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Lousicidal, ovicidal and repellent efficacy of some essential oils against lice and flies infesting water buffaloes in Egypt

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ABSTRACT

The lousicidal and repellent effects of five essential oils were investigated for the first time against the buffalo louse, *Haematopinus tuberculatus*, and flies infesting water buffaloes in Qalyubia Governorate, Egypt.

For the *in vitro* studies, filter paper contact bioassays were used to test the oils and their lethal activities were compared with that of *d*-phenothrin. Four minutes post-treatment, the median lethal concentration, LC50, values were 2.74, 7.28, 12.35, 18.67 and 22.79% for camphor (*Cinnamomum camphora*), onion (*Allium cepa*), peppermint (*Mentha piperita*), chamomile (*Matricaria chamomilla*) and rosemary oils (*Rosmarinus officinalis*), respectively, whereas for *d*-phenothrin, it was 1.17%.

The lethal time (50) (LT50) values were 0.89, 2.75, 15.39, 21.32, 11.60 and 1.94 min after treatment with 7.5% camphor, onion, peppermint, chamomile, rosemary and *d*-phenothrin, respectively. All the materials used except rosemary, which was not applied, were ovicidal to the eggs of *H. tuberculatus*.

Despite the results of the *in vitro* assays, the *in vivo* treatments revealed that the pediculicidal activity was more pronounced with oils. All treated lice were killed after 0.5–2 min, whereas with *d*-phenothrin, 100% mortality was reached only after 120 min.

The number of lice infesting buffaloes was significantly reduced 3, 6, 4, 6 and 9 days after treatment with camphor, peppermint, chamomile, onion, and *d*-phenothrin, respectively. Moreover, the oils and *d*-phenothrin significantly repelled flies, *Musca domestica*, *Stomoxys calcitrans*, *Haematobia irritans* and *Hippobosca equina*, for 6 and 3 days post-treatment, respectively. No adverse effects were noted on either animals or pour-on operators after exposure to the applied materials.

Consequently, some Egyptian essential oils show potential for the development of new, speedy and safe lousicides and insect repellents for controlling lice and flies which infest water buffaloes.

1. Introduction

The buffalo louse, *Haematopinus tuberculatus*, Nitzsch (Phthiraptera: Haematopinidae) is principally an ectoparasite of Carabao, water buffalo, *Bubalus bubalis* L. (Lancaster and Meisch, 1986; Bastianetto and Leite, 2005; Lemcke, 2006). *H. tuberculatus* has been recorded where buffaloes have been introduced and domesticated in Egypt, the Philippines, Australia, Madagascar, China and Myanmar (Lancaster and Meisch, 1986). It has also been found on cattle, in close association with buffalo (Lancaster and Meisch, 1986), on camel (Lawal et al., 2007) and on wild ruminants (Marley and Conder, 2002). Buffalo lice cause anaemia and loss of body condition. They need to be controlled, particularly if the animals' condition is affected (Lemcke, 2006). The itch caused by *H. tuberculatus* is responsible for the

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low milk and meat productivity (Bastianetto and Leite, 2005). Damage caused by blood-sucking lice involves blood loss, and in serious cases, abortion and death (Lancaster and Meisch, 1986). Small calves can build up high numbers of lice, making control necessary (Lemcke, 2006).

The severity of the infestations and the potential for transmission of rinderpest may make such louse an important pest for control measures (Woodworth, 1922). In addition, flies infesting animals cause great economic losses and transmit many diseases (Roberts and Janovy, 2005).

Among lousicidal treatments, most cattle–lice formulations are effective against the buffalo louse (Colwell, 2002; Marley and Conder, 2002; Hussain et al., 2006; Lemcke, 2006). The need for novel solutions to control pediculosis has been intensified due to the emergence of resistance (Chosidow et al., 1994; Levot, 2000), environmental pollution (Gassner et al., 1997) and insecticidal residues in milk (Gottschall et al., 2005).

Essential oils have been used for centuries as insecticides and insect repellents, as well as for treating and preventing infestations by lice (Priestley et al., 2006; Williamson, 2007; Williamson et al., 2007). The constituents of plant volatile oils have long been known to affect the behavioural responses of pests; their monoterpenoid components appear to be the most useful as insecticides or anti-feedants (Veal, 1996).

The aims of this study were to determine the *in vitro* and *in vivo* lousicidal efficacy of some essential oils in comparison to *d*-phenothrin, as well as their ovicidal and repellent effects against flies when applied as pour-on solutions to water buffaloes.

2. Materials and methods

2.1. Lice

The buffalo louse *H. tuberculatus* was collected from infested water buffaloes at the farms of Faculties of Agriculture and Veterinary Medicine at Moshtohor, Benha University, Qalyubia Governorate, Egypt.

2.2. Tested substances

Five essential oils were tested, namely camphor (*Cinnamomum camphora*), onion (*Allium cepa*), peppermint (*Mentha piperita*), chamomile (*Matricaria chamomilla*) and rosemary (*Rosmarinus officinalis*). All oils, obtained from El-Kaptain Company, Egypt, were authorised by the Egyptian Ministry of Health for different human uses.

The use of 0.4% *d*-phenothrin (Item®, Mash Co., Egypt) as an anti-lice shampoo is authorised in Coryne, Monaco, France. It is also authorised by the Ministries of Health in France and Egypt for the treatment of adults and eggs of human head lice, *Pediculus humanus* var *capitis*.

2.3. Testing for in vitro pediculicidal activity

The filter paper contact, concentration–response bioassay was chosen because it is more representative of what could occur in nature. Lice would directly contact compounds, as they do in the filter paper imitating field circumstances. Consequently, *in vitro* assays were useful for pre-screening of the efficacy of materials before field application. The method used to assess the pediculicidal activity was adapted from the World Health Organization (WHO, 1981) protocol and was according to Priestley et al. (2006) methodology.

Preliminary experiments were conducted to determine suitable experimental parameters, such as dilution factors for tested substances and the duration of their exposure to lice. Bioassays were performed at 27 ± 2 °C and $75 \pm 5\%$ relative humidity (RH). The finalised direct contact assay was carried out as follows. Each test substance was diluted in water to different concentrations. The applied concentrations were 0.9%, 1.4%, 1.8%, 7.5% and 30% for camphor oil; 1.8%, 4.7%, 7.5%, 15% and 30% for onion oil; 3.8%, 7.5%, 15%, 22.5% and 30% for peppermint oil; 7.5%, 15%, 19%, 22% and 30% for chamomile oil; 3.8%, 15%, 22%, 30% and 60% for rosemary and 0.23%, 0.45%, 0.9%, 2.7% and 30% for *d*-phenothrin. A few drops of Tween 80 were added as an emulsifier. A volume of 600 μl of the diluted sample was distributed evenly over a 9-cm diameter filter paper held in the lower half of a 9-cm glass Petri dish. After 15 min, the liquid had spread out, the filter paper was fully impregnated and no excess moisture was left in the dish. Ten buffalo lice, males and females, were placed on the top of each filter paper disc. The control groups were treated with distilled water and Tween 80. Ten replicates were used for each concentration.

Lice were examined under a dissecting microscope at different time intervals (1, 2, 4, 8, 10, 15, 20, 30 and 60 min). Death was defined as the lack of limb and gut movement, and the failure to respond when the legs were stroked with a forceps (Priestley et al., 2006). The number of fatalities was recorded, and the lethal concentrations, LC50, LC90 and LC95, were subsequently calculated.

2.4. Testing for in vitro lethal time (LT)

The time–response bioassay was similar to the standard concentration–response bioassay with the following exception; lice were exposed to a single concentration for each trial. The mortality was initially assessed 1 min after being subjected to test materials, followed by mortality assessment at 2, 4, 8, 10, 15, 20, 30 and 60 min. The used concentrations were 0.9%, 1.4%, 1.8% and 7.5% for camphor oil; 1.8%, 4.7%, 7.5% and 15% for onion oil; 3.8%, 7.5%, 15% and 22.5% for peppermint oil; 7.5%, 15%, 19% and 22.5% for chamomile oil; 3.8%, 7.5%, 15%, 22.5% and 60%; and 0.23%, 0.45%, 2.7% and 7.5% for *d*-phenothrin.

2.5. In vitro assessment of the ovicidal effect

The discriminating doses (DDs) of the *in vitro* bioassays were calculated according to the method of Kristensen et al. (2006) and were approximately twice the lethal dose that kills 95% of the insects, that is, the LC95 (Table 1).

The ovicidal activity was assessed according to the method of Priestley et al. (2006). The DDs were prepared and used to fill 20-ml glass bottles. Twenty-five eggs were immersed in each test substance for 10 min. After this time, the eggs were removed and blotted on a medical