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Journal of Physics: Conference Series

PAPER • OPEN ACCESS

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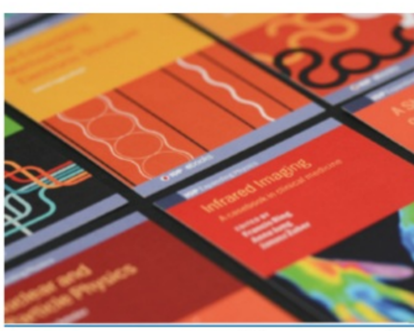
To cite this article: N Subekti and P Indrawati 2020 *J. Phys.: Conf. Ser.* **1567** 042063



Insecticidal activity of essential oils compared with chemical insecticide against stored-grain pest *Sitophilus oryzae*

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Abstract. Stored-grain pest *Sitophilus oryzae* causes loss of rice in quantitative and qualitative. Until recently, the most frequent ways used to control insect pest is synthetic chemicals that harmful to human health, causing resistance to the pest, and pollute the environment. An alternative solution is offered by natural insecticide. The aim of the research is to analyze the effect of toxicity of three essential oils compared with a chemical insecticide toward rice weevil *(Sitophilus oryzae)*. Bioassay was carried out by fumigation. The essential oil of *Myristica fragrans* 40 µL g^{-1} was the most insecticidal than essential oil of *Cymbopogon citratus* and *Eucalyptus* sp. with 100% mortality after 48 h exposure. Chlorpyriphos 2 µL g^{-1} as chemical insecticide generally used had 100% mortality after 24 h exposure. In spite of natural insecticide had longer time and higher concentration to kill insect pest, but it can be used for integrated management of stored-grain pest rice weevil *(Sitophilus oryzae)* that more safety for human and environment.

1. Introduction

Government in 2017 was only able to store an internal target to 2.14 from 3.74 million tonnes of rice (57.2%). As a result, the stock of rice in early 2018, less than 1 million tonnes of rice [1]. The infestation of stored-grain pest rice weevil *(Sitophilus oryzae)* plays an important role in rice production caused limited power. Loss of rice due to insect infestation during storage is a serious problem. Damage in tropical countries achieves 5 – 10% production [2]. In addition, infestation of pest causes about 30 – 40 % weight losses and may have up to 90% demage potential within 5 – 6 months after infection [3]. The challenge of food security caused by infestation of rice weevil *(Sitophilus oryzae)* is occured not only in Indonesia as a developing country, but also many developed countries over the world [4]. This problem is dificult to be solved because the population of rice weevil *(Sitophilus oryzae)* can easily increase in warm condition, especially when rice is stored. The total time needed for development of rice weevil *(Sitophilus oryzae)* from egg to adult in rice is about 37 days [5].

The control of rice weevil *(Sitophilus oryzae)* as grain-stored pest is very important to ensure the food supply of national rice. Until now often carried out fumigation using methyl bromide and phosphine. Other synthetic insecticides to control grain infestation from pests are chlorpyrifos, malathion and pyrethroids [6]. The chemical is highly toxic to humans, resistant to insect, damage the ozone layer, may leave residues on foodstuffs and hazardous to health. Research validated the time to 99.9% knockdown ($KT_{99.9}$) value for the 5 mg/lier against field-derived populations of rice weevil

(Sitophilus oryzae) [7]. Fumigation in unsealed storages, combined with high fequency of weak resistance were found to be the main criteria that led to the development of strong resistance in rice weevil *(Sitophilus oryzae)*. Independent development, rather than gene flow via migration, apears to be primary responsible for the geographic incidence of strong resistance to phosphine in rice weevil *(Sitophilus oryzae)* [8].

The adverse effects of chemical insecticides have led researchers to develop integrated pest management with a low level toxic to humans and capable of maintaining the environment on the storage system. Natural insecticide in the form of essential oil (EO) on integrated pest management program to reduce the use of chemical insecticides are highly recommended [9]. EO is a compound that is synthesized by plants and play an important role in the signaling processes including interest also pollinators and other beneficial insects [10].

This study focused on the analysis of some essential oils, namely *Eucalyptus* sp., *Myristica fragrans,* and *Cymbopogon citratus* as natural insecticides were compared to chemical insecticides chlorpyrifos against rice weevil *(Sitophilus oryzae)*. Therefore, at the end of this study will be known kind of EO with a particular concentration that has the most capability closer to chemical insecticides for mortality of rice weevil *(Sitophilus oryzae)*.

2. Methods

The research was carried out in the Laboratory of Biology, Semarang State University in 2019. The insects used in this study was rice weevil *(Sitophilus oryzae)* were obtained from rearing in the laboratory. The study was designed with a complete random design with 14 groups and 3 replications.

2.1 Rearing

Insects rice weevil *(Sitophilus oryzae)* maintained using medium rice in a jar covered gauze to keep aeration. Each jar contains 20 unsexed adult insects. The first generation of rice weevil *(Sitophilus oryzae)* aged 7 – 14 days used for this study. This results in the insect of the same age for the test.

2.2 Extraction

The leaves of *Eucalyptus* sp., nutmeg *(Myristica fragrans)*, and leaves of lemongras *(Cymbopogon citratus)* were dried in the shade for 24 h, cut, weighed, and hydrodistilled using Clevenger-type apparatus for 24 h. EO obtained was stored at 4 °C for use in bioassays.

2.3 Bioassay

Insecticidal activity was tested with fumigation assay using EO of *Cymbopogon citratus*, *Myristica fragrans*, and *Eucalyptus* sp. with doses of 5, 10, 20, and 40 μL/ g. Chemical insecticide used in the assays was chlorpyrifos 20 μg/ g in accordance with the doses used in the market generally. Each insecticide was dissolved in 20 mL butanol, then applied to 20 g of rice. After the rice was submerged by insecticides, dried rice-aired so that butanol is lost. A total of 10 unsexed insects are added to 20 g of rice in 100 mL sealed glass jar. Live and dead insects wa calculated for 24 and 48 h by the formula mortality by Abbott (1925), as follows:

$$\%\, mortality = \frac{total\ of\ dead\ insects}{total\ of\ tested\ insects}\, x\ 100\%$$

2.4 Data Analysis

Quantitative data were expressed as mean ± SE and ANOVA were used to determine significance at the 5% level.

3. Results and Discussion

The study was conducted to test the insecticidal activity of the three kinds of EO with various concentrations compared to chemical insecticides against stored-grain pest rice weevil *(Sitophilus oryzae)*. Results of the research have gradually analyzed using mean comparisons between the treatment groups.

The first analysis is a comparison between kind of EO with dose variation using Two-way ANOVA. Retrieved from EO of *M. fragrans* with a dose of 40 μL/ g has the highest insecticidal against rice weevil *(Sitophilus oryzae)* compared other EO. The precentage of mortality up to 70%

after 24 h exposure, then increased to 100% after 48 h exposure. Rice weevil *(Sitophilus oryzae)* mortality in the group of EO of *C. citratus* occupies the second place before *Eucalyptus* sp., wich is 56.67% at a dose of 5 μL/ g after 48 h exposure. The lowest mortality is EO of *Eucalyptus* sp. with the highest percentage in this EO is 33.33% after 48 h exposure at the dose of 40 μL/ g. The significant value between EO and between doses less than 0.05, then the average mortality on the type of EO as well as variations in the doses are not the same. Likewise, between EO and dose have a significant value of less than 0.05, then there is an interaction between the EO and dose. The results of insecticidal activity on a different type of EO with the variation of dose for 24 and 48 h exposure presented in Table 1.

Table 1. Insecticidal Activity Between Treatment of EO

Times (h)	Doses (μL/ g)	Corrected Mortality (%) *Eucalyptus* sp.	*M. fragrans*	*C. citratus*	Sig. EO	Doses	EO*Doses
24	5	0.00	0.00	43.33	0.03	0.02	0.00
	10	6.67	6.67	0.00			
	20	0.00	16.67	0.00			
	40	13.33	70.00	0.00			
48	5	0.00	0.00	56.67	0.00	0.01	0.00
	10	0.00	20.00	0.00			
	20	0.00	90.00	0.00			
	40	33.33	100.00	3.33			

Factors that affect these things one of which is the type and levels of active compounds contained in the EO. A mixture of volatile hydrocarbons, especially monoterpenoid widely available in the EO. Monoterpenoid useful as fumigant insects with strong toxicity due to volatile and have a lipophilic nature which can penetrate into the insect's body, thus affecting physiological functions of insects. Volatile compounds are responsible for the strong odor that can block the insect tracheal respiration. An important mechanism of action of EO in insects is the inhibition of acetylcholinesterase (AChE), which is a key enzyme to end the transmission of nerve impulses through the sympathetic pathway, causing paralysis and death [11].

The main compound on EO of *Eucalyptus* sp. is cineole, is included monoterpenoid [12]. Another research showed that the formulated nanoemultion of eucalyptus oil can be an alternative to harmful chemical pesticides against rice weevil *(Sitophilus oryzae)* with LC_{50} 0.795 μL/cm^2 [13]. The active compounds in the EO of *M. fragrans* are Myristicin, Sabinene, 4-Terpineol, α-Pinene, and Limonene. A compound of Myristicin is the most active compound with the highest toxicity as adulticidal and larvacidal *Cx. pipiens* and *Ae. Aegypti* [14]. Eugenol, Methyleugenol, Elemicin, and Myristicin isolated from *M. fragrans* repellent activity against fleas tobacco *(Lasioderma serricorne)*. In addition, Elemicin is also claimed as a compound that has the most contact toxicity to *L. serricorne,* in addition to the compound Methyleugenol, Eugenol, and Safrole isolated from EO of *M. fragrans* [15]. While the EO of *C. citratus* contains Neral and Geranial has been reported to have insecticidal activity on *Anopheles* [16].

EO of *M. fragrans* which have insecticidal activity is highest among the other EO against rice weevil *(Sitophilus oryzae)* then compared with chemical insecticide chlorpyrifos. Based on the research results that chlorpyrifos has higher insecticidal activity of the EO of *M. fragrans*. The value of significance between the EO of *M. fragrans* 40 μL/ g with chlorpyrifos is $0.032 < 0.05$. This means that the average mortality is not the same, there is an interaction between the kind of insecticide, time, and between types of insecticides with time. Exposure for 24 h already provides lethal effects on chemical insecticide chlorpyrifos, whereas in nature insecticide from EO of *M. fragrans* recently gave the effect of mortality after 48 h exposure. Comparison of the insecticidal activity of EO of *M. fragrans* 40 μL/ g that in fact have the highest insecticidal activity among other EO with chlorpyrifos are presented in Table 2.

Table 2. Insecticidal Activity of EO from *M. fragrans* 40 µL/ g and Chlorpyrifos

Times	Corrected Mortality (%)		Sig.		
(h)	*M. fragrans* 40 µL/ g	Klorpirifos	Group	Times	Group*Times
24	70.00	100.00	0.032	0.032	0.032
48	100.00	100.00			

As we know that a chemical compound organophosphorus pesticide chlorpyrifos is used in various fields of agriculture, industry and domestic. The resulting residue of the insecticide was found in the environment as well as food and drinks. Chlorpyrifos acts as an inhibitor of AChE that can limit muscle contractility and respiration patterns [17]. The function is the same as the EO of *M. fragrans*. Nevertheless, however, an insect has the ability quickly to the resistance of chemical insecticides [18].

4. Conclusion

In conclusion, our present results evidenced the comparison of the insecticidal activity of nature insecticide from various EO with chemical insecticide chlorpyrifos against rice weevil *(Sitophilus oryzae)*. EO of *M. fragrans* 40 µL/ g as nature insecticide that had highest insecticidal activity than other nature insecticide based on the results of this study had a lower insecticidal activity then chemical insecticide chlorpyrifos, although the effect on both is same as an inhibitor of AChE. However, nature insecticide can be an alternative of ingredients of insecticide to reduce the use of chemical insecticides that tend to give the effect of resistant to insect pests, adverse effects on human health and the environment

Acknowledgments

We gratefully acknowledge the support of this work by Ministry of Research, Technology and Higher Education of the Republic of Indonesia (KEMENRISTEKDIKTI). We also wish to thank Semarang State University for the support.

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