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Evaluation of effect of essential oils (eucalyptus and lemon grass) against the darkling beetle, *Alphitobius diaperinus*

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Abstract
The lesser mealworm (*Alphitobius diaperinus)*, also known as darkling beetle, is responsible for huge economic losses in poultry production. This insect has fully adapted to poultry houses and commonly found in high density in poultry litter. It is a potential transmitter of bacteria, viruses, fungi, protozoa and particularly tapeworms. Most strategies used to control this pest are based on the application of chemical insecticides. However, the widespread and inappropriate application of the chemical insecticides against these darkling beetles produced the risk of developing resistance to almost all the compounds introduced in the market. To overcome this problem, a study was conducted to evaluate the effect of essential oils (eucalyptus and lemon grass) at different concentrations against larvae and adult darkling beetles. The essential oils were mixed with acetone at three different concentrations viz., 5, 10 and 20%. Twenty adult beetles / larvae were placed into the glass vials and their mouth covered with muslin cloth. The filter papers impregnated with different concentrations of essential oils were placed into a bottle. The glass vials with beetles were hung at the geometrical centre of glass bottles, which were then closed with air-tight lids. Mortality/viability of insects was recorded after every 30 min., 24hrs and 48 hrs. The results of the current study showed that none of the beetles died even at the higher concentration of both the essential oils. The present study suggests that eucalyptus and lemon grass essential oils are not effective against the larvae and adult darkling beetles dwelling in poultry litter.

Keywords: *Alphitobius diaperinus*, Eucalyptus, lemon grass, Essential oils, Poultry litter

1. Introduction
The lessor mealworm beetle, *Alphitobius diaperinu*s is the major pests in poultry houses in India. It belongs to tenebrionid family. It may cause considerable economic loses if not adequately controlled because it can rapidly spread in poultry houses. The beetles affect bird performance when consumed by poultry and have been implicated in the transmission of over thirty avian diseases including turkey corona virus (TCV) [1]. In addition, Chickens feeding on beetle larvae show poor weight gain and increased mortality [2]. *Alphitobius diaperinus* inhabits poultry droppings and they are known vectors and reservoirs for a number of serious poultry diseases viz., Aspergillosis, Marek's disease, infectious bursal disease (IBD), and Newcastle disease [3] and can also act as an intermediate host for nematodes, cestodes and protozoa [4]. In addition, both larvae and adults can transmit some food-borne pathogens such as *Salmonella* and *Campylobacter* to broilers [5-7]. In particular, chicks are more likely to be infected by eating larvae than adult beetles [8]. It is also a vector for *Eimeria sp* (protozoa) that cause coccidiosis in birds [9] and fowl tapeworms such as *Choanotaenia infundibulum* and the nematodes, *Subulura brumpti* and *Hadjelia truncate* [10].
A various types of insecticides are being used for the control of darkling beetles in poultry houses. However, *Alphitobius diaperinu*s (lessor mealworm beetle) has likely developed resistance to insecticides such as DDT [11], 2, 2-dichlorovinyl dimethyl phosphate (DDVP) and nicotinoid [12]. These problems have highlighted the need for the development of new types of selective insect-control alternatives with fumigant action [13]. It is believed that essential oils have the advantage over conventional fumigants in terms of low mammalian toxicity, rapid degradation and local availability [14]. They do not leave residues toxic to the environment and have medicinal properties for humans [15]. These are the by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites [16]. Because of the intensity of plant-insect interactions, the plants have well developed defense mechanisms against pests and are excellent sources of new insecticidal substances.

Therefore, this study was aimed to evaluate the efficacy of essential oils against the darkling beetles dwelling in poultry litter.

2. Materials and methods

2.1 Insect sampling

The darkling beetles were collected directly by handpicking from the manure of poultry houses located in and around Veterinary College and Research Institute, Namakkal. The darkling beetles were maintained in insect collecting vials at room temperature at Department of Veterinary Parasitology, Veterinary College and Research Institute, Namakkal until the experiment was performed.

2.2 Preparation of essential oils and Cypermethrin

Essential oil was extracted from the fresh leaves of Eucalyptus (*Eucalyptus globules*) and Lemon grass (*Cymbopogon citratus*) by hydro-distillation using Clevenger apparatus at 80° C for 3 - 4 hrs. One hundred gram of fresh leaves yielded maximum of 1 to 2 ml of oils. Cypermethrin (Sigma-Aldrich) contains 1 mg/ml was used in this study.

2.3 Fumigant toxicity of essential oils

The darkling beetles were kept in the insect collecting glass vial (4×2 ×2 cm) (Tarson). Essential oils were mixed with acetone at three different concentrations viz., 5, 10 and 20%. Twenty adult beetles / larvae were placed into the glass vials and their mouth was covered with muslin cloth (Fig.1). The filter papers (What man No. 1, cut into 4 × 5 cm paper strip) impregnated with different concentrations of essential oils were placed into a bottle. After that, the glass vials with beetles were hung at the geometrical centre of glass bottles, which were then closed with air-tight lids (Fig.2). In the control bottles, only acetone was applied on the filter papers. Each experiment was replicated three times for each concentration. All tests were done at room temperature.

Fig 1: Twenty adult *Alphitobius* beetles / few larvae were placed inside the glass vials

Fig 2: Fumigant toxicity assay against adult beetles of *Alphitobius diaperinus* and their larvae

2.4 Measurement

In all cases, the exposure times were 30 min., 24hrs, and 48 hrs. Mortality rate of insects were recorded manually. The comparison between treated and control group were analyzed.

3. Results and Discussion

The effective control of the lessor mealworm beetles (*Alphitobius diaperinus*) is solely on the application of insecticides. Alternatively, entomopathogenic fungi is one of the most promising agents for biological control of beetles, because it persists in the host population for a longer period leading to high mortality rates in larvae and adults, however, the action of entomopathogenic fungi is slow and needs appropriate conditions of temperature and humidity to maintain its viability and pathogenicity [17]. The susceptibility level of beetles is dependent on active ingredients, population treated, formulation, surface treated and timing of observation [18].

The mortality rates for larvae and adult darkling beetles after exposure with different concentrations of essential oils are shown in the Table 1 and 2. The darkling beetles in the treated group were alive during the entire period of the experiment. None of the larvae or adult beetles was died in the untreated control group also. The results of the present study showed that there was no mortality of both adults and larvae of darkling beetles after applying with increasing concentrations of essential oils even after 72 hours and suggesting that these essential oils are not effective against the darkling beetles dwelling in poultry litter. In addition, the adult beetles and larvae in the treated group were highly aggressive and trying to move away from glass vials, however, all adult beetles and larvae in cypermethrin-treated groups were died after 60 minutes of exposure.

Table 1: Mortality rates for adult beetles of *Alphitobius diaperinus* over times after applying with eucalyptus oil at various concentrations at different times

Time	Control	Concentration (%)		
		5	10	20
30 min	0/20	0	0	0
24 hrs	0/20	0	0	0
48 hrs	0/20	0	0	0

Table 2: Mortality rates for adult beetles of *Alphitobius diaperinus* over times after applying with lemon grass oil at various concentrations at different times.

Time	Control	Concentration (%)		
		5	10	20
30 min	0/20	0	0	0
24 hrs	0/20	0	0	0
48 hrs	0/20	0	0	0

Many commercial products have low effectiveness in the control of *A. diaperinus*, since the target organism develops resistance [19]. This phenomenon was also described by Chernaki-Leffer *et al*. (2011) [20], who found high resistance of *A. diaperinus* to cypermethrin. Resistance to organophosphate insecticides and pyrethroids by *A. diaperinus* has been reported frequently in the literature [21]. The mode and timing of insecticide application affects the action of the insecticide. For cypermethrin, cases of re-infestation are common. The use of higher doses of cypermethrin against *A. diaperinus* causes ecotoxicological effects. In addition to its environmental effects, cypermethrin causes toxic effects to broilers that may manifest as biochemical, histopathological and/or clinico-hematological changes [22].

In contrary to what was observed in our study, essential oils of eucalyptus and lemon grass were highly efficacious against

larvae, pupae and adult house flies at different concentrations (50, 100, 150, and 200 µl) and efficacy was found to be proportional to the concentration of the oils [23, 24]. These differences in effectiveness could be due to variation in application methods, behavioural resistance of larvae and adult beetles and variations in insecticidal activity of essential oils among different species of insects. We observed that these essential oils had more repellent activity than fumigant toxicity. In our previous study, we found that cypermethrin was highly effective against the darkling beetles and their larvae even at low concentration of 50 pm [25]. Nowadays, many studies have been done for evaluation of susceptibility of insect pests of stored product specially *Callosobruchus maculatus* to plant essential oils. For example, the anti-feedant activities of *Citrus reticulate* Blanco, *Citrus limon* L. and *Citrus aurantium* L. essential oils against eggs, larvae and adults of *C. maculatus* and found that the effect of different concentrations of the essential oil vapours on egg hatchability as well as larval and adult mortality was found to be significant [26]. Futher, they observed that the essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis* against *C. maculatus* was evaluated and the results revealed that *E. globulus* oils were more effective than *E. camaldulensis* oils, by significantly decreasing the RGR, RCR and ECI. Both of plant essential oils, with the same activity, increased FDI as the oil concentration was increased, showing high feeding deterrence activity against *C. maculatus*.

4. Conclusion

In vitro study on insecticidal efficacy of essential oils of Eucalyptus and Lemon grass at different concentrations against larvae and adult darkling beetles (*Alphitobius diaperinus)* was evaluated. The present study indicates that these essential oils are not effective against the adult darkling beetles and their larvae, therefore these oils cannot be used for strategic control of beetles dwelling in poultry litter.

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6. References

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