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Insecticidal activity of some plant essential oil extracts against *Alphitobius diaperinus* pest causing *Avian influenza*

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Abstract. *Alphitobius diaperinus* is a type of pest insects found in animal feed and chicken manure that the direct contact with chickens causes this pest to be used as a vector for various pathogens like *Avian influenza* virus. *Alphitobius diaperinus* control which still using chemical pesticides is dangerous for health and the environment. The study aims to examine the insecticidal activity of some plant essential oil extracts (*Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus*) against *Alphitobius diaperinus*. Test insects used were adult *Alphitobius diaperinus* propagated in the laboratory. Tests carried out are insecticidal tests. Observations were made every hour after treatment for seven days. The two-way ANOVA with the Tukey follow-up test were employed to analyze the data. The results revealed that *Mentha piperita* at the ninth hour and *Syzygium aromaticum* at the 10 hour showed against *Alphitobius diaperinus* insecticidal properties. Whereas *Cymbopogon citratus* and *Melaleuca leucadendra* did not give a mortality effect for four days. Thus, *Mentha piperita* and *Syzygium aromaticum* essential oil extracts can be an environmentally friendly natural pest control for *Alphitobius diaperinus*.

5 Introduction

Avian influenza is a disease caused by *Avian influenza virus type A* [1]. Cases of *Avian influenza (A H5N1)* globally, from January 2003 to June 2019 there were a total of 861 cases that infect humans, and as many as 455 of them are fatal cases [2]. In Indonesia, the case began to spread since 2005. From 2005 to 2018, is greatest in 2006. A total of 55 cases of which 45 died, then every year decline, which in 2014 and 2015 there were two cases of 2 died. Until 2018, as many as 200 cases of *Avian influenza* that occurred in Indonesia, with a total of 168 cases of fatal cause of death [3]. According to the latest data of WHO in September 2019, the last case occurred in Nepal on 30 April 2019.

Avian influenza can cause economic losses in the poultry sector [4], the impact of the availability of food and the dangers of disease transmission to humans which can cause death [5]. *Avian influenza* infection can occur by direct contact or contact with contaminated environment dirt or liquid excretions / secretions poultry or through a vector.

Alphitobius diaperinus constitute one of the vectors of *Avian influenza* [6]. *Alphitobius diaperinus* is one type of insect pest found in animal feed. These pests are found in warehouse storage of food products and is considered a pest [7]. The presence of these pests around the farm is quite disturbing directly, and can also inadvertently by a chicken when you're eating. This is why the presence of this pest in the livestock industry to be a problem that harm.

Alphitobius diaperinus or better known as Frenki lice are insect pests in poultry [8]. In its life cycle, these insects undergo complete metamorphosis from egg then into larvae, pupae and adult fleas. Adult



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lice can live between 3 and 12 months. The eggs hatch in 4 to 7 days, and become adult beetles back after 40-100 days based on food reserve and temperature [9]. These pests serve as vectors for pathogens, especially bacteria, protozoa and viruses. *Alphitobius diaperinus* can affect body weight of broiler chickens, especially in young chickens [10].

Currently control of *Alphitobius diaperinus* done using synthetic chemical pesticides. However, using chemical pesticides might harm human health, it is not environmentally friendly, and harmless to animals and nontarget plants [11]. Therefore, the necessary control *Alphitobius diaperinus* that are natural and do not cause pollution to the environment using essential oils of some plants with biotechnology.

Some of the compounds in the essential oil on tropical plants contain compounds monoterpenoid main form, which can act as a natural insecticide that is safe for the environment [12]. *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus* is a type of tropical plant that contains metabolites for insect control. Some plant compounds known to contain secondary metabolites, ie tannins, coumarins, terpenoids, lectins, phenolic, polyphenols, quinone, and flavonoids [13]. Secondary metabolites function as plant protection, antibacterial, antifungal, antiviral and insecticides. In this study aims to analyze the content of the active compound *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus*.

2. Methods

2.1. Preparation of *Alphitobius diaperinus*

The study was conducted at the Biology laboratory, Mathematics and Natural Sciences, Universitas Negeri Semarang. *Alphitobius diaperinus* obtained from the farm Semarang. Taken a sample of 1 kg of infected cattle feed *Alphitobius diaperinus* stored in containers, left and reproduced in the laboratory for two months, at a temperature 30°C - 33°C and humidity of 90%. *Alphitobius diaperinus* used is the adult pests has multiplied the age, weight and the same size. Total pests *Alphitobius diaperinus* used as many as 300 fish mature.

2.2. Plant material

The samples of *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus* diluted with ethanol up to 10%. Only the aerial chunk was used; the apical parts and the leaves were sun-dried in the lee for eight days at 25 °C.

2.3. Microwave-assisted hydrodistillation

The hydro distillation was done using an assembly involving of a domestic microwave which is straightly connected to an extractor (Clevenger-type) and a cooling system to continuously curtail the distillate. The condensed water was then refluxed to the extraction ask to revive the water into the plant. This distillation was worked under the ideal extracting time, microwave power, and ratio of water per plant material. 100 gr of *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus*. Samples were settled in a two liters distilled water (200 ml), fired by the microwave. The mixture was burnt at a fixed power of 600 W until all essential oils was extracted. The essential oils from other extractions are dried by anhydrous sodium sulfate and kept in the dark until ready for analysis.

2.4. Hydrodistillation by clevenger

The essential oils of *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus* were extracted through hydrodistillation under optimum conditions, 100 g of *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus* was settled in 800 ml of distilled water. The set was put in a balloon heater which is connected to a refrigerator to condense the essential oils in three hours. At the final of the distillation, two phases were examined, the organic phase (essential oil) and the aqueous phase (aromatic water), lighter

than water. The oil was accumulated, dried on anhydrous sodium sulfate, and kept in sealed vials in the dark, at 4°C.

2.5. Insecticidal test [14]

Tests carried out by immersing Whatman filter paper with essential oils *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus*, Then allowed to stand for one to two hours to sink into Whatman filter paper. *Alphitobius diaperinus* prepared on a petri dish as much as 20 birds, with the concentration of each extract essential oils 50%. Observations were made every hour for seven days. Two-way ANOVA analysis and Tukey test were engaged to analyze the data.

3. Result and Discussion

Insecticidal activity of essential oils *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus*, tested *Alphitobius diaperinus* been seen at one hour after treatment, that is characterized by pest mortality test.

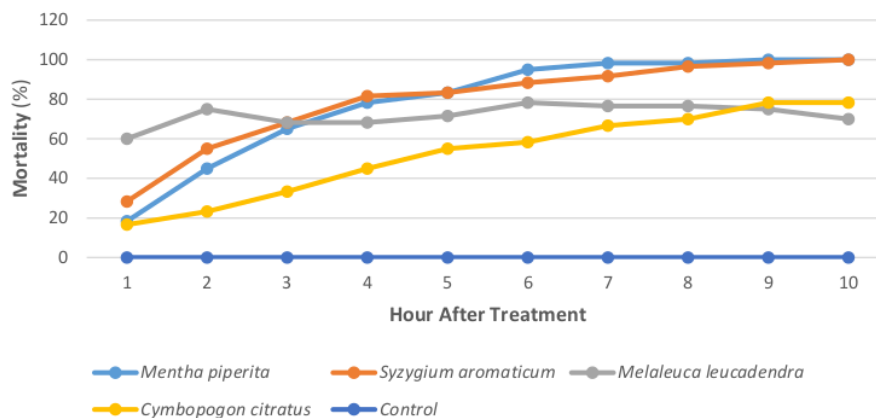


Figure 1. Mortality *Alphitobius diaperinus* concentration of 50% using the essential oil *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus*

The mortality rate is highest *Alphitobius diaperinus* on *Mentha piperita* essential oil with a mortality rate of 100% within 9 hours. On the control does not occur *Alphitobius* mortality *diaperinus* up to 168 hours of observation. The main compound in *Mentha piperita*, among others: Menthol 53.28%, 15.10% menthyl acetate, menthofuran 11.18%, and 6.69% cineole [15]. The content of toxic compounds contained in *Mentha piperita* was tested for acetylcholinesterase activity and as an antioxidant enzyme system in some insects, among others on *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). Menthone and menthol which is a major component is among pesticides. As fumigant. A dominant component appeared 100% mortality at 75.0mL / L of water also 100.0mL / L of water against *Tribolium castaneum* and *Sitophilus oryzae* at 24 h exposure [16].

Effect of *Syzygium aromaticum* essential oil on *Alphitobius diaperinus* 100% mortality occurred within 10 hours. The active compounds contained in *Syzygium aromaticum* among other things: 75.41% eugenol, caryophyllene 15.11%, 3.78%; caryophyllene oxide; 1.13% and 0.84% cardinene. Eugenol is the main component of essential oils *Syzygium aromaticum* [17]. Eugenol can function as a bactericide, fungicide and can attack the nervous system of insects [18, 19] and as a pesticide in some types of pests [20]. In a similar study, mortality rate was directly proportional to the dosages of both oils. The inhibitory potential of the oils, however, was only observed at the highest dose (150µl / L)

for the adult stage, where low amylase activity was recorded. On the whole, amylase activity was higher in the insect stages fumigated with *Syzygium aromaticum* oil. Similarly, significant positive linear correlation exists only between the mortality rate and the dosages of *Syzygium aromaticum* oil ($r = 0.994$, $N = 4$, $P = 0.006$). The results of the Gave cues on the action of the essential oils via some stress-related physiological activities in the insect stages that culminated in the killing of the insects. Upon exposure to toxic substances, changes in enzymatic reactions have been reported to be generally developmental stage-specific and in a number of insects including *Tribolium* spp. In these species, a higher enzyme activity is an occurrence commonly recorded in the adults more than in the larvae. Accordingly, the findings in this research, the which reveal higher levels of enzyme activity in the adult *Tribolium castaneum* more than in the larvae, appear to be well correlated with the reported findings. higher proportion of toxicity to the insects relative to the other doses tested, it is most likely that this high-dose contains enough amounts of inhibitory compounds that propagated the decline recorded in the amylase activity.

Moreover, the cytotoxic effects of the essential oils on the epithelial cells of the midgut of *Tribolium castaneum* have been implicated in the reduced α -amylase activity. the which reveal higher levels of enzyme activity in the adult *Tribolium castaneum* more than in the larvae, appear to be well correlated with the reported findings. higher proportion of toxicity to the insects relative to the other doses tested, it is most likely that this high-dose contains enough amounts of inhibitory compounds that propagated the decline recorded in the amylase activity [21].

Effect of Essential Oil *Melaleuca leucadendra* on mortality *Alphitobius diaperinus* 100% occurred within 96 hours. *Melaleuca leucadendra* among others: 22 1,8-cineole, 45%, terpineol 12:45%, 6.95% caryophyllen, pinene 5.74%, and 5.25% pinene. The content of 1,8-cineol on *Melaleuca leucadendra* function as an insecticide, as evidenced in research shows the active compound can sineol *Pediculus humanus* cause mortality [22]. Research on the active compound doses *Melaleuca leucadendra*. The LD50 values for *Tribolium castaneum* and *Sitophilus zeamais* in contact toxicities were 0.062 and 0.143 mL of stored-product insects. Further investigation should be done for other stored-product insects [23].

Meanwhile, the effect of essential oils *Cymbopogon citratus* on mortality *Alphitobius diaperinus* 100% occurred within 96 hours. *Cymbopogon citratus*, among others: trans-citral (geranial) 46.6%, cis-citral (neral) 34.1%, myrcene terpineol 6.8% and 1.5%. The main components of essential oils are compounds sitral *Cymbopogon citratus* [24]. Sitral compound is a natural combination of two isomeric aldehydes. Trans-sitral known as sitral A or geranial. Cis-sitral known as sitral B or neral, namely geranial isomer (α -citral) and neral (β -citral). Sitral has the effect of pheromones in insects, as an antifungal, and as a basic ingredient of pesticides [25]. Research on *Tribolium castaneum* and *Podisus nigrispinus* that the dose LC50 value of the extract was 0.75 mg / 20 mL of methanol with an LT50 of 71.10 h. The methanol extract of *Cymbopogon citratus* showed the highest mortality compared to the less toxic powder. The study revealed that *Cymbopogon citratus* products are encouraging insecticides and can be effectively enforced in the *Tribolium castaneum* management in storage of pluses and grains. The terpenoids in lemongrass essential oil showed a negative effect on the *Podisus nigrispinus* respiration rate. The reduction of the respiratory rate of the third-instar *Podisus nigrispinus* nymphs after being exposed to geranyl and citral acetate may be due to muscle paralysis, oxidative phosphorylation processes disruption and the breathing activities dysregulation [26].

Some studies using essential oils in *Cinnamomum aromaticum* to control *Tribolium castaneum* at a concentration of 8% is effective to kill *Tribolium castaneum* 100% within 24 hours [27]. The content of essential oil *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus* that is volatile or volatile, so it can be toxic to the respiratory and nervous systems of insects. Symptoms *Alphitobius diaperinus* observed mortality is inverted body, short legs do not come out (contract), or the movement of the limp body, and his body is shrinking. In nymphs insects exposed to the essential oil terpenoids constituents advanced low respiration rates, which farther provoke to the organism physiology imbalance, as described for *Sitophilus granarius*L (Coleoptera: Curculionidae) and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). Essential oils can result in sub lethal effects

namely increased heart rate, changes in the cAMP levels in the nervous system and decreased binding to octopamine receptors, as detailed for *Periplaneta americana* L. [28].

4. Conclusion

Essential oils affect mortality *Alphitobius diaperinus*. Essential oils are most effective in a row is *Mentha piperita*, *Syzygium aromaticum*, *Melaleuca leucadendra* and *Cymbopogon citratus* with the main component eugenol. Results showed the toxicity of EO associations with the prevention of AChE activity accompanied by imbalance of oxidation. Therefore, *Mentha piperita* Noteworthy EO has a role in insecticidal properties and could be proposed as an eco-friendly alternative to synthetic insecticides.

Acknowledgments

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