FRUIT FLY *Bactrocera* spp. RESPONSES TO ODOUR OF CURRY LEAVES, *Murraya koenigii* L. SPRENG

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ABSTRACT

Bactrocera spp. fruit fly is a major pest for various horticultural crops, particularly fruit trees. The intensity of fruit fly attacks in several Indonesia areas shows a fairly large variation and it needs an intensive control program. Moreover, this research was conducted to determine the semiochemical compounds of curry leaves for controlling fruit flies. The results showed that the volatile compounds of curry leaves from the GC-MS analysis were dominated by compounds of the terpene group, namely caryophyllene compounds (36.9%), hexadecene compounds (22.72%) and α-selinene compounds (20.43%). Therefore, the results of the odor detection test carried out on the Y-tube olfactometer showed that male fruit flies were more attracted to the aroma of curry leaves around 80% than female fruit flies around 57.5%. The results of this research can be developed as attractant of male fruit flies and trap them so that later it is expected to reduce fruit fly pest populations.

Keywords: Fruit fly, volatile compounds, GC-MS analysis, curry leaves

ABSTRAK

Lalat buah *Bactrocera* spp merupakan perosak utama bagi pelbagai tanaman hortikultur, terutama pokok buah-buahan. Kekuatan serangan lalat buah di beberapa kawasan di Indonesia menunjukkan variasi yang agak besar dan memerlukan kawalan yang serius. Kajian ini dijalankan untuk menentukan komposisi semiokimia daun kari untuk pengawalan lalat buah. Hasil kajian menunjukkan sebatian yang mengandungi daun kari hasil analisis GC-MS didominasi oleh sebatian daripada kumpulan terpene iaitu sebatian caryophyllene (36,9%), sebatian hexadecene (22,72%) dan α-selinene (20,43%). Hasil kajian bagi ujian pengesanan bau yang dijalankan pada olfaktometer tiub-Y menunjukkan lalat buah jantan lebih tertarik

kepada aroma daun kari (80%) berbanding lalat buah betina (57.5%). Maklumat ini boleh digunakan untuk perangkap lalat buah jantan sehingga hasilnya nanti dapat mengawal populasi perosak lalat buah.

Katakunci: Lalat buah, sebatian meruap, analisis GC-MS, daun kari

INTRODUCTION

Bactrocera sp. fruit fly is a major pest on various fruit crops. There are 4000 species of fruit flies in the world and 35% of them are important pests on fruits, including commercial fruits that have high economic value. In the western part of Indonesia, there are 90 species of fruit flies that are indigenous namely *Bactrocera albistrigata*, *B. carambolae*, *B. dorsalis*, *B. papayae, B. umbrosa, B. cucurbitae,* dan *Dacus longicornis* (Sodiq et al. 2015). Fruit fly species can be grouped into complex *dorsalis* and non-complex *dorsalis* groups. There are several examples of species belonging to the complex dorsalis group are *Bactrocera affinidorsalis*, *B. bimaculata*, *B. carambolae*, while the example of species that are not classified as complex dorsalis are *Bactrocera albistrigata, B. calumniata, B. cucurbitae* and other species (Sodiq et al. 2015; Suputa et al. 2010; Tripathi et al., 2018).

The symptoms of fruit fly attacks on fruit are visible from puncture marks of the fruit fly ovipositor in the form of black dots (Lubis et al. 2020). Fruit flies insert their ovipositor into the fruit to lay their eggs. Nevertheless, lack of yield due to fruit fly attacks varies between 30-100% depending on environmental conditions and the vulnerability of the type of fruit it attacks (Badii et al. 2015; Siwi et al. 2006). The intensity of fruit fly attacks in several Indonesian areas shows a fairly big variation. Bali is one part of the area that has fruit fly pests attack on jackfruit, starfruit, water apple, and guava around 100% (Astriyani et al. 2016). In Salahutu District, Central Maluku Regency, fruit fly attacks on chili plants are around 41- 49% and on starfruit approximately 70% (Segura et al., 2018). Sodiq et al. (2015) stated that the intensity of fruit fly attacks on mangoes ranged from 14.8-23%. However, it is not infrequently the damage caused by fruit flies such as the damage that occurs on starfruit and guava approximately 100%.

There are several ways conducted to control fruit flies are fruit bagging, using attractant trapping, spraying insecticides, and biological control. One of the control techniques to manage fruit fly pests is using by semiochemical compounds. Methyl eugenol trap is one example of the use of semiochemical compounds in controlling fruit fly pests. Curry leaf (*Murraya koenigii*) is one of the most famous plants in Indonesia. Local people use these leaves for Indonesian cuisine because the leaves have a strong odor. Several studies have shown that the leaves have insecticidal, anti-bacterial, and anti-fungal effects (Beloti et al. 2017; Kamiji et al. 2018; Miyazaki et al. 2018; Sahetapy et al. 2019). The results of the GC-MS of curry leaf extract are dominated by hydrocarbon compounds α-pinene and Caryophyllene, where these compounds are compounds that act as repellents to insects (Kamiji et al., 2018). The strong volatile aroma of curry leaves is suspected play an important role in insect activity, whether it will function as an attractant or repellent on the fruit fly *Bactrocera* spp. Therefore, this study aims to determine whether the volatile aroma of curry leaf extract can be used as a trapping to control the fruit fly *Bactrocera* sp.

MATERIALS AND METHODS

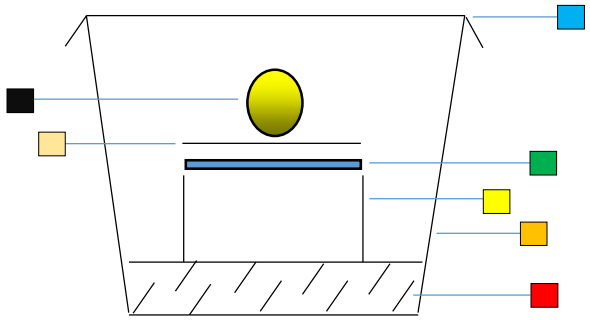
The research was conducted in the Pest and Plant Diseases Laboratory, Faculty of Agriculture, University of Muhammadiyah North Sumatra (UMSU) Medan, Indonesia. Curry leaf extract

was made in the Laboratory of Organic Chemistry, FMIPA, University of North Sumatra. Identification of volatile compounds in the GC-MS machine was carried out at the Palm Oil Research Center (PPKS) Medan laboratory.

a. Preparing Insects Samples

This study used the fruit fly samples collected from guava that had been infected with fruit flies and reared in the laboratory. Next, the infected fruit was placed into a plastic container measuring 32x23x17 cm. The breeding container was set up with sand to a height of approximately 2 cm, a small plastic container, and cloth. Fruit that has been attacked by fruit flies is placed on a small box that has been welded by cloth. Late larval stage jump out from the fruit, burrowing into the sand to pupate (Figure 1). After the formation of pupae, the sand was sifted to obtain fruit fly pupae.

The fruit fly pupae obtained were placed in a petri dish and put into a rearing box 30x30x30 cm. The fruit fly rearing box was made of wire mesh cages where one side of the cage was covered with rubber tires and a hole was made with a diameter of ±11cm (or as wide as the diameter of a mineral water cup). On top of the cage was placed a foam moistened with water to maintain moisture and also cotton moistened with a 10% honey solution as food for fruit fly adult.



Legend
- Sand
- Big Box
- Small Box
- Wire
- Black cloth
- Cloth
- Fruit samples infected with fruit flies

Figure 1. Rearing container set up of test insects (larval stage)

b. Preparation of Curry Leaf Extract

Curry leaf extract was prepared using the maceration method (Patel et al. 2019). Curry leaves were washed and then air-dried at room temperature. After the leaves were dry, 500 g of the dried leaves were taken then mashed with a blender and macerated in 3 liters of 95% methanol for 96 hours at room temperature. The liquid extract was then concentrated using a Vacuum Rotary Evaporator (VRE) with the brand Heidolph VV 2000 at 55°C and pressure of 3×10^{-1} mbar. This thick extract was then tested on a GC-MS machine to see the volatile compounds contained in the curry leaves.

c. Semiochemical Testing

Semiochemical testing of fruit flies was carried out using an olfactometer, which consisted of a Y-tube with each branch connected to an Erlenmeyer tube. At each end of the arm of the Y

tube, a small fan is placed which functions to circulate airflow to each arm of the Y tube. Semiochemical experiments were carried out with; a) Inserting 1 male fruit fly adult emerged from guava at the bottom of the Y tube and repeated 10 times for each experimental unit. The experiment was carried out by comparing one arm of the Y tube containing the smell of curry leaf extract and the other arm being empty (control), b) Fruit flies are categorized as choosing the odor if the fruit fly moved to one of the arms and stayed there for 5 minutes, and if the fruit fly still stayed at the bottom of the Y tube for 5 minutes without making a choice, then indicates that the fruit fly does not choose the given smell.

RESULTS

GC-MS Analysis

The results of GC-MS analysis showed that the three main peaks of volatile compounds found in curry leaves (*M. koenigii*) were caryophyllene, hexadecene and alpha selinene (Figure 2). In addition, there were several other compounds detected from the GC-MS results, most of which were compounds from the terpenoid group shown in Table 1.

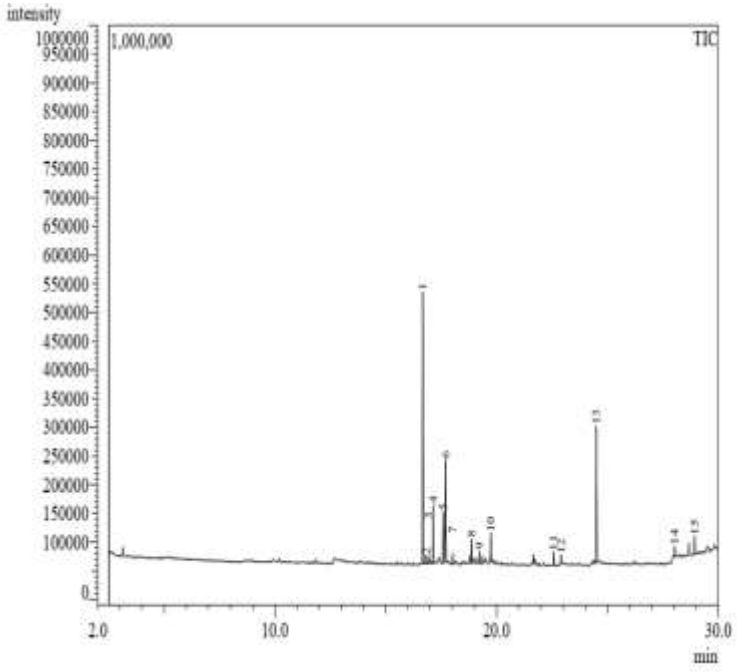


Figure 2. Chromatographic results of volatile compounds from curry leaves

Table 1. Volatile compounds from GC-MS curry leaves

No.	RT (Minutes)	Molecular Formula	Compound Name	% Area
1	16,667	$C_{15}H_{24}$	Trans-Caryophyllene	33.47
2	16,817	$C_{15}H_{24}$	Trans-Alpha-Bergamotene	1.71
3	16,933	$C_{15}H_{24}$	(+)-Aromadendrene	1.57
4	17,142	$C_{15}H_{24}$	Alpha-Humulene	7.30
5	17,591	$C_{15}H_{24}$	Beta-Selinene	6.99
6	17.702	$C_{15}H_{24}$	Alpha-Selinene	13.44
7	18.018	$C_{15}H_{24}$	Delta-Cadinene(-)	1.38

8	18,877	$C_{15}H_{24}O$	(-)-Caryophyllene oxide	3.43
9	19,233		Junipercamphor	1.76
10	19,748		Junipercamphor	3.58
11	22,578	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	1.72
12	22,912	$C_{16}H_{32}O_2$	Hexadecanoic acid (CAS) Palmitic acid	1.42
13	24,503	$C20H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*-E]-(CAS) Phytol	18.05
14	28.035	$C_{19}H_{38}O$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS) 2-Monopalmite	1.53
15	28,939	$C_{13}H_{15}IN_2O_2$	IH-Indol, 3-Tert.Butyl-1-Methyl-2-Phenyl	2.65

Olfactometry Test

The results of the curry leaf odor test on the olfactometer tube showed that 80% of male fruit flies chose curry leaf aroma (Figure 3), while only 57.5% of female fruit flies prefer the scent of curry leaves (Figure 4).

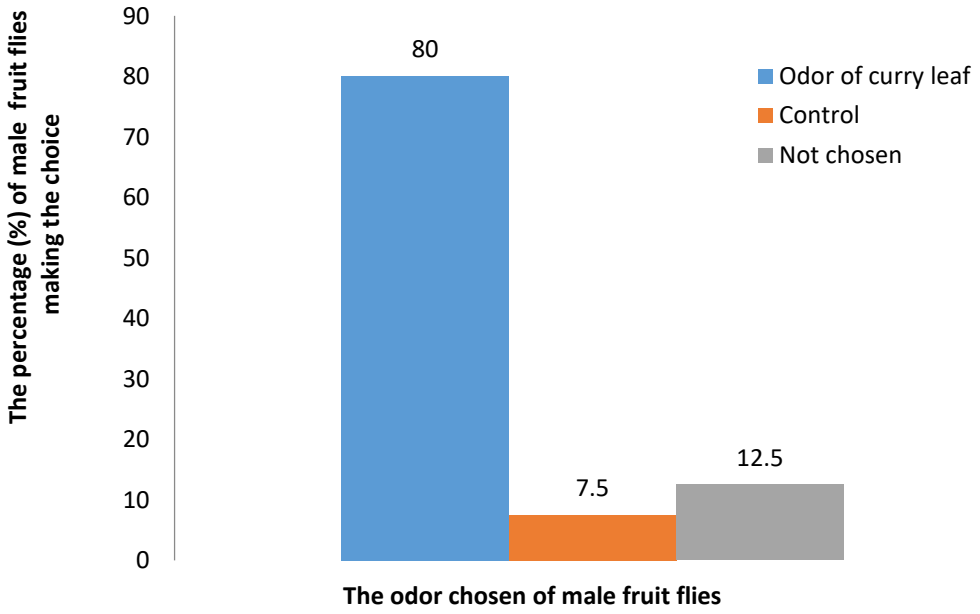


Figure 3. Selection of the smell of curry leaves by adult male fruit flies

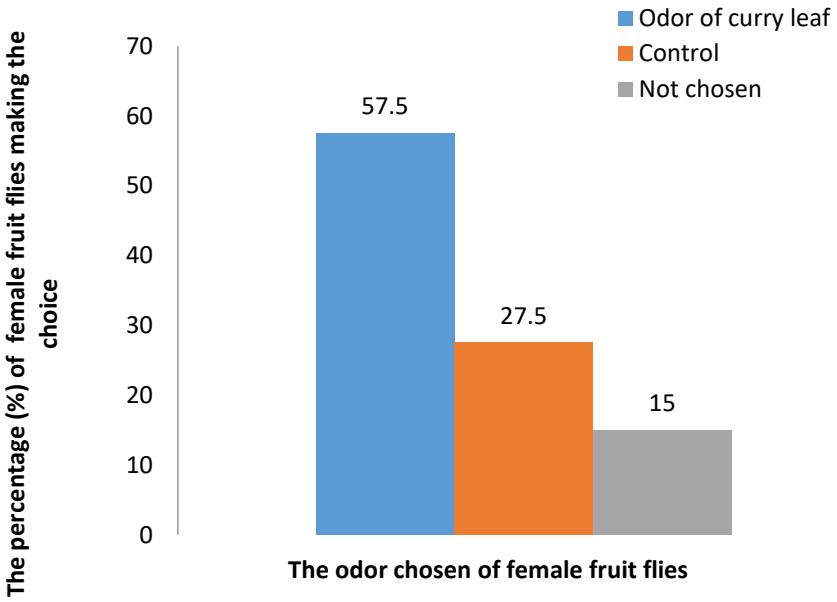


Figure 4. Selection of the smell of curry leaves by adult female fruit flies

DISCUSSION

Volatile compounds have a lot of use as an alternative for controlling pests, they can be able to pest control and as an attractant or repellent. Moreover, insects can communicate between species and intra-species utilizing chemical compounds which are often referred to as semiochemicals (El-ghany, 2019). The volatile compounds that have the highest peaks from curry leaves as a result of GC-MS analysis are caryophyllene and hexadecene compounds. This compound is a compound of the terpene group. Caryophyllene compounds are compounds found in a lot of herbs and vegetables such as cloves, black pepper, and various other plants. This compound has many benefits, including being used as an antimicrobial (Jaleel et al. 2019; Zeni et al. 2021), and also used as a repellent (Adjalian et al. 2015 ;Góngora et al. 2020; Kamiji et al. 2018) or attractant to various types of insects (Lunau, 2000).

The behavior of phytophagous insects in selecting host plants involves a series of signaling processes between plant volatile organic compounds and insect sensor systems. Detection of plant metabolites by insects occurs through the response of various receptor organs; olfactory, gustatory, tactile, and visual (Malwal et al. 2009; Mustanir et al. 2019; Malwal et al., 2009). Olfactory receptor neurons are receptors that are responsible for receiving signals from volatile compounds emitted by plants. The signal is processed and interpreted by a combination of several receptors and influences the insect's decision to choose food sources, lay eggs, and insect growth and development (Sharma et al. 2017).

Volatile compounds from the essential oil group formed from secondary metabolites of many plants can function as attractants or insecticides depending on the dose used (Zeni et al., 2021). Keyta & Zuharah (2021) explained that cashew nut liquid extract can be a repellent for *Bactrocera dorsalis* fruit flies that attack papaya. According to Ghosh et al. (2021) caryophyllene compounds found in Albizia trees can function as attractants and also be used as

insecticides for several types of insects, while the results of this study Adjalian et al. (2015) stated that the caryophyllene and humulene compounds contained in the leaves of *Premma angolensis* and *Premma quadrifolia* were repellent and insecticidal in *Sitotraga cerealella* larvae. This statement similar to Jaleel et al. (2019) explained that caryophyllene and humulene compounds were attractants for female fruit flies *Bactrocera dorsalis* and *Bactrocera correcta*. The role of volatile compounds, whether they will act as a repellent, attractant, or insecticide on insects, depends on the dose of volatile compounds used (Zeni et al., 2021).

The olfactometry experiments showed that male flies were more attracted to the volatile aroma of curry leaves than female fruit flies (Figure 2). This is because caryophyllene is the dominant volatile compound obtained from the GC-MS analysis of curry leaves. This compound is a combination between the compound terpene group and the compound of the eugenol group. Eugenol is a compound that required by male fruit flies to carry out copulation activity (Widihastuty et al. 2021). Methyl eugenol compounds which are commonly used as compounds for fruit fly traps are derived compounds from the eugenol group. Methyl eugenol is one of the stimuli to increase the success of fruit fly mating. Methyl eugenol attractant is classified as a food lure (Hasyim et al. 2020). The response of male fruit flies to methyl eugenol can fluctuate. The results of Wee & Wei Hee (2020) research found that male fruit flies respond more to methyl eugenol in the morning than in the afternoon. Male flies will try to get methyl eugenol before mating. However, Kamiji et al. (2018) stated that *Bactrocera correcta* male flies responded more to caryophyllene compounds than methyl eugenol (ME) compounds for a faster sexual maturation process.

In addition, caryophyllene being an attractant, these compounds are thought to be able to act as insecticides for several types of insects (Adjalian et al. 2015; Ghosh et al. 2021). Therefore, fruit flies who are attracted to the aroma of curry leaves and taste the curry leaf extract, and look to lose their flight balance during observation. This is in line with the statement of Zeni et al. (2021) that volatile compounds obtained from secondary metabolites of plants can function as attractants and insecticides depending on the dose used.

CONCLUSION

The volatile compounds based on the GC-MS analysis from curry leaves (*M. koenigii*) are compounds from the terpene group, namely caryophyllene, hexadecene, and α-selinene. Compounds of this group have a function as attractants and as insecticides. Male fruit flies were attracted to the smell of curry leaves more (80%) than female fruit flies which were only 57.5%. The interest of fruit flies in the aroma of curry leaves has opportunities to use curry leaves as an alternative for controlling the fruit fly pest *Bactrocera* sp.

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AUTHORS DECLARATIONS

Funding Statement

Funding statement has been explained in acknowledgement

Conflict of Interest
The authors declare that they have no conflict of interest.

Ethics Declarations
No ethical issue required for this research.

Data Availability Statement
My manuscript has data related to Widihastuty et al. research on Fruit Fly *Bactrocera* spp. responses to odour of curry leaves *Murraya koenigii* L. SPRE**NG** data.

Authors Contributions
W, DA and SU conceived this research and designed experiments;W, AM, and KH participated in the design and interpretation of the data; W, DA, and SU performed experiments and analysis; W and AM wrote the paper and participated in the revisions of it. All authors read and approved the final manuscript. All authors read and approved the final manuscript.

REFERENCES

Adjalian, E., Sessou, P., Odjo, T., Figueredo, G., Kossou, D., Avlessi, F., Menut, C., & Sohounhloué, D. 2015. Chemical Composition and Insecticidal and Repellent Effect of Essential Oils of Two *Premna* Species against *Sitotroga cerealella Journal of Insects* 319045: 1–6.

Astriyani, N.K.N.K., Supartha, I.W. & Sudiarta, I.P. 2016. Kelimpahan populasi dan persentase serangan lalat buah yang menyerang tanaman buah-buahan di Bali. *Journal of Agricultural Science and Biotechnology* 5(1): 19–27.

Badii, K.B., Billah, M.K. & Nyarko, G. 2015. Review of the pest status , economic impact and management of fruit-infesting flies (Diptera :Tephritidae in Africa. *African Journal of Agricultural Research* 10(12): 1488–1498.

Beloti, V.H., Santos, F., Alves, G.R., Bento, J.M.S. & Yamamoto, P.T. 2017. Curry leaf smells better than citrus to females of *Diaphorina citri* (Hemiptera : Liviidae). *Arthropod-Plant Interactions* 11(5): 709–716.

El-ghany, N.M.A. 2019. Semiochemicals for controlling insect pests. *Journal of Plant Protection Research* 59(1): 1–11.

Ghosh, A., Majumder, S., Saha, S., Chakraborty, S. & Bhattacharya, M. 2021. Leaves and barks of Albizia shade trees in tea plantation shows both insect attractant and pesticidal properties: A GC-MS based investigation. *Asian Journal of Agriculture* 5(2): 84–89.

Góngora, C.E., Tapias, J., Jaramillo, J., Medina, R., Gonzalez, S., Casanova, H., Ortiz, A. & Benavides, P. 2020. Evaluation of terpene-volatile compounds repellent to the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Curculionidae). *Journal of Chemical Ecology* 46(9): 881–890.

Hasyim, A., Lukman, L. & Setiawati, W. 2020. *Teknologi Pengendalian Hama Lalat Buah*. Badan Penelitian dan Pengembangan Pertanian. Jakarta: IAARD Press.

Jaleel, W., He, Y. & Lü, L. 2019. The response of two *Bactrocera* species (Diptera: Tephritidae) to fruit volatiles. *Journal of Asia-Pacific Entomology* 22(3): 758–765.

Kamiji, T., Kaneda, M., Sasaki, M. & Ohto, K. 2018. Sexual maturation of male *Bactrocera* correcta (Diptera: Tephritidae) and age-related responses to β-caryophyllene and methyl eugenol. *Applied Entomology and Zoology* 53(1): 41–46.

Keyta, S. & Zuharah, W. F. 2021. Repellent Action of cashew (*Anacardium occidentale* L.) Nut Shell Liquid (Cnsl) On adult papaya fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Serangga* 26(2): 312–324.

Lubis, E., Susanti, R. & Nurhajizah. 2020. Sosialisasi teknologi pengendalian lalat buah *Bactrocera* sp yang ramah lingkungan di Desa Kubu Colia Kecamatan Dolat Rakyat. *Journal Prodikmas Hasil Pengabdian Kepada Masyarakat* 5(3): 21–25.

Lunau, K. 2000. The ecology and evolution of visual pollen signals. *Plant Systematics and* .

*Evo*lution 222: 89-111.

Malwal, M., Sarin, R., Shakeet, P. & Bakshi, S. 2009. Natural insect controlling agents from *Murraya koenigii* (L .) Spreng. *Journal of Herbal and Toxicolog*y 3(2): 161–162.

Miyazaki, H., Otake, J., Mitsuno, H., Ozaki, K., Kanzaki, R., Chui-Ting Chieng, A., Kah-Wei Hee, A., Nishida, R. & Ono, H. 2018. Functional characterization of olfactory receptors in the Oriental fruit fly *Bactrocera dorsalis* that respond to plant volatiles. *Insect Biochemistry and Molecular Biology* 101: 32–46.

Mustanir, M., Al-Qarana, T.R., Gusvianna, H. & Saidi, N. 2019. Analisa Potensi ekstrak daun kari (*Murraya koenigii* L. Spreng). *Talenta Conference Series: Science and Technology* (ST) 2(1): 1–8.

Patel, K., Panchal, N. & Ingle, P. 2019. Techniques adopted for extraction of natural products extraction methods: Maceration, percolation, soxhlet extraction, turbo distillation, Supercritical fluid extraction. *International Journal of Advanced Research in Chemical Science* 6(4): 1–12.

Sahetapy, B., Uluputty, M.R. & Naibu, L. 2019. Identifikasi lalat buah (*Bactrocera* spp.), pada tanaman cabai (*Capsicum annum* L.) dan belimbing (*Averrhoa carambola* L.) di Kecamatan Salahutu Kabupaten Maluku Tengah. *Agrikultura* 30(2): 63–74.

Segura, D.F., Belliard, S.A., Vera, M.T., Bachmann, G.E., Ruiz, M.J., Jofre-Barud, F., Fernández, P.C., López, M.L. & Shelly, T.E. 2018. Plant chemicals and the sexual behavior of male Tephritid fruit flies. *Annals of the Entomological Society of America* 111(5): 239–264.

Sharma, S., Kooner, R. & Arora, R. 2017. Insect pest and crop losses. In Arora, R. & Shandu, S. (eds). *Breeding Insect Resistant Crops for Sustainable Agriculture*, pp. 45–66. Singapore: Springer Nature Singapore Pte Ltd.

Siwi, S., Hidayat, P. & Supua. 2006. *Taksonomi dan Bioekologi Lalat Buah Penting Bactrocera* spp. Jakarta: Balai Besar Penelitian Dan Pengembangan Bioteknologi Dan Sumberdaya Genetik Pertanian, Departemen Pertanian.

Sodiq, M., Sudarmadji & Sutoyo. 2015. Efektifitas atraktan terhadap lalat buah belimbing di Jawa Timur. *Agrotop* 5(1): 71–79.

Suputa, Trisyono, Y.A., Martono, E. & Siwi, S. S. 2010. Update on the host range of different species of fruit flies in Indonesia. *Jurnal Perlindungan Tanaman Indonesia* 16(2): 62–75.

Tripathi, Y., Anjum, N. & Rana, A. 2018 Chemical composition and in vitro antifungal and antioxidant activities of essential oil from *Murraya koenigii* (L.) spreng. leaves. *Asian Journal of Biomedical and Pharmaceutical Sciences 8*(65): 6–13.

Wee, S.L. & Wei Hee, A.K. 2020. Diurnal attraction of fruit flies (Diptera: Tephritidae) to Methyl eugenol in a village ecosystem in Tanjung Bungah, Penang, Malaysia. *Serangga* 23(2): 83–91.

Widihastuty, Ardilla, D. & Tarigan, D.M. 2021. Pembuatan bioatraktan dari daun cengkeh (*Syzigium aromaticum*) Untuk mengendalikan hama lalat buah *Batrocera* sp. *Jurnal Masyarakat Mandiri* 5(6): 5–11.

Zeni, V., Benelli, G., Campolo, O., Giunti, G., Palmeri, V., Maggi, F., Rizzo, R., Lo Verde, G., Lucchi, A. & Canale, A. 2021. Toxics or lures? Biological and behavioral effects of plant essential oils on tephritidae fruit flies. *Molecules* 26(19): 1–42.