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LABORATORY AND FIELD EVALUATION OF ESSENTIAL OILS FROM *Cymbopogon nardus* AS OVIPOSITION DETERRENT AND OVICIDAL ACTIVITIES AGAINST *Helicoverpa armigera* Hubner ON CHILI PEPPER

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ABSTRACT

The fruit borer (*Helicoverpa armigera* Hubner) is one of the key pests of chili pepper in Indonesia. Yield loss due to this insect pest may reach up to 60%. Chemical treatment for controlling this insect pest is ineffective and eventually leads to environmental pollution. More environmentally safe insecticides are developed based on natural plant ingredients as their active compound such as essential oils. This study aimed to assess the potential of citronella oil for managing *H. armigera* on chili pepper. The experiments were conducted at the Indonesian Vegetables Research Institute from April 2009 to March 2010 and in Cirebon, West Java from November 2009 to March 2010. A field experiment was designed in a randomized complete block design with five treatments and replicated five times. Citronella oil was extracted by steam distillation from *Cymbopogon nardus*. The oil was then chemically characterized by using GC-MS and its efficacy (ovicidal and feeding deterrent) against *H. armigera* was tested both in laboratory and field conditions. The GC-MS result showed that major chemical compounds of the citronella oil used were citronella (35.97%), nerol (17.28%), citronellol (10.03%), geranyle acetate (4.44%), elemol (4.38%), limonene (3.98%), and citronnellyle acetate (3.51%). The laboratory experiment revealed that the highest concentration (4,000 ppm) of citronella oil reduced egg laying by 53-66%. Ovicidal activity was concentration dependent, and egg hatchability decreased by 15-95% compared to control. The field experiment showed that treatment of citronella oil at 2.0 mL L^{-1} significantly reduced fruit damage by *H. armigera* similar to the plots treated with spinosad at the recommended dose (60 g ai ha^{-1}). Application of citronella oil significantly reduced fruit damage by 72% and increased quality of the chili pepper. Because oviposition and feeding deterrent properties are key factors in controlling the pest, therefore this study revealed that citronella oil has potential to be incorporated into the controlling program of *H. armigera* on chili pepper.

[***Keywords***: *Capsicum annuum*, *Cymbopogon nardus, Helicoverpa armigera*, oviposition deterrent, ovicidal activities]

INTRODUCTION

Chili pepper is an important vegetable crop in Indonesia since it is grown year-round. It is cultivated mainly by small farmers both in highland and lowland areas under rainfed as well as irrigated conditions. In 2008, total planted areas of chili pepper was 109,178 ha producing 695,707 t of fresh fruit with an average yield of 6.37 t ha^{-1} (Statistics Indonesia 2009).

A wide range of insects attack chili pepper and causes significant yield loss. Among the pests, fruit borer (*Helicoverpa armigera* Hubner) is the most destructive insect pest, not only on chili pepper but also in other vegetable crops, especially during the dry season. *H. armigera* caused yield loss of chili pepper up to 60% (Luther *et al*. 2007). The pest is difficult to be eradicated because it has many alternative host plants, such as tobacco, maize, sorghum, cotton, potato, okra, cabbage, green peas, chrysanthemum, tomato, carrot, lettuce, eggplants, and other horticultural crops (Kalshoven 1981).

Most of farmers applied synthetic chemical insecticides to control insect pests on chili pepper. Some farmers mixed different types of synthetic insecticides to get satisfied control level of the pests. Number of spray application during a season was around 21 times (Adiyoga 2007). Despite its high insecticide use, the average yield loss due to insects is still high. Study in some countries indicated that *H. armigera* is resistant to major conventional groups of pesticides, such as pyrethroids, organophosphates, organochlorines, and carbamates (Ahmad *et al.* 1997; Ahmad *et al.* 2001; Torres-Vila *et al.* 2002; Ramasubramanian and Regupathy 2004; Ahmad 2007; Chaturvedi 2007).

Many plant essential oils show a broad spectrum of activities against insect pests and plant pathogenic fungi ranging from insecticidal, antifeedant, repellent, oviposition deterrent, growth regulatory, and antivector activities. These essential oils are, therefore, potential to be used as an alternative pesticides for controlling insect pests. Cost-effective, safe, and ecologically-sound strategies for controlling these pests are essential to maintain production efficiency and quality.

Recently, scientists have become more interested in the utilization of plant materials as environmentally-friendly botanical pesticides because they often minimize the adverse effects on beneficial insects, reduce the need for prohibitively expensive chemicals, reduce the development of resistance, and are less polluting the environment. Among these botanical pesticides, citronella oil has been most extensively studied in the last decades. The efficacy of citronella oil against various insect species has been noted as a repellent, an antifeedant, and an oviposition deterrent. Some studies indicated that citronella oil is effective in repelling mosquito *Aedes aegypti* (Jantan and Zaki 1999), *Spodoptera frugiperda* (Labinas and Crocomo 2002), and as antifungal and antibacterial (Nakahara *et al.* 2003; Pattnaik *et al*. 2006). Citronella oil is extracted from *Cymbopogon nardus* (also known as *Andropogon nardus*) of the Gramineae (Poaceae) family. Citronellal, trans-geraniol, carvone, and limonene were active compounds as anti-microbial (Simic *et al*. 2008), citronellal and linalool as an antifungal (Nakahara *et al.* 2003), whereas menthone, trans-geraniol, and citronellal showed a strong inhibitory effect (JIRCAS 2005). The present study aimed to evaluate the potential of citronella oil for oviposition deterrence and ovicidal activity against *H. armigera* on chili pepper.

MATERIALS AND METHODS

H. armigera larvae were collected from chili pepper in Lembang District, West Java, Indonesia. The larvae were then reared in the laboratory of the Indonesian Vegetables Research Institute (IVEGRI). Late larvae were separated to cannibalism sites. Emerging adult moths were transferred to cages at a ratio of 1:1 and fed on a 10% sucrose solution for oviposition. The cage was then covered with paper towel for egg laying. The paper towel containing eggs was removed daily then moistened and kept in plastic containers to allow hatching. One day-old adults were used for oviposition deterrent activity. All experiments and mass rearing were carried out at ambient temperature (27°C ± 2°C).

C. nardus leaves were collected in April and May 2009 from a field experiment of IVEGRI. The leaves were transported in plastic bags to the laboratory where they were put to dry at room temperature (28°C) on the same day. After 4 days, the leaves were chopped before extraction. The plant was identified and authenticated by a plant taxonomist.

For plant extraction, a simple laboratory quick fit apparatus with 2,000 mL steam generator flask, a distilling flask, a condenser, and a receiving vessel were used for steam distillation. The flask was heated with a gas burner. One hundred grams of air dried and chopped leaves of *C. nardus* were subjected to steam distillation.

Coupled gas chromatography mass spectrometry (GC-MS) was performed on a Hewlett Packard 5973 mass selective detector (70 Ev), coupled to a Hewlett Packard gas chromatograph equipped with a split/splitless Programmed Temperature Vaporization (PTV) injection system (CIS 4: Gerstel, Mûlheim an der Ruhr, Germany). Injections were done in split mode only (1 µL). The column was a 30 m EC-5 fused silica column, 0.25 mm ID, and 0.25 µm film thickness (Alltech/Applied Science BV., Breda, the Netherlands). Conditions were: carrier gas, helium, constant head pressure at 0.6 bar; temperature programming at 50°C for 2 minute hold to 300°C for 5 minute hold at 8°C min^{-1}, injector temperature at 250°C, and transfer line temperature at 300°C.

The efficiency of the citronella oil at different concentrations was determined in IVEGRI laboratory from June to October 2009. For choice test, five pots were sprayed with one of the tested citronella oil concentrations (4,000, 3,000, 2,000, and 1,000 ppm plus 0.05% of Tween 20) using a hand sprayer of 1 L capacity. Another five pots were sprayed with water and emulsifier (Tween 20) as an untreated check. The pots (four treated + check in random) were then placed in a wooden cage (100 cm x 100 cm x 100 cm) and covered with a screen. For no-choice test, five pots were sprayed with one of the tested citronella oil concentrations (4,000, 3,000, 2,000, and 1,000 ppm) using a hand sprayer of 1 L capacity. Another five pots were sprayed with water and emulsifier as an untreated check. Five pots of each treatment were placed in a wooden cage (100 cm x 100 cm x 100 cm), respectively.

Ten pairs of emerged moths were introduced into the cage with the sprayed hot pepper potted plants and the control. Each test was replicated five times. The experiment was laid out in randomized complete block design (RCBD). The number of eggs deposited on treated or untreated plants was counted daily from each test. The percent effective repellency for each leaf extract concentration was calculated using the following formula:

$$ER\ (\%) = \frac{NC - NT}{NC} \times 100\ (\%)$$

Where
ER = percent effective repellency
NC = number of eggs in control
NT = number of eggs in treatment.

Ovicidal activity was evaluated by exposing 20 eggs of *H. armigera* to five concentrations of citronella oil (4,000, 3,000, 2,000, 1,000 ppm and 0 ppm plus 0.05% of Tween 20) and allowed to hatch. The experiment was carried out with five treatments in five replications using RCBD.

Field experiment was carried out at a farmer's field at Pabedilan Village in Cirebon, West Java, from November 2009 to March 2010. Plot size was 60 m^2. Each treatment consisted of 400 plants. The experiment was designed in a RCBD with five treatments, including one untreated plot, and replicated five times. The treatments consisted of citronella oil (2.0 mL L^{-1}), spinosad at recommended dose (60 g ai ha^{-1}), citronella oil (2.0 mL L^{-1}) alternated weekly with spinosad at recommended dose (60 g ai ha^{-1}), conventional control (imidacloprid 200 g L^{-1} + fenitrothion 500 g L^{-1}), and untreated control treatment. Applications of all treatments were done eight times during the experiment with one-week interval between applications. Data on fruit quality (percentage of damaged fruits or percentage of unmarketable fruits) and yield (undamaged or marketable fruits) were evaluated at each harvest.

The data were analyzed using Genstat for Windows 11th edition. The comparison of means was done using Duncan's multiple range test at 0.05 level.

RESULTS AND DISCUSSION

Chemical Composition of Essential Oils

GC-trace and chemical composition of essential oils of *C. nardus* are presented in Figure 1 and Table 1. The major chemical compounds were citronella (35.97%), nerol (17.28%), citronellol (10.03%), geranyle acetate (4.44%), elemol (4.38%), limonene (3.98%), and citronnellyle acetate (3.51%). The composition of essential oils resulted in this study was significantly different from that previously reported by Mahalwal and Ali (2003). The major components of essential oils from *C. nardus* cultivated in India were citronellal (29.7%), geraniol (24.2%), terpineol (9.2%), and cissabinene hydrate. It was reported that the citronellal content varied among the cultivated citronella varieties. Citronella oil from Sri Lanka contained 40.5-60.7% of citronellal, whereas that from India contained much smaller amounts of citronellal, i.e. 17.2-33.2% (Mahalwal and Ali 2003). Additionally, the composition of essential oils is affected by many factors, including the cultivation conditions of the plants and isolation techniques (Janssen *et al.* 1987). The other constituents of *C. nardus* Mahapengiri variety at 6 months after planting are given in Table 1.

Oviposition Deterrent

In laboratory oviposition deterrent tests, citronella oil concentrations greatly reduced the number of eggs

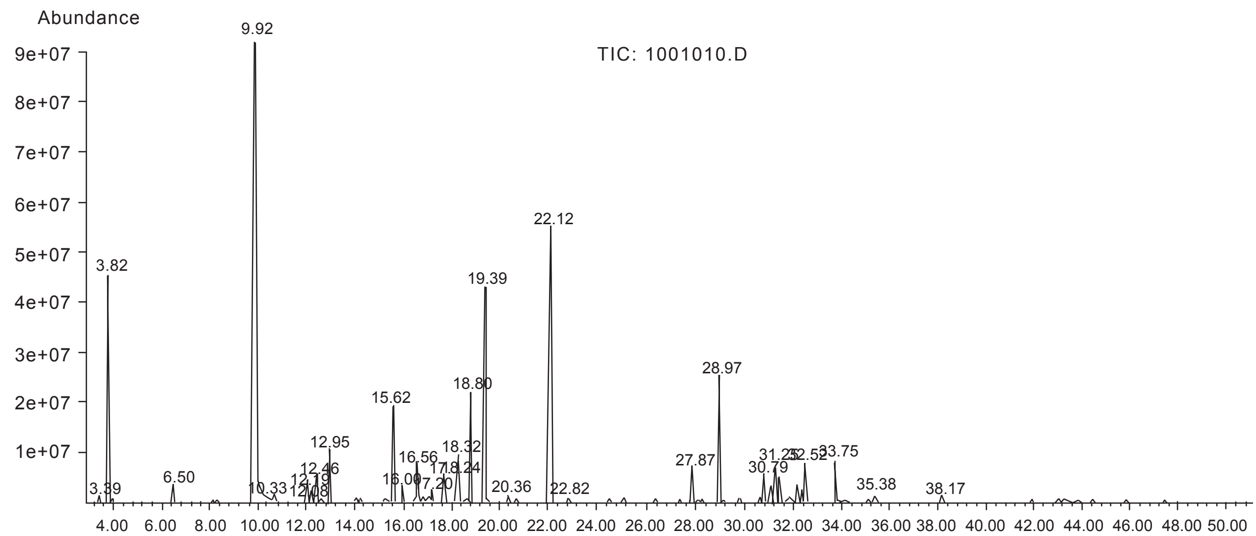

Fig. 1. GC- trace of *Cymbopogon nardus.*

Table 1. Chemical compositions of essential oils of *Cymbopogon nardus.*

Components	Relative percentage (%)
Beta-myrcene	0.10
Limonene	3.98
2.6-dimetil-5-heptenal	0.44
Citronella	35.97
Citronella	0.18
Linalool	0.64
Isopulegol	0.44
(-)-isopulegol	0.90
Beta-elemene	1.80
Citronnellyle acetate	3.51
Z-citral	0.59
Germacrene D	1.52
Delta δ guaiena	0.21
Geraniol formate	0.21
Alfa α muurolena	0.49
Citral	1.01
Bisiklo (4.4.0) dec-1-en, 2 isoprophy	0.96
Delta δ cadinena	1.76
Geranyle acetate	4.44
Citronellol	10.03
Nerol	0.20
Nerol	17.28
5-nitrobenzofuran -2-carboksil acid	0.15
Endo-1-bourbonanol	1.28
Elemol	4.38
Patchouli alcohol	0.99
Beta-patchoulena	0.39
Alfa-cadinol	0.54
Eugenol	1.29
T-muroolol	0.56
Alfa-copaena	0.06
Alfa-eudesmol	0.37
Beta-eudesmol	0.39
T. cadinol	1.24
Siklobutene, 1,2,3,4-tetrametil	1.41
Geranil linalool isomer	0.16
7-acetil-2-hidroksi-2-metil	0.13

layed by *H. armigera* (Table 2 and 3). In no-choice test, the egg laying capacity gradually decreased with the increase in citronella oil concentration level. The maximum reduction in egg laying was noticed in treatment of essential oil concentration of 4,000 ppm where only 73 eggs were laid by *H. armigera* on the treated plant as against 152 eggs in the control. There was no significant difference in the number of eggs laid by the female of *H. armigera* on the plants of varying treatments in the choice test. Choice and no-choice tests revealed that highest concentrations of citronella oil reduced egg laying by 53-66%. Lower concentrations also had deterrent activity both in no-choice and choice tests.

Table 2. Oviposition deterrent activity of essential oil (citronella) of *Cymbopogon nardus* on female *Helicoverpa armigera* in a no-choice treatment.

Concentration (ppm)	Number of eggs	Effective repellency (%)
4,000	73.0d	53
3,000	95.2c	38
2,000	97.2c	33
1,000	113.4b	26
0 (untreated)	152.4a	-

Means followed by the same letters are not significantly different according to DMRT at $\alpha = 0.05$.

Table 3. Oviposition deterrent activity of essential oil (citronella) of *Cymbopogon nardus* on female *Helicoverpa armigera* in choice treatment.

Concentration (ppm)	Number of eggs	Effective repellency (%)
4,000	154.0a	66
3,000	178.2b	61
2,000	180.8b	60
1,000	184.8b	60
0 (untreated)	452.0b	-

Means followed by the same letters are not significantly different according to DMRT at $\alpha = 0.05$.

The potential of plant essential oils as a source of insecticide has been worked out and reported with reference to various pests. Aerts and Mordue (1977), working with triterpenoids extracted from *Azadirachta indica*, verified high deterrent activity on *Spodoptera littoralis* larvae and *Schistocerna gregaria* nymphs. Rosemary oil was effective against certain insect and mite (Papachristos and Stampoulos 2004) and *Ocimum americanum* essential oil was effective on *Agrotis ipsilon* (Sadia *et al.* 2007).

The result of our study showed that essential oils from *C. nardus* were effective against *H. armigera*. These findings are in accordance with Paranagama *et al*. (2003, 2004) who found that citronella oil was effective against *Callosobruchus maculatus* and *Sitophilus oryzae*.

The effect of citronella oil on the oviposition preference of *H. armigera* within the treatments showed a similar pattern. These results have confirmed that the use of essential oils did not affect the oviposition preference of *H. armigera* where eggs were distributed over all parts of chili plants in both choice and no-choice test (Fig. 2 and 3). However, leaves were more preferred by *H. armigera* for oviposition than other plant parts, with 60-98% of the total eggs were laid on leaves compared to stem, flower, and

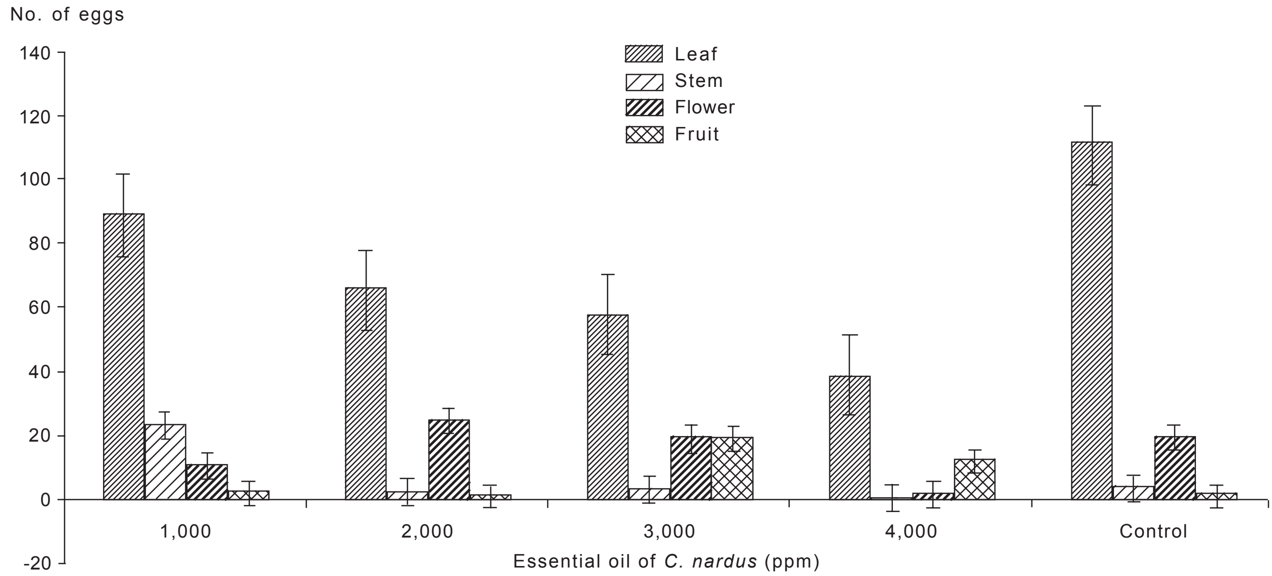

Fig 2. Effect of citronella oil concentration on distribution of *Helicoverpa armigera* eggs laid on chili pepper plant (mean ± SE) in a no-choice treatment.

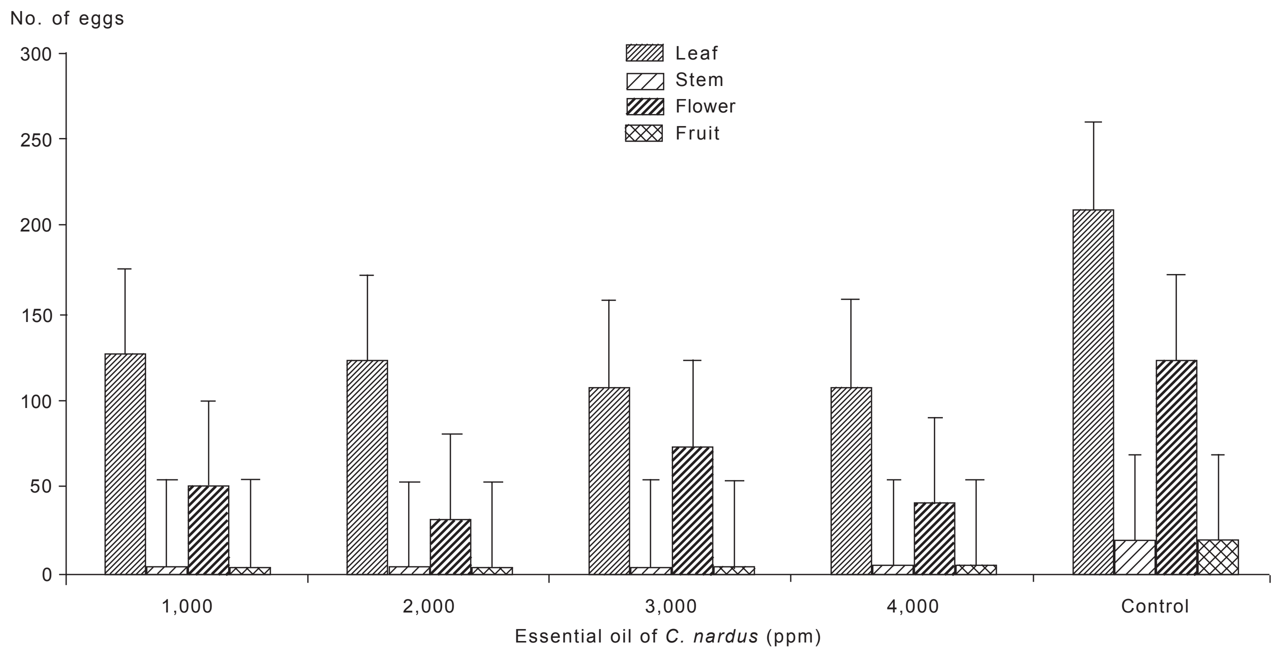

Fig 3. Effect of citronella oil concentration on distribution of *Helicoverpa armigera* eggs laid on chili pepper plant (mean ± SE) in a choice treatment.

fruit. A similar result was observed in all treatments. Jallow *et al.* (2001) reported that female *H. armigera* preferred to lay its eggs on leaves of tomato, maize, okra, and pepper, whereas on eggplant the insect preferred flowers and fruits. Shape, color, texture, size, and chemical composition of these plants may influence oviposition preference (Courtney and Kibota 1990). Thomson (1987) and Javed *et al.* (2009) stated that the females prefer to lay eggs on plant parts with high trichome density and high concentration of stimulatory chemicals.

The result of ovicidal assay is illustrated in Table 4. The data show that ovicidal activity was concentration dependent. Citronella oil concentrations of 2,000 ppm, 3,000 ppm, and 4,000 ppm decreased egg hatchability by 15-95% compared to the control. The

Table 4. Effect of citronella oil concentration on the egg viability of *Helicoverpa armigera.*

Concentration (ppm)	Egg viability (%)	Reduction (%)
4,000	5.0d	95
3,000	40.0c	60
2,000	85.0b	15
1,000	100.0a	0
0 (control)	100.0a	-

Means followed by the same letters are not significantly different according to DMRT at $\alpha = 0.05$.

observed mortality from the lower concentrations was comparable to the control. The finding of the present experiment revealed that citronella oil possesses oviposition deterrent and ovicidal activities against *H. armigera*. The toxic effect of this oil could be attributed to major constituents such as citronella, linalool, and geranyle acetate. High toxicity of these compounds was reported against the rice weevil *Sitophilus oryzae* and *Rhyzopertha dominica* (Rozman *et al.* 2007). Boeke *et al*. (2004) reported that the volatile oils of *C. nardus* caused most of the eggs not to develop into adult (abnormality in egg development to adult). The chemical composition and broad spectrum of biological activity of essential oils can vary with plant age, plant tissue, geographical origin of plant, organ used in distillation process, type of distillation, and species and age of targeted pest organism (Janssen *et al*. 1987; Chiasson *et al*. 2001).

Field Evaluation of Citronella Oil

Field observation showed that five insect pest species were found in chili pepper plots throughout the growing season, i.e. thrips (*Thrips parvispinus*), *Empoasca* sp., whitefly (*Bemisia tabaci*), fruit fly (*Bactrocera dorsalis*), and fruit worm (*H. armigera*). However, repellence effects were observed only for *H. armigera*. At the initial growing period, there was no phytotoxicity symptoms appeared on chilli pepper plants treated with citronella oil, therefore the oil is safe to the plant.

The highest and statistically similar reduction of fruit damage was observed in plots treated with spinosad insecticide alone (10.39%) and with citronella oil alone (13.45%). However, when citronella oil was applied alternately with spinosad, its efficacy was not as high as expected. This indicates that weekly alternate application of citronella oil with spinosad had some antagonistic effect against *H. armigera*. Therefore, conventional control by using synthetic insecticide alone proved to be the least effective. The reduction of fruit damage over untreated control ranged from 54.70% to 78.92%. The highest percent reduction of fruit damage was recorded with spinosad (78.92%) followed by citronella oil (72.71%) (Table 5).

Treatment of the citronella oil on chili pepper plant significantly affected yields (Table 5). The conventional control had an average yield of 6.54 t ha^{-1} and the citronella oil treatment yielded an average of 7.15-10.90 t ha^{-1}. The results demonstrated that the use of citronella oil reduced fruit damage by *H. armigera* as well as increased yields.

Efficacy of citronella oil against *H. armigera* did not significantly differ from spinosad insecticide at recommended dose (60 g ai ha^{-1}). Spinosad is a fermentation by-product based compound derived from a naturally occurring soil actinomyces bactsporaerium (*Saccharopolyspora spinosa*) and is a mixture of spinosyn A and spinosyn D (Thompson *et al*. 1997). As reported, spinosad has two unique modes of action, i.e. acting primarily on the insect's nervous

Table 5. Fruit damage and yield of chili pepper treated with citronella oil, Cirebon, West Java, 2009-2010.

Treatment	Fruit damage infested by *H. armigera* (%)	Yield	
		kg $plot^{-1}$	t ha^{-1}
Citronella oil (2 mL L^{-1})	13.45 cd	65.39a	10.90
Spinosad (60 g ai ha^{-1})	10.39 d	51.67b	8.61
Citronella oil (2 mL L^{-1}) applied alternately with spinosad (60 g ai ha^{-1})	16.37 c	42.88c	7.15
Conventional control (imidacloprid 200 g L^{-1} + fenitrothion 500 g L^{-1})	22.33 b	39.26c	6.54
Control (untreated plot)	49.29 a	23.98d	4.00

Means followed by the same letters within the same column are not significantly different according to DMRT at $\alpha = 0.05$.

system at the nicotinic acetylcholine receptor, and exhibiting activity at GABA receptor (Salgado 1997). Spinosad has relatively broad-spectrum activities, and has been effectively used for controlling many species of insect pests, such as *S. litura, H. armigera*, and *T. parvispinus* (Setiawati 2009).

CONCLUSION

Choice and no-choice tests revealed that the highest concentration of citronella oil reduced egg laying of *H. armigera* by 53-66%. The oil also set as ovicidal which decreased egg hatchability up to 95% compared to control. Eggs were distributed over all plant parts of chili pepper in both choice and no-choice test, however, leaves were more preferred by *H. armigera* for oviposition than other plant parts, with 60-98% of the total eggs were laid on leaves compared with stem, flower, and fruit. The use of citronella oil alone reduced fruit damage by *H. armigera*, therefore no synthetic insecticide is required. This indicates that citronella oil can be incorporated in pest control program for chili pepper without adversely affecting yield or fruit quality compared with conventional control based on using synthetic insecticide.

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