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Article

In vitro acaricidal effect of *Melia azedarach* ripe fruit extract against *Dermanyssus gallinae* (Acari: Dermanyssidae)

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ABSTRACT
The aim of this study was to evaluate acaricidal activity of *Melia azedarach* L. ripe fruit extract against *Dermanyssus gallinae* (De Geer, 1778) under laboratory conditions. For this purpose, hexanic ripe fruit extract of this tree at concentrations 0.25, 0.5, 1, 3, 5 and 10% were prepared with acetone as solvent and treatment groups exposed to these concentrations using Petri dish residual film bioassay method. The positive control was carbaryl while negative control was acetone. The bioassay was repeated five times. The mortality rate of mites in treatment and control groups were assessed 24 h post exposure. All treatment groups showed significantly higher mortality compared to the negative control group ($P < 0.05$). All concentrations of extract showed moderate or high miticidal effects on *D. gallinae* as mean mortality of exposed mites to concentrations 0.25, 0.50, 1, 3, 5 and 10% were 40, 43, 60, 62, 79 and 100%, respectively. Carbaryl as a positive control caused 100% mite mortality at 1 mg/ml concentration. The LC50, 90, 99 (lethality concentrations for 50, 90 and 99%) values of this extract were calculated and were 1.78, 5.93 and 9.32%, respectively. The results of the current study revealed that the ripe fruit extract of *M. azedarach* was highly toxic to *D. gallinae*.

KEY WORDS: Acaricidal activity; biopesticide; natural acaricide; Persian lilac; poultry red mite.

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INTRODUCTION

The poultry red mite, *Dermanyssus gallinae* (De Geer, 1778) is an obligatory bloodsucking ectoparasite of both domestic and wild birds. This mite is the most important ectoparasite of laying caged hens and has become an increasing economic problem for the poultry industry worldwide (Chauve 1998). It poses economic losses by a reduction in egg production, egg down-grading, anemia and increase in mortality of infested birds (Sparagano *et al*. 2009; 2014).

Synthetic acaricidal compounds have been used up to now against this mite in infested farms. These compounds are used widely for controlling *D. gallinae* but extensive and repeated usage of them have resulted in lack or decrease in their efficacy probably due to developed resistance (Marangi *et al*. 2009; Sparagano *et al*. 2014). Beside this, chemical residues in human's food,

stricter legislation and increase demand for organic products have led the researchers to investigate alternative *D. gallinae* control methods (George *et al*. 2014; Sparagano *et al*. 2014). In recent years, a lot of interests and attentions have been drawn to the natural-based products such as biopesticides and plant-derived products. In some European countries, spinosad as a highly active biopesticide has been approved for using in laying hen poultry farms. Neem-based product (Mitestop, Felema, Switzerland) and a garlic-based acaricide are two plant-derived products which commercially are available for *D. gallinae* control (Sparagano *et al*. 2014).

Melia azedarach or Persian lilac is a multipurpose tree in the family Meliaceae which commonly known by many names such as Persian lilac, Indian lilac, and Chinaberry tree. Aside from being an ornamental tree, its leaves extract has insecticidal properties and its seed is the most medicinal products of this tree. Traditionally, its seed extract is used for ringworm and scabies treatment. It is also useful in malaria fever and leprosy (Ramya *et al*. 2009). Seed oil of Persian lilac has shown promising insecticidal effects on *Anopheles stephensi* (Hadjiakhoondi *et al*. 2006).

The aim of this study was to evaluate acaricidal effects of ripe fruit extract of *M. azedarach* against *D. gallinae* under laboratory conditions.

MATERIALS AND METHODS

Mite collection and preparation of fruit extract

Dermanyssus gallinae mites were collected from a naturally infested caged laying poultry farm and were kept at 25 ℃ and 75% RH (16:8 h light: dark cycle).

The fruits of *M. azedarach* were collected from trees growing on the campus of Ferdowsi University of Mashhad (Mashhad, Iran) and were dried in the shade and powdered. Dried powder (500 g) was extracted using a Soxhlet apparatus with two liters of hexane. Hexane was evaporated under vacuum in the flash evaporator. The extract was a dark green oily viscous liquid and extraction yield was found to be 5.8% v/w.

Bioassay

Petri dish residual film method was employed to evaluate the toxicity of the aforementioned extract to *D. gallinae.* Bioassay was performed with *D. gallinae* adult mites using concentration of 0.25, 0.5, 1, 3, 5 and 10% *M. azedarach* ripe fruit extract that were prepared with acetone as solvent (Banchio *et al.* 2003; Borges *et al.* 2003; Sousa *et al.* 2011). The negative control solution was prepared as above, but just without the extract. Carbaryl (85% WP) at concentration 1mg/ml was used as positive control and prepared with carbaryl and acetone as solvent. Before running each test, clear glass Petri dishes (90 mm diameter and 15 mm depth) with tight fitting lids were washed by ethanol 100% and dried to remove any surface residues. The internal surface of the base and lid of each Petri dish was sprayed with two ml of different concentrations of examined extract and control solutions. Afterwards, Petri dishes were left uncovered for one hour to dry, then 20 adult mites were transferred into each Petri dish and the Petri dish was sealed using parafilm after the lid was placed on top.

Treatment and control groups were kept in an incubator set at 25 ℃ and 75% RH for 24 h. After that, mites were examined under a dissecting microscope for assessment of mite mortality. Each mite was considered to be dead if it did not show any sign of movement when it was agitated with an entomological pin. There were five replicates for each concentration and control group.

Statistical analysis

The data were analyzed using SPSS software ver. 22.0 for Windows (SPSS Inc., Chicago, Illinois). Kruskal–Wallis and Mann-Whitney tests were used for comparing mortality means achieved by different concentrations of examined extract and control groups. The statistical

difference was considered significant when P value was less than 0.05. The lethal concentrations (LC) 50, 90 and 99% were determined using the program XLSTAT-PRO ver.19.02 for Windows (Addinsoft).

RESULTS

All treatment groups showed significantly higher mortality compared to the negative control group ($P < 0.05$). All concentrations of extract showed moderate or high miticidal effects on *D. gallinae* as mean mortality of exposed *D. gallinae* to concentrations 0.25, 0.50, 1, 3, 5 and 10% were 40, 43, 60, 62, 79 and 100 %, respectively. Statistical difference between the mortality rates of *D. agallinae* obtained by different concentration has been presented in Table 1.

The mortality rate of mites had a direct relation with the concentration and this relationship was statistically significant ($P < 0.05$).

Carbaryl as a positive control caused 100% mite mortality at 1 mg/ml concentration.

The LC50, 90, 99 (lethal concentrations for 50, 90 and 99%) values of this extract were calculated and were 1.78, 5.93 and 9.32%, respectively.

Table 1. Acaricidal activity of *Melia azedarach* ripe fruit extract at different concentrations against *Dermanyssus gallinae* 24 h post exposure. According to Mann-Whitney test, means that showed by the same letter are not significantly different ($P > 0.05$).

Concentration (%)	Number of mites exposed	Mortality, % (Mean ±SE)	CI for mean mortality	LC50 (CI)	LC90 (CI)
0	20	1 ± 0.44^{a}	−0.24–2.24		
0.25	20	40 ± 6.124^{b}	23–57		
0.50	20	43 ± 7.84^{b}	21.23–64.77	1.78 (1.08–2.52)	5.93 (4.67–8.39)
1	20	60 ± 9.35^{bc}	34.03–85.97		
3	20	62 ± 7.00^{bc}	42.50–81.44		
5	20	79 ± 6.96^{cd}	59.66–98.34		
10	20	100 ± 0.00^{d}	100–100		

CI: 95% confidence interval.

DISCUSSION

In this study, the acaricidal potency of *M. azedarach* ripe fruit extract against *D. gallinae* was investigated and its acaricidal effects on exposed mites was revealed under laboratory conditions. The results of this study showed that the mortality rate was dependent on concentration and the highest mortality was obtained at concentration 10% that was able to kill 100% of exposed *D. gallinae* mites. These results coincide with those observed by Borges *et al*. (2003) when they evaluated efficacy of hexanic ripe fruit extract of *M. azedarach* against the tick, *Boophilus microplus* (Canestrini) and observed 98% mortality in tested tick larvae (Borges *et al.* 2003). The acaricidal activity of the fruit extract of this tree has been shown against two-spotted spider mite, *Tetranychus urticae* Koch (Yanar *et al.* 2011). Some authors have reported larvicidal effects of this fruit/seed extract against larvae of *Anopheles stephensi* Liston, *Aedes aegypti* L. and *Culex quinquefaciatus* Say, 24 h after treatment (Hadjiakhoondi *et al*. 2006; Selvaraj and Mosses 2011).

We used whole extract in this study because according to some researches active constituents rarely have the same degree of activity as the unrefined extract, probably due to absence of interacting substances present in the extract. A further advantage of whole extract is that many plants contain substances that inhibit multi-drug resistance (Rasoanaivo *et al.* 2011).

Some well-known components of fruit extract of *M. azedarach* include a wide range of triterpenoids such as meliartenin, meliacaprin, meliacin, meliantrol, melianol, salanin, nimbin, and azaderachine. These components exert their acaricidal/insecticidal activities by acting as anti-

feedent, growth regulator, repellent and/or inhibiting egg production and embryogenesis (Isman 2006; Castillo *et al*. 2010). This extract also contains lignans with insecticidal activity such as pinoresinol bis-epi-pinoresinol and diacid that have anti-molting activity (Cabral *et al.* 1995). The acaricidal/insecticidal properties of fruit and seed extract of *M. azedarach* result from aforementioned bioactive compounds but no component with immediate lethal effect has so far been identified in this extract (Arias *et al*. 1992; Gajmer *et al.* 2002; Banchio *et al.* 2003; Borges *et al.* 2003; Trudel and Bomblies 2011). However, our work showed that the *M. azedarach* ripe fruit extract could kill *D. gallinae* mites upon 24 h after exposure that indicating for possible immediate lethal effects. Further experiments are required to verify such toxic ingredients that cause this immediate lethal effect.

Although, in this study promising results have been obtained but natural acaricide such as this plant extract may be harmful to humans and birds. Therefore, more work needs to be done regarding safety of this extract and its practical application in poultry farms.

It is concluded that the ripe fruit extract of *M. azedarach* demonstrated acaricidal activity against *D. gallinae*.

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ارزیابی آزمایشگاهی اثر کشندگی عصارهٔ میوهٔ رسیدهٔ درخت زیتون تلخ روی *Dermanyssus gallinae* (Acari: Dermanyssidae)

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چکیده

این مطالعه با هدف بررسی اثر کشندگی عصارهٔ میوهٔ رسیدهٔ زیتون تلخ روی *Dermanyssus gallinae* (De Geer, 1778) در شرایط آزمایشگاهی انجام گرفت. به این منظور، عصارهٔ میوه‌های رسیده با کمک هگزان استخراج شد و غلظت‌های ۰/۲۵، ۰/۵، ۱، ۳، ۵ و ۱۰ درصد از این عصاره در استون به عنوان حلال تهیه شد. غلظت‌های تهیه شده با روش زیست سنجی لایهٔ باقیمانده در تشتک پتری روی جرب‌های زنده آزمایش شد. آزمایش روی جرب‌ها با هر غلظت پنج بار تکرار شد و در هر تکرار گروه شاهد مثبت (کاربارل) و گروه شاهد منفی (استون) وجود داشت. میزان مرگ و میر جرب‌ها ۲۴ ساعت پس از مواجهه در هر گروه مورد بررسی قرار گرفت. میزان مرگ و میر جرب‌ها در گروه درمان نسبت به گروه شاهد منفی به صورت معنی داری بیشتر بود ($P < 0.05$). میانگین مرگ و میر جرب‌ها در مواجهه با غلظت های ۰/۲۵، ۰/۵، ۱، ۳، ۵ و ۱۰ درصد عصاره استفاده شده به ترتیب ۴۰، ۴۳، ۶۰، ۶۲، ۷۹ و ۱۰۰ درصد بود. کاربارل با غلظت یک میلی گرم در میلی لیتر باعث مرگ ۱۰۰ درصد جرب‌ها در گروه شاهد مثبت شد. غلظت های کشنده ۵۰، ۹۰ و ۹۹ درصد این عصاره به ترتیب ۱/۷۸، ۵/۹۳ و ۹/۳۲ درصد محاسبه شد. نتایج مطالعه حاضر نشان داد عصارهٔ میوهٔ رسیدهٔ زیتون تلخ اثرات کشندگی شدیدی روی کنهٔ *D. gallinae* دارد.

واژگان کلیدی: فعالیت کنه‌کشی؛ آفت‌کش زیستی؛ کنه‌کش طبیعی؛ زیتون تلخ ایرانی؛ کنهٔ قرمز طیور.

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