or annual factors.

In the current study a chemical pesticide (Carbaryl 85%WP) was used as a marker for evaluating efficacy of two examined plants. This compound has been used for several years against *D. gallinae* in infested aviary farms in Iran. In 2011 Iranian plant protection organization banned production, distribution, recommendation and application of Carbaryl in Iran. Interestingly, the L50 of tobacco extract was very close to LC50 of Carbaryl in this study.

In conclusion thyme essential oil showed low acaricidal activity but tobacco was more effective against *D. gallinae*. If tobacco side-effects and its safety concerns for mammalian and bird hosts will be investigated and solved, it can be one of the alternatives that we look for.

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**References**


