EFFICACY OF INSECTICIDES ON BLACK-HEADED STEM BORER, Chilo polychrysus WALKER (LEPIDOPTERA: PYRALIDAE) IN GLASSHOUSE CONDITION

Nur Atiqah Mohd Khari^{1,2*} & Suhaila Ab Hamid¹

 ¹ School of Biological Sciences,
Universiti Sains Malaysia, 11800 Penang, Malaysia
²Malaysia Agriculture Research and Development Institute, MARDI Seberang Perai, 13200,
Kepala Batas, Penang, Malaysia.
*Corresponding author: *atiqahmk@mardi.gov.my*

ABSTRACT

Three groups of insecticides; two from diamide and one from phenylpyrazole exemplified by chlorantraniliprole, flubendiamide, and fipronil, respectively, are the most widely used conventional insecticides in Malaysia to control several pests of lepidopterans in rice, including black-headed stem borer, Chilo polychrysus. However, the development of insecticide resistance in the rice stem borer has been raised up in many other countries. Therefore, this study aims to verify the efficacy of fipronil, flubendiamide and chlorantraniliprole to the susceptible colony of C. polychrysus as the initiative for the resistance monitoring. A seedling dip bioassay method was adopted to compare the efficacy and lethal concentration (LC₅₀) of the three conventional insecticides. The insecticide efficacy (E_t) was determined from the mortality percentage of C. polychrysus. The initial efficacy (at 24 hours) of fipronil, flubendiamide and chlorantraniliprole were 87.5%, 15.8% and 21.7% respectively. The effectiveness of the insecticides is determined by the mortality percentage or the final efficacy (Et). The final efficacy of fipronil was recorded at 72 hours of observation, followed by flubendiamide (96 hours) and chlorantraniliprole (120 hours). The lethality index (LI) of fipronil was the highest, (96.7%) that caused rapid effect to the rice stem borer. Result of toxicity effect showed that LC₅₀ for fipronil was the most effective against *C. polychrysus* with 16.12 mg/L followed by chlorantraniliprole (43.25 mg/L) and flubendiamide (76.43 mg/L). The LC₅₀ value of fipronil was significantly different to flubendiamide but not to chlorantraniliprole. Thus, fipronil is the most toxic and most effective insecticide to control C. polychrysus in glasshouse.

Keywords: Chilo polychrysus, chlorantraniliprole, flubendiamide, fipronil, insecticide efficacy

ABSTRAK

Tiga kumpulan racun serangga; dua racun phenylpyrazole dan satu diamide antaranya chlorantraniliprole, flubendiamide, dan fipronil,setiap satu adalah antara racun serangga yang paling banyak digunakan di Malaysia untuk mengawal serangga perosak untuk tanaman padi

terutama Lepidoptera termasuklah pengorek batang padi, Chilo polychrysus. Kawalan kimia ke atas serangga perosak ini menjadi lebih sukar kerana peningkatan kerintangan serangga terhadap kebanyakan racun serangga komersial. Justeru, kajian ini dijalankan untuk menentukan keberkesanan fipronil, flubendiamide dan chlorantraniliprole ke atas larva pengorek batang kepala hitam, C. polychrysus bagi tujuan mengawal kerintangannya. Kaedah bioasai rendaman anak pokok dijalankan untuk membandingkan keberkesanan dan kepekatan yang mematikan 50% populasi (LC₅₀) untuk tiga jenis racun serangga. Keberkesanan racun serangga (Et) ditentukan daripada peratus kematian larva C. polychrysus. Nilai keberkesanan permulaan (Et) pada 24 jam untuk fipronil, flubendiamide dan chlorantraniliprole masingmasing adalah 87.5%, 15.8% dan 21.7%. Keberkesanan akhir racun serangga ditentukan oleh peratus kematian atau keberkesanan akhir (Et). Nilai keberkesanan akhir (Et) fipronil direkodkan pada 72 jam diikuti pada 96 jam oleh flubendiamide dan 120 jam oleh chlorantraniliprole. Indeks kematian (LI) tertinggi direkodkan pada fipronil dengan LI 96.67% yang boleh menyebabkan kesan yang pantas kepada C. polychrysus. Ujian penentuan ukuran dasar kerintangan C. polychrysus ini mendapati LC₅₀ untuk fipronil adalah 16.12 mg/L diikuti oleh chlorantraniliprole (43.25 mg/L) dan flubendiamide (76.43 mg/L). Keseluruhannya didapati fipronil adalah paling toksik dan berkesan untuk mengawal C. polychrysus di dalam rumah kaca.

Kata kunci: *Chilo polychrysus*, chlorantraniliprole, flubendiamide, fipronil, keberkesanan racun serangga

INTRODUCTION

Rice stem borer, *Chilo polychrysus* Walker (Lepidoptera: Pyralidae) is an important rice pest in Asia, including Malaysia (Ooi 2015). The stem borer infests the paddy plants from the vegetative until reproductive stages (Suharto & Usyati 2005; Yaakop et al. 2020). The larvae bore into the paddy stem and cause deadheart and whitehead. The symptoms cause the central leaf of the plants to wilt during the vegetative stage and the grain become unfilled over the reproductive stage (Shamik 2020). The damage caused by the rice stem borer contribute to a significant yield loss (Rahman et al. 2004).

There are many methods in controlling the rice stem borer such as biological approach, cultural practices, resistance varieties, mechanical and chemical control for a sustainable management (Frank et al. 2018). However, current control practiced in reducing the rice stem borer infestation is solely by using chemical insecticides (Rahaman & Stout 2019). The control of rice stem borer by using chemical insecticides become popular among the farmers because it gives fast effect, more practical and have various choices in the market (Chen & Klein 2012). Due to that, heavy application of insecticides in the field has gone beyond the recommended dose. This has cause many problems at all angles including economy, health, environment pollution and even to the development of insecticide resistance in the insect body as mentioned by Aktar et al. (2009) and Gill and Gard (2014).

The development of insecticide resistance become one of the factors that influences the insecticide efficacy (Zhu et. al 2016). Many studies have been conducted in Malaysia to measure the efficacy of insecticides against insect pests, e.g by Noor Aslinda Ummi et al. (2019) to control the *Aedes aegypti*. The insecticide is defined effective when it consistently reduces pest numbers or damage to a commercially acceptance level when applied in accordance to label directions (Damalas & Eleftherohorinos 2011).

In Malaysia, the damages caused by the rice stem borer has become more severe as reported by Noor Ainon (2019a) where a total of 1127 out of 4584 hectares were affected by the rice stem borer infestation in Seberang Perak, Malaysia. The economic threshold level (ETL) reported was 10, 15, 40 and 40% at Blok A, B, C and D in IADA Seberang Perak, respectively. A total 0f 800 farmers were burden with yield loss and unseen incomes. The farmers complaint that the conventional insecticides was less effective to control the rice stem borer (Noor Ainon 2019b). Therefore, the performance of the insecticides required to be reevaluate. As an initiative for insecticide resistance monitoring, the efficacy of certain insecticides was evaluated on the susceptible population. The susceptible population with uniform resistance status of the insect pest promises a non-bias result and more precise. The efficacy of certain insecticides is expecting to reflect the optimum effect with the susceptible population. Therefore, this study was performed to evaluate the efficacy of selected insecticides against black-headed stem borer, *Chilo polychrysus* in the glasshouse.

MATERIALS AND METHOD

Insect Sampling and Rearing

The third instar larvae of *C. polychrysus* were obtained from glasshouse rearing for the bioassay purpose. The third instar were identified by the head capsule (Calvo & Molina 2008), larval body size including the length and the weight of the instar from the preliminary study. The individual number of head capsules in the paddy stems showed the number of instar stage of the larvae. The insect was collected from the rice plot in MARDI Seberang Perai, Penang, Malaysia by using sweep net for the adult and hand picking for the larvae and were reared in the glasshouse for ten generations. The methodology followed in the present study was adapted from Iranipour et al. (2010) and Manikanda et al. (2016). This study was conducted in glasshouse cages at temperatures ranging from 29°C to 32°C and relative humidity from 65% to 81% (Manikanda et al. 2016). The host plants used were the TN1 rice varieties (Taichung Native 1) until they reached 25 cm in height (Wu 2014) or approximate 30 days old and were placed in a cage (34 cm (L) x 20 cm (W) x 24 cm (H)) to prevent pest infestation.

Insecticide Preparation

All insecticides were purchased from the local market in Kepala Batas, Penang Three conventional insecticides with different active ingredients were chosen with two insecticides are from diamide group and one from phenylpyrazole group. The following three insecticides listed with their maximum label rates were tested as formulated material: clorantraniliprole 5SC (DuPont Crop Protection, 5% of active ingredients, 95% of inert materials of suspension concentrate) 9 ml (AI)/10L and flubendiamide 20WG (Agriculture Chemicals (M) Sdn. Bhd., 20% of active ingredients, 80% of inert materials of wettable granule) 5.4 g (AI)/10L and fipronil 5SC (Bayer Crop Science, 5% of active ingredients, 95% of inert materials of suspension concentrate), 10ml (AI)/10L Each insecticide was prepared in volume 250 ml at four different concentrations according to a series of concentration levels; 0.5x, x, 1.5x and 2.0x where x was the rate stated on the label. The preparation of the insecticide solutions was followed by the equation of Amarasekare and Shearer (2013).

Seedling-dip Bioassay Technique

The host plants used were rice variety TN1 (Taichung Native 1) seedlings. Approximately, 30 grains of rice seeds were planted in each pot and grown until thirty days old before used in the experiment. Method for growing and maintaining the rice Othman et al. (2008).

The 30 days old rice seedling played as the medium for the seedling dipping method for insecticide monitoring bioassay. A perfect single seedling with roots cut into 6 cm stem and soaked in the 250 ml insecticide solutions for 10 seconds (Gao et al. 2013). After the stem was air-dried, 10 individuals of the third instars larvae were introduced to the stem. The infested stem was transferred into a plastic container with 5 cm diameter x 10 cm in height with good ventilation and covered with a lid and represented as one replication. During the bioassay, the paddy stem was supplied with sufficient water at the bottom of the container. There were three replications for each treatment. The observation was recorded until day 5. The number of dead larvae were counted and recorded daily. The larvae were considered dead when no movement was observed when stimulated gently with a brush. This seedling dip method was used to measure the efficacy of the selected insecticides against the *C. polychrysus* in glasshouse conducted at the MARDI Seberang Perai, Penang, Malaysia from 2019 to 2020. It is situated in the northern zone of Malaysia at $5.5172^{\circ}N$ latitude and $100.4315^{\circ}E$ longitude.

Insecticide Efficacy and Lethality Index

The insecticide efficacy (E_t) was measured by the percentage mortality of the *C. polychrysus* larvae over the time observed. The observation was recorded at 24 (E_0), 48 (E_1), 72 (E_2), 96 (E_3) and 120 (E_4) hours. The initial efficacy (E_0) which was at 24 hours represent the first respond of *C. polychrysus* larvae in terms of the percentage mortality to the treatments. It was classified as low ($E_0 \le 10$ %), moderate 10 % < $E_0 < 90$ % or high ($E_0 \ge 90\%$) (Norhayu & Nurnisa Nabilah 2020). The two ends; $E_0 \le 10$ % and $E_0 \ge 90\%$ was established to represent the classified insecticides into the level of very low and highly harmful insecticides to the initial efficacy of the *C. polychrysus* larvae. In addition, the changes over 120 hours or the efficacy change (E_4 - E_0) were classified as stable, increasing or decreasing (Norhayu & Nurnisa Nabilah 2020). The insecticide was classified as stable when efficacy change was within ±10% from the initial efficacy, E_0 , increasing ((E_4 - E_0) >10%) or decreasing ((E_4 - E_0) <10%). The maximum range for the initial efficacy (E_0) to be increased or decreased was less than (>10%) from the E_0 itself (Leskey 2012). According to Leskey (2012) a lethality index (LI) was established to compare the changes of *C. polychrysus* in respond to the insecticide effect over the observation period after the insecticide treatment. The index was calculated by the formula below:

 $LI = \sum [(Number of alive larvae x 0.0) + (Number of dead larvae x 1.0)] / (Number of total larvae x Number of observations) x 100$

The equation appoints 0.0 and 1.0 for alive and dead individuals respectively. The lower value of the index indicates that the insecticides have slower effect to the insects.

Lethal Concentration (LC₅₀)

Toxicity study of the tested insecticides was performed to determine the effect of the insecticides to the mortality of the *C. polychrysus* larvae. The value was presenting in LC_{50} which was the lethal concentration to kill 50% of the tested population. A pesticide with a lower LC_{50} is more toxic than a pesticide with a higher value of LC_{50} . The lethal concentration was evaluated by using Polo Plus software [Robertson et al. 2003)] The LC_{50} obtained from the Probit analysis established the insecticidal activity in term of toxicity effect of the insecticides. The toxicity between the treatments was considered significantly difference when no overlapping of LC_{50} value at 95% confidence interval (CI).

Data Analysis

ISSN 1394-5130

The mortality data were analysed using analysis of variance (ANOVA) Complete Randomized Design (CRD) by Statistical Analysis System (SAS) programme software version 9.4 and treatment means were separated using Duncan's multiple range test (DMRT) at the 5% and 1% (p<0.05 and p<0.01) level of significance (Gomez &Gomez 1984).

RESULTS

Insecticide Efficacy and Lethality Index

The results indicated that the fipronil possessed the most potent insecticidal activity, exhibiting the greatest mortality effect on the third instar larvae of *C. polychrysus* followed by flubendiamide and chlorantraniliprole (Figure 1). The insecticides crossed the 50 % mortality lines as early as 24 hours presented by fipronil with 87.50% of mortality in front of the flubendiamide, (60.83%) and chlorantraniliprole, (53.33%) at 48 hours.

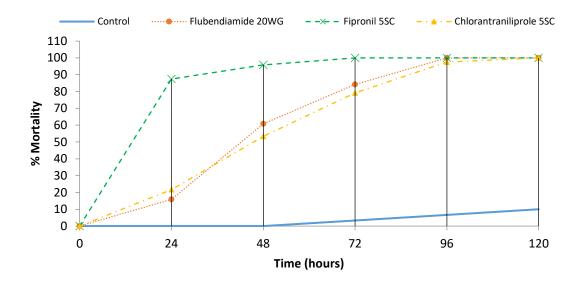


Figure 1. Effect of insecticides on the mortality of the *C. polychrysus* larvae within 120 hours (5 days) of observation

		Insectic	ide Efficacy (E _t), %	6				
Concentrations (%)	Time (hours)							
	24 h (E ₀)	48 h (E ₁)	72 h (E ₂)	96 h (E ₃)	120 h (E ₄)	Mean±SE		
	<u>Fipronil</u>							
0.5x	73.3	93.33	100	100	100	77.77±8.77a		
1.0x	83.33	96.67	100	100	100	79.44±8.80a		
1.5x	93.33	96.67	100	100	100	81.67±8.90a		
2.0x	100	100	100	100	100	83.33±9.04a		
	Flubendiamide							
0.5x	16.67	43.33	80	100	100	56.67±9.70c		
1.0x	16.67	56.67	86.67	100	100	60b±9.84c		
1.5x	13.33	63.33	90	100	100	61.11±10.25b		
2.0x	16.67	80	80	100	100	62.78±9.83bc		
			<u>Chloran</u>	<u>traniliprole</u>				
0.5x	6.7	36.67	60	93.33	100	49.44±9.75d		
1.0x	10	46.67	83.33	96.67	100	56.11±10.07c		
1.5x	40	63.33	83.33	100	100	64.44±8.86b		
2.0x	30	66.67	90.00	100	100	64.44±9.71b		
	Control							
Control	0	0	3.3	6.7	10	3.3±1.40e		
	Mean							
Insecticides	E_0	E_1	E_2	E_3	E_4			
Fipronil 5SC	87.50±3.51a	95.83±1.49a	100.00a	-	-			
Flubendiamide 20WG	15.83±3.12b	60.83±6.57b	84.17±3.36b	100a	-			
Chlorantraniliprole 5SC	21.67±5.88b	53.33±6.31b	79.17±5.43b	97.5±1.80a	100a			

Table 1. Insecticides treatments efficacy on Chilo polychrysus recorded after 24, 48, 72, 96 and 120 hours

Values are expressed as mean \pm SD x ._x Means with the same letters within a column are not significantly different.

The insecticide efficacy (E_t) study was determined by the percentage mortality of the *C*. *polychrysus* larvae after being treated with insecticides for 24, 48, 72, 96 and 120 hours (Table 1). The initial efficacy (E_0) (24 hours) of fipronil was 87.5%, flubendiamide (15.8 %) and chlorantraniliprole (21.7 %). The final efficacy of fipronil was recorded at 72 hours of observation, followed by flubendiamide (96 hours) and chlorantraniliprole (120 hours). The E_0 of fipronil was significantly differed (p<0.050) to flubendiamide and chlorantraniliprole until E_2 (72 hours). However, flubendiamide and chlorantraniliprole efficacy did not differed significantly from 24 hours until 96 hours.

The initial efficacy of fipronil, flubendiamide and chlorantraniliprole were classified as moderate, because the initial efficacy value was more than 10% and less than 90%, $(10\% < E_0 < 90\%)$ (Table 2). The value of the efficacy changes determined the differential of the final efficacy to the initial efficacy. The changes over 120 hours or the efficacy changes (E₄ - E₀) of all three insecticides were classified as increasing when the values obtained were greater than 10% with the fipronil recorded 12.5% followed by chlorantraniliprole (78.33%) and flubendiamide (84.17%).

The lethality index (LI) presented the strength rate of the insecticides to kill the insect. This indicated that fipronil gave the fastest effect to the mortality of *C. polychrysus* larvae. The following insecticides, flubendiamide and chlorantraniliprole, respectively recorded 72.17% and 70.33% LI with slower effect on the *C. polychrysus* larvae as presented in Table2.

Rank	Conventional Insecticides	Initial efficacy Efficacy Change (E ₄ -Lethality			
		(\mathbf{E}_0)	E ₀)	Index (%)	
1	Fipronil	Moderate	Increasing	96.67	
2	Flubendiamide	Moderate	Increasing	72.17	
3	Chlorantraniliprole	Moderate	Increasing	70.33	

Table 2. Lethality index of insecticides, initial efficacy and efficacy change over 120 hours of observation

 E_0 = the percentage of dead insects at 24 hours.

Low for $E_0 \le 10\%$; **Moderate** for $10\% < E_0 < 90\%$; **High** for $E_0 \ge 90\%$.

Increasing for $(E_4 - E_0) > 10\%$; **Decreasing** for $(E_4 - E_0) < -10\%$; **Stable** for $-10\% \le (E_4 - E_0) \le 10\%$.

Lethal concentration (LC₅₀)

Fipronil was more toxic compared to flubendiamide. Fipronil showed the lowest number of LC_{50} (16.12 mg/L) followed by chlorantraniliprole (43.25 mg/L) and flubendiamide (76.43 mg/L) (Table 3). Fipronil required the lowest amount of insecticide to kill 50% of population compared to the other two insecticides. The LC_{50} of fipronil was 16.12 mg/L which was lower than 0.5x concentration level (25 mg/L). In addition, there was a significant difference between fipronil and flubendiamide where no overlapping of LC_{50} value at 95% CI was recorded.

Insecticides	Number of populations	Slope ± SE	$\chi^2(df)$	LC ₅₀ (mg/L) (95% CI)
Fipronil	120	2.548±0.757	2.346 (2)	16.12 (4.16-24.49)
Flubendiamide	120	1.487±0.526	0.925 (2)	76.43 (24.62 -109.72)
Chlorantraniliprole	120	1.378±0.527	0.335 (2)	43.25 (18.78-67.79)

Table 3.The value of LC₅₀ for every insecticides by using seedling-dip technique

SE: Standard Error; χ^2 : Chi-squared value for the lethal concentration with significant level at P<0.05; CI: Confidential Interval

The LC₅₀ value of chlorantraniliprole was slightly lower than the 1.0x (recommended rate) which was 45 mg/L. This mean at 43.25 mg/L, the concentration can kill 50% of the insect population. However, Chlorantraniliprole did not differed significantly in terms of toxicity in comparison to fipronil and flubendiamide. The 95% CI recorded overlapping in LC₅₀ value in both chlorantraniliprole-fipronil and chlorantraniliprole-flubendiamide .

The highest LC_{50} was flubendiamide (76.43 mg/L) and the LC_{50} value was lower than the recommended rate which was 108 mg/L. With the application of insecticide at 76.43 mg/L, 50% of *C. polychrysus* larvae population can be reduced. The obtained LC_{50} value was listed in different range of 95% CI with no overlapping to the fipronil range. There was a significant difference on the toxicity level observed between flubendiamide and fipronil.

DISCUSSION

Insecticide efficacy of the three insecticides against the susceptible population of the, *C. polychrysus* was determined from the seedling-dip bioassay in the glasshouse study. The three insecticides were chosen because they were produced in single active ingredients and well-known insecticides used by farmers in Malaysia (Jafar et al. 2013). From the results, fipronil showed the capability to control *C. polychrysus* by killing 100% of the treated larvae. Fipronil showed the most excellent performance on the E_0 , E_t , LI and LC₅₀. This is still parallel to the finding since late 1990s that fipronil is among the excellent conventional insecticides in controlling stem borer. Saljoqi et al. (2002) in his study have confirmed that fipronil was one of the effective insecticides to control rice stem borer, *Tryporyza incertulas*. Furthermore, the incidence of rice stem borer was reducing effectively on the Basmathi rice also by using fipronil (Roshan 2006). In a study by Gunasekara et al. (2007), fipronil was found to give more efficient control against Chironomid insects for 9 to 14 days after their appearance on rice grains at 12.5 g of active ingredient per hectare than malathion at 300 g of active ingredient per hectare.

The mortality of *C. polychrysus* was recorded approximately 87.5% mortality within 24 hours (E₀) and reached 100% mortality within 72 hours (E₄). It shows that fipronil can kill the targeted insect in a high number within a short period of time. This was proven by the highest value of the LI, which was 96.67% if treated by fipronil. This indicated that fipronil had strong knockdown effect that might due to the unique action mechanism of the insecticide. Fipronil is the novel phenylpyrazole insecticide that gave prominent control effect against the rice stem borers. Fipronil formula, (\pm)-5-amino-1-(2,6-dichloro-a,a,a-triflfluorop-tolyl)-4-triflfluoromethylsulfi finylpyr-azole-3-carbonitrile, blocks GABAA-gated chloride channels in the stem borer central nervous system (Grant et al. 1998). Disruption of the GABAA receptors by fipronil could prevents the uptake of chloride ions resulting in excess neuronal stimulation and death of the target insect (Cole et al. 1993 & Grant et al. 1998). Mansoor et al. (2019) also found fipronil toxicity was higher than other tested insecticides for the field strain insects

(LC₅₀=16.12 mg/L). The mode of action of fipronil has make the insecticide works faster in a low concentration (Gunasekara et al. 2007). In this study, fipronil was observed to be more toxic compare to the other two insecticides from diamide group. Midges (*Chironomus tepperi*), which are common pests in rice fields, are also highly toxic to fipronil with the LC₅₀ and LC₉₀ values for midges were 0.43 and 1.05 mg/l, respectively (Stevens et al. 1998). Fipronil was also said to be very effective against a number of mosquito species. Ali et al. (1999) reported that *Culex quinquefasciatus* mosquito larvae were killed with fipronil at LC₉₀ = 0.90 lg/l and (C₅₀ = 0.35 lg/l within 24 hours. In addition, the LCs₅₀ for *Aedes aegypti* mosquito larvae at 24 and 48 hours were 11.7 mg/l and 7.14 mg/l respectively (Aajoud et al. 2003).

In line to fipronil, flubendiamide performed similar effect on the final percentage mortality of C. polychrysus. However, flubendiamide was recorded the lowest E₀ which was 15.83% on 24 hours of observation. This might due to the mode of action of diamide insecticide which occurred in the ingestion system where it took a longer time to kill the insect compare to fipronil. Flubendiamide plays a role as a ryanodine receptor (RyRs) activator, causing uncontrolled release of calcium and cause death by paralysing the insect muscular (Hannig et al. 2009). Flubendiamide targeted the insects with chewing feeding type because the ingestion by the insect can contributed to the stomach poison and an oral intoxicant. Interestingly, flubendiamide showed very low knockdown, but, at the same time, mortality was high even at short exposure intervals. In the current study, flubendiamide was observed to perform 100% mortality within 4 days (final efficacy), a day later than fipronil. With a non-parametric approach, the lethality index provides a standardised knockdown-to-mortality scaling. However, because this "standardised" approach is based on the weight of specific averaged observations, it concealed possible differences between insecticide categories with different knockdown patterns (Agrafioti et al. 2015). This result revealed that flubendiamide had a high lethality index which was 72.17% although the initial knockdown was very low. In addition, this foliar applied insecticide with lipophilic character that made flubendiamide insecticide fast acting, long lasting and has limited plant penetration and systemicity (Raminderjit et al. 2008). Apart from that, flubendiamide was observed to have the highest value of LC_{50} . In this study, it was observed that flubendiamide was significantly different on the lethal concentration value compare to fipronil (LC₅₀=76.43 mg/L). This emphasis that, flubendiamide required a higher amount (76.43 mg/L) of active ingredients compare to fipronil (16.12 mg/L) to kill 50% of the C. polychrysus larvae. Besides, this indicate that flubendiamide was less toxic and potentially considered to be a suitable agent for controlling lepidopterous insects because it was very safe for non-target species (Tohinishi et al. 2005). As studied by Jhansi Lakshmi et al. (2010), flubendiamide was least toxic to green mirid bug, an important predator in rice ecosystem compared to acephate and other insecticides. The current results are consistent with those of Tohinishi et al. (2005) and Ameta and Ajay Kumar (2008), who found that flubendiamide was substantially superior and highly successful in reducing *H. armigera* and *S. litura* populations. According to Mallikarjunappa et al. (2008), flubendiamide 20 WG @ 35 g a.i./ha was the most effective insecticide in reducing the incidence of rice stem borer, Scirphophaga incertulas (Walker), and leaf folder Cnaphalocrosis medinalis (Guen.) while also increasing yield. The finding by Venkataiah et al. (2015) also proven that flubendiamide was the most effective insecticide against Spodoptera litura in groundnut (Arachis hypogaea L.) with the highest yield; 2650 and 2100 kg/ha during 2010-11 and 2011-12, respectively. From the result, flubendiamide killed 100% of the C. polychrysus larvae in four days, high lethality index and less toxic. Based on low initial efficacy, flubendiamide hit the final efficacy on day four, just a day after fipronil, which had an aggressively high initial efficacy. This insecticide characteristic has made it the best candidate to be included in the integrated pest management strategy.

As a dimide insecticide, chlorantraniliprole has posed a low initial efficacy and that was 21.67%, in line to flubendiamide but in opposite to fipronil. This is due to the mode of action between the insecticides. The mode of action of chlorantraniliprole reflecting flubendiamide by showing the approximate percentage of C. polychrysus larvae mortality that explained by the LI. There was a high LI recorded, (70.33%) resulting in 100% final efficacy on day 5. Chlorantraniliprole reached the efficacy target at a longer time because most larvae were paralyzed but low mortality was reported. During the knockdown effect, the larvae were observed to be weak and less moving. Chlorantraniliprole (3-bromo-N-(4-chloro-2-methyl-6-((methylamino) carbonyl)phenyl)-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide) can cause an imbalance release of calcium by opening the ryanodine receptor modulator that impaired regulation of insect muscle contraction, which leads to rapid paralysis and eventual death (Ashfaq et al. 2011; Whalon et al. 2008). The selectively target characteristics of chlorantraniliprole to the RyRs in muscle fibers of insects made it a great fighter against the caterpillars (Lepidoptera) and some species in other insect orders such as Coleoptera, Hymenoptera, Diptera, and Hemiptera (Cordova et al. 2006; Lahm et al. 2007). In this study, chlorantraniliprole showed a moderate toxicity level ($LC_{50}=43.25 \text{ mg/L}$) with no significant difference observed between all insecticides. Both diamides insecticide have selective target (Huang et al. 2011). This was explained by Cordova et al. (2006) that the vertebrate RyRs are 400 to 3000 times less sensitive to the susceptible insect and non-toxic to them. The sensitivity of chlorantraniliprole to the ddifferential RyR is the reason for the active respond of the insecticide to attack some groups of insects than others (Isaacs et al. 2012), low toxicity to bees (Gradish et al. 2011; Larson et al. 2013) and to most families of predatory and parasitic insects that contribute to biological control (Brugger et al. 2009; Whalen et al. 2016).

The three insecticides were successfully control the susceptible black-headed stem borer *C. polychrysus* in the glasshouse study at different level of concentrations. Fipronil (systemic activity insecticides), flubendiamide and chlorantraniliprole with translaminar (Bostian et al. 1996) and systemic activity insecticides (Troczka et al. 2016) were absorbed into the plants after the application. All insecticides targeted the larval stage of the rice stem borer because it was the most active life stage that consumed the plant parts. Insecticides absorbed by plants entered the body of the insect from the chewing habit of the larvae and were digested in their stomach. After entering the insect body, the insecticides reacted to the nervous system of the insects and caused the death. While the adult stage of the rice stem borer, which was a moth, did not directly consume the plant parts to survive and was not affected by the diamide insecticides. The adult moth, on the other hand, had to be killed because it was the reproductive agent, and insect populations expanded when the moth's egg successfully hatched. Choosing the right insecticides was therefore important in controlling this pest.

CONCLUSION

As the conclusion, all insecticides were effective to control the susceptible population of blackheaded stem borer, *C. polychrysus* with 100% mortality. In this study fipronil showed the most excellent performance in most of the analysis. However, chlorantraniliprole also was proven effectively to control the black-headed stem borer but with moderate initial efficacy (E_0), final efficacy (E_t) and moderate in lethality index (LI) and toxicity value (LC₅₀). Flubendiamide was observed to take longer time in controlling the black-headed stem borer, *C. polychrysus* based on its low initial efficacy (E_0), less toxic and moderate in the final efficacy (E_t) and lethality index (LI) results.

ACKNOWLEDGEMENTS

This research was supported in part by Geran Universiti Penyelidikan (RUI) from Universiti Sains Malaysia (1001/PBIOLOGI/8011033) given to the second author. We are grateful to Malaysia Agriculture Research and Development Institute (MARDI) Seberang Perai, Kepala Batas, Pulau Pinang especially En. Amiruddin Mokhtar, Director of MARDI Pulau Pinang and Mdm. Maisarah Mohamad Saad, Senior Research Officer for giving permission and providing the facilities and place to conduct the *C. polychrysus* experiments. Many thanks to Rice and Paddy Research Centre MARDI especially to the Pest and Disease Programme for the research inputs of *C. polychrysus* studies. We thank Mr Badruhadza and Mr. Erwan Shah, MARDI Senior Research Officer for the advice on the technical parts and statistical analysis. Thanks to all assistants especially Nurul Syifa binti Mazlan, Ahmad Hadri bin Jumat, Siti Hamidah binti Ismail and Mr. Hazizi for their assistance in this experiment.

REFERENCES

- Aajoud, A., Ravanel, P., Tissut, M. 2003. Fipronil metabolism and dissipation in a simplified aquatic ecosystem. *Journal of Agricultural and Food Chemistry*. 51:1347–1352
- Agrafioti, P., Athanassiou, C.G., Vassilakos, T.N., Vlontzos, G. & Arthur, F.H. 2015. Using a lethality index to assess susceptibility of *Tribolium confusum* and *Oryzaephilus surinamensis* to insecticides. *PLoS One* 10(11): e0142044.
- Aktar, W., Sengupta, D. & Chowdhury, A. 2009. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology* 2:1–12.
- Ali, A., Chowdhury, M.A., Hossain, M.I., Ameen, M.U., Habiba, D.B. & Aslam, A.F.M. 1999. Laboratory evaluation of selected larvicides and insect growth regulators against fieldcollected *Culex quinquefasciatus* larvae from urban Dhaka, Bangladesh. *Journal of the American Mosquito Control Association* 15:43–47.
- Amarasekare, K.G. & Shearer, P.W. 2013. Laboratory bioassays to estimate the lethal and sublethal effects of various insecticides and fungicides on *Deraeocoris brevis* (Hemiptera: Miridae). *Journal of Economic Entomology* 106(2): 776–785.
- Ameta, O.P. & Ajay, K. 2008. Efficacy of flubendiamide 480SC against *Helicoverpa armigera* (Hubner) and *Spodoptera litura* (Fab.) in chilli. *Pestology* 32(5): 26-29.
- Ashfaq, A.S., Jay, F.B. & Stephen, F.G. 2011. Biochemical characterization of chlorantraniliprole and spinetoram resistance in laboratory-selected obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae). *Pesticide*. *Biochemistry and Physiology*. 99: 274-279.
- Bostian, A.L., Swasdichai, S. & Darus, L. 1996. Activity of fipronil on diamondback moth. Proceedings of the Third International Workshop: The Management of the Diamondback Moth and Other Crucifer Pests, pp.195-198.
- Brugger, K.E., Cole, P.G., Newman, I.C., Parker, N., Scholtz, B., Suvagia, P., Walker, G. & Hammond. T.G. 2009. Selectivity of chlorantraniliprole to parasitoid wasps. *Pest Management Science* 66:1075–1081.
- Calvo, D. & Molina, J.M. 2008. Head capsule width and instar determination for larvae of *Streblote panda* (Lepidoptera: Lasiocampidae). *Annals of the Entomological Society of America* 101: 881–886.
- Chen, R.Z. & Klein. M.G. 2012. Efficacy of insecticides against the Rice Stem-borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), and use of sex pheromones to time accurately the yearly application. *International Journal of Pest Management* 58: 354–360.
- Cole, L.M., Nicholson, R.A. & Casida, J.E. 1993. Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pesticide Biochemistry and Physiology*. 46:47–54.

- Cordova, D., Benner, E.A., Sacher, M.D., Rauh, J.J., Sopa, J.S., Lahm, G.P., Selby, T.P., ...et al....2006. Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pesticide Biochemistry and Physiology* 84:196– 214.
- Damalas, C.A. & Eleftherohorinos, G.E. 2011. Pesticide exposure, safety issues, and risk assessment indicators. *International Journal of Environmental Research and Public Health* 8 (5): 1402–1419.
- Frank, S., Bradley, L.K. & Moore. K.A. 2018. Chapter 8: Integrated pest management. In: Moore, K.A. & Bradley, L.K. (eds.). North Carolina Extension Gardener Handbook. Raleigh, NC: NC State Extension http://content.ces.ncsu.edu/8-integrated-pest-management-ipm [2 March 2021]
- Gao, C., Yao, R., Zhang, Z., Wu, M., Zhang, S. & Su, J. 2013. Susceptibility baseline and chlorantraniliprole resistance monitoring in *Chilo suppressalis* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*. 106: 2190-2194.
- Gill, H.K. & Garg, H. 2014. Pesticides: Environmental impacts and management strategies. In: Marcelo, L. (ed.). *Pesticides–Toxic aspects*, pp. 187–230. Croatia: InTech
- Gomez, K.A. & Gomez, A.A. 1984. Statistical procedures for agricultural research. NewYork: John wiley and sons.
- Gradish, A.E., Scott-Dupree, C.D., Shipp, L., Harris, C.R. & Ferguson, G. 2011. Effect of reduced risk pesticides on greenhouse vegetable arthropod biological control agents. *Pest Management Science* 67:82–86.
- Grant, D.B., Chalmers, A.E., Wolff, M.A., Hoffman, H.B., Bushey, D.F., Kuhr, R.J. & Motoyama, N. 1998. Fipronil: Action at the GABA receptor. In. Kuhr, R.J. & Motoyama, N. (eds). *Pesticides and the Future: Minimizing Chronic Exposure of Humans and the Environment*, pp. 147–156. Amsterdam: IOS Press.
- Gunasekara, A.S., Truong, T., Goh, K.S., Spurlock, F. & Tjeerdema, R. 2007. Environmental fate and toxicology of fipronil. *Journal of Pesticide Science* 32(3):189-199.
- Hannig, G.T., Ziegler, M. & Marçon, P.G. 2009. Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Management Science*. 65: 969 - 974.
- Huang, J., Wu, S.F. & Ye., G.Y. 2011. Evaluation of lethal effects of chlorantraniliprole on *Chilo suppressalis* and its larval parasitoid, *Cotesia chilonis*. *Journal of Integrative Agriculture*. 10: 1134 -1138.
- Iranipour, S., Pakdel, A. K. & Radjabi, G. 2010. Life history parameters of the sunn pest, *Eurygaster integriceps*, held at four constant temperatures. *Journal of Insect Science* 10: 1–9

- Isaacs, A.K.J., Qi, S., Sarpong, R. & Casida. J.E. 2012. Insect ryanodine receptor: Distinct but coupled insecticide binding sites for [N-C(3)H(3)]chlorantraniliprole, flubendiamide, and [(3) H]ryanodine. *Chemical Research in Toxicology* 25:1571–1573.
- Jafar, W.N.W., Mazlan, N., Adam, N.A. & Omar, D. 2013. Evaluation on the effects of insecticides on biodiversity of arthropod in rice ecosystem. *Acta Biologica Malaysiana*. 2(3):115-123.
- Jhansi Lakshmi, V., Krishnaiah, N. V., Katti, G. R., Pasalu, I. C and Chirutkar, P. M. (2010). Screening of insecticides for toxicity to rice hoppers and their predators. *Oryza*. 47(4): 295-301.
- Lahm, G.P., T.M. Stevenson, T.P. Selby, J.H. Freudenberger, C.M. Dubas, B.H. Smith, D. Cordova, et al. (2007). Rynaxypyr®: A new anthranilic diamide insecticide acting at the rynanodine receptor. pp. 111–120. In: H. Ohkawa, H. Miyagawa, and P.W. Lee (Eds.). Pesticide Chemistry, Crop Protection, Public Health, and Environmental Safety. Wiley-VCH, Weinheim, Germany.
- Larson, J.L., C.T. Redmond, and D.A. Potter. (2013). Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns. PLOS ONE. 8(6):e66375.
- Leskey, T. C., Lee, D., Short, B. D. & Wright, S. E. (2012). Impact of insecticides on the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae): Analysis of insecticide lethality. *Journal of Economic Entomology*. 105(5): 1726-1735.
- Mallikarjunappa, S., Kendappa, G.N. and Ganesh Bhat, U. (2008), Flubendiamide 20% WG-a novel insecticide for the control of rice stemborer, *Scirphophaga incertulas* and leaf folder *Cnaphalocrosis medinalis*. In Coleman memorial National Symp. on Plant prot., 4-6, December, Univ. Agric. Sci., GKVK, Bangalore.
- Manikanda, N., Kennedy, J. S. & Gutthalakshmi, V. 2016. Effect of elevated temperature on life history parameters of rice yellow stem borer (*Scirpophaga incertulas* Walker). *Current science* 110(5):851-854.
- Mansoor, M.M., Raza, A.B.M., Afzal, M.B.S. (2019). Fipronil resistance in pink stem borer, Sesamia inferens (Walker) (Lepidoptera: Noctuidae) from Pakistan: Cross-resistance, genetics and realized heritability. Crop Protection 120, 103-108.
- Noor Aslinda Ummi, A.B., Azman, S., Lailatul Nadhirah, A., Khadijah, K. (2019). Resistance status of *Aedes aegypti* towards different insecticides in selected dengue outbreak area in Petaling district (Diptera: Culicinae). *Serangga*. 24(2):41-48
- Noor Ainon, M. (2019a, Disember 28). IADA Seberang Perak serius tangani ulat putih. Sinar Harian Online. Access on 22 February 2021 from https://www.sinarharian.com.my/artiCIe/63703/EDISI/Perak/IADA-Seberang-Perakserius-tangani-ulat-putih
- Noor Ainon, M. (2019b, Disember 28). Padi musnah dirosakkan ulat putih. Sinar Harian
Online.OnlineAccessOn22February2021from

https://www.sinarharian.com.my/artiCIe/63626/EDISI/Perak/Padi-musnahdirosakkan-ulat-putih

- Norhayu, A. & Nurnisa Nabilah, M. 2020. Effect of six insecticides on oil palm pollinating weevil, *Elaeidobius Kamerunicus* (Coleoptera: Curculionidae). *Serangga* 25(2):1-9
- Ooi, P.A.C. 2015. Common insect pests of rice and their natural biological control: An illustrated guide to the insect pests that feed on rice plants and the organisms that feed on and control those pests. *UTAR Agriculture Science Journal* 1(1): 49-59.
- Othman, O., Abu Hassan, D., Alias, I., Ayob, A.H., Azmi, A.R., Azmi, M., Badrulhadza, A., Maisarah, M.S., Muhamad, H., Saad, A., Sariam, O., Siti N.M., Syahrin, S. & Yahaya, H. 2008. *Manual Teknologi Penanaman Padi Lestari*. Serdang: MARDI
- Rahaman, M.M. & Stout, M.J. 2019. Comparative efficacies of next-generation insecticides against yellow stem borer and their effects on natural enemies in rice ecosystem. *Rice Science* 26(3): 157-166.
- Rahman, M.T., Khalequzzaman, M., Khan, M.A.R. 2004. Assessment of infestation and yield loss by stem borers on variety of rice. *Journal of Asia-Pacific Entomology* 7: 89-95.
- Raminderjit, S.B., Baljeet, S., Rubaljot, K. & Balwinder, S. 2008. Simple and efficient method for the estimation of residues of flubendiamide and its metabolite desiodo flubendiamide. *Journal of Agricultural and Food Chemistry* 56 (7): 2299-2302.
- Robertson, J.A., Preisler, H.K. & Russell, R.M. 2003. LeOra Software. Polo-Plus: Probit and Logit analysis. POLO for windows. Petaluma, CA.
- Roshan, L. 2006. Effect of fipronil on the incidence of stem borers in Basmathi rice. *Pesticide Reseach Journal* 18 (2): 146-149.
- Saljoqi, A.U.R, Khan, M., Abdullah, K. & Latif, A. 2002. Evaluation of fipronil for the management of rice stem borer. *Sarhad Journal of Agriculture* 18(1):59-61
- Shamik, D. 2020. Stem borers, an important yield reducing insect pest complex of rice in India: A review. *Journal of Entomology and Zoology Studies* 8(5): 786-789.
- Stevens, M.M., Helliwell, S. & Warren, G.N. 1998. Fipronil seed treatments for the control of chironomid larvae (Diptera: Chironomidae) in aerially-sown rice crops. *Field Crop Research* 57:195–207.
- Suharto, H. & Usyati, N. 2005. The stem borer infestation on rice cultivars at three planting times. *Indonesian. Journal of Agricultural Science* 6(2):39–45.
- Tohinishi, M., Nakao, H., Furuya, T., Seo, A., Kodama, H., Tsubata, K., Fujika, S., Hirooka, T. & Nishimastu, T. 2005. Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. *Journal of Pesticide Science* 30(4): 354-360.

- Troczka, B.J., Williamson, M.S., Field, L.M. & Davies, T.G.E. 2016. Rapid selection for resistance to diamide insecticides in *Plutella xylostella* via specific amino acid polymorphisms in the ryanodine receptor. *Neurotoxicology* 60: 224–233.
- Venkataiah, M., Anil Kumar, B. & Sreedhar, C. 2015. Efficacy of newer insecticides against Spodoptera litura in groundnut (Arachis hypogaea L.). Journal of Oilseeds Research 32(2): 152-154.
- Whalen, R.A., Herbert, D.A., Malone, S., Kuhar, T.P., Brewster, C.C. & Reisig, D.D. 2016. Effects of diamide insecticides on predators in soybean. *Journal of Economic Entomology* 109: 2014–2019.
- Whalon, M.E., Mota–Sanchez, D. & Hollingworth, R.M. 2008. Analysis of global pesticide resistance in arthropods. In Whalon, M.E. (ed.). *Global Pesticide Resistance in Arthropods*, pp. 5-31. Wallingford, United Kingdom: CABI.
- Wu, M., Zhang, S., Yao, R., Wu, S., Su, J. & Gao, C. 2014. Susceptibility of the rice stem borer, *Chilo suppressalis* (Lepidoptera: Crambidae), to Flubendiamide in China. *Journal of Economic Entomology* 107(3): 1250–1255.
- Yaakop, S., David-Dass, A., Shaharuddin, U.S., Sabri, S., Badrulisham, A.S. & Che Radziah Che Md Zain. 2020. Species richness of leaf roller and stem borers (Lepidoptera) associated with different paddy growth and first documentation of its DNA barcode. *Pertanika Journal of Tropical Agricultural Science* 43 (4): 523 – 535.
- Zhu, F., Lavine, L, O'Neal, S., Lavine, M., Foss, C. & Walsh, D. 2016. Insecticide resistance and management strategies in urban ecosystems. *Insects* 7: 2.