THE EFFECT OF INSECTICIDES ON POLLINATING WEEVILS, *Elaeidobius kamerunicus* ON FLOWER VISITATION AND NEWLY-DEVELOPED WEEVILS

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ABSTRACT

Overuse of insecticide could be detrimental to pollinators and reduce pollination efficiency. This study evaluated the effect of four commercial insecticides on oil palm pollinating weevil, *Elaeidobius kamerunicus*, with special focus on flower visitation and the emergence of newly developed weevils in a seven year-old oil palm estate. In general, total number of weevils visiting the male inflorescences reduced with the use of insecticides. Visiting weevils on the spikelets of male inflorescence were confined, counted, and stored at room temperature for the emergence of new adults. The total number of visiting weevils and newly developed weevils were recorded. This study found that all insecticides tested had varying degrees of adverse effect on the number of weevils visiting oil palm male inflorescences. On average, without any insecticide application a male inflorescence would receive 43 weevils throughout the anthesis development and the flower visitation by the weevils reached its climax on the third day of anthesis. However, when treated with insecticides, noticeable reduction (49% - 83%) in flower visitation was observed, especially in Fipronil and Diflubenzuron treated plots. In Fipronil treated plot, the reduction of weevils' flower visitation was the sharpest, 83% and the number of newly developed weevils emerged from the spent inflorescences was only 1.68±0.89 per spikelet, which is the lowest among all treatments. In the study, it was observed that both insecticides Chromafenozide and Pyridalyl were found to be friendlier to weevil and less reduction in flower visitation and newly emerged weevils was recorded. As the mode of action of Chromafenozide and Pyridalyl is different, they can be recommended to be used alternately to provide a broader defense against pest in oil palm estates to reduce risk of resistance development.

Keywords: Chromafenozide, oil palm, pollinator, male inflorescences, fipronil

ABSTRAK

Penyalahgunaan racun serangga perosak berpotensi merbahayakan kemandirian serangga pendebunga dan mengugat keberkesanan pendebungaan. Kajian ini bertujuaan untuk menilai kesan empat jenis racun serangga perosak di pasaran terhadap agen pendebunga kelapa sawit, *Elaeidobius kamerunicus* dengan memberi tumpuan khas terhadap bilangan agen pendebunga melawat bunga dan pendebunga baru yang muncul dari bunga kelapa sawit di ladang kelapa sawit yang berusia tujuh tahun. Secara umumnya, jumlah kumbang yang melawati bunga jantan berkurang dengan penggunaan insektisid. Hasil kajian ini mendapati kesemua racun serangga perosak yang dikaji memberi kesan negatif terhadap jumlah bilangan individu pendebunga yang melawat bunga jantan kelapa sawit, tetapi masing-masing pada tahap yang berlainan. Secara purata, tanpa penyemburan racun serangga perosak, sebanyak 43 ekor pendebunga akan melawat sekuntum bunga jantan kelapa sawit dan bilangannya mencecah kemucaknya pada hari ketiga pendebungaan. Walau bagaimanapun, apabila bunga jantan kelapa sawit dirawat dengan racun perosak, didapati jumlah serangga pendebunga yang melawat bunga jantan tersebut berkurangan dengan ketara, iaitu 43% hingga 83%, terutamanya bagi bunga jantan yang dirawat dengan racun Fipronil dan Diflubenzuron. Penurunan jumlah serangga pendebunga yang melawat bunga jantan adalah paling ketara pada kelapa sawit yang dirawat Fipronil iaitu sebanyak 83% dan rawatan ini juga menyebabkan penurunan yang paling mendadak bagi jumlah bilangan serangga pendebunga baru yang muncul dari bunga jantan kelapa sawit yang sudah layu di kalangan kesemua rawatan, iaitu hanya 1.68±0.89 per spikelet. Racun serangga Chlorantraniliprole dan Pyridalyl didapati kurang kesan negatif terhadap *E. kamerunicus* maka lebih mesra terhadap serangga pendebunga. Mekanisma tindakan Chromafenozide dan Pyridalyl adalah berbeza, kedua-dua racun ini boleh digunakan secara bergilir untuk mencapai liputan pertahanan terhadap serangan serangga perosak yang lebih luas di ladang kelapa sawit. Di samping itu, mengurangkan risiko pembentukan tahap kerintangan di kalangan serangga perosak.

Kata Kunci: Chromafenozide, kelapa sawit, pendebunga, bunga jantan, fipronil

INTRODUCTION

It was conservatively estimated that Penisular Malaysia has 7834 species of seed plants with 220 families (Kiew et al. 2010) and approximately 94% of floral species are animal-pollinated plants in the tropics (Ollerton et al. 2011). Relationships between plant and pollinator form one of the most ecologically important animal and plant interactions for the continuity of the ecosystem. Plants need pollinators to complete the cycle of pollination, seed setting and reproduction, whereas pollinators need nectar and other incentives from plants.

In an oil palm ecosystem, the pollinating weevils ensure proper fruit set formation of oil palm fruit bunches. However, due to the increase in pest occurrence such as bunch moth, *Tirathaba mundella,* which renders the application of insecticides ineluctable, the population of insect pollinators is threatened (Klein et al. 2017; Potts et al. 2016). Many studies have shown that insecticides whose concentration is deemed safe have significantly reduced cognitive abilities, foraging performance and, subsequently, the survival of exposed pollinators (Klein et al. 2017). The reduction of pollinators' population would defile the ecosystem services provided by them. To this end, European Commission banned the use of three neonicotinoid insecticides namely; clothianidin, imidacloprid, and thiamethoxam for all field crops in 2018 (Stokstad 2018). In Malaysia, organochlorine insecticides, which include dichlorodiphenyl-trichloroethane (DDT), aldrin, amitrole, chlordane, dieldrin, lindane, mirex are listed as persistent organic pollutants and their usage are prohibited (DOA n.d.).

Owing to several factors such as the rise in the number of resistant pests, increasing food demand, and the need for increased agricultural productivity, the insecticide market is perpetually expanding in the Asia-Pacific region. The European Food Safety Authority (EFSA 2013) recommended the examination on the short-term and long-term effects of exposure to the market available insecticides on the behavior and survival of pollinators. Few studies have reported the effect of insecticide usage on *Elaeidobius kamerunicus* (Mohd Najib et al. 2019; 2012; Yusdayati & Hamid 2015) and these were carried out in lab scale.

In this study, the effect of four commercial insecticides on oil palm pollination weevil, *Elaeidobius kamerunicus*, was evaluated with a particular focus on flower visitation and numbers of newly emerged weevils. These four insecticides, namely Diflubenzuron, Fipronil, Chromafenozide and Pyridalyl are commonly used by oil palm farmers in Sarawak to control oil palm bunch moth, *T. mundella* (Su et al. 2021a,b) and they have different modes of action in controlling the pests' population.

MATERIALS AND METHODS

Experimental Design and Insecticides Treatments

The effect of insecticides on oil palm pollinating weevil on flower visitation and reproduction were investigated based on field trial that was conducted between April 2019 to July 2019 in a 30 hectare seven years old oil palm estate established on peat land (N 4º 02' 57.660" E 114º 13' 10.380") in Miri, Sarawak, Malaysia. A total of 25 male inflorescences at pre-anthesis stage were randomly selected from a hectare area for the experiment. Four insecticides (treatments) namely; Pyridalyl 32.5% w/w, Fipronil 80% w/w, Diflubenzuron 25.0% w/w and Chromafenozide 30% w/w, were tested under a complete randomized design with four replicates. Water was used as the control treatment.

Insecticide Application and Sampling of Male Inflorescence

Six stages of oil palm male inflorescence anthesis were recorded in the field (Figure 1 A-F). Twenty-five oil palm male inflorescences at stage 0 of the anthesis (Figure 1A) were randomly selected from a seven year-old oil palm estate. The male inflorescence bracts were opened at this point. The inflorescences were randomly divided into five treatment groups, each treatment groups containing five male inflorescences as replicat. Male inflorescences in each group were sprayed with one liter of a selected insecticide assigned to the treatment and the inflorescences in the control treatment group were sprayed with water only. The treatment application rate was summarized in Table 1. The spraying was accomplished using PB 16 knapsack sprayer attached with 60 elbow and liquid spray adjustable solid cone red nozzle with 0.70-0.85L flow rate per minute. The spraying was carried out early in the morning, between 7:00 am to 9:00 am. The percentage of active ingredient concentration adopted in this study for Diflubenzuron, Fipronil, Chromafenozide and Pyridalyl per liter basis were 0.25%, 0.048%, 0.33% and 0.61% respectively, which are equivalent to the recommended rate per hectare. The rate per hectare adopted in this trial for Diflubenzuron, Fipronil, Chromafenozide and Pyridalyl were 150g, 19g, 1L and 200 mL respectively. When flowers began opening at the base of the spikelets (Figure 1B), treated male inflorescences were collected and this sampling process continued till the end of anthesis (Figure 1F). Sampling of male inflorescence was carried out in the evening, after 5:00 p.m. on each sampling day. This is because adult weevils were more likely to stay on the spikelets for mating and oviposition at dusk, which explained why *E. kamerunicus* was found to be more settling and made less flight after 5:00 p.m. GMT 8 (Chiu 1984; Yue et al. 2015).

Eighteen spikelets were sampled from each male inflorescence for *E. kamerunicus* sampling. The spikelets were selected from six positions in a male inflorescence, which were inner basal, outer basal, inner medium, outer medium, inner distal and outer distal of male inflorescence. Three spikelets were collected at each position and hence a total of 18 spikelets were sampled from each inflorescence. This method will considerably reduce the average percent error (Chiu 1984). All sampled spikelets were kept in modified plastic containers with mesh wire as a cap prior to *E. kamerunicus* counting in the laboratory.

Figure 1A. Day 0- There was no sign of anthesis. No weevil was spotted on the spikelets of the male inflorescence

Figure 1B. Day 1 – Anthesis stage I. Florets at the base of the spikelet began to open. A small number of weevils began to visit spikelets of male inflorescence

Figure 1C. Day 2 – Anthesis Stage II. More florets began to open and gradually moving upwards with mild aniseed smell. The number of weevils visiting the male inflorescence gradually increased

Figure 1D. Day 3 – Anthesis Stage III. Full bloom of the male inflorescence with a strong aniseed smell. Abundant weevils had been found at this stage on the spikelets of male inflorescence

Figure 1E. Day 4 – Stage IV. Male inflorescence retained its full blooming, but the aniseed scent began to fade away, as did the color of the pollen. Pollen was beginning to shed. The number of weevils found on the spikelets was noticeably reduced

Figure 1F. Day 5 – Stage V. The anthesis fully over. No more pollen and aniseed scent were detected. White fungi would often grow on the surface of the spikelets

Table 1. Treatments for weevil topical spraying trial and the rate used

Treatment	Product Rate/ha	Rate/Liter On Each Inflorescence	% ai of Product/Liter
(T1) Control	-	-	-
(T2) Diflubenzuron 25% w/w	150g	1.0 g	0.25
(T3) Fipronil 80% w/w	19.00g	0.06 g	0.048
(T4) Chromafenozide 4.9% w/w	1000.00 ml	6.67 mL	0.33
(T5) Pyridalyl 45.5% w/w	200.00 ml	1.34 mL	0.61

Counting For Trapped Adult Weevils Visiting Male Inflorescence

The containers that contained spikelets were covered with a big transparent plastic bag. The containers were given several gentle shakes to provoke the weevils that lodged on the spikelets to fly out. The container cap was slowly lifted up to allow the weevils in the spikelet and container flew into the plastic bag. The plastic bag that confined the visiting weevils was stored in the -20^{o}C freezer for 30 minutes to freeze the weevils. Then the total number of weevils present on each spikelet was counted and recorded to determine the effect of insecticides on the number of visiting adult weevils in male inflorescence. The containers with spikelets were put away at 25^{o}C for another 21 days to allow the weevils' egg that remained in the spikelets to hatch.

Counting for Newly Emerged Adult Weevils

The containers with spikelets were kept at room temperature. The containers were given several gentle shakes each day to agitate the newly hatched weelvils to leave the spikelets. The newly emerged weevils were captured in a transparent plastic bag that covered the container when the container cap was removed. The first adult weevil emerged after one week of sample collection from the field was observed. Subsequently, the newly emerged adult weevils were continually counted and recorded for 21 days. This formed raw data for the total number of newly emerged adult weevils from the spent male inflorescence spikelets.

Statistical Analysis for Weevil Count

Number of visiting adult weevil per spikelet of male inflorescence and newly emerged adult weevil were subjected to logarithmic transformation in order to make data conform more closely to the normal distribution. After transformation, the total weevil count for different treatments were analyzed using one-way analysis of variance (ANOVA) in the statistical analysis system (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA). Duncan's multiple range test (DNMRT) at $P \leq 0.05$ was used to separate the treatment means. Correlation coefficient between total number of visiting weevils and newly emergence weevils for all treatments was also analysed using statistical analysis system (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Our results showed that the total number of weevils visiting the male inflorescences reduced with the use of insecticides (Table 2). It was estimated that without any insecticide application, the oil palm male inflorescence would have received 8-103 weevils per spikelet (Saharul et al. 2021a,b), while in this study, the inflorescences from insecticides treated plots had received below five weevils. Oil palm male inflorescences treated with Chromafenozide and Pyridalyl were the least affected among the four insecticides tested while the male inflorescences sprayed with Fipronil experienced 80% reduction in flower visitation by weevils. This indicates that Fipronil had the most substantial adverse effect on flower visitation by the pollinating weevil. This is similar to what was reported by Noor Farehan et al. (2020) in their lab based bioassay study.

Table 2. Mean number of visiting weevils per spikelet per day

Treatment	**Mean number of visiting weevils per spikelet**
(T1) Control	9.58±1.72[a]
(T2) Diflubenzuron 25% w/w	2.78±0.45[bc]
(T3) Fipronil 80% w/w	1.55±0.44[c]
(T4) Chromafenozide 4.9% w/w	3.96±0.41[b]
(T5) Pyridalyl 45.5% w/w	4.80±1.23[b]

Mean values followed by the same letter within column are not significantly different at P = 0.05, based on DNMRT (Mean±SE; n=4).

The highest number of visiting weevils was observed in the medium outer part of the spikelet (Table 3). Most of the florets in this part opened on Day 2-3 of anthesis (Figure 1C & 1D). Peak of pollinating weevil's visitation to the male inflorescence occurred on Day 2-3. Table 4 indicates that the number of visiting weevils per spikelet peaked at anthesis stage 3 in all the treatments.

Distal position had the least number of visiting weevils. It was observed that the distal florets opened towards the end of anthesis when the aniseed scent began to fade away. The diminishing chemical attractant to the weevils could contribute to less weevils' visitation. On top of that, the distal spikelets were significantly shorter than the medium and basal spikelets, as also noted by Saharul et. al. (2021a) and Chiu (1984). Shorter spikelets would have less florets that are the main breeding site and food source for the weevils. Hence, this could be another possible reason that less number of visiting weevils was noted at the distal spikelets.

The number of newly emerged adult weevils indicates reproducibility of weevils. New batch of weevils emerged as adults a week after the first day of anthesis. The control plot (without insecticide application) had an mean of 43 newly emerged adult weevils per spikelet (Table 5). The male inflorescences treated with Chromafenozide and Pyridalyl had a statistically similar number of newly emerged adult weevils per spikelet, to the control treatment. However, the mean number of newly emerged adult weevils per spikelet was significantly lower in both Diflubenzuron and Fipronil insecticides. Furthermore, in comparison with the control, the number of newly emerged adult weevils per spikelet in the Fipronil treatment was exceedingly low. Such a situation is alarming as the weevil population can be significantly reduced if either Fipronil or Diflubenzuron is to be used continuously in the oil palm plantation.

Fipronil disrupts the central nervous system of the target insect (Robea et al. 2018) by blocking GABA-gated chloride channels and glutamate-gated chloride (GluCl) channels. Due to its high affinity to GABA insect receptors, Fipronil is target-specific to insects and appears harmless to mammals that do not have GluCl channels (Amrith & Tresca 2007). The findings of this study reveal that Fipronil, a member of the phenylpyrazole chemical family, could be detrimental to the weevils, *E. kamerunicus*. Moreover, in the study, only very few adult weevils were found pollinating the treated male inflorescence during sampling. In addition, clumps of dead oil palm pollinating weevils were found at the base of the male inflorescence stalk treated with Fipronil. This indicates that Fipronil may instantly or gradually kill the weevils upon visiting the treated male inflorescence.

On the other hand, Diflubenzuron with active ingredient 1-(4-chlorophenyl)-3-(2,6-difluoro-benzoyl)-urea, is known to inhibit insect growth by hindering the synthesis of chitin, a component of arthropod exoskeletons (Andreotti et al. 2015). Diflubenzuron is claimed to be less effective against adult pest (Castro et al. 2012).

However, many studies i.e Andreotti et al. (2015), Grosscurt & Tipker (1980) and Grosscurt (1980) have shown that Diflubenzuron, like other benzoyl phenyl urea derivatives, has larvical and ovicidal activity in which the compound would disrupt the reproductive process of the target insects, in particular Diptera and Lepidoptera insects. From the current study (Table 2 & Table 4), application of Diflubenzuron reduced the number of visits to male inflorescence and newly developed adult weevils, which could impact pollination efficiency and the size of the weevil population when prolonged use is practiced.

Both Chromafenozide and Pyridalyl insecticides showed a milder adverse effect on *E. kamerunicus*. Even though the number of visiting weevils in these treatments had a marginal decrease, the number of newly developed adult weevils was not affected. Chromafenozide is a non-steroidal agonist of the insect molting hormone 20-hydroxyecdysone (Ahmed et al. 2015) effective in controlling various lepidopterous pests, for example Pyralidae, Tortricidae, Noctuidae and Heteropteran bugs on different crop plants like rice, tea, apple, cabbage, among others (Ahmed et al. 2015; Mikio et al. 2006). Chromafenozide acts relatively fast, stopping the insect from feeding within 10-12 hours of exposure to the toxin (Mikio et al. 2006). It is nontoxic for a wide variety of coleopteran, homopteran, orthopteran, hemipteran, dipteran and mite pests by means of an extra selective procedure for action against lepidopteran pests (Mikio et al. 2006). This supports present findings for which application of Chromafenozide did not adversely affect *E. kamerunicus* visits to oil palm male inflorescence and the number of newly developed weevil. Thus, Chromafenozide is deemed to be the more promising insecticide to be used in oil palm estates.

Pyridalyl, on the other hand, is a highly selective insecticide against various lepidopteran insects (Ishaaya & Casida 1974; Sakamoto et al. 2005; Shinya et al. 2007), Diptera and Thysanopterous insect pests (Powell et al. 2011; Sakamoto et al. 2005). Pyridalyl inhibits protein synthesis in the target insect cell growth (Sakamoto et al. 2003) and causes oxidative stress in the affected cells, whose result is protein degradation, necrosis and eventual cell death (Powell et al. 2011). In this study however, Pyridalyl was safe to use in oil palm estate as it did not harm the oil palm pollinator, *E. kamerunicus*.

In absence of insecticide intervention throughout the anthesis period, the total number of weevils that visited the male inflorescence per spikelet was approximately 43 individuals (Table 5). This result agrees with the findings reported in Saharul et al. (2021b) and Su & Bong (2017). The number of weevils that visited the inflorescence was not correlated with the number of newly developed weevils in all the treatments (Table 6).

Table 3. Mean number of visiting weevils per spikelet by position

Treatment	**Spikelet Section**					
	Basal In	**Basal Out**	**Middle In**	**Middle Out**	**Distal In**	**Distal Out**
(T1) Control	11.37±2.21[ab]	10.67±3.64[ab]	9.57±3.45[ab]	13.28±2.28[a]	5.24±1.83[c]	7.37±2.25[bc]
(T2) Diflubenzuron 25% w/w	2.92±1.26[ab]	3.75±0.81[a]	2.27±0.43[ab]	3.35±0.39[ab]	2.07±0.69[b]	2.30±0.46[ab]
(T3) Fipronil 80% w/w	1.12±0.27[bc]	1.78±0.25[abc]	2.07±0.95[ab]	3.28±1.42[a]	0.25±0.13[c]	0.78±0.26[bc]
(T4) Chromafenozide 4.9% w/w	2.88±0.67[c]	5.29±1.12[ab]	3.45±0.52[bc]	5.85±0.91[a]	2.50±0.89[c]	3.78±0.45[bc]
(T5) Pyridalyl 45.5% w/w	5.15±1.35[ab]	4.57±1.28[bc]	4.95±1.18[b]	7.32±2.20[a]	2.57±0.76[c]	4.27±1.76[bc]

Mean values followed by the same letter within row are not significantly different at *P=0.05*, based on DNMRT (Mean±SE; n=4).

Table 4. Mean number of visiting weevils per spikelet by stages

Treatment	**Anthesis Stage(s)**				
	S1	**S2**	**S3**	**S4**	**S5**
(T1) Control	3.74±1.66[b]	10.06±1.54[b]	22.67±6.08[a]	9.93±2.17[b]	1.51±0.18[b]
(T2) Diflubenzuron 25% w/w	1.06±0.19[c]	2.67±0.40[b]	6.86±0.95[a]	2.50±0.63[b]	0.79±0.17[c]
(T3) Fipronil 80% w/w	0.49±0.17[c]	1.97±0.56[b]	3.88±1.16[a]	1.07±0.24[bc]	0.33±0.08[c]
(T4) Chromafenozide 4.9% w/w	1.71±0.33[bc]	3.75±0.46[b]	10.03±1.47[a]	2.31±0.46[bc]	1.14±0.15[c]
(T5) Pyridalyl 45.5% w/w	2.03±0.42[bc]	5.76±1.53[b]	10.65±2.87[a]	4.43±1.58[bc]	1.14±0.30[c]

Mean values followed by the same letter within row are not significantly different at *P=0.05*, based on DNMRT (Mean±SE; n=4).

Table 5. Mean number of newly emergence weevils per spikelet per day

Treatment	**Number of newly emerged weevils per spikelet**
(T1) Control	43.45±6.34[a]
(T2) Diflubenzuron 25% w/w	16.58±2.71[bc]
(T3) Fipronil 80% w/w	1.68±0.89[c]
(T4) Chromafenozide 4.9% w/w	39.49±4.74[a]
(T5) Pyridalyl 45.5% w/w	29.21±11.22[ab]

Mean values followed by the same letter within column are not significantly different at *P=0.05*, based on DNMRT (Mean±SE; n=4).

Table 6. Correlation coefficient between total number of visiting weevils and newly emergence weevils among different treatment

Treatment	***P≤0.05***
(T1) Control	0.66
(T2) Diflubenzuron 25% w/w	0.09
(T3) Fipronil 80% w/w	0.09
(T4) Chromafenozide 4.9% w/w	0.97
(T5) Pyridalyl 45.5% w/w	0.44

CONCLUSION

The consideration of selecting suitable insecticides that are safe for oil palm natural pollinators, *E. kamerunicus,* while maintaining the effectiveness of insect pests control such as *T. mundella* in the fields becomes an urgent need in ensuring healthy growth of the oil palm ecosystem. Based on the findings of this study, it is recommended the use of either Pyridalyl or Chromafenozide as the promising insecticides in oil palm estates as both insecticides were found to be friendly to natural pollinators of oil palms. Furthermore, it is also recommended that both insecticides to be used alternately in order to provide a more comprehensive protection coverage against insect pests due to their different modes of action.

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