BIOCHEMICAL STUDIES ON VOLATILE OILS OF THYME, CARAWAY AND CORIANDER PLANTS AND THEIR EFFECTS ON VARROA CONTROL

By

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ABSTRACT

This work was carried out to find a local effective alternative varroacide which can be used for varroa mite control in Egyptian honey bee apiaries. Essential oils of thyme, caraway and coriander plants or their individual components were screened and evaluated for their acaricidal effects. Chemical composition of each essential oil was studied. Also, the study aimed to find a suitable inert carrier material which can be used for formulate a commercial varroacides preparations. Thymol residues in honey and wax were also studied. The major components of steam distilled thyme essential oil were thymol (34.27%) followed by caryophyllene (10.79 %), and p-cymene (8.97%). However, the major components were thymol (16.95%) followed by caryophyllene (8.71%), p-cymene (8.38%) and γ-terpinene (7.05%) in the hydrodistilled one. In caraway, the major components were S (+)-carvone (58.58%) and limonene (27.36%) for steam distilled essential oil and were limonene (47.57%) and S (+)-carvone (22.0%) followed by carvol (10.8%) in the hydrodistilled one. Linalool (35.06%) and citral (11.61%) were the main components of the steam distilled coriander essential oil. The percentages in hydrodistilled one were (38.38%) and (7.96%) for linalool and citral, respectively. The most effective treatment against varroa was caraway essential oil (71.7± 5.9%) followed by thyme essential oil (65.9 ± 8.5 %) and coriander essential oil (43.1± 9.4%). Carriers study indicated that thick paper is the most suitable inert carrier material followed by vermiculite and thin paper at thymol dosage rate of between 2.5 and 5 g. The efficacy of thymol dosage rate of 10 g per colony was 97.8%, 95.75% and 13.9% for thymol-vermiculite preparation, thymol-thick paper preparation and control group, respectively. However, at thymol dosage rate of 5 g per colony it was 92.6%±5.0, 94.7%±1.9 and 36.9%±5.9 for thymol-vermiculite preparation, thymol-thick paper preparation and control group, respectively. At thymol dosage rate of 10 g, thymol residues in honey were 2.10 ± 0.22 mg/kg for thymol-vermiculite preparations group, 2.02 ± 0.12 mg/kg for thymol-thick paper preparations group, and 1.32 ± 0.08 mg/kg for control group, respectively. The residues were higher in wax 371.5 ± 165.4 mg/kg for thymol-vermiculite preparations, 475.0 ± 140.9 mg/kg for thymol-thick paper preparations, and 87.5 ± 3.2 mg/kg for control group.
Key Words: Varroa, thymol, thyme, caraway, coriander, essential oil.

DEDICATION

I dedicate this work to whom my heart felt thanks; to my brother **Hamdi**, *all my friends, as well as to my parents and my wife* **Nadia** *for all the support they lovely offered along the period of my post graduation. I also dedicate this work to my lovely sons* **Zead** *and* **Hazem** *as well as to my nephew* **Hazem Hamdi.**

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INTRODUCTION

Varroa destructor (Anderson and Ttrueman, 2000) is the most destructive ectoparasitic mite of honey bee (*Apis mellifera* L.). It was first described on the Asian honey bee, *Apis cerana* Fabr (Oudemans, 1904). The new host infestation (*Apis mellifera*) occurred in the 1950, when European productive bees were introduced to Asia (Ian Tsin-He, 1965). Now, it is infested honey bees in most parts of the world. Infestations of this mite in North America have reached epidemic proportions, with annual losses of untreated colonies soaring as high as 50-80% in some areas of United States (Kraus and Page, 1995; Finley *et al*., 1996). In 1995-1996, the U.S. reported epidemic losses of managed bee colonies, ranging from 25 to 80% (Finely *et al*., 1996). Most commercial colonies die following 1-2 years of consecutive infestation without treatment (Martin *et al*., 1998; Downy and Winston, 2001). It damages bee colonies by feeding on haemolymph of honey bee larvae and pupae (Anderson and Ttrueman, 2000), resulted in severe deformation of wings and legs, reduced worker longevity, smaller population size, and colony death if left untreated (De Jong, 1990; Genc and Aksoy, 1992).

The first *V. distructor* mite detection in Egypt was in September 1987 at El-Arish region (Yousif-Khalil, 1992). By September 1989 this mite had become widespread, and by the autumn 1990 a heavy infestation was found in many parts of Egypt, and many apiaries were nearly destroyed (Abou-Zeid and Ghoniemy, 1993).

Varroa control requires that treatments must be mite selective (*i.e.,* fatal to mites at doses that are harmless to bees), and leave no or minimal residues in honey and wax (Ferrer-Dufol *et al*., 1991).

In the past few years, several studies have focused on the potential use of essential oil applications in biological control of different insect pests. The essential oils may be more rapidly degraded in the environment than synthetic compounds, and some have increased specificity that favors beneficial insects (Pillmoor *et al*., 1993). Botanical products such as essential oil constituents and organic acids have demonstrated potential for mite control. They are inexpensive to produce and many such products have been exempted from the costly registration process in the United States and Canada of their low mammalian toxicity (Quarles, 1996).

Different products, chemical and natural, are currently used for controlling this mite infestation. However, this mite still causes severe damage in apiaries because of its higher reproduction rate than its host (Ifantidis, 1983 and DE Ruijter, 1987), and the adaptation between the mite and the host's life cycle (Haenel and Koeniger, 1986). Additionally, *V. destructor* are developing resistance to the most common chemical acaricides fluvalinate and comaphose (Elzen *et al.*, 1998; Milani, 1999 and Elzen *et al.*, 2000). Other synthetic Varroa treatment such as flumethrine and bromopropylate leave toxic residues (Lodesani *et al.*, 1992). Thus, there is an urgent need for an alternative treatment such as organic acids and essential oil constituents that are effective, and do not leave toxic residues in honey and wax. Essential oil constituents such as thymol (5-methyl-2-isopropyl phenol) have demonstrated a varroacidal activity not only in laboratory assays, but also in field in Europe (Imdorf *et al.*, 1999), and in North America (Caledrone, 1999). Thymol comes from the herb thyme (*Thymus vulgaris* L.). It has the same molecular weight and formula as carvacrol (Prakash, 1990 and Leung and Foster, 1996). Thymol is a white or colorless crystal or powder and one gram is soluble in alcohol (1ml), olive oil (1.7ml) or water (1L) (Budavari, 1996). Thymol is used as an antioxidant and has activity against bacteria, molds and fungi (Panizzi *et al*., 1993; Shapiro *et al*., 1994; Eldosch and Hassanien, 2007). It also has larvicidal activity, antiseptic and anthelimintic properties (especially for hookworms and nematodes), and is used as a meat preservative at 0.0003 % (Leung and Foster, 1996).

Different thymol-based formulations have been studied to improve acaricide effectiveness, attempting to control the phenol release by different carriers. Thymol formulations significantly reduced the levels of the mite infestation of adult bees and sealed brood (Floris *et al.*, 2004). The environmental conditions, colony condition, and the carrier of the active ingredient play an important role in the thymol effectiveness against Varroa (Baggio *et al.*, 2004). Some of these formulations such as Apilife VAR, Thymovar, and Apiguard are registered as a control of Varroa mite in different countries.

The choice of formulation is influenced by several factors, such as the physical, chemical, and biological properties of the pesticide; the mode of application; the crop to be treated; and agricultural practices. Economic considerations also have to be taken into account, particularly when repeat field applications are necessary to maintain pest control. Controlled-release formulations allowing smaller quantities of pesticide to be used more effectively over a given time interval seem to be the best choice to meet these multiple demands of efficacy, suitability to mode of application, and minimization of environmental damage (Kydonieus, 1980). Currently, the use of illegal, unauthorized synthetic acaricides such as Dicofol is the most common control tactic for the Varroa mite in Egypt.

The aim of this study was to screening and evaluating several plant essential oils and/ or their individual components that may have acaricidal effects with little or no hazardous effects on honey bees with their advantages of low cost and low health and environmental hazards for both consumers and bee keepers. Also, the study aimed to find a suitable inert carrier material which can be used for formulate a commercial varroacides preparations. Thymol residues in honey and wax were also studied.

In this work, three essential oils of different chemical composition were first formulated as emulsion oil in water (o/w) and tested in apiary trials to determine their efficacy against varroa mite. The most promising oils and/or one of their constituents were then formulated as tables and strips as a potential new tool for varroa mite control.

REVIEW OF LITERATURE

1. Essential oil content of thyme and chemical composition of thyme essential oil

Thyme (*Thymus vulgaris* L.), belonging to the Lamiaceae family (Davis, 1982). The genus *Thymus* L. consists of about 215 species of herbaceous perennials subshrubs. The Mediterranean region can be described as the center of the genus (Stalil-Biskup and Saez, 2002).

T. vulgaris, also known as common thyme, a plant native to the Mediterranean region (Spain, Italy, France, Greece, *etc*.), has long been used as a source of the essential oil (thyme oil) and other constituents (*e.g.* thymol, flavanoid, caffeic acid and labiatic acid) derived from the different parts of the plant (Leung and Foster, 1996). The pharmacological properties of the plant and of its different extracts, in particular the essential oils, has been thoroughly studied and afforded the many industrial (mainly as food additive) and medical applications of the plant (Soulier, 1995).

Evaluations of the oil composition extracted from different parts of the plant or upon variable environmental, cultivation, and/or storage conditions have also been reported (Gouyon and Vernet, 1986; Venskutonis *et al*., 1996; Guillen and Manzanus, 1998).

In addition to their numerous traditional uses, the plant (herb) and its essential oil have found diverse applications in pharmacy and medicine (Soulier, 1995; Leung and Foster, 1996). The oil was reported to have antimicrobial (bacteria and fungi) (de Bouchberg *et al*., 1976; Ferhout *et al*., 1999; Chao *et al.,* 2000; Horne *et al*., 2001), carminative