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Study on the utilization, chemical composition, and insecticidal activity of nutmeg essential oil (*Myristrica fragnans* Houtt) against fruit flies, *Bactrocera* spp. (Diptera: Tephritidae)

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ABSTRACT

This study aims to determine the activity of the essential oil nutmeg (*Myristrica fragrans* Houtt) against fruit flies Bactrocera spp. The attractant treatments used were 1) pure nutmeg essential oil (NEO), 2) petrogenol (800 g/l methyl eugenol) and 3) sterile water as a control. The variables observed were the type of species, sex, number of fruit flies captured, and activity period of NEO and Petrogenol in the field scale. The chemical composition of NEO was investigated by Gas Chromatography-Mass Spectometry (GC-MS). The results of the research indicate the period of activity of NEO and Petrogenol in the field is more than 43 days with an average of fruit fly attracted was 12.22 adults/day and 17.04 adults/day respectively. There are three species of fruit flies attracted namely *Bactrocera carambolae, B. dorsalis*, and *B. umbrosa*. Among those species, *B. carambolae* and *B. dorsalis* were dominantly attracted. GC-MS analysis shows that the highest chemical composition of NEO is obtained by 4-allyl-1,2-dimethoxybenzene-carboxylate (Methyl eugenol) (8.33%) which has the potential to be developed as a fruit fly attractant.

Key words : *Natural attractant, Essential oil, Fruit fly, Methyl eugenol, Biopesticides.*

Introduction

Fruit flies (Diptera: Tephritidae) are one of the main pests that can harm the productivity of fruit and vegetable commodities in Indonesia. Loss of fruit caused by the fruit fly maggots eating the fruit pulp rots the fruit before it ripens (Radonjic *et al.,* 2019). Fruit flies can cause direct damage to fruits and vegetables which will cause loss of agricultural production by up to 90-100% depending on the fruit fly population, locality, variety, and season (Sarwar *et al.,* 2014). In general, the loss of agricultural output due to fruit flies can vary between 30-100% (Dhillon *et al.,* 2005). About 35% of species attack soft-textured fruit, including fruit cultivated by farmers (White and Elson-Harris 1992). In India, about 50% of the Cucurbitaceae are attacked by *Bactrocera cucurbitae* (Jaleel *et al.,* 2020). Furthermore, reported

by Siwi *et al.* (2006) there are 16 species of fruit flies that are considered important pests of vegetable and fruit commodities in Indonesia.

To date, various attempts have been made to control fruit flies in Indonesia. However, the use of pesticides is still predominantly carried out on a field scale by many fruit and vegetable farmers (Ngowi *et al.*, 2007; Thanh Mai *et al.*, 2018; Wilson and Tisdell 2001). The unwise use of pesticides is considered an unsustainable effort for the environment (Aktar *et al.*, 2009); Prastika *et al.*, 2021; Afifah *et al.*, 2020), environmental hazards such as harmful side effects on beneficial insects (Sanchez-Bayo 2011), and human health (Singh *et al.*, 2018); Ibrahim *et al.*, 2020a; Ibrahim *et al.*, 2020b).

Essential oil (EO) is a potential source that can be developed as a promising bioinsecticide because the chemical content of secondary metabolites is dominated by monoterpenes, sesquiterpenes, phenylpropanoids, and hydrocarbons (Setiawan *et al.*, 2016; Butnariu and Sarac 2018; Kasman *et al.* 2020) Recently, the development of essential oils isolated from plants has great promise for use in pest control. Plants that have the potential to be used as this material are Nutmeg *(Myristrica fragrans Houtt).* Since ancient times, local people in Indonesia have used *Myristrica fragrans* seeds as a food spice, flavor, and traditional medicine for headaches and fevers (Muchtaridi *et al.*, 2010). Nutmeg essential oil also has antimicrobial, antiseptic, antiparasitic, anti-inflammatory, and antioxidant properties (Muchtaridi *et al.*, 2010; Baser and Buchbauer 2015). The report also shows that the essential oil concentration in nutmeg ranges from 5 - 15% and its secondary metabolite components are dominated by terpene hydrocarbons, oxygenated terpenes, and aromatic ethers (Gupta *et al.*, 2013; Lanari *et al.*, 2018; Baser and Buchbauer 2015).

The use of attractants or natural essential oils (EO) is one component of an integrated pest management system that is environmentally friendly and sustainable (Damos *et al.*, 2015). This method is useful as an effective, efficient, and environmentally friendly way to control fruit flies in the field. Until now, this type of synthetic attractant has been widely sold in Indonesia under the brand name Petrogenol 800 g/L methyl eugenol. Apart from synthetic attractants, there are also attractants sourced from *Ocimum* spp. plant. According to Kardinan *et al.* (2020) controlling fruit flies using *Ocimum* spp. as an attractant can increase production by up to 73%.

Based on this, this study was conducted to determine the activity of the essential oil nutmeg *(Myristica fragrans* Houtt*)* against fruit flies *(Bactrocera dorsalis* Complex*)*. This research was conducted to develop natural attractant materials that have the potential to be developed in the biological control of the agricultural sector.

Materials and Methods

Study area

The experiment was conducted in the Laboratory of Integrated Pest Management, Faculty of Agriculture, Udayana University and field the experiment was conducted in Denpasar City, Bali, Indonesia from May to September 2020.

Extraction of Esential Oils (EOs)

The nutmeg oil extract was made using the Steam Distillation method. In short, the nutmeg which consists of seeds, mace, and pulp is uniformly cut and dried for 5 - 7 days. After drying, the nutmegs are put into a kettle that has been filled with water as high as 5 cm. The kettle is tightly closed and heated. The heating process lasts for 24 hours. The essential oil and water produced are separated using a separating funnel. The essential oil produced in this distillation process is used to test (bioassay) and analyze bioactive materials by GC-MS.

GC-MS Analysis

The bioactive ingredients contained in essential oil extracts were analyzed using Gas chromatography-mass spectrometry (GC-MS) based on company protocol (Bernardi *et al.*, 2016).

Treatment and experimental design

The treatment tested pure nutmeg essential oil (NEO) and Petrogenol (800 g/l methyl eugenol) as an attractant and sterilized water as a control. The research was arranged to randomize block design with 10 replicates. Each two of the treatment units were put diagonally in the city area of Denpasar. Modified Steiner traps made from transparent plastic bottles (measuring 8 cm in diameter and 32 cm in length) (Fig. 1) were used to collect the fruit flies attracted on NEO and Petrogenol in the field (International Atomic Energy Agency, 2003).

Into trap 1 cotton was inserted containing 1.5 ml

sterilized water, while trap-2 cotton was inserted containing 1.5 ml NEO, and into trap-3 cotton was inserted containing 1.5 ml Petrogenol. Each trap was hung on the part of the plant about 2 to 3 meters above the soil surface. The distance of the inter-treatment trap (trap-1, trap-2, and trap-3) was about 40 m to 50 m, while the distance of inter replication traps was about three km. The observation of fruit flies-captured by the traps was conducted every day (24 hours after treatment).

Fig. 1. Types of fruit fly trap applied in this study

The parameters observed

The variables observed were the type of species and composition, number of fruit flies captured, and activity period of NEO and Petrogenol in the field. The analysis of the chemical composition of NEO was also done in this research using GC-MS.

Data analysis

The result of the parameters observed were statistically analyzed with SPSS program version 23.0 by ANOVA and comparing means by LSD test (Rem *et al.*, 2020).

Results and Discussion

Attractants are substances formulated to attract flies. In these studies, we used Petrogenol and NEO as attractants. They had a prominent effect on attracting flies, especially for the Tephritidae family. Average of fruit flies captured per day on the traps with Petrogenol and NEO as an attractant is 17.04 adults and 12.22 adults, respectively. These effects are significant compared with control *(P<0.05)* (Table 1). All of the adult fruit flies captured on the traps were male. There are three species of fruit flies identified that are *Bactrocera carambolae, B. dorsalis,* and *B. umbrosa* (Fig. 2). This phenomenon indicated that nutmeg essential oil can strongly attract fruit flies.

Based on *Gas Chromatography-Mass Spectrophotometer* (GC-MS) analysis indicated that one of the chemicals components of NEO was 8.33% methyl eugenol (Table 2). According to de Kogel *et al.* (2007), the chemical substance of methyl eugenol was able to attract around 17 species of fruit flies such as *B. araceae, B. carambolae, B. dorsalis, B. exornata, B. indonesiae, B. latilineola, B. muiri, B. nigrita, B. omlipitalis, B. papayae, B. platamus, B. raiensis, B. sulawesiae, B. thailandica, B. unimaculata,* and *B. verbascifoliae.* Vargas *et al.* (2000) stated that males of *B. dorsalis* were very attracted to methyl eugenol. According to Ghanim, (2013), many species of Bactrocera males after consumption of the methyl eugenol can enhance their mating competitiveness.

Table 1. Average of fruit flies captured per day on each attractant

Treatment	Average of fruit flies captured per day (adults)
Petrogenol	17.04 [a]
NEO	12.22 [b]
Distillated water	0.00 [c]

Note: NEO : *Nutmeg Essential Oil*. Different letter notations in the column indicated a significant difference between treatments ($P < 0.05$) based on the LSD test.

Figure 2 showed that species of *B. carambolae* and *B. dorsalis* were dominantly captured than *B. umbrosa*. This phenomenon was caused by both of the species having wider host plant range available in the field compare with *B. umbrosa*. According to Clarke *et al.* (2005); Maryati *et al.* (2008); Sauers-Muller, (2005) that *B. carambolae* has the widest host range and wide geographic distribution. As reported by Chinajariyawong *et al.* (2000), *B. carambolae* and *B. papayae* have the host plants more than *B. umbrosa.*

The activity period of NEO and Petrogenol attracting fruit flies in the field of more than 43 days is shown in Figure 3. The results showed that the number of fruit flies caught in traps with Petrogenol

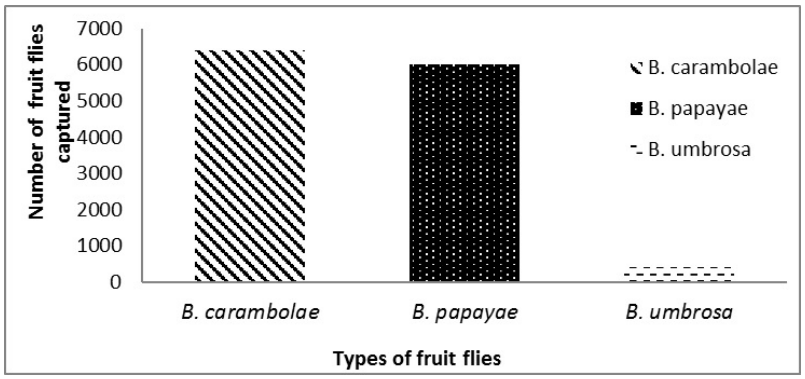

Fig. 2. Type and composition of species fruit flies captured on the traps contain Petrogenol and NEO treatments

was higher than NEO. This phenomenon can be caused by the higher content of methyl eugenol in Petrogenol treatment 800 g/l when compared to NEO treatment which is only 8.33% (Table 2). The higher content of Methyl eugenol Petrogenol had a stronger effect on attracting male fruit flies than NEO.

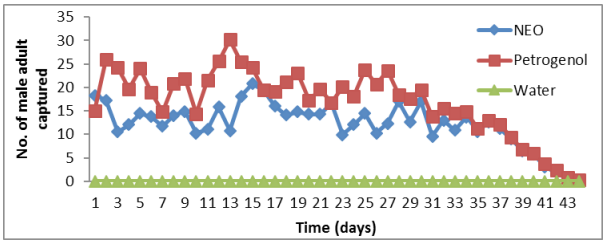

Fig. 3. Activity period of attractant to attract fruit flies number per day in the field study

Conclusion

The nutmeg essential oil *(Myristrica fragrans)* has the ability to attract only the male fruit flies *(Bactrocera dorsalis* complex*)*. There are three species of fruit flies identified that are *Bactrocera carambolae, B. papayae*, and *B. umbrosa*. Among the those species, *B. carambolae* and *B. dorsalis* were dominantly attracted. The results of GC-MS analysis showed that the compound 4-allyl-1,2-dimethoxybenzene-carboxylate (Methyl eugenol) was identified as a fruit fly attractant with a percentage of 8.33%. The activity period of nutmeg and petrogenol essential oil to attract fruit flies in the fields for more than 43 days shows that the number of fruit flies caught in the traps with petrogenol is higher than the essential oil of nutmeg.

The NEO material in this study has good development prospects in future research and still requires a lot of field trials so that it can reduce the use of synthetic pesticides.

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Table 2. Chemical composition of NEO analyzing with GC-MS

No.	Formula of molecule	Chemical components	Percentage (%)
1	$C_{10}H_{16}$	Camphene	0.89
2	$C_{10}H_{16}$	(+)-4-Carene	3.33
3	$C_{10}H_{14}$	1-methyl-4-(1-methylethyl)benzene (Camphogen)	2.18
4	$C_{10}H_{16}$	1-methyl-4-(1-methylethylidene)cyclohexene (Terpinolene)	2.91
5	$C_{10}H_{10}O_2$	5-(1-propenyl)1,3-benzodioole	1.13
6	$C_{15}H_{24}$	á-cubebene	1.11
7	**$C_{11}H_{14}O_2$**	**4-allyl-1,2-dimethoxybenzene-carboxylate (Methyleugenol)**	**8.33**
8	$C_{15}H_{24}$	Caryophyllene	0.62
9	$C_{11}H_{14}O_2$	1,2-dimethoxy-4-(1-propenyl)benzene (Methylisoeugenol)	5.02
10	C11H12O3	4-methoxy-6-(2-propenyl)1,3-benzodioxole (Myristicine)	6.58
11	C12H16	1,2,3-trimethyl-5-(2-propenyl)benzene	3.0

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