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Original article

The effects of clove oil on the enzyme activity of *Varroa destructor* Anderson and Trueman (Arachnida: Acari: Varroidae)

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ABSTRACT

Varroa destructor, a key biotic threat to the Western honey bee, has played a major role in colony losses over the past few years worldwide. Overuse of traditional acaricides, such as tau-fluvalinate and flumethrin, on *V. destructor* has only increased its tolerance to them. Therefore, the application of essential oils in place of traditional pesticides is an attractive alternative, as demonstrated by its high efficiency, lack of residue and tolerance resistance. To study the acaricidal activity of essential oils, we used clove oil (*Syzygium aromaticum* L.), a typical essential oil with a wide range of field applications, and examined its effects on the enzyme activities of Ca^{2+}-Mg^{2+}-ATPase, glutathione-S-transferase (GST) and superoxide dismutase (SOD) and its effects on the water-soluble protein content of *V. destructor* body extracts after exposure to 0.1 μl and 1.0 μl of clove oil for 30 min. Our results showed that the water-soluble protein content significantly decreased after the treatments, indicating that the metabolism of the mites was adversely affected. The bioactivity of GSTs increased significantly after a low dosage (0.1 μl) exposure but decreased at a higher dosage (1.0 μl), while the activities of SOD and Ca^{2+}-Mg^{2+}-ATPase were significantly elevated after treatments. These results suggest that the protective enzyme SOD and detoxifying enzymes Ca^{2+}-Mg^{2+}-ATPase and GST contributed to the stress reaction of *V. destructor* to the essential oils and that the detoxification ability of *V. destructor* via GST was inhibited at higher dosages. Our findings are conducive to understanding the physiological reactions of *V. destructor* to treatment with essential oils and the underlying mechanisms behind the acaricidal activities of these natural products.

1. Introduction

The currently declining health status of the Western honey bee (*Apis mellifera*) has caused great concern globally over the past few years (Neumann and Carreck, 2010). *Varroa destructor* Anderson and Trueman (Arachnida: Acari: Varroidae), an ectoparasite mite of honey bees, is considered the most severe threat to colony health (Dietemann et al., 2012). *V. destructor* feeds on the haemolymph of honey bees, causing several detrimental effects to its host, including attenuated lifespan, disorientation and decreased immunity (Amdam et al., 2004; Kralj et al., 2007; Rosenkranz et al., 2010; Nazzi et al., 2012). More grave is that these adverse effects of *V. destructor* are compounded by bee viruses, such as DWV and IAPV, thus proving a fatal role in colony collapse (Oldroyd, 2007; Martin et al., 2012). Previous research has shown that untreated varroa-infested colonies usually die within six months to two years (Le Conte et al., 2010). To maintain control of the *V. destructor* population within domesticated *A. mellifera* colonies, various acaricides have been applied in the past: organophosphate coumaphos; pyrethroids, such as tau-fluvalinate and flumethrin; and formamidine amitraz (Milani and Barbattini, 1988; Milani and Iob, 1998). While these acaricides were effective for a time, *V. destructor* mites have rapidly grown resistant to these drugs due to their overuse and the single active composition of traditional miticides (Elzen et al., 1999; Thompson et al., 2002). Furthermore, wide attention has been drawn to the fact that traces of these pesticides have been found

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in bee products, causing additional risk to human health (Bogdanov et al., 1998; Wallner, 1999).

Essential oils are secondary metabolism products that are produced during the course of plant growth (Wallace, 2004). They facilitate the primary metabolism of plants and provide certain defences against herbivores, pests and pathogens to keep the plants healthy (Isman, 2000). Previous research has shown that over 150 of these essential oils have demonstrated varied efficacies in terms of controlling *V. destructor* (Lindberg et al., 2000; Gashout and Guzmán-Novoa, 2009; Su et al., 2012). As an alternative to traditional acaricides, essential oil products present certain advantages, such as high toxicity to mites, low toxicity to bees, few residues in bee products and no drug resistance (Rosenkranz et al., 2010).

The efficacy of clove *Syzygium aromaticum* (L.) merr. et perry (Myrtaceae) oil as a treatment for *V. destructor* mites has been previously demonstrated both in the laboratory and in the field. In a 20-ml glass-vial residual test, 0.75 mg of clove oil caused a 96% mortality rate in *V. destructor* (Gashout and Guzmán-Novoa, 2009). A 2012 study by Su et al. showed that clove oil caused a 60% mortality rate in *V. destructor* mites at a dosage of 1.0 μl for 48 h, a level that is considered safe for bees. Additionally, eugenol, the principal constituent of clove oil, was discovered in beeswax during a two-week period under semi-field conditions, which supports the idea that medicinal treatment with clove oil is both stable and sustainable (Girisgin et al., 2014). Mahmood et al. (2014) also demonstrated the effectiveness of clove oil and regarded it as a promising alternative to treat *V. destructor* in the field. Despite the promising results of prior studies, the underlying acaricidal mechanisms of treatment with essential oils have yet to be elucidated. In this study, we determined the effects of clove oil on the enzyme activities of Ca^{2+}-Mg^{2+}-ATPase, glutathione-S-transferase (GST) and superoxide dismutase (SOD), as well as on the total protein content in the body extracts of *V. destructor* mites. Our aim is to understand the physiological reactions of *Varroa* mites to essential oils and the associated underlying mechanisms behind its acaricidal effects.

2. Materials and methods

2.1. Extraction of clove essential oil

Clove leaves were purchased from Tongrentang Chinese Medicine-Since. Essential oil was extracted according to steam distillation methods described in Chinese Pharmacopoeia (edition 2010). Briefly, 500 g of air-dried clove leaf powder and 5 L of distilled water were combined in a round-bottom distillation flask. The flask was connected to a condenser tube, and heated to a steady boil for five hours. The extracted essential oil was collected into Eppendorf tubes (1.5 ml) and stored at 4 °C until use. According to our previous study, this essential oil consists of 62.28% eugenol, 20.79% caryophyllene, 2.48% α-caryophyllene and 6.03% phenol-2-methoxy-4-(2-propenyl)-acetate (Su et al., 2012).

2.2. Mites collection

A. mellifera colonies that were highly infested with *V. destructor* mites and had been untreated for approximately six months prior served as the *V. destructor* donor colonies. Adult female mites from capped worker or drone brood combs were collected with a small soft brush, placed on wet tissue paper and fed five fifth instar worker bee larvae. Treatments on these mites began within one hour after feeding.

2.3. Exposure to clove oil

A group of *V. destructor* mites (100 mg, ≈350 mites) was gathered and placed into a petri dish (Ø = 60 mm) and fed five fifth instar worker bee larvae (Fig. 1A, B). Wet tissue papers were laid at the bottom of the petri dishes to maintain humidity. A piece of gauze was placed inside the cover of the petri dish. We then applied some clove oil to a small piece of filter paper and secured it between the gauze and the cover (Fig. 1C). According to Su et al. (2012), 1.0 μl clove oil is the maximum dosage that does not cause

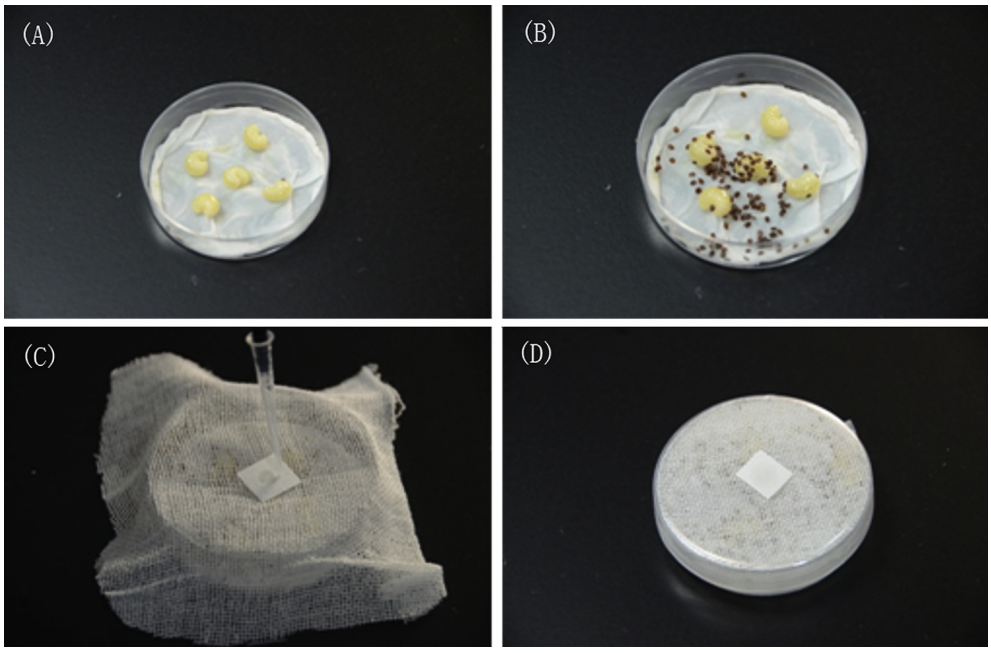

Fig. 1. Experimental setup of the study. (A) The varroa mites were fed five fifth instar worker bee larvae in a petri dish. (B) A group of *V. destructor* mites (100 mg, ≈350 mites) were collected and placed in a petri dish. (C) Clove oil was dropped on a small piece of filter paper and secured between the gauze and the cover of the petri dish. (D) The petri dishes containing varroa mites were sealed with a membrane to prevent the volatilization of essential oils.