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SHORT COMMUNICATION

Evaluation of the efficacy of essential oils of *Lavandula angustifolia* and *Eucalyptus globulus* for the control of *Varroa destructor* in *Apis mellifera*: A randomised field study

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ABSTRACT. *Varroa destructor* is the most harmful and widespread parasite that spreads disease in bees. *Eucalyptus spp* essential oils (EOs) have proved effective against *V. destructor*. Additionally, *Lavender spp* EOs treatment has caused mite mortality rates of 95% to 97% for the same parasite. In this study, 20 mL of each oil or the placebo were distributed on two sheets of papier-mâché located on the frames of the brood chamber inside each hive. The miticidal effects of *Lavandula angustifolia* and *Eucalyptus globulus* EOs were analysed. Parasitic load and mite fall were evaluated under field conditions. The mean infestation rate obtained from each of the three treatment groups at the beginning of the study was less than 3.6%. Then, the infestation rate increased gradually in each group until day 36. The infestation rate in the group treated with *L. angustifolia* was lower than in the control group by over two per cent and never exceeded 10%; the differences between the control group and the *L. angustifolia* group were statistically significant ($P<0.05$). In conclusion, *L. angustifolia* EO provided effective parasite control starting at the second treatment dose. However, *E. globulus* EO did not show a consistent parasite control. Further studies should consider the evaluation of EOs for the control of *V. destructor* in different weather conditions and other treatment delivery systems.

Key words: bees, parasite, mite, varroosis, ecological control.

INTRODUCTION

As pollinators, bees participate in the global economy and their contribution has been valued at between 235 and 285 billion US$ per year (Lautenbach *et al.*, 2012). Currently, a general weakening of honeybee populations, represented by colony losses, has been reported raising public concern and, in turn, the costs of managing bee colonies and pollination services have increased (Calderone, 2012).

The decline in pollinators has been associated with multiple factors such as natural disasters, environmental pollution, and a variety of pathologies (Potts *et al.*, 2010). According to Neira *et al.* (2004) varroosis is the most serious parasitic disease in bees. A large number of products have been tested to control this disease, and their repeated and improper use has resulted in the production of contaminated honey. There is a current trend towards the use of natural products, creating a constantly increasing demand for them. For this reason, varroosis diagnostic and treatment methods have been the subject of studies in several countries to improve its control (Gonzalez-Acuña *et al.*, 2005).

Current methods used for *V. destructor* control are the application of acaricides such as fluvalinate and coumaphos. Both were initially effective, but their recurrent use has led to the development of resistance in the mites (Milani, 1999).

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Chemicals applied by beekeepers against varroosis are a source of bee product contamination. There are maximum residue limits for authorised chemical substances. The contamination of bee products by acaricides can be minimised through careful use of chemotherapeutic products, however, the use of unauthorised products to control varroosis could become a major problem (Karazafiris *et al.*, 2011).

Cruzat & Baasch (2010) established that the control of varroosis has been managed mainly with artificially synthesised chemical products such as fluvalinate, flumethrin, amitraz, bromopropylate, and cymiazole. Nevertheless, these products can have dangerous consequences due to the accumulation of their residues in honey, wax, and propolis. In addition, their improper and repeated use can lead to significant resistance against these products in *V. destructor*.

EOs generally represent a less-expensive and safer alternative for both humans and bees (Calderone, 2012). Also, they are classified as food supplements that are safe for human consumption (Quarles, 1996). EOs can alter the behaviour, growth, and development as well as the ecdysis, mating and oviposition of insects (Khater, 2012). Additionally, the insecticidal activities of the components of lavender EO have been related to acetylcholinesterase inhibition, and eucalyptus EO has been shown to exhibit octopaminergic agonist activity (Rattan, 2010).

In Chile, a limited range of veterinary products has been authorised for apicultural use by the Agricultural and Livestock Service (SAG, 2019) and registered under the names Bayvarol®, Verostop® and Apilife Var®. The active molecule of the first two products is flumethrin, while the third product contains thymol, levomenthol, eucalyptus oil and camphor.

On the other hand, the *Chilean National System for the Certification of Organic Products* created under Decree No. 36 of 2006 of the Ministry of Agriculture allows, according to its Technical Standard Annex A List 5, the treatment of pests and diseases that affect beekeeping with natural treatments such as phytotherapy, aromatherapy, etheric EOs (camphor, eucalyptol, menthol, thymol), sulfur, oxalic acid, lactic acid, acetic acid, and formic acid (SAG, 2019).

Neira *et al.* (2004) established that applying lavender and laurel EOs under laboratory conditions resulted in approximately 90 to 100% mite fall. In addition, the mite mortality rates reached average values of nearly 40%.

Several studies on the use of EOs against *V. destructor* have been conducted under controlled laboratory conditions and have demonstrated miticidal effects (Imdorf *et al.*, 1999). Eucalyptus EO, which is rich in 1-8-cineol, has proved effective against *V. destructor* (Ghasemi *et al.*, 2011). Additionally, thyme, sage, rosemary, marjoram, dillsun and lavender EOs at concentrations of 1% and 2% (w/w) resulted in mite mortality rates ranging between 95% and 97%, respectively, and peppermint at 2% (w/w) killed more than 97% of *V. destructor* (Ariana *et al.*, 2002).

The effectiveness of different synthetic (amitraz, Apivar®) and natural (formulated from Api Life Var®, thymol oil and thymol alcohol) products authorised for the control of *V. destructor* were evaluated in a field study. All these treatments reduced the infestations of *V. destructor*, although they did not eliminate the parasite. However, the effectiveness of the treatment depended on the apiary to which it was applied. The variability in effectiveness detected among different apiaries represents a challenge for the identification of the significant factors that influence miticide effectiveness (Gracia *et al.*, 2017).

To our knowledge, no studies have currently investigated the residual effects of EOs or any long-term application protocols for them in production apiaries, specifically with regard to the dose, duration, residual effect, or application time and frequency. EOs selection was based on preliminary trials with unpublished data which evaluated the effects of *Syzygium aromaticum, Citrus sinensis*, *Lavandula angustifolia* and *Eucalyptus globulus* EOS on total mite fall, observing the best results with *Lavandula angustifolia* and *Eucalyptus globulus.* The study aimed to evaluate the effects of *Lavandula angustifolia* and *Eucalyptus globulus* EOs for varroosis control in *A. mellifera* in a 48-day, double-blind field trial.

MATERIAL AND METHODS

LOCATION

The research was conducted in the experimental apiary at the School of Agricultural and Veterinary Sciences, Universidad Viña del Mar, Chile, under regular production management practices during the months of March and April 2018. The climate conditions were Mediterranean temperate, with median temperatures of 19°C and 16°C in March and April, respectively (33°04’06”S, 71°33’27”W).

ESSENTIAL OIL APPLICATION

Twenty-four Langstroth-type hives naturally infested with *V. destructor* were randomly allocated to three groups of 8 beehives. The treatments applied were a) 5% *E. globulus* EO (group E) (1.8-cineole 60.2%; α-pinene 15.26%) and b) 5% *L. angustifolia* EO (group L) (linalool 36.53%; linalyl acetate 32.8%), both diluted in vegetable glycerine, and c) glycerine as the placebo (control group, C). Both essential oils are of commercial origin (Ac Es Eucalipto Org 5 ml; Ac Es Lavanda Org 5 ml, Sociedad Comercial Katmandú SpA, Chile).

During the treatments, 20 mL of each oil or the placebo were distributed on two sheets of papier-mâché located on the frames of the brood chamber inside each hive. Four applications were performed, on days 1, 11, 22 and 33 of the experimental period, to cover two consecutive reproductive periods of the bees and the parasite. The treatments were applied with a double-blind treatment design.

COLONY INFESTATION RATE

The colony infestation rate was evaluated in a sample of 200 bees from the brood frames of each colony. The frames were placed in a container with water and non-foaming detergent and were covered and stirred for two minutes. After settling for 10 minutes, the contents of each container were poured through a double screen. The first screen retained the bees and the second collected the *V. destructor* individuals. The bees and *V. destructor* individuals were then counted. Finally, the infestation rate was calculated according to the following formula (De Jong *et al.*, 1982):

$$\textit{Infestation rate (\%)} \\ = (\textit{V. destructor number}) / (\textit{bees number per sample}) \times 100$$

To measure the effects of the treatments and compare them with the effect of the placebo, the basal parasitic load was determined by the double-sieve sampling protocol at the beginning of the study (day 1, before the first treatment) and every 9 days thereafter (days 9, 18, 27, 36 and 45).

TOTAL MITE FALL

In each hive, from days 2-45 of the experiment, a daily mite fall count was performed as a complementary method for determining the parasitic load of the hive. A piece of white cardboard was placed on the bottom board of the hive and covered with glycerine to trap fallen *V. destructor* individuals. The cardboard was removed and replaced daily.

The infestation rate and total number of mite fall were tabulated and analysed for significant differences between treatments with *Friedman's* and *Dunn's* tests ($P<0.05$).

RESULTS AND DISCUSSION

The mean infestation rates obtained from each group at the beginning of the study were lower than 3.6%. Then, a gradual increase in infestation rate was observed in each of the three groups until day 36 (Group C 11.1%; Group L 9.4%; Group E 9.8%). At this point, the infestation rates decreased, reaching similar levels to those on day 18, especially in the groups treated with EOs.

Group L presented significantly lower parasitic loads than group C ($P<0.05$), as evidenced by the infestation rates during the 45 days of the study. Furthermore, the lower total drop of mites observed in group L responds to the lower infestation rate in the same group compared to groups L and C. Nonetheless, group E showed non-significant differences in the parasitic loads compared to group C (figures 1 and 2).

According to Gracia *et al.*, (2017) parasitic infestations are almost impossible to eradicate, therefore, only partial control of parasites is possible. Our results are consistent with those of these authors and demonstrate effective, easy and safe mite control to infestation rates lower than 8% with lavender EO. These effects were modulated by the local environmental, genetic and production conditions, which determine the efficiency of parasitic control (Bounous & Boga, 2005). Thus, EOs can control varroosis and are not considered contaminants by the technical standards of the National System of Certification of Organic Products of Chile (SAG, 2019).

The analysis of the parasitic infestation of the bee colonies showed that until the second dose (day 11), the infestation rates in the three groups did not show linear behaviour. However, after the second treatment, the infestation rates in group L was consistently lower than in group C (figure 1).

In this study, *L. angustifolia* EO was an efficient treatment because it stopped the sustained growth of the infestation rate in the treated hives. In contrast, in the control group, the infestation rate increased continuously. These results are consistent with those of Jean-Prost & Conte, 2007), who established that sustained infestation rate growth occurs because the multiplication of the parasite is associated with bee reproduction and the absence of antiparasitic treatments. In addition, the effects of *L. angustifolia* EO on the infestation rates and total mite fall could be due to the application in late summer and early autumn because the bee reproduction begins to decrease during this period (Avitabile, 1978). The decrease in bee reproduction rate leads to a decline in the adult mite population, resulting in a low infestation rate at the beginning of spring, which represents an improvement in the sanitary condition of the hive.

In total, during the 45 days of the study, 1,132 adult mites fell in group L, 1,802 adult mites fell in group E, and 2,019 adult mites fell in group C. There were statistically significant differences in mite fall between groups C and L as well as between groups L and E ($P<0.05$) (figure 2).

Daily measurements of adult mite fall were performed during the study as a complementary method of determining the degree of infestation in each group. However, this approach was not intended to measure the effect of each

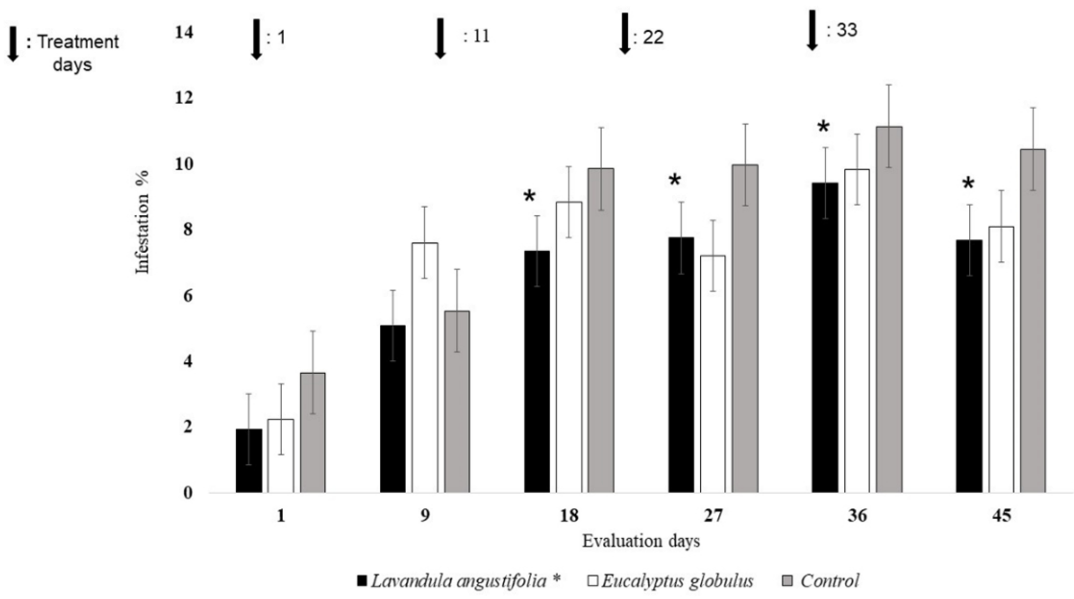

Figure 1. Mean infestation rate and standard error for each treatment group at different sampling times. Arrows indicate the treatment application times.

* indicates statistically significant differences between L and C according to Friedman's and Dunn's tests ($P<0.05$).

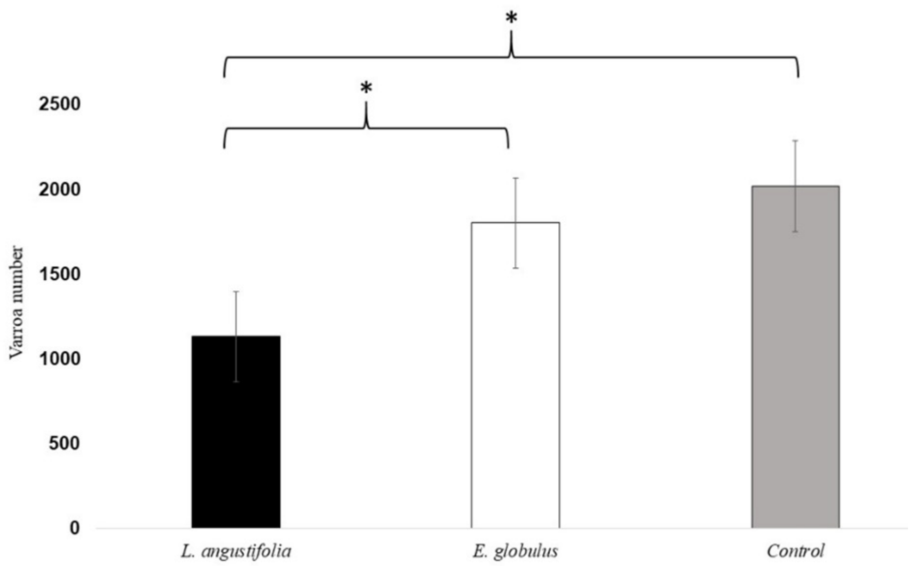

Figure 2. Total mite fall and standard error for each treatment group.
* indicates statistically significant differences according to Friedman's and Dunn's tests (*P*<0.05).

treatment since mite detachment can be caused by different factors, such as the size of the colonies, the typical handling of the hives, the grooming capacity of the families, the internal temperature of the hives, and the environmental temperature, as well as by the treatments applied (Spivak & Reuter, 1998).

Some limitations of our study included active behavioural defences of the hive that could affect the infestation rates individually, favourable weather conditions for mite reproduction such as temperature, humidity, or the availability of pollen and nectar, and the wrong entry of the bees in other hives that can affect the infestation rates. In addition, further studies should consider the evaluation of EOs for the control of *V. Destructor* in different weather conditions and other treatment delivery systems.

In conclusion, *L. angustifolia* EO provided effective parasite control. The infestation rates in the *L. angustifolia* EO treatment group were consistently lower than those in the control group starting at the second treatment dose due to the reproductive cycles of both species *Varroa destructor and Apis mellifera*. However, *E. globulus* EO did not show a consistent parasite control.

COMPETING INTERESTS STATEMENT

The authors declare that there is no conflict of interests regarding the publication of this article.

AUTHOR CONTRIBUTIONS

M.A., J.L.M. and A.C. designed research; M.A., J.L.M., G.B., C.S. and Y.O. performed research; M.A., J.L.M., G.B., C.S. and Y.O. contributed to acquisition of data; M.A., J.L.M., and H.M. analysed data; M.A., J.L.M., and H.M. wrote the paper.

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