The effect of pesticide residue on mortality and fecundity of *Elaeidobius kamerunicus* (Coleoptera: Curculionidae)

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Abstract. The effect of pesticide residue (acaricide, insecticides, herbicides, and fungicides) commonly used in oil palm plantations was assessed to pollinating weevil, *Elaeidobius kamerunicus* under laboratory condition. The study showed that all insecticides and acaricide in the spikelet were harmful to the weevil, causing mortality ranged from 37% to 100% application. Dimehypo, carbosulfan and deltamethrin cause 100% mortality at 10 hr after treatment (HAT) followed by fipronil, acephate, pyridaben, imidacloprid and *Bacillus thuringiensis* cause 87%, 83%, 60%, 40% and 37% mortality at 10 HAT respectively. Our study also revealed that all herbicides recidu in the spikelet had a negative impact on the weevil. Metsulfuron-methyl caused the highest mortality (83%) of the weevils death, followed by triclopyr, ammonium glufosinate, glyphosate, and fluroxyphyr caused 63%, 43%, 33% and 30% at 10 HAT, respectively. Fungicides also had negative impact with lower extent. Exposing mancozeb was causing 27% mortality, significantly higher than benomyl (13%). Further analysis showed that acaricide and insecticides significantly reduce fecundity of the weevil except for *B. thuringiensis*, herbicides, and fungicides. The study suggests that choosing correct active ingredients would maintain sustainable number of weevil in oil palm plantation and would avoid the declining regeneration effect of pollinating weevil.

1. Introduction

The oil palm (*Elaeis guineensis*) is an important crop in Indonesia that has separate male and female flowers, thus requiring pollen to be transferred by wind or insects [1]. The pollinating weevil of oil palm, *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae) was introduced to Peninsular Malaysia from Cameroon, West Africa in 1981. It was then released to Indonesia in 1983 [2, 3, 4]. The weevil is very efficient pollinator because they carry abundant pollen and have good searching ability on male inflorescence [5, 6]. Weevils are very dependent on the male inflorescence of the oil palm. Adult weevils consume oil palm pollen and lay eggs in anthesis male inflorescences [7].

After the introduction, weevils spread rapidly and develop big populations in all oil palm plantations in Indonesia, the pollinating weevil is so widespread [4, 8, 9]. The introduction of this weevil improved fruit set resulting in a higher bunch yield of oil palm [4, 10]. Pollinating weevil led to 36% – 80% improvement of fruit set, 12% increase in fruit-to-bunch ratio and 28% – 54% increase in mean bunch weight, the oil-to-bunch ratio increased 9% and 43% improvement in the kernel-to-bunch ratio [11].

Pesticides are commonly used in oil palm plantation from nursery to the field, which includes acaricides, insecticides, herbicides, and fungicides. The application of pesticides can minimize...
because of integrated pest management (IPM) which is proved can manage population of pest in the field [12, 13]. The implementation of IPM in oil palm plantation such as for leaf-eating caterpillars and rhinoceros beetle [14, 15] leads to decrease population of those pests. However, sometimes outbreak still occurs in certain areas and intervention using insecticides is unavoidable [16]. The other case application of herbicide to control weed is widely adopted by oil palm company where regular spraying focus to the circle and harvesting path at 4 mo interval. Furthermore, the fungicides and acaricides mostly used in oil palm nursery to control the mites and leaf spot disease. The bioassay of residue those pesticides on the weevil is necessary. However, to date, the side effects from all kind of pesticides that commonly used in oil palm plantation and residue to the flower to the weevils remain unclear.

The pollinating weevil is known very sensitive to some insecticides. Some studies reported that insecticides included lambda-cyhalothrin and cypermethrin directly affecting the activity and breeding performance of pollinator insects [17]. Meanwhile, the treatment of chlorantraniliprole and flubendiamide and Bacillus thuringiensis did not affect the development of pollinating weevil in the laboratory [18]. Therefore, evaluation the effect of residue common pesticides used in oil palm plantations on the mortality of weevil and fecundity in laboratory conditions should be addressed.

2. Methods

2.1. Study sites, experimental design and treatment

The experiment was carried out at Entomology Laboratory of Crop Protection Department, SMART Research Institute (SMARTRI), Riau Province, Indonesia from December 2017 to March 2018. There were 16 treatments (Table 1) and 3 replicates using Complete Randomized Design (CRD). Each plot consists of 5 pairs of E. kamerunicus. The experiment was conducted at a room temperature 28.8 ± 0.6 °C and 52.7 ± 2.4% relative humidity.

Table 1. Treatments and concentration of pesticides used in the study.

| No | Pesticide Type | Active Ingredient | Group          | Conc. of Pesticide (%)^a |
|----|----------------|-------------------|----------------|--------------------------|
| 1  | Control        | -                 | -              | -                        |
| 2  | Acaricide      | Pyridaben 135 g/L | Pyridazinone   | 0.1                      |
| 3  | Insecticide    | Fipronil 50 g/L   | Fenil-pirazol  | 0.3                      |
| 4  |               | Imidacloprid 200 g/L | Neonicotinoid | 0.2                      |
| 5  |               | Acephate 75%      | Organophosphate| 0.2                      |
| 6  | Insecticide    | Dimehypo 400 g/L  | Neristokin     | 0.3                      |
| 7  |               | Deltamethrin 25 g/L | Pyrethroid    | 0.2                      |
| 8  |               | Carbosulfan 5%    | Carbamate      | 0.3                      |
| 9  |               | B. thuringiensis 4.1 x 10^9 colony/g | Bacterium | 0.5                      |
| 10 | Herbicide      | Glyphosate 480 g/L | Glycine        | 0.3                      |
| 11 |               | Am. Glufosinate 150 g/L | Glutamine | 0.6                      |
| 12 | Herbicide      | Metsulfuron-methyl (MMS) 20% | Sulfonylurea | 0.025                    |
| 13 |               | Fluroxypyr 290 g/L | Pyridine       | 0.125                    |
| 14 |               | Triclopyr 670 g/L | Pyridine       | 0.3                      |
| 15 | Fungicide      | Benomyl 50 g/L    | Benzimidazole  | 0.2                      |
| 16 |               | Mancozeb 80 g/L   | Dithiocarbamate| 0.2                      |

^aDetermining of pesticide concentration based on commercial application.
2.2. Collection of adult weevil
Post-anthesis male flower was cut from the oil palm bunch and brought to the laboratory. It then put in a rearing cage (50 cm x 50 cm x 75 cm). After about 1 wk, adult weevils that emerged from the flower were used for the study. Before the study started, pollen was supplemented in the rearing cage as a feed source for adults. The adult weevils that emerged from a bunch post-anthesis flower assumed has the same age.

2.3. Bioassay of residue pesticides on adult weevil
The male flower of oil palm was bagged before anthesis using a pollination bag to avoid colonized by pollinating weevil during anthesis period. Once anthesis, the male flower was cut and brought to the laboratory. The spikelet of anthesis male flower was cut and then sprayed 3 times by pesticides based on treatment (Table 1) and air dried for 5 min. The sprayed spikelet then put into a glass tube (18 cm height and 5 cm diameter) with the lower and upper-end is closed by muslin fabric. Five pairs of adult weevils were then put in the glass tube to expose treated spikelets and then closed with a muslin cloth. To evaluate the side effects of pesticides tested by feeding the weevil with the contaminated food and then mortality percentage and number of generation weevil were counted [19, 20]. Mortality of weevils was recorded hourly until 10 HAT (hr after treatment). After observation finished, the spikelets then moved into difference glass tubes to observe fecundity or generation produced by weevil in each treatment. Evaluation for fecundity was carried out after 14 d since spikelets were put into the new glass tube to count the number of weevils emerge.

2.4. Data analysis
Data was subjected to the analysis of variance (ANOVA) and Duncan (DMRT) at 95% confidence level for different weevil mortality and fecundity. All analyses were performed using SAS version 9.4 program (SAS Institute 2013).

3. Results and discussion
The effect of pesticides residue in spikelets to the weevil showed by accumulated mortality hourly. Pyridaben (Acaricide) was still harmful to the weevil where 60% mortality was observed at 10 hr after treatment (HAT) (Fig. 1a). For insecticides dimehypo, carbosulfan and deltamethrin caused 100% mortality, whereas 87%, 83%, 40% and 37% mortality recorded for treatment fipronil, acephate, imidacloprid and B. thuringiensis respectively at 10 HAT, respectively (Fig. 1b). Among herbicides tested, metsulfuron-methyl showed the highest mortality where 83% of weevils death after exposed to sprayed spikelets. Triclopyr, ammonium glufosinate, glyphosate and fluroxyphyr cause 63%, 43%, 33% and 30% mortality respectively at 10 HAT (Fig. 1c). Fungicide had the lowest mortality compared to other pesticides type, while mancozeb caused 27% mortality at 10 HAT. Lower mortality was observed from benomyl treatment where only 13% mortality and almost similar to control treatment (Fig. 1d).

Pesticides applied on the palm may not contact directly to pollinating weevil. However, it does not mean that there is no side effect to non-target pollinating weevil. The residue of pesticides can be residue in all parts of the palm including male flowers. Pesticides are readily taken up by the plant roots or incorporated into the tissues of the growing plants as they develop [21], so the insect that comes to eat them ingest a lethal dose and might die [22]. This study is one of investigation in laboratory condition against pollinating weevil. The overall study indicated that residue pesticides cause a significant problem to mortality and fecundity of pollinating weevil.

The first observation with pyridaben (acaricide) which commonly used to control mites in the nursery. It is still harmful to the weevil where 60% mortality was observed at 10 HAT. It is suggested that this acaricide is also toxic to the pollinating weevil. Some studies also reported that pyridaben is harmful to non-target insects and may decline the population of beneficial species such as scale parasitoid [23], beneficial spiders [24] and caused 100% mortality of parasitic wasp Aphidius colemani.
24 hr after application [25]. However, lower toxicities were observed for pyridaben to the predatory mites *Amblyseius cucumeris* and *Phytoseiulus persimilis* [26].

Chemical insecticides have a strong residual effect as mentioned earlier and can cause high mortality. Dimehypo, carbofuran, deltamethrin, fipronil, and achepate are systemic insecticide that has a strong effect [17, 18, 27] compared to contact and bio-pesticides insecticides. Contact insecticides like imidacloprid cause high toxicity if applied directly to the target [28, 29]. Contact insecticides act usually in single exposures (e.g. spray droplets, pulse contamination after spraying, etc.) and have the highest effects immediately after application [27]. Imidacloprid also reported can reduce the population of pollinating weevil but not severely [30]. Bio-insecticide tested containing *B. thuringiensis* showed the lowest mortality compared to systemic insecticides. *B. thuringiensis* did not affect weevil populations visiting anthesis oil palm inflorescences [17]. Furthermore, another report revealed that *B. thuringiensis* does not cause a significant reduction for weevil populations [31].

Herbicides may give physiologically effect or kill the non-target organisms especially insects at the time of herbicide application [32-34] although herbicides designed specially to control weeds. Other report reported the population of spiders and carabid beetle declined after application of glyphosate in the field [35], although glyphosate reported harmless to non-target organisms, such as a common spider of agricultural [36] and also had no effect on survival of the water hyacinth weevils *Neochetina eichhorniae* Warner (Coleoptera: Curculionidae) and *N. bruchi* Hustache (Coleoptera: Curculionidae)
Another result showed that glufosinate-ammonium was highly toxic to mite *Amblyseius womersleyi* Schicha (Acari: Phytoseiidae), *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae), and *Tetranychus urticae* Koch (Acari: Tetranychidae) [32]. Some different results of herbicides effects are dependent on the active ingredient, formulation, pesticide application, and insect species [33, 34, 38].

Fungicide reported has the lowest mortality compared to insecticides and herbicides where mancozeb cause 27% mortality at 10 HAT. Lower mortality reported from benomyl treatment where only 13% mortality reported and similar to control treatment. A fungicide is a specific type to control and killing the fungus causing the disease. Some reports also showed that fungicide has a sub-lethal effect on pollinators [39, 40]. Pesticides not only cause direct killing for the weevils but also has subtle effects that reduce thrive ability, survival rate and oviposition preference [41, 42].
Bacillus thuringiensis had no negative impact on weevil and did not affect the egg of weevil [17, 18]. The number of generation weevil on herbicides and fungicides affect treatment has similarity, but actually they have significant difference compared to insecticides treatment. That condition indicates residue insecticide (except B. thuringiensis) and acaricide not only have a strong effect on mortality but also can reduce weevil fecundity significantly.

To demonstrate the further impact of residue pesticides, investigation of the fecundity of weevil was carried out. Here the number of weevil generations that emerged from spikelets (Fig. 2). The study showed that chemical insecticides and acaricide tested (fipronil, acephate, carbosulfan, dimethoate, imidacloprid, deltamethrin, and pyridaben) could affect the generation ability. Furthermore, the reduction of fecundity would result in lower number of weevil generation. They only produced less than 3 weevils per spikelet. Except for bio-insecticide B. thuringiensis which had the lowest effect on weevil fecundity, which produced a higher population (18 weevils per spikelet) compared to chemical insecticides. For the other kind of pesticides, herbicides and fungicide could reduce the generation number of weevil but still had high fecundity (ranged from 15 to 22 weevils per spikelet) compared to chemical insecticides. Further analysis also showed that mortality percentage had a strong relationship with the weevil fecundity with p<0.01, R² = 0.662 (Fig. 3).

A side effect of some pesticides for oil palm plantation results in unfortunate consequences to pollinating weevil E. kamerunicus. Pesticides give a negative effect to weevil population that causing direct losses of population. In order that it can losses of oil palm yield because of lack of adequate pollination indirectly. One of the important steps before making a pesticide application is knowledge active ingredient of pesticide, target, and timing. Some pesticides are more environmentally friendly and may be selected for the special target. The selective pesticides will help to conserve pollinator population and sustainable agriculture.

4. Conclusion
Residue pesticides cause a significant problem to pollinating weevil where high mortality was observed when the weevils were exposed to sprayed spikelets. Among several pesticides type tested, insecticides were most harmful to weevil, followed by herbicides and fungicides. Among insecticides, bio-insecticide B. thuringiensis showed the lowest mortality than chemical insecticides. Weevil in B. thuringiensis treatment also produces more generation than chemical insecticides. This finding suggests that the bio-insecticides could be an ideal alternative to be used to deal with oil palm pests such as leaf-eating caterpillars to minimize side effects to the weevil. The study also suggests that herbicides could give negative effects to weevil. Regular training and specific equipment (cover the nozzle) for pesticide operator are needed when spraying on the immature palm to ensure no drift of pesticide substance reaching oil palm flower especially at immature stage.

Acknowledgments
The results presented are part of pollinating weevil and oil palm production project, financially supported by PT. Smart Tbk. We would also thank Rini Lestari and Agus Priono as the part of insectarium of Smartri and Sri Imriani Pulungan for reviewing this paper.

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