J. Trop. Plant Pests Dis.
Vol. 22, No. 1, March 2022
Pages: 16–22

ISSN: 1411-7525
E-ISSN: 2461-0399
DOI : 10.23960/j.hptt.12216-22

RESEARCH PAPER

The effect of methyl eugenol from *Ocimum minimum* on the sticky trap to the direction and daily activity of fruit flies (*Bactrocera* spp.)

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Manuscript received: 8 July 2021. Revision accepted: 8 October 2021. Available online: 9 March 2022.

ABSTRACT

The fruit fly *Bactrocera* spp. is a major pest in the horticulture. It caused several important commodities to rot and fall which lead into large loss, or even crop failure. The research objective was to obtain information regarding the activity of fruit fly during night and day and the most attractive direction of sticky trap to trap the fruit flies. The research consisted of 3 activities, namely (1) analysis of active ingredient of Ocimum plant, (2) study on the effect of ocimum sticky traps on the direction of fruit flies, and (3) study on the effect ocimum sticky traps on the daily activity of fruit flies. The study on the effect of time and direction of trap consisted of 4 treatments (North, South, East and West) and 6 replications. A sticky trap contained methyl eugenol as active ingredient, smeared on a yellow plastic surface measuring 10 × 20 cm and attached to 2 m high wooden poles, each facing East and West, as well as North and South (as opposed to one wood). Observations were made every hour starting from 03:00 am to 07:00 pm (until there was no fruit flies get trapped in each treatment). The results showed that sticky trap used contain Ocimum oil containing 76% of methyl eugenol used as an attractant for fruit flies. Fruit flies active during the day where the highest/peak activity of the fruit flies population occured at around 07:00 am. The direction of trap to East and West catched more fruit flies number than to North and South.

Key words: attractant, *Bactrocera* spp., control, orchard, period

INTRODUCTION

One of the important pests in the horticulture field is fruit fly, which is a very harmful pest in the world, especially in Asia and has spread to almost all parts of the world (Liu et al., 2019). Fruit flies that are widely found in Indonesia are from the genus *Bactrocera*. Research conducted by Suputa et al. (2010) showed that there were 40 species of *Bactrocera* that already present in Indonesia. Among these many species, one of the most important and malignant species is *Bactrocera dorsalis* (Diptera: Tephritidae) Hendel Complex (Siwi et al., 2006). *B. dorsalis* is polyphagous, which has about 26 hosts, such as star fruit, guava, tomato, red chili, melon, apple, jackfruit, and mango which causes young infected fruit rotten and eventually fall off, thereby reducing production by 30–40%, even in certain commodities can result in crop failure (Susanto et al., 2017).

The ability to lay eggs of a female fly in optimum conditions is very large, around 3000 eggs during her life, while in field conditions it can produce between 1200 to 1500 eggs, so the potential to produce a new generation population is quite high (Weems et al., 2019). Even the fruit fly infestation rate on mango plants in Myanmar from 2016 to 2018 increased every year, where the highest number was recorded at 78.2 ± 6.5% with the population reaching its peak in June every year (Maung et al., 2019).

Several control techniques have been developed, including the use of GA (gibberellic acid), which makes the fruit look immature, so that fruit flies are reluctant to lay eggs on fruit (Greany et al., 1991). Spraying fruit with kaolin will reduce the attack rate of fruit flies (Mozhdehi & Kayhanian, 2014) or spraying with coconut oil which will act as an anti-ovipositant (suppressing eggs on fruit) (Hidayat et al., 2018). Fruit flies control using toxic baits (insecticides) is considered to kill more fruit flies (Varikou et al., 2014; Stonehouse et al., 2002). In addition, the application technique for releasing sterile insects (especially barren male insects) has been widely used and has yielded satisfactory results. Other techniques that have been successfully developed in Australia are foliage baiting (use of toxic bait), cover spraying (spraying plants and their fruit with insecticides) and trapping (trap with attractants), in addition to maintaining garden sanitation (Sarwar, 2015; Broughton & De Lima, 2002). Researchers in

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Germany developed a genetic engineering technique (Genetically Modified Organism) on male flies, so that male flies normally mate with females, but the eggs that are formed will not become larvae. This method is considered to be better than the sterile insect technique, which results in male that have been spayed often unable to compete with normal male in nature for females, so they are considered less effective (Leftwich et al., 2014). The wrap technique is also often used by the farmers. The fruit will be wrapped in plastic, paper or other material to protect it from the fruit flies. It is a simple physical barrier to the oviposition but it has to be applied well before the fruit is attacked. But this traditional technique is considered inconvenient sometimes because it is quite difficult to reach all the fruit on the tree, although it is quite effective. In several studies, the fruit wrapping technique has been shown to reduce the level of fruit fly infestation (Swibawa et al., 2003; Mondal et al., 2015; Zulina et al., 2020). It can be also resulted in increasing weight of fruit, earlier fruit maturity, and better fruit quality (in terms of colour and glossiness) (Mondal et al., 2015).

One of the fruit fly control techniques is the use of botanical attractant (fruit fly lures with the active ingredient of methyl eugenol – $C_{11}H_{14}O_2$) which can reduce the use of synthetic chemical pesticides up to 75–95% (Vargas et al., 2015; Lengkong et al., 2011). Ocimum minimum that is known to produce several aromatic compounds such as methyl eugenol, eugenol, and cavicol (Telci et al., 2009). Methyl eugenol has been used as a strong insect attractant, especially to fruit flies (*Bactrocera* species) for years (Tan & Nishida, 2011). The use of this attractant is usually used in conjunction with traps with certain colours such as yellow which are attractive to fruit flies (Sikandar et al., 2017; Said et al., 2017; Tarwotjo et al., 2019; Susanto et al., 2020; Mulyadi et al., 2021). The height of the trap is also one of the factors that affect the success of trapping. It is usually adjusted to the plants around the trap, starting from 0.25–0.50 m (Said et al., 2017) up to 1–3 m above ground level (Tarwotjo et al., 2019). The objective of the research was to obtain an updated information regarding the activity of fruit fly in Bogor Regency, during night and day and the most attractive direction of sticky trap to trap fruit fly, so that these information could be used as a basis for developing control techniques.

MATERIALS AND METHODS

Research Site. The research was conducted from December 2020 to January 2021 at the Orchard in Bogor, West Java. The *O. minimum* came from the plant collection in ISMECRI. The analysis of the content of the active ingredient (Methyl eugenol–$C_{11}H_{14}O_2$) contained in the *O. minimum* (bush basil) oil was carried out in the Indonesian Spice and Medicinal Crops Research Institute Laboratory.

Preparation of *O. minimum* Essential Oil. The plant material in the form of *O. minimum* leaves and flowers was distilled to produce essential oil. The essential oil was analyzed by Gas Chromatography (GC) to determine the content of the active ingredient, namely methyl eugenol (C11H14O2).

Gas Chromatography Analysis. The analysis was carried out in the Indonesian Spice and Medicinal Crops Research Institute (ISMECRI) Laboratory with GC under certain operating conditions (instrument: Agilent 6890 N; detector: ionization flame (FID); column: capillary, 3 m long, 0.25 mm in diameter, contains carbowax 20 M; propulsion gas (carrier): nitrogen; flow rate: 1 mL/min; column temperature: 60–200 ºC; rate: 30/min; injector temperature: 220 ºC; temperature detector: 250 ºC; and injection volume: 0.2 µL).

Application of *O. minimum* Essential Oil. Ocimum essential oil was mixed with glue with a concentration of 10%. The glue was in the form of mousetrap glue (PT Megasari Makmur, Indonesia) which was first diluted with gasoline (with a ratio of 7:3) before being mixed with ocimum oil (the ratio of glue to ocimum oil is 9:1). Furthermore, the glue was applied evenly on four yellow plastic surfaces measuring 10 × 20 cm, each facing north, south, east and west, then was placed in a relatively open place about 2 m high, surrounded by fruit trees such as guava, jackfruit, *rambutan*, mango and other fruit trees (Figure 1).

Effect of *O. minimum* Sticky Traps on the Direction and Daily Activity of Fruit Flies. The study was designed in a randomized block, 4 treatments and 6 replications. The treatment consisted of yellow plastic facing 4 directions (North, South, East, and West) measuring 10 × 20 cm which was smeared with transparent glue containing methyl eugenol, then was attached to a wooden pole 2 m high above the ground, each in opposite directions (North and South, East, and West). Observations on the number of fruit flies trapped were counted every hour starting at 3:00 am until there were no more fruit flies trapped.

RESULTS AND DISCUSSION

Methyl Eugenol ($C_{11}H_{14}O_2$) Content. The results of GC analysis could be seen in Figure 2. It showed that methyl eugenol ($C_{11}H_{14}O_2$) was the most dominant chemical compounds found in ocimum oil covering up to 76.30%. This data quite compatible with other research by Hadipoentyanti & Wahyuni (2008) which stated that *O. minimum* had a high methyl eugenol content of 68.00%. In other reasearch done by Telci et al. (2009), the content of methyl eugenol in *O. minimum* was quite low (2–3%). But, this could be due to plant phase, time of collection, extraction methods, and many other factors. These factors may affect the essential oils percentages (Skalicka-Wozniak et al., 2009).

In nature environment, when any part of plant (especially the leaves) was damaged, many male fruit flies were attracted to the damaged part. This indicated the release of methyl eugenol (Tan & Nishida, 2011). A lot of studies showed that methyl eugenol was a strong attractant for male fruit flies. It considered as one of food sources needed by male fruit flies to increase their fitness level before copulation thus making the copulatory success rate getting higher. Research by Reyes-Hernandez et al. (2018) stated that the whole body protein (as an important indicator to male quali-

Figure 1. Ocimum sticky trap facing 4 directions.

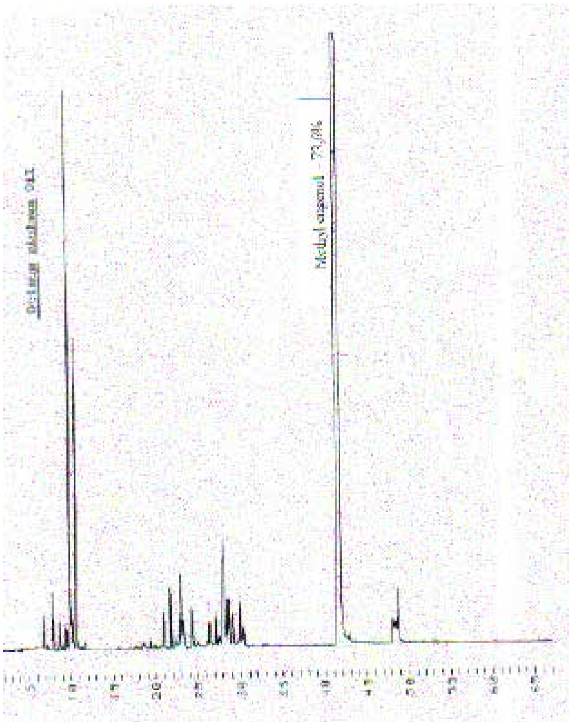

Figure 2. Ocimum essential oil chromatogram.

ty) in males with access in methyl eugenol was higher compared to males without access to methyl eugenol. This suggested that methyl eugenol affected the protein metabolism or protein synthesis. This higher metabolism can have positive impacts to male mobility and sexual behaviour, meanwhile the higher male quality can make the female more attracted so that the copulatory rates will get higher.

Therefore, immediately after detecting methyl eugenol, male fruit flies will come to the source trying to consume the methyl eugenol. This male fruit flies feel the need of methyl eugenol. The attracted males even often demonstrated aggression or territorial behaviour on other alighted males at the same methyl eugenol source (chasing away and/or head-on fighting against other intruding males). This can be translated as if they are protecting a resource of good value (Wee et al., 2017). When this sticky trap is combined with a certain trap colour (Mulyadi et al., 2021) and other integrated fruit flies management strategies, it will be more effective at catching more male fruit flies .

Several of male fruit flies (*Bactrocera dorsalis* and *Bactrocera papayae*) even more sensitive to methyl eugenol and responded at a younger age before mating. This will make the fruit flies control program more effective because it can trap the males before they mate. This will reduce the numbers of potential mates from population, therefore will cut the numbers of gravid females from causing the damage on fruits (Wee et al., 2002).

Effect of *O. minimum* Sticky Traps on Daily Activity of Fruit Flies. The results showed that there were 2 waves of fruit fly population activity. The fruit fly began to be trapped at 4:00 am with 1 fly/trap, then was increased to about 2 flies/trap at 05:00 am and around 14 flies/trap at 06:00 am. The highest number of catches was obtained at 07:00 am, which was about 35 flies/trap and began to decrease at 08:00 am to 03:00 pm. This showed that fruit flies were very active in the morning around 07:00 am when the atmosphere and the air temperature was warm. However, at 04:00 pm, there was an increase in the number of catches from about 3 flies/trap at 03:00 pm to around 10 flies/trap at 04:00 pm. It was presumed that the atmosphere was shady with warm temperature, namely at 04:00 pm like the situation in the morning around 07.00 am, so the fruit flies were active again and after that they began to decline until at 07:00 pm was only trapped in the below 1 fly/trap and no more were trapped at 08:00 pm (Figure 3).

Insect activity is generally grouped into nocturnal, diurnal and crepuscular. One of these activities is influenced by biotic factors (competition, predators, and others) (Gottlieb et al., 2005). Insects in several ways try to avoid predators, such as through mimicry, disguise, chemical defense and other means. Insects regulate their activity patterns to survive and avoid predators, so that their activities do not coincide with the time of their predatory activities (Fournet et al., 2004; Barbosa & Castellanos, 2005). The other factor that influenced insect activity is the weather. This research activity was carried out in rainy season where the weather conditions on that day were as follows: temperature= 22–33 °C, humidity 70%, wind velocity= 10 km/hour, and wind direction= south (BPBD, 2021; BMKG, 2021). We calculated rainfall of 4 mm. These climatic factors had been shown by several studies to affect fruit fly. Minimum and maximum temperature had positive correlation with fruit fly population

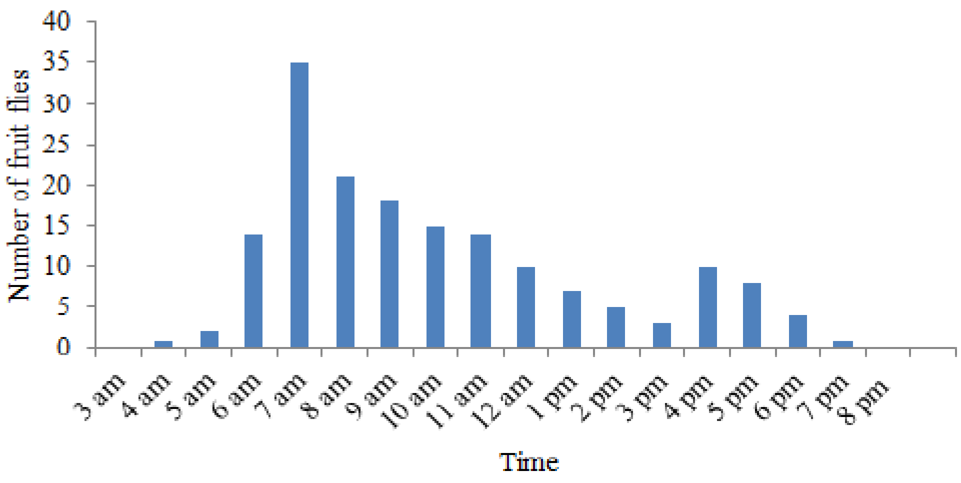

Figure 3. The number of fruit flies trapped per trap.

(Barma & Jha, 2011; Raghuvanshi et al., 2012; Das et al., 2017; Bansode & Patel, 2018; Vignesh et al., 2020). A study showed that rainfall had positive correlation with fruit fly population (Barma & Jha, 2011). But another study showed that rainfall had negative correlation with fruit fly population (Das et al., 2017; Vignesh et al., 2020). Morning humidity had positive correlation (Das et al., 2017), meanwhile afternoon or evening humidity had negative correlation (Das et al., 2017; Vignesh et al., 2020). A study by Bansode & Patel (2018) also showed that an increase wind velocity would increase the fruit fly population. However, the magnitude of this climate effect was also influenced by other factors such as the fruit fly species. A study by Math et al. (2018) showed that *B. dorsalis* had no significant relationship with these weather parameters. Meanwhile the *B. correcta* and *B. zonata* had a significant positive relationship with minimum temperature. This variation might be due to host food availability and stage of fruits.

The results of this study indicated that fruit flies were diurnal. Previously stated that the activity of female fruit flies in oviposition increase during early and late of the day more than the mid-day (Abbas, 2018). This makes the males also came at these times. Our result was also in accordance with the results shown by Manurung et al. (2012); Wee & Hee (2018); and Saputra et al. (2019). Although the most active hours of fruit flies were slightly different, this could be caused by different weather, temperature, and environmental conditions when the study was conducted. The most active time for fruit flies in this study was 07:00–09:00 am. This was in accordance with research by Wee & Hee (2018) which showed the most active time for fruit flies at 07:30–09:30 am. While other studies showed almost the same hours. The most active time in chili cultivation was 10:00 am until 02:00 pm (Saputra et al., 2019) while in citrus plantation it was 10:00 until 12:00 am (Manurung et al., 2012).

Effect of *O. minimum* Sticky Traps on the Direction. The results showed that at 06:00 am the number of fruit flies trapped was relatively the same in all treatments with a relatively low number, because fruit flies were not fully active at 06:00 am. At 09.00 am, it was seen an increase in the number of catches of fruit flies, namely traps facing to the East trapping more (35 flies/trap) and significantly different from the number of traps (facing south, north and west), followed by traps facing west with the number of fruit flies trapped of 27 flies/trap which was significantly different from the number caught by traps facing north and south (Table 1).

Traps facing west starting at 12:00 am showed the ability to trap a large number of fruit flies, thus equaling the number of fruit flies trapped in traps facing to the east and significantly different from the number of fruit flies trapped in traps facing north and south. This continued until the next observation, namely at 03:00 pm and 06:00 pm where the number of fruit flies trapped in the traps facing east and west showed no significant difference, but significantly different from the number of fruit flies trapped in the traps facing towards North and South (Table 1).

This presumably due to the light rays. The traps that pointed to the East get the morning sun, meanwhile the traps facing west received sunlight during the day. Both of them reflected the light, made it more attractive to fruit flies. It was quite compatible with other research by El-Gendy (2012) which stated that west wass the best direction for placing traps to receive the highest number of peach fruit flies (*B. zonata*). Other different result stated by Abbas (2018), where the north side of guava trees recorded the highest level by *B. zonata* infestation compared to the other sides during 2013 season.

Table 1. The total number of fruit flies trapped per trap according to the direction of the trap

Direction of the trap	Time				
	06:00 am	09:00 am	12:00 am	03:00 pm	06:00 pm
North	4 a	14 a	30 ab	32 a	39 ab
South	3 a	16 a	23 a	25 a	34 a
East	4 a	35 c	38 bc	47 b	49 c
West	7 a	27 b	40 c	42 b	46 bc

Numbers followed by the same letter at the same column are not significantly different at 5.00% DMRT.

CONCLUSION

O.minimum essential oil contained 76% of methyl eugenol ($C_{11}H_{14}O_2$) used as an attractant for fruit flies. Fruit flies were active during the day where the highest/peak activity of the fruit flies population occured at around 7:00 am. The direction of trap to East and West catched more fruit flies number than to North and South. Further research was needed to find out more about the effect of some weather parameters on the fruit flies population.

ACKNOWLEDGMENTS

Thank you and much appreciation to Galih Perkasa and Nurbetti Tarigan as Entomology research technicians at the Entomology Laboratory (ISMECRI) who have helped carry out the research from preparation, implementation to observation.

FUNDING

This research was independently funded.

AUTHORS' CONTRIBUTIONS

AK: Designed the study and statistical analysis. AK and PM: Carried out the preparation and observation, data interpretation, manuscript writing, and manuscript editing. All authors have read and approved the final manuscript.

COMPETING INTEREST

The authors state that there is no conflict of interest regarding our publication.

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