Exp Appl Acarol (2009) 48:31–41
DOI 10.1007/s10493-009-9254-2

In vitro efficacies of oils, silicas and plant preparations against the poultry red mite *Dermanyssus gallinae*

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Received: 15 December 2008 / Accepted: 6 February 2009 / Published online: 20 February 2009

Abstract The aim of this study was to test the effectiveness of physically acting substances (oils and silicas) and plant preparations for the control of the poultry red mite *Dermanyssus gallinae* (De Geer 1778). Reproduction and survival of fed *D. gallinae* females were evaluated in vitro for a total of 168 h using the "area under the survival curve" (AUC) to compare survival of the mites between treatments. Four oils (two plant oils, one petroleum spray oil and diesel), one soap, three silicas (one synthetic amorphous silica, one diatomaceous earth (DE) and one DE with 2% pyrethrum extract) and seven plant preparations (derived from *Chrysanthemum cinearіaefolium*, *Allium sativum*, *Tanacetum vulgare*, *Yucca schidigera*, *Quillaja saponaria*, *Dryopteris filix-mas*, and *Thuja occidentalis*) were tested at various concentrations. All the oils, diesel and soap significantly reduced *D. gallinae* survival. All silicas tested inhibited reproduction. DE significantly reduced mite survival, but amorphous silica was less effective in vitro. Except for pure *A. sativum* juice and the highest concentration of *C. cinearіaefolium* extract, the plant preparations tested resulted in statistically insignificant control of *D. gallinae*.

Keywords *Dermanyssus gallinae* · Ectoparasite · Control · Silicas · Oils · Plant extracts

Introduction

The poultry red mite *Dermanyssus gallinae* (De Geer 1778) is regarded as the most important ectoparasite of laying hens in organic as well as conventional egg production in Europe (Maurer et al. 1993; Höglund et al. 1995; Fiddes et al. 2005). The haematophagous mite is a nocturnal feeder and spends the daylight hours in refugia in the vicinity of the hens. At high population densities *D. gallinae* can cause severe anaemia and associated

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mortality (Kirkwood 1967). Even low mite populations can irritate hens to the extent that they refuse to use the henhouse or rest on the perches (Maurer et al. 1995; Kilpinen et al. 2005). Production can also be affected by reductions in egg production and egg quality, where mites may cause staining of the egg shell surface (Cencek 2003). Since *D. gallinae* may also act as a vector for numerous pathogens of medical and veterinary importance, spread of disease is another problem associated with this mite in poultry systems (Chirico et al. 2003). *D. gallinae* causes nuisance and dermatitis in people working in heavily infested poultry houses (Rosen et al. 2002).

Dermanyssus gallinae are typically controlled by treating the poultry house installations rather than the hens themselves (Hoop 2008). Several synthetic acaricide classes are widely used for mite control (organophosphates, pyrethroids, carbamates), but *D. gallinae* has developed resistance against some of these compounds (Zeman and Zelezny 1985; Beugnet et al. 1997; Nordenfors et al. 2001). In addition, some compounds are unsuitable for food safety and environmental reasons (Chauve 1998). On organic farms, synthetic acaricides may be used as a last resort, but *D. gallinae* control should primarily be achieved by preventive measures and acaricides of natural origin according to national and international regulations (e.g. the Council Regulation (EC) No 834/2007; EC 2007). A three-stage control system is widely applied on Swiss organic farms. The concept includes management practices as a first stage (such as cleaning and disinfection of the empty house after each cycle). As a second stage, physically acting substances (such as oil and desiccant dusts) are used during flocks. As a third and final stage, acaricides of natural origin are selectively applied to highly infested sites in the house. Physically acting substances such as oils and dusts offer an attractive alternative to synthetic acaricides because resistance is less likely to occur. Acaricides of natural origin have been researched for several areas of pest management with encouraging results (Isman 2006). *Varroa destructor* and the tracheal mite *Acarapis woodi* are examples for parasitic mite species which can successfully be controlled with acaricides of natural origin (Rice et al. 2002). Some work has already been conducted with natural plant preparations for their effect on *D. gallinae* where recent studies have focused on in vitro effects of oriental medicinal plant extracts (Kim et al. 2007) and plant essential oils (Kim et al. 2004; George et al. 2008a, b).

With both natural plant preparations and physically acting substances showing promise for *D. gallinae* management, it is important that comparisons are made between these methods to maximize the effectiveness of existing or potential non-synthetic *D. gallinae* control strategies. This paper deals with the in vitro effectiveness of oils, silicas, and selected plant preparations for the control of *D. gallinae* with the aim of comparing the efficacy of these different non-synthetic control options.

Materials and methods

Dermanyssus gallinae

Fed *D. gallinae* females were obtained from a naturally infested poultry house at the Research Institute of Organic Agriculture, Ackerstrasse, Switzerland. The mites were collected in traps consisting of a u-shaped aluminium-profile containing a strip of fabric in a zigzag fold fixed under the perches during one night (Maurer et al. 1993). All mites were used for the tests within 1 day of collection.

Test substances

Table 1 gives an overview of the test substances, their origin, and concentration tested. A total of four oils (two plant oils, one petroleum spray oil and diesel), one soap, three silicas (one synthetic amorphous silica, one diatomaceous earth (DE) and one DE with 2% pyrethrum extract) and plant preparations based on *Chrysanthemum cinearіaefolium*, *Allium sativum*, *Tanacetum vulgare*, *Yucca schidigera*, *Quillaja saponaria*, *Dryopteris filix-mas*, and *Thuja occidentalis* were tested. Six of these test substances were tested at various concentrations and/or mixtures.

In vitro assay

Oils and plant preparations

Five fed adult female mites were transferred into plastic vials (ø 33 × 16 mm) with a tightly closing lid containing a filter paper disk (ø 27 mm) saturated with the test substance. The following controls were used: untreated (water) for water extracts and alcohol (EtOH 10%) for EtOH extracts.

Silicas (powders and liquid formulation)

Five fed adult female mites were transferred into the same type of plastic vials containing 0.05 or 0.005 g of the powders (9 or 0.9 mg/cm^2) or a filter disk treated with the amount of liquid test product containing 0.05 g of dry matter and air dried before the test. Untreated vials served as controls.

Numbers of replicates used per concentration are indicated in Table 1. Vials were placed together to assure a similar macro-environment. Treatments were randomly assigned to the vials. The mites were kept at 27°C in dark during the experiments. Surviving mites were counted after 4, 24, and 168 h under a binocular microscope. Mites showing no symptoms as well as stricken mites (movements, but no locomotion) were classified as "alive". Mites were considered "dead" if no movement was visible even after a gentle touch with a paint brush. The presence of offspring (eggs, larvae and/or unfed protonymphs) was qualitatively recorded at each observation interval.

Data analysis

For statistical comparison of *D. gallinae* survival, the integral of the survival curves was estimated for each vial (trapezoidal integration). The calculated "area under the curve" (AUC) has units of "percent-hours" (Campbell and Madden 1990). For each vial, AUCs were calculated for the periods from 0 to 4 h and from 0 to 168 h after treatment, respectively (herein after referred to as AUC4 and AUC168). The non parametric Kruskal–Wallis test was used to provide estimates of the global difference between groups separately for: oils (nine treatment groups including the control), silicas (five treatment groups), water-based plant preparations (13 treatment groups) and ethanol-based plant preparations (three treatment groups). In case of a significant result of the global test, pair-wise comparisons were made between the treatment groups using the Mann–Whitney rank sum test. *P*-values of the pair-wise comparisons where adjusted for multiple comparisons according to the formula:

$$p \text{ - value} = \alpha \times 2/k(k-1)$$

Table 1 Test substances used in in vitro assay with *Dermanyssus gallinae*: origins, concentrations and numbers of replicates

Scientific name	Common name	Plant part/extraction method	Supplier	Specification/brand name of commercial product	Active ingredient in commercial product (g/l or g/kg)	Concentrations tested (g/l)	Replicates (number of vials)
Oils/soap							
H_2O	Water, control					1,000	10
	Rapeseed oil		Local shop	Food-grade	1,000	1,000	10
	Orange oil		Wigger, Althäusern (CH)	Parasitex®	50	50, 5, 2.5	10, 15, 10
	Diesel (fuel)		Local shop	Fuel	1,000	1,000	15
	Petroleum spray oil		Omya, Oftringen (CH)	Mineral Oil Omya®	990	990	10
	Soap		Biocontrol, Grossdietwil (CH)	Natural®	1,000	1,000, 100, 10	10
Silicas							
	Empty vial, control					0	20
	Synthetic amorphous silica		Evonik Degussa, Frankfurt (DE)	Indispron D110®	1,000	0.005[a]	10
	Diatomaceous earth		Biovet, Grossdietwil (CH)	Gallo-Sec®	1,000	0.005, 0.0005[a]	10
	Diatomaceous earth & pyrethrum extract		Agro-Hygiene AG, Wald (CH)	FLY-END Acaricide powder®	9,980 & 20	0.0005[a]	10
Plant preparations							
Commercial product, water extracts and mechanically pressed juices							
H_2O	Water, control					1,000	50
Chrysanthemum cinerariaefolium	Pyrethrum		Biovet, Grossdietwil (CH)	Pyri-Fly®	20	2, 0.2, 0.02	10
Allium sativum	Garlic	Bulb/mechanically pressed fresh juice	Local shop	Organic production		1,000, 100, 10	10, 5, 5
Tanacetum vulgare	Tansy	Superficial parts; start of full bloom/water extract	In house production			1,000	10

Table 1 continued

Scientific name	Common name	Plant part/extraction method	Supplier	Specification/brand name of commercial product	Active ingredient in commercial product (g/l or g/kg)	Concentrations tested (g/l)	Replicates (number of vials)
Yucca schidigera	Mohave yucca	Leaves/mechanically pressed, thermally condensed	Desert King International, San Diego (USA)			1,000, 100	10, 10
Quillaja saponaria	Soapbark tree	Bark/water extract	Desert King International, San Diego (USA)			1,000, 100	20, 10
Yucca:Quillaja 1:1	See above	See above	Desert King International, San Diego (USA)			500 : 500	10
Ethanol extracts							
EtOH	Ethanol, control				960	100	10
Dryopteris filix-mas	Male-fern	Leaves/ethanol extract	In house production			100	10
Thuja occidentalis	Arborvitae	Leaves/ethanol extract	In house production			100	10

[a] g/vial

(Bonferroni Correction; Lu and Fang 2003), where k = number of comparisons and α = agreed chance of falsely positive result (here 0.05). Reproduction data was analysed separately for oils, silicas and water-based plant preparations using logistic regression models. All data was analysed using STATA® 9.0 (StataCorp LP, 4905 Lakeway Drive, TX 77845, USA) software.

Results

Table 2 presents the proportion of vials with reproduction and the AUC of the oil, silica, and plant preparation treatments. In all control vials reproduction occurred during the experiment and AUCs were close to or at the maximum possible values.

Oils

Plant (rapeseed and orange) as well as petroleum spray oil and diesel reduced *D. gallinae* reproduction and the AUCs, including AUC4, which indicates a rapid effect of those products. Treatments with the petroleum spray oil and diesel significantly reduced the AUC168 values by 95% and more. Orange oil was significantly effective at concentrations of 5% only. Mites treated with 100% soap did not reproduce, but the effect on survival was significant only after 1 week (AUC168).

Silicas

Female mites did not produce eggs in any of the silica treatments. DE with or without additional pyrethrum increased mortality of the mites later than 4 h after treatment, reflected by high AUC4 and low AUC168. Synthetic amorphous silica was not effective.

Plant preparations

Egg production was observed in all treatments except for the highest dose of pyrethrum and the higher doses of garlic juice. Pure garlic juice was the only plant preparation which quickly killed *D. gallinae*, as reflected by a significantly reduced AUC4 by 50%. Of the several preparations tested, only pyrethrum 0.2 and 0.02% and garlic juice 10 and 100% significantly reduced AUC168 by more than 50%. A dose-response of the pyrethrum treatment was seen in the % vials with reproduction as well as in the AUC168 values.

Discussion

Untreated mites reproduced and their survival was close to the maximum possible value. This indicates that the experimental conditions used were favourable for survival and reproduction of the fed *D. gallinae* females. George et al. (2008b) suggest that *D. gallinae* are more susceptible to the effects of plant essential oils after starving for 3 weeks than recently fed mites. Our experimental conditions using fed females therefore represent a

Table 2 Area under the curve (AUC) of *Dermanyssus gallinae* 4 h (AUC4) and 7 days (AUC168) after treatment with potential acaricides

Name (brand name or specification)	Concentration (g/l)	Reproduction		AUC4		AUC168	
		Proportion of replicates with juveniles	Difference to control (%)	Mean (percent-hours[b])	Difference to control (%)	Mean (percent-hours[b])	Difference to control (%)
Oils/soap							
Control	–	1		400		16,470	
Rapeseed oil	1,000	0.4	−60*	284	−29.0*	2,632	−84.1*
Orange oil	50	0	−100**	256	−36.0*	2,012	−87.8*
Orange oil	5	0.66	−34	336	−16.0	8,021	−51.4
Orange oil	2.5	1	0	392	−2.0	13,976	−15.4
Diesel	1,000	0	−100**	205	−48.7*	232	−98.6*
Petroleum spray oil	990	0	−100**	304	−24.0	824	−95.0*
Soap	1,000	0	−100**	324	−19.0	3,568	−78.4*
Soap	100	0.7	−30	344	−14.0	7,880	−52.3
Silicas							
Control (empty vial)	–	1		400		16,152	
Synthetic amorphous silica	0.005[a]	0	−100**	400	0.0	7,796	−51.7
Diatomaceous earth	0.005[a]	0	−100**	352	−12.0	1,112	−93.1*
Diatomaceous earth	0.0005[a]	0	−100**	352	−12.0*	1,194	−92.6*
Diatomaceous earth & Pyrethrum extract	0.0005[a]	0	−100**	400	0.0	1,892	−88.3*
Plant preparations							
commercial product, water extracts and mechanically pressed juices							
Control (water)	–	1		400		16,470	
Chrysanthemum cinerariaefolium	2	0	−100**	400	0.0	1,400	−91.5*
Chrysanthemum cinerariaefolium	0.2	0.33	−67*	346	−13.5	5,581	−66.1*
Chrysanthemum cinerariaefolium	0.02	1	0	400	0.0	1,450	−11.7
Allium sativum	1,000	0	−100**	200	−50.0*	200	−98.8*

Table 2 continued

Name (brand name or specification)	Concentration (g/l)	Reproduction		AUC4		AUC168	
		Proportion of replicates with juveniles	Difference to control (%)	Mean (percent-hours[b])	Difference to control (%)	Mean (percent-hours[b])	Difference to control (%)
Allium sativum	100	0	−100**	400	0.0	5,008	−69.6*
Allium sativum	10	1	0	400	0.0	15,936	−3.2
Tanacetum vulgare	1,000	1	0	400	0.0	15,504	−5.9
Yucca schidigera	1,000	1	0	400	0.0	11,432	−30.6*
Yucca schidigera	100	1	0	400	0.0	14,352	−12.9
Quillaja saponaria	1,000	1	0	392	−1.2	1,244	−24.4*
Quillaja saponaria	100	1	0	400	0.0	13,056	−20.7
Yucca:Quillaja 1:1	1,000	1	0	400	0.0	10,320	−37.3*
Ethanol extracts							
Control (EtOH 10%)	100	1	0	400		16,512	
Dryopteris filix-mas	100	1	0	400	0.0	14,456	−12.5
Thuja occidentalis	100	1	0	400	0.0	16,665	+0.9

[a] g/vial

[b] Campbell and Madden (1990)

* $P < 0.05$; ** $P < 0.01$

more severe test of product efficacy than tests with starved mites as proposed e.g. by Thind and Ford (2006).

The effects of oils on plant pest insects and mites have been investigated in some detail (e.g. Agnello et al. 2003; Fernandez et al. 2005). Mineral oil was more toxic to adult phytoseiid mites than plant oil (Momen et al. 2006). A study on the effects of oils on *D. gallinae* showed that a mineral oil developed for agricultural use (OPPA) caused 100% mite mortality after 2 h of exposure when sprayed directly on the mites (Guimaraes and Tucci 1992). None of the oils tested in the current study acted as quickly and completely, probably because the contact of the mites with the test substances in the experimental setup was reduced as compared to that in the aforementioned study, where mites where completely covered by means of spraying. Diesel reduced the AUC4 by 50% in the current study, and the AUC168 by almost 100%. However, odour and associated risk of egg contamination are a serious drawback of diesel, which is not recommended for use in poultry houses on this basis (Hoop 2008). Slightly higher AUC168s were attained by the odourless and relatively cheap petroleum spray oil and rapeseed oil, making these interesting alternatives to diesel. The effects of the undiluted product containing 5% orange oil were similar to those of diesel and petroleum spray oil. The disadvantage of this treatment is the high cost of the effective concentration.

Inert dusts based on silica are commonly used as desiccating agents against stored-product pests (Collins 2006; Palyvos et al. 2006). Silicas act physically and their activity is not dependent on metabolic pathways. In our experiment, neither the treatment dose (mg/vial) nor the addition of Pyrethrum extract improved upon the already favourable efficiency of DE. Mortality from DE exposure is mainly a result of desiccation (Saez and Fuentes Mora 2007), and arthropods are therefore not expected to develop genetic resistance. However, insects have been shown to develop behavioural responses to avoid contact with such products (Ebeling 1971), and this may also be the case for mites. Drawbacks of DE are the formation of dust during treatment and the decrease of efficacy due to high humidity. Therefore, substantial efforts have been put into the development of liquid formulations (Lamina and Kruner 1966) such as the synthetic amorphous silica used in the present study. In the in vitro setup used in the current work, the liquid formulation completely suppressed reproduction of *D. gallinae* females, but AUC168 was not significantly different from the control. This result suggests insufficient efficiency of the amorphous silica compared to DE. However, field experiments performed by Maurer and Perler (2006) in heavily infested layer houses revealed a longer residual effect of the liquid amorphous silica compared to DE.

Garlic is often used in folk medicine, but scientific studies on the effects of *A. sativum* on mites are scarce. A study by Birrenkott et al. (2000) showed that repeated topical applications of garlic juice (10%) on hens heavily infested with the Northern fowl mite *Ornithonyssus sylviarum* significantly reduced the level of infestation. The authors suggest that this was mainly due to a repellent effect preventing re-infestation and not to direct acaricidal effects. In our in vitro test, direct effects on oviposition and on survival of the closely related species *D. gallinae* have been demonstrated. This indicates that fresh garlic juice can have both, direct and indirect effects on gamasid mites. From the literature, it remains unclear whether garlic oil has the same insecticidal properties as fresh juice. A study of Amonkar and Reeves (1970) demonstrated that partly purified garlic oil had a higher toxic effect on mosquito larvae than the crude extract. In contrast, a 10% concentrate made from commercial chopped garlic provided control of whiteflies, but commercial garlic oil gave little or no control in an experiment by Flint et al. (1995).

Before garlic can be considered as a valuable component of a strategy against *D. gallinae*, the question of conservation and standardisation of the crude extract has to be solved.

Except for pure garlic juice and the highest concentration of pyrethrum extract, the plant preparations tested in our study resulted in <70% or no significant reduction of *D. gallinae*. Therefore, and also supported by observations of others (George et al. 2008b), it may be more suitable to use plant preparations as a treatment for starved mites in the poultry houses between layer flocks, rather than to apply them during flocks when mites have the opportunity to recently feed. In contrast, our experiment showed that physically acting products such as rapeseed oil, petroleum spray oil, soap or DE were effective on fed mites. These products therefore seem better suited for application in cases of severe *D. gallinae* infestations during flocks.

Acknowledgments The authors gratefully acknowledge funding from the European Community financial participation under the Sixth Framework Programme for Research, Technological Development and Demonstration Activities, for the Integrated Project QUALITYLOWINPUTFOOD, FP6-FOOD-CT-2003-506358. The views expressed in this publication are the sole responsibility of the author(s) and do not necessarily reflect the views of the European Commission. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the information contained herein. Mention of a proprietary chemical or a trade name does not constitute a recommendation or endorsement.

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