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TOXICITY OF GERANIOL SOLUTION *IN VITRO* TO THE POULTRY RED MITE, *DERMANYSSUS GALLINAE*

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Summary:

Geraniol is a bioactive component found in Palmarosa essential oil which has previously demonstrated good repellence to cattle ticks, mosquitoes and sand flies. The toxicity of geraniol to *Dermanyssus gallinae*, the most economically deleterious ectoparasite of laying hens in Europe, was studied *in vitro* in the laboratory. Exposure of *D. gallinae* to 0.5, 1 and 2 % concentrations of geraniol (equating approximately to 0.02, 0.04 and 0.07 mg/cm^2 geraniol, respectively) over a period of 24 hours resulted in 100 % mortality of *D. gallinae* at all doses used.

KEY WORDS : *Dermanyssus gallinae*, geraniol, poultry, mite, ectoparasite, control.

Résumé : TOXICITÉ DU GÉRANIOL CONTRE L'ACARIEN DES POULES, *DERMANYSSUS GALLINAE*

Le géraniol est un des composants actifs de l'huile essentielle de Palmarosa. Il a déjà montré son efficacité comme répulsif contre les tiques et les piqures de moustiques ou de phlébotomes. La toxicité de ce géraniol contre Dermanyssus gallinae*, l'un des ectoparasites les plus importants économiquement pour l'élevage de poules pondeuses en Europe, a été étudiée au laboratoire. L'exposition de* D. gallinae *à des concentrations de 0.5, 1 et 2 % de géraniol (correspondant approximativement à des concentrations de 0,02, 0,04 et 0,07 mg/cm^2) pendant une période de 24 h a entraîné dans chaque cas une mortalité de 100 % de ces ectoparasites.*

MOTS CLÉS : Dermanyssus gallinae*, géraniol, ectoparasite, acarien, volaille, lutte.*

The poultry red mite, *Dermanyssus gallinae* (De Geer), is currently the most economically deleterious ectoparasite of laying hens in Europe (Chauve, 1998). Infestations of *D. gallinae* can result in significant stress to hens with subsequent reductions in bird condition, growth rate, egg quality and egg production (Chauve, 1998). In extreme cases, mite population levels may be so high as to cause anaemia, and even death of hens (Cosoroaba, 2001). Furthermore, *D. gallinae* may serve as a vector for numerous poultry pathogens (Chirico *et al*., 2003).

Control of *D. gallinae* has typically been achieved through synthetic acaricides. The continued use of these products, however, may be hampered by issues of mite resistance (Kim *et al*., 2004; Fiddes *et al*., 2005) and reduced product availability as a result of more stringent legislation regarding existing and proposed active ingredients (Fiddes *et al*., 2005). It is therefore becoming increasingly important to identify alternative approaches for the management of *D. gallinae* in poultry production systems. One such approach focuses on studying the toxic effects of plant essential oils for managing *D. gallinae* (Kim *et al*., 2004; George *et al*., 2009). Whilst the acaricidal effect of numerous essential oils against *D. gallinae* may be encouraging, the use essential oils *per se* may be hampered by their inconsistence in efficacy, which has been reported as a reason for the poor market penetration of botanical insecticides to date (Isman, 2008).

Various environmental factors, such as dust, humidity and temperature, may affect essential oil toxicity to varying degrees (George *et al.* 2009). In addition, the chemical composition may differ between oils from the same or taxonomically similar species (Cimanga *et al*., 2002) due to external factors. These differences will in turn effect the biocidal activity of oils (Miresmailli *et al*., 2006), making it difficult to recommend any essential oil for its pesticidal qualities simply by taxonomy alone. A possible means of resolving this problem might be to isolate active components from essential oils with acaricidal potential and develop these for use as acaricides. Geraniol is a monoterpene commonly found in essential oils, and it is already available for use in pest control (Fulltec®, Zug, Switzerland) where results have found good product repellence to cattle ticks at 1% (Khallaayoune *et al*., 2009). Geraniol has also shown promise as a pest repellent elsewhere, outperforming other essential oil components (citronella and linalool) when used to protect humans from mosquito and sand fly bites (Müller *et al*., 2008). It is there-

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fore possible that geraniol could hold promise as a *D. gallinae* acaricide. As such, the aim of this study was to assess the toxicity of geraniol to *D. gallinae*.

METHOD

Geraniol was used to impregnate filter papers (Whatman No. 2, 4.25 cm diameter) according to methods used by George *et al.* (2009) and using solutions of 2 %, 1 %, 0.5 % and 0 % geraniol in 50 µl of ethanol, where control filter papers received 50 µl of ethanol only (equating approximately to 0.07, 0.04, 0.02 and 0.00 mg/cm^2 expressed in pure geraniol applied to the filter paper, respectively). These solutions were prepared and used on the 26 May 2009 from a stock solution of commercial product ("Fulltec Insect Killer Concentrate 30 % Geraniol", Fulltec® and Tecnofirm). Once impregnated, filter papers were added to Petri-dishes (4.8 cm diameter × 1.2 cm height). After three minutes storage time in a fume cupboard to allow for evaporation of the ethanol, approximately 25 adult female *D. gallinae*, previously collected from a free range poultry unit, were added to each dish using a pooter and sealed therein using polyethylene film (Clingfilm®) to prevent air exchange between the dish interior and surrounding environment. Mites were used five days after collection. The Petri-dishes were stored for a period of 24 hours at 22 °C with a 16:8 light:dark cycle after which mortality was assessed under magnification. A mite was considered dead if no movement was observed following repeated agitation with an entomological pin (*i.e.* a purpose-made fine-pointed needle fitted with a handle). Four replicates were done for each concentration.

The percentage mortality of mites under each treatment was calculated (dead mites/total mites × 100) and compared between all treatments using a Kruskal-Wallis test (due to the non-normal and non-continuous nature of the data). *Post hoc* testing of differences between individual treatments was not possible as all data points were equal (*i.e.* 100 % mortality) whenever geraniol was used.

RESULTS

There was a significant difference in the mortality of *D. gallinae* between treatments (adjusted for ties; $H = 14.66$, $P < 0.01$). *Post hoc* tests could not be conducted, but it is likely that this difference was caused by significantly higher mortality in all geraniol treatments as compared to the control (Fig. 1). Control mortality in the current study was consistently low (averaging only 2 % and ranging between 0 % and 4 % across the four replicates), whereas exposure to geraniol at all concentrations resulted in 100 % *D. gallinae* mortality (Fig. 1).

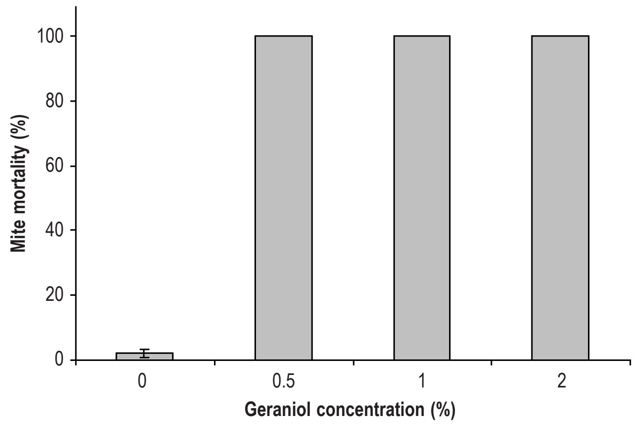

Fig. 1. – Mean percentage mortality of adult female *Dermanyssus gallinae* after exposure to different concentrations of geraniol over a period of 24 hours. Error bars show ± SE. N = 4 for all means.

DISCUSSION

Numerous whole essential oils have been shown to have potential for use as acaricides against *D. gallinae* (Kim *et al.*, 2004; George *et al.*, 2009). However, the use essential oils *per se* may be hampered by their inconsistence in efficacy (Isman, 2008), which is likely the result of chemical variation between seemingly identical oils (Cimanga *et al.*, 2002). In order to address this issue of variability when employing natural products for pest control, it is possible that single biocidal components could be isolated from essential oils so that they could be used at known concentrations and application rates. Nevertheless, such components need to be shown to be toxic to pests in their own right as work exists to suggest that this cannot necessarily be assumed. Miresmailli *et al.* (2006), for example, have shown that the acaricidal effect of complete essential oils is greater than combinations of their primary components, possibly because "inactive" components act synergistically with "active" components to achieve maximum toxicities.

According to the results of the current study, geraniol can be considered an 'active' essential oil component against *D. gallinae*, where 100 % mortality of these mites was observed at all geraniol concentrations used. This suggests that further experimentation using "Fulltec Insect Killer Concentrate 30 % Geraniol" against *D. gallinae* is warranted. Examination of lethal dose (LD) levels (where according to the results presented 100 % mortality *in vitro* can be expected with the method used at concentrations lower than 0.5 %), repellence, residual toxicity and stability *in vivo* would all be beneficial to the future development of this existing commercial product for use in poultry units to manage *D. gallinae*. Out of a range of 50 pure essential oils tested against *D. gallinae*, the lowest LD_{99} reported was 0.06 mg/cm^2 (George *et al.*, 2009), suggesting that pure geraniol may be more effective than any of the whole oils tested (where at ~ 0.02 mg/cm^2, *i.e.* 0.5 %, exposure to geraniol resulted in 100 % mite mortality in the current study). Should further experimentation continue to yield such favourable results, assessment of *in vivo* efficacy of geraniol against *D. gallinae* will also be needed. Environmental variables may affect the toxicity of at least some essential oils to *D. gallinae* (George *et al.* 2009), so it could be expected that the same may be true of their chemical components, such as geraniol.

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