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Response of citrus fruit flies (*Bactrocera* spp.) to the application of citronella oil as a pest repellent

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Abstract: The fruit fly is one of the major citrus pests. The study aimed to evaluate the repellency of citronella oil against *Bactrocera* spp. in citrus. The two experiments were carried out in a farmer's citrus orchard in Junrejo, East Java, Indonesia from August to December 2022. In the first experiment, a randomized block design with five treatments and four replications was employed in the field to evaluate the potential repellency of citronella against *Bactrocera* spp. The treatments consisted of various mixtures of citronella and methyl eugenol. In the second experiment, the effect of one and two applications of 2 mL /L of citronella per week and synthetic pesticide on the population of *Bactrocera* spp. were compared. The results showed that citronella had a repellent capacity against *Bactrocera* spp. The number of pests trapped in citronella traps was lower than those captured in Methyl Eugenol traps only. The effectiveness of citronella as repellent for ten days. The one spraying per week of citronella was more efficient and effective in controlling *Bactrocera* spp. than other treatments. The use of citronella as a repellent could be integrated with the existing technologies to form an environmentally friendly package of fruit fly control approach.

Index Terms: Citronella oil, Repellent, *Bactrocera* spp., Citrus.

Resposta de moscas-de-frutas-cítricas (*Bactrocera* spp.) à aplicação de óleo de citronela como repelente de pragas

Resumo: A mosca-da-fruta é uma das principais pragas das culturas cítricas. Este estudo teve como objetivo avaliar o efeito repelente do óleo de citronela contra *Bactrocera* spp. em frutas cítricas. Dois experimentos foram realizados em um pomar de citros de um agricultor, em Junrejo, East Java, Indonésia, de agosto a dezembro de 2022. No primeiro experimento, um delineamento em blocos casualizados, com cinco tratamentos e quatro repetições, foi empregado em campo para avaliar a potencial repelência de citronela contra *Bactrocera* spp. Os tratamentos consis-

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tiram em diversas misturas de citronela e metileugenol. No segundo experimento, os efeitos de uma e duas aplicações de 2 mL/L de citronela por semana e pesticida sintético, na população de *Bactrocera* spp., foram avaliados. Os resultados mostraram que a citronela apresentou capacidade repelente contra *Bactrocera* spp. O número de indivíduos capturados em armadilhas de citronela foi menor do que aqueles capturados em armadilhas de metileugenol. A eficácia da citronela como repelente é de dez dias. Uma única pulverização semanal de citronela foi mais eficiente no controle de *Bactrocera* spp. do que outros tratamentos. O uso da citronela como repelente poderia ser integrado às tecnologias existentes para formar um pacote ecologicamente correto no controle da mosca-da-fruta.

Termos para indexação: Óleo de citronela, Repelente, *Bactrocera* spp., Citrus.

Introduction

Fruit fly (*Bactrocera* spp. Macquart (Diptera: Tephritidae) is a significant pest of citrus (*Citrus nobilis* Lour.). Fruit fly larvae feed on the citrus fruit and the damaged fruits eventually fall. These destructive attacks diminish fruit output in both quality and quantity. In Indonesia, the intensity of fruit fly attack varies by commodity; for example, it can reach 100% on starfruit (*Averrhoa carambola* L.) and guava (*Psidium guajava* L.), and ranges from 35-52% on citrus crop (WIJAYA et al., 2018).

Several control approaches are currently in use, including (a) fruit wrapping, (b) sanitation (collecting and destroying damaged fruits), (c) employing Methyl Eugenol (ME) as a trap for male fruit flies, (d) releasing sterile males, (e) utilizing natural enemies, and (f) insecticides application. However, fruit fly attack remains a significant obstacle in citrus production. Since the population of fruit fly correlates with the percentage of fruit fly attacks (ASTRIYANI et al., 2016), thus reducing the fruit fly numbers should be a top priority to prevent fruit fly attacks in the field.

Manipulating the insect's chemical ecology to reduce the pest preference on the crop has been used as an innovative approach to control fruit flies. The method could eventually reduce the fruit fly population. Citronella oil is one of the essential oils that has been utilized to modify the chemical ecology of the fruit fly. This oil acts as a repellent to fruit flies. Previous research have shown that citronella oil can prevent insect pests such as *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) (SAAD, MOHAMAD ROFF and IDRIS 2017), *Aedes aegypti* Linnaeus (Diptera: Cullicidae) (WANY et al., 2013), *Ephestia cautella* Walker (Lepidoptera: Pyralidae) (HASYIM et al., 2014), *Drosophila melanogaster* Meigen (Diptera: Drosophillidae) (ANGGRAENI et al., 2018), *Ceratitis capitata* Wiedemann, (Diptera: Tephritidae) (ARANCIBIA et al., 2013), *Bactrocera zonata* Saunders (Diptera: Tephritidae) (FOUDA et al.,2017) and *Bactrocera carambolae* Drew & Hancock, (Diptera: Tephritidae) (MURYATI et al., 2012). Citronella oil consists of three main compounds including citronellal, citronellol, and geraniol, all of which have insect repellent properties (MAHMUD et al., 2022). However, until recently, limited studies have been conducted on the use of citronella oil as a citrus pest control agent. Therefore, this research aimed to evaluate the effectiveness of citronella oil as a repellent in reducing *Bactrocera* spp. attacks on citrus in field conditions.

Material and Methods

Experiment site and period

The study was conducted in a farmer's 5-year-old citrus orchard in Tlekung Village, Junrejo District, Batu City, East Java, Indonesia (950 m asl; 7°54'19.5''S 112°32'07.6"E; Figure 1) from August to December 2022. The location citrus orchad selected was bearing fruits and endemic fruit fly attacked.

Figure 1. Research location.

Preparation of insect traps.

Two chemical compounds were used in the study, i.e. fruit fly attractant, commercial Methyl Eugenol (ME), acquired from the nearest agricultural store and laboratory formulation of Citronella Oil (SW). Citronella oil was extracted from lemon grass in the entomology laboratory of Brawijaya University using steam distillation procedures. Evaluation of SW oil as a fruit fly repellent refers to ME's ability to attract the presence of male *Bactrocera* spp. in the field. Insect traps were constructed using 600 ml mineral water bottles (Figure 2).

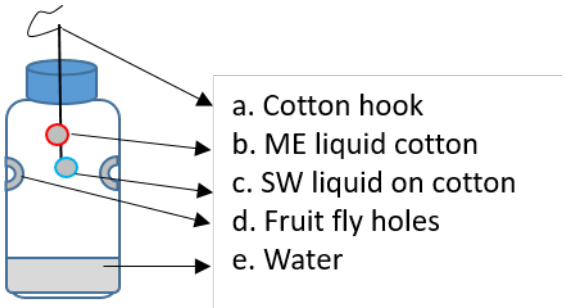

Figure 2. Fruit fly trap.

Four 0.8 cm diameter holes were drilled in the cardinal directions, and a cotton hook was inserted into the bottle lid. The chemical compounds were put on unscented cotton that was hung from a cotton hook. Both test materials used were pure materials without any addition of solvents/surfactants.

Evaluation of the capacity of citronella oil as repellent against *Bactrocera spp.*

The purpose of this experiment was to determine the potential of citronella oil as a repellent against *Bactrocera* spp. by disrupting the aroma of the male fruit fly attractant, methyl eugenol (ME). A randomized block design with 5 treatments and 4 replications was set up in the field. The treatments consisted of five combinations of citronella oil (SW) and methyl eugenol (ME), i.e. (A) 10 µL SW + 60 µL ME, (B) 20 µL SW + 60 µL ME, (C) 40 µL SW + 60 µL ME, (D) 60 µL SW + 60 µL ME, and (E) 60 µL ME only as control treatment. The materials tested were then applied into the odorless cottons that were placed within trap container. Trap containers were placed in each plot according to the treatments. The trap containers were hung on fruit-bearing citrus plants with around 10-15 meters between treatment traps. ME and SW were applied once on a day before the initial observation. Observations were conducted every day for 19 days and the number of *Bactrocera* spp. that captured in the trap container were counted daily.

Chemical components in citronella oil were identified using Gas Chromatography Mass Spectrophotometry (GC-MS) technique at the Essential Institute of Brawijaya University, in Malang, East Java. Citronella oil contains ten major compounds, including citroinella, Cyclohexene, 1-methyl-4-(1-methylethenyl)-, geranyl acetate, citronellyl acetate, E-citral, trans-caryophyllene, germacrene D, Z-Citral, Alpha. -Terpinolene and cyclohexane, 1-ethenyl-1-methyl 2,4-bis (1- successively starting with the largest concentration. The largest content of citronella oil was Citronellal (86.206%), while the content of other compounds ranged from 0.6-3.9% (Figure 3) and Table 1.

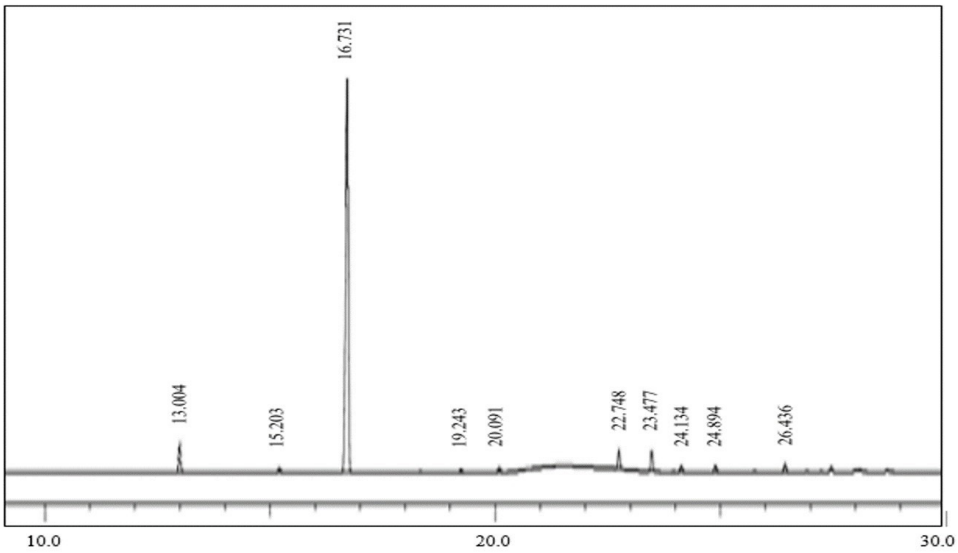

Figure 3. The compounds content of citronella oil.

Table 1. Compounds contained in citronella oil.

No.	Compound	R.Time	m/z	Area	Height	Conc	UnitR	Index
1	Cyclohexene, 1-methyl-4-(1-methylethenyl)-	13.004	68.00	538175	181666	3.887	%	0
2	Alpha.-Terpinolene	15.203	43.00	80550	20613	0.582	%	0
3	Citronellal	16.731	41.00	11934500	2967389	86.206	%	0
4	Z-Citral	19.243	41.00	86637	27960	0.626	%	0
5	E-Citral	20.091	41.00	171085	52632	1.236	%	0
6	Citronellyl acetate	22.748	43.00	316898	102373	2.289	%	0
7	Geranyl acetate	23.477	69.00	462168	145319	3.338	%	0
8	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-	24.134	41.00	42581	15675	0.308	%	0
9	trans-Caryophyllene	24.894	41.00	107077	33742	0.773	%	0
10	Germacrene D	26.436	41.00	104461	29957	0.755	%	0

Evaluation of the effectiveness of citronella oil in suppressing fruit fly attacks in citrus farm

The purpose of this experiment was to assess the efficacy of citronella oil as a repellent of *Bactrocera* spp. in the field using spray application. A randomized block design (RBD) with three treatments and ten replications was established in the 5-year-old citrus trees. The treatments were (a) once, (b) twice applications of citronella oil per week, and (c) farmer control measures were established in the field. The concentration of 2 mL/L citronella oil was used in the study as suggested by previous study on the effect of citronella oil to various fruit plant pests. So that citronella oil can form an emulsion with water, surfactant with ingredient Alkilaril poliglikol eter 400 g/L was added with concentration 2 mL/L water (ISTIANTO; EMILDA, 2021). Farmers normally apply insecticides (1-2 mL abamectin and deltametrin/L water) to control *Bactrocera* spp. every two weeks. The 1.5 L solution/tree was sprayed directly onto the citrus tree, including the fruits. The citrus farm that consisted of 150 citrus tree was used for the evaluation, and it was divided into 10 blocks that each block served as a replication and each citrus tree served as the unit sample. The number of fruits attacked by *Bactrocera* spp. was recorded every week for 3 months started from the fruit ripening period until harvesting period. Plants maintenance included fertilization, irrigation, weed removal, trimming, and disease treatment were conducted to maintain crops performances. Citronella essential oil used in the study was obtained from Batusangkar, West Sumatra.

Data analysis

Minitab 17 software was used to analyze observational data. The effect of citronella oil dosages on fruit fly repellency was examined in data analysis. The analysis of variance (ANOVA) method was employed to determine the significance of each treatment's effect. To understand the duration effect of citronella oil as repellent to *Bactrocera* spp,analysis of variance using randomized block design with split plots, concentration oil as main plot and period infestation of fruit fly as subplot was used. If there was a significant effect on the treatment, Tukey's test at 5% level was performed.

Result

Evaluation of the citronella repellency against *Bactrocera* spp.

The result of field test showed that citronella oil has the ability to repel the presence of *Bactrocera* spp.. This is shown in the different numbers of *Bactrocera* spp., in bottle traps that were given citronella oil and those without citronella oil (Figure 4).

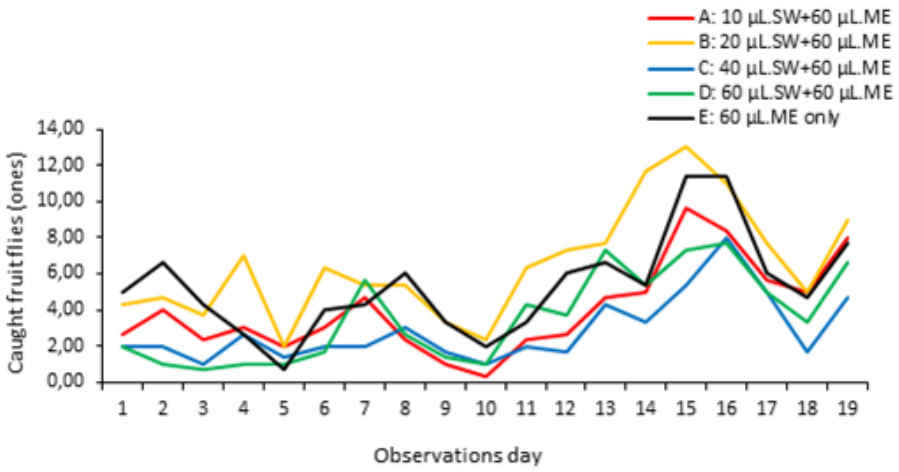

Figure 4. The number of *Bactrocera* spp. caught in trap containers.

During the 19 days of observation, the number of *Bactrocera* spp. caught in bottle trap fluctuated and there were differences among treatments. The Citronella oil treatments with volumes of 40 µL and 60 µL mL was relatively consistent in reducing the number of *Bactrocera* spp. trapped in bottle. This number was lower than the number of *Bactrocera* spp. trapped in bottle trap containing only methyl eugenol (control). The data also informed that effectiveness of citronella oil as repellent to *Bactrocera* sp for ten days. After period ten days, the number of fruit flies captured in bottle traps were higher significantly (Table 2). The 40 µL of Citronella oil more effective as a repellent to *Bactrocera* spp than other tretments but it was not different significantly with volume 60 µL (Table 3).

Table 2. The number of fruit flies caught in the bootle trap in different observation periods ($\bar{X}$ ± StDev).

No	Periods	Means
1.	1st-5th	2.79 ± 2.18 b
2.	6th-10th	3.03 ± 1.88 b
3.	11th-15th	5.91 ± 4.14 a
4.	16th19th	6.57 ± 3.50 a

Note: Means followed by the same letter in the same observation show no significant difference based on the Tukey test at a 5% level.

Table 3. The number of fruit flies caught in the bootle trap in different oil treatments ($\bar{X}$ ± StDev).

No	Volume oil treatments (µL)	Means
1.	10 µL SW + 60 µL ME	4.17 ± 2.77 bc
2.	20 µL SW + 60 µL ME	6.56 ± 4.80 a
3.	40 µL SW + 60 µL ME	2.98 ± 2.37 c
4.	60 µL SW + 60 µL ME	3.72 ± 2.91 bc
5.	60 µL ME	5.44 ± 3.11 ab

Note: Means followed by the same letter in the same observation show no significant difference based on the Tukey test at a 5% level.

Evaluation of the effectiveness of citronella oil in suppressing *Bactrocera spp.* attacks on citrus farm.

Figure 5 shows that the number of fruits attacked by fruit flies on citrus crops that were sprayed with citronella oil (A and B) were lower than on plants that were sprayed with synthetic pesticides once in 2 weeks (C). During the period of study, the number of citrus fruits attacked by fruit flies on plants that were applied with citronella oil was continually lower than the control. The was no significant difference of *Bactrocera* spp., attacks between the once and twice application of citronella oil per week. There were less than 2 fruits attacked by fruit flies in the citronella oil application plot, and it was considerably low compared to a range of 4 to 16 fruits damaged found in farmer approach plots. For the frequency of application, it is more efficient to do it once a week at a dose of 2 mL/L of water.

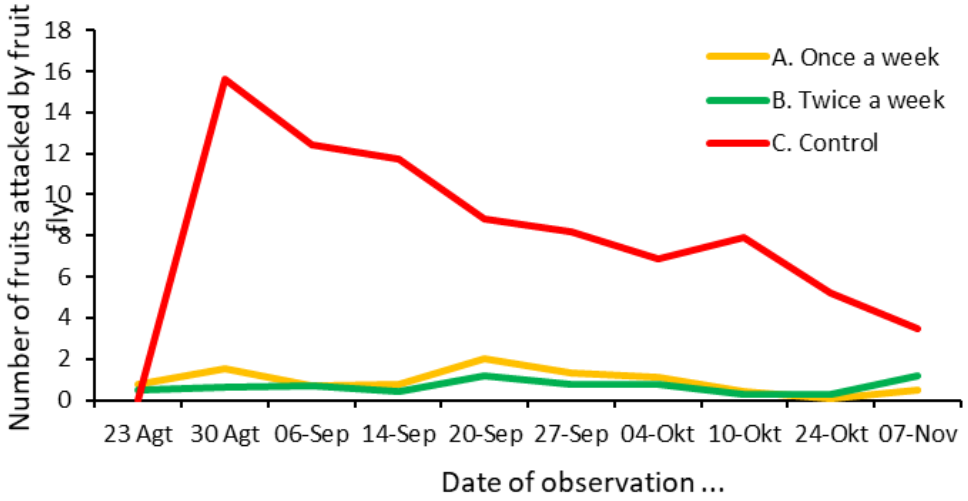

Figure 5. Number of citrus fruits attacked by fruit flies.

Discussion

The evaluation of *Bactrocera* spp. behavior revealed that they disliked the aroma of citronella oil. The study found a lower number of fruit flies that were trapped into bottle traps containing Methyl Eugenol and citronella oil compared to those in bottle traps containing methyl eugenol only. This suggested that citronella oil has repellent activity against fruit flies. Previous studies revealed the repellency effect of citronella oil againts *Tribolium castaneum* herdst. (Coleoptera: Tenebrionidae) and *Lasioderma serricorne* Fabricius (Coleoptera: Ptinidae) (PANG et al., 2021), *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) (OLAYINKA et al., 2022), *Aedes albopictus* Skuse, (Diptera: Culicidae) (LIAKAKOU et al., 2021); *Sitotroga cerealella* Olivier (Lepidptera: Gelechiidae) (ADJALIAN et al., 2015). and barn beetles (*Tribolium castaneum* Herdst (Coleoptera: Tenebrionidae) (WONG et al., 2005). It is caused citronella oil has volatile compounds content that have insect repellent properties, such as citronellal, citronellol, and geraniol (MAHMUD et al., 2022). Based on the GC-MS analysis. the citronella oil used in this study contained ten dominant compounds (see Table 1). Almost all these compounds have potential as insect repellents (WONG et al., 2005); (PLATA-RUEDA et al., 2020); (LIAKAKOU et al., 2021); (OLAYINKA et al., 2022). The exposure of citronella essential oil, geranyl acetate, or citral could lessen the insect' respiration rates, reduced locomotor activity, and generated avoidance responses (PLATA-RUEDA et al., 2020).

The higher volume of citronella oil should have a stronger repellency effect. Unfortunately, the finding showed the inconsistent repellency effect of citronella oil. This could be caused by the mixture of methyl eugenol and citronella odor. The mixture of methyl eugenol and citronella oil odor greatly determined the behavior of fruit flies to be attracted to or to leave the trap container. Insects could have different responses while exposed to a mixture of volatile compounds and a single compound (BEYAERT; HILKER, 2014). The dynamics of the composition of the mixture of methyl eugenol and citronella oil odor in the air as shown by the Plant Odor Plume (POP) concept may explain variations of fruit fly, responses as shown by the different number of individuals entering the traps. According to the concept, an insect can follow numerous POPs to their source, and may abandon a trail of one POP and switch to another if its scent provides a more reliable signal or signals a better suitable resource (BEYAERT; HILKER, 2014). (DEISIG et al., 2006) indicated that the proportion of compounds in the odor mixture is significant in determining insect reaction than single compound components (elemental processing). Interactions between chemicals in a combination can result in a diminished perception of a molecule relative to its individual components, a process known as "mixture suppression" (ACHE et al., 1988).

The current field study confirmed that the presence of insect repellent properties in the citronella oil compounds could deter the fruit flies attack on citrus crops.The repellent capacity is considered as one of citronella oil' mode of actions in suppressing fruit fly attack in the field. The odor of citronella oil interfered with the attractant compounds released by the host plants and made the plants less detectable by fruit flies. There are different compounds in different plants as fruit fly attractants. There are three compounds that commonly found related to the fruit flies' development, i.e. limonene content account largely for ovipositional responses of fruit flies on sweet orange (IOANNOU et al., 2012), 2-furan methanol that could attract male of *B. dorsalis* (PATONI et al., 2022). Volatile compounds released by chili fruits included β-cis-ocimene were correlated with oviposition incidence of *Bactrocera dorsalis* Hendel (Diptera: Tephritidae) (SYAMSUDIN et al., 2022). These three compounds (limonene, 2 furan methanol and β-cis-ocimene) were not detected in citronella oil, In the other hand, citronelol, one of citronella oil compounds (BURDOCK, 2009) was a moderately repellent for *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) that attacked soft fruits (RENKEMA et al., 2017). Additionally. citronellal repelled *flies* via olfactory pathways. but not directly as feeding deterrence through direct activating Transient Receptor Potential Ankyrin 1 (TRPA1) (DU et al., 2015). Citronellal is a direct agonist for *Drosophila*, (FOUDA et al., 2017) found that citronella oil had repellent activity against the fruit flies *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) and *Bactrocera zonata* Saunders (Diptera: Tephritidae) under field conditions. Thus, citronella oil could be developed as an potential repellent to a *Bactrocera* spp. The once a week application of 2mL citronella oil/L water is effective enough to control *Bactrocera* spp. attack on citrus. It was also showed by previous research that citronella oil was effective to control ant and thrips on mangosteen (ISTIANTO; EMILDA, 2021) and *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) on papaya (ISTIANTO et al., 2024) at the same concentration and frequency. In addition, citronella extract applied once a week was able to control *Nilaparvata lugens* Stal, (Hemiptera: Delphacidae), *Hieroglyphus banian* Fabricius (Orthoptera: Acrididae) and *Oebalus pugnax* Fabricius, (Hemiptera: Pentatomidae) in rice plants (TELAUMBANUA et al., 2021).

Application once a week of citronella oil as repellent was also supported by the result of first experiment of this reserach that informed the effectiveness of citronella oil as repelent for ten days.

Conclusion

The study found that citronella oil has repellent capacity against fruit flies and has the potential to suppress *Bactrocera* spp. attacks on citrus in the field. The effectiveness of citronella oil as repellent to *Bactrocera* spp for ten days. The spray of 2 mL citronella oil/L water once a week was considered as the most effective and efficient *Bactrocera* spp., control method.

For the future work it is necessary (1) to test the effectivity of citronella oil to control fruit flies in other areas (multi location test) (2) to understand compatibility citronella oil with other existing technologies. (3) to know other mode of action of citronella oil besides as repellent in order this technology can be applied more effective.

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