COMPARATIVE TOXICITY OF SIX INSECTICIDES ON THE RHINOCEROS BEETLE (COLEOPTERA: SCARABAEIDAE)

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Abstract

Strategus aloeus (Linnaeus, 1758) (Coleoptera: Scarabaeidae) is a dangerous pest of oil palms in the Americas, because the adults cause several kinds of damage and kill palm trees. Effective methods for pest management are needed urgently. Bioassays were conducted to compare the toxicity to *S. aloeus* of the insecticides: fipronil, imidacloprid, lambda-cyhalothrin, spinosad, thiacloprid and thiamethoxam. The toxicity of each insecticide to the adults of *S. aloeus* was determined as: (1) the LC_{50} and LC_{90} under laboratory conditions, after exposure of six concentrations of each insecticide applied in a semi-solid diet and used to feed each insect and (2) the mortality under semi-controlled field conditions after applications of insecticides into the beetle galleries in the oil palm tree. The mortality of *S. aloeus* was higher with fipronil, imidacloprid, lambda-cyhalothrin and thiamethoxam, while spinosad and thiacloprid were less effective. Higher mortalities were obtained with concentrations of 12.5, 25, 50 µL mL^{-1} for determining LC_{50} values and 50, 100 µL mL^{-1} for determining LC_{90} values during 72 h. The mortalities of *S. aloeus* had similar tendencies under laboratory and semi-controlled field conditions. Fipronil, imidacloprid, lambda-cyhalothrin and thiamethoxam caused substantial mortality in *S. aloeus* and, thus, can be used rotationally in integrated pest management programs (IPM) against this pest in the oil palm plantations.

Key Words: Fipronil, imidacloprid, lambda-cyhalothrin, thiamethoxam, insect pest, neurotoxic insecticide, oil palm

Resumen

Strategus aloeus es una plaga peligrosa de la palma de aceite en América porque los adultos causan diversos daños y matan las palmas. Esto sugiere la necesidad de buscar métodos efectivos para el control de plagas. Se llevaron a cabo bioensayos para comparar la toxicidad del fipronil, imidacloprid, lambdacialotrina, spinosad, tiacloprid y tiametoxam sobre *S. aloeus*. La toxicidad de cada insecticida sobre adultos de *S. aloeus* fue determinada: (1) la concentración letal CL_{50} y CL_{90} en condiciones de laboratorio, después de la exposición de seis concentraciones de cada insecticida aplicadas en una dieta semisólida y utilizada para alimentar cada insecto y (2) mortalidad en condiciones semi-controladas de campo con la aplicación de insecticidas sobre galerías hechas por los insectos en árboles de palma de aceite. La mortalidad de *S. aloeus* fue mayor con fipronil, imidacloprid, lambdacialotrina y tiametoxam, mientras spinosad y tiacloprid fueron menos eficaces. Altas mortalidades se obtuvieron con concentraciones de 12.5, 25, 50 µL mL^{-1} para la CL_{50} y CL_{90}, 100 µL mL^{-1} para la CL_{90} durante 72 horas. La mortalidad de *S. aloeus* fue similar en las condiciones controladas de laboratorio y de campo. La acción insecticida de esos compuestos puede ser producido debido a su capacidad de penetrar rápidamente en el cuerpo del insecto y afectar el sistema nervioso. Los insecticidas como fipronil, imidacloprid, lambdacialotrina y tiametoxam causaron mortalidad en *S. aloeus* y, por lo tanto, pueden ser utilizados en programas de Manejo Integrado de Plagas (MIP) de esa plaga en plantaciones de palma de aceite.

Palabras Clave: Fipronil, imidacloprid, lambdacialotrina, tiametoxam, insecticidas neurotóxicos, insecto plaga, palma de aceite

The rhinoceros beetle, *Strategus aloeus* (Linnaeus, 1758) (Coleoptera: Scarabaeidae) is a pest in oil palm (*Elaeis guineensis* Jacquin; Arecales: Arecaceae) plantations in the Americas. *Strategus aloeus* feeds on members of Arecaceae family such as *Bactris gasipaes* Kunth, *Cocos nucifera* (L.), *Copernicia cerifera* (Mart.), *Elaeis oleifera* (Kunth) and *Phoenix dactylifera* (L.) (Ratcliffe 1976; Genty et al. 1978; Chinchilla 2003). *Strategus aloeus* has a geographical distribution from the southern USA through Central America up to South America (Ratcliffe 1976; Genty et al. 1978). This beetle is nocturnal, and feeds and mates between 18:30 and 05:30 h and 02:00 and 05:00 h, respectively (Pallares et al. 2000).

Adult *S. aloeus* colonize oil palm plantations in the replanting areas of 25-year-old oil palm stands. During replanting old palm trees are felled and replaced by young palms (Boyé & Aubry 1973; Corley & Tinker 2003). The eggs of *S. aloeus* are laid in dead tissues of the palms; the larvae live in dead trunks or in the soil, and feed on decaying organic matter (Genty et al.1978; Ahumada et al. 1995). The adults attack 1-3 yr old palms by tunneling into the soil near the palm, and then boring their way into the plant just above the roots. Often, the growing point is reached and plant death occurs (Mariau 1994; Corley & Tinker 2003).

Various control methods such as application of entomopathogenic fungi and capture of adults by pheromone-baited traps have not been effective in protecting replanted crops where high populations of *S. aloeus* exist (Genty et al. 1978; Ahumada et al. 1995 Pallares et al. 2000). Other control procedures, such as collection of the immature stages on dead oil palms has a prohibitively high cost in commercial plantations (Bedford 1980; Corley & Tinker 2003). Insecticides such as carbamate and organophosphate compounds are the main method of control for *S. aloeus* (Genty et al. 1978; Ahumada et al. 1995). Carbamates and organophosphates act through contact and ingestion and cause neurotoxicity, which may be lethal (Bloomquist 1996; He 2000; Gilbert & Gill 2010). To protect oil palm crops, insecticides are applied inside the galleries of *S. aloeus* in the soil. Application of insecticides to the soil is an efficient method in managing the pest's populations by reducing palm tree damage and lessening the risk to human health and the environment (Darus & Basri 2000; Hartley 2002; Martínez 2013). Oil palm pests, such as *Oryctes rhinoceros* (L.) and *Scapanes australis* (Boisduval) (Coleoptera: Scarabaeidae), have been controlled successfully with insecticides applied in the soil galleries (Bedford 1976; Norman & Mohd Basri 1997; Corley & Tinker 2003).

Insecticides can be effective in protecting oil palm plantations, because they rapidly decimate dense insect pest populations (Mariau 1993; Nordin et al. 2004). New insecticidal compounds that are achieving registration within the current regulatory environment of the US Environmental Protection Agency (EPA) include chemical compounds that have reduced-risk and less immediate toxicity to the pest insects than the conventional insecticides (EPA 2011). These types of insecticides are potential new tools for use against the oil palm pest in the commercial plantations because of their suitability for inclusion in IPM programs.

This study compared the toxicity of 6 insecticides on *S. aloeus* adults under laboratory and field-controlled conditions, in order to contribute new control measures to IPM programs against *S. aloeus* in oil palm plantations.

Materials and Methods

Insects

In the field, 2,683 pupae of *S. aloeus* (♂ = 1314, ♀ = 1369) were hand collected from dead palm stems in 2 yr-old commercial plantations of the oil palm in the municipality of Puerto Wilches, Santander, Colombia (N 07° 20' -W 73° 54'), with average conditions of 28.46 °C, 75-92% RH, 1,450-2,250 h sunshine/year and 2,168 mm annual rainfall. The insects were placed in metallic boxes (70 × 70 × 80 cm) covered with a nylon mesh and transferred to the Entomology Laboratory of the Brisas Oil Palm Plantation, Puerto Wilches, Santander, Colombia. Pupae of *S. aloeus* were isolated in plastic containers (15 × 10 cm) containing soil and a moistened cotton ball. The insects were maintained at 28 ± 1 °C, 75 ± 5% RH and 12:12 h L:D photoperiod until adult emergence.

Adults were placed in glass containers (30 × 30 × 30 cm) covered with a nylon mesh with a 15 cm deep soil layer maintained at 80% RH by periodic watering rather by a sprinkler. The beetles were fed on semi-solid diet daily (10 g of stem palm meristem + 10 g of sugarcane + 10 g of banana + water in a 5:5:2:1 ratio) (Pallares et al. 2000). The diet ingredients were cut, chopped and mixed in a laboratory blender (Waring LBC15; 3,500-20,000 rpm). The semi-solid diet was sterilized with ultraviolet light for 30 min and stored at -5 °C. Photophase and scotophase periods were simulated with white and red lights (IRO 110V 60W; Toshiba Lighting and Technology Corp., Tokyo, Japan), respectively. Healthy *S. aloeus* adults without amputations or malformations were used in the bioassays.

Insecticides

Six different neurotoxic insecticides were used in this study. The following commercial insecticides were tested at their maximum label rates: fipronil (Clap® SC, Bayer, Germany), 200 g L^{-1}; imidacloprid (Confidor® SL, Cyanamid, Belgium), 200 g L^{-1}; lambda-cyhalothrin (Karate®

EC, Zeneca, Wilmington, USA), 25 g L^{-1}; spinosad (Tracer® SC, Dow Agrosciences, USA), 120 g L^{-1}; Thiacloprid (Calypso® SC, Cyanamid, Belgium), 480 g L^{-1}; and thiamethoxam (Actara® SC, Syngenta, Canada), 240 g L^{-1}. These insecticides were diluted in 1 L water to produce a stock solution by adjusting 100 g L^{-1} per insecticide and to obtain the required concentrations.

Insecticide Bioassay

Insecticide efficacy was determined by calculating the lethal concentrations (LC_{50} and LC_{90}) and lethal time (LT_{50} and LT_{99}) values under laboratory conditions for each formulation. Six concentrations of each insecticide besides the control (distilled water), were adjusted in 1 mL stock solution (insecticide and distilled water): 3.12, 6.25, 12.5, 25, 50 µL and 100 µL. For each insecticide, aliquots were taken from the stock solution and mixed with distilled water in 5 mL glass vials. Different concentrations of the insecticides were applied in 1 µL solution onto 5 g of semi-solid diet and used to feed each insect. Fifty insects were used per concentration and were placed individually in plastic containers (15 × 10 cm) with a perforated lid and maintained in the dark. The number of dead insects in each cage was counted after insecticide exposure at intervals of 2 h over 6 days.

Assays for Mortality under Field Conditions

One hundred, 6-month-old oil palms ('Tenera' cv, 'Deli' × 'Ghana') and 100 adults of *S. aloeus* were used for each insecticide in the controlled field test. These palms were planted into soil in polyethylene bags and maintained in the greenhouse at a temperature of 27 ± 2 °C, 75-80% RH and 25% shadow. One insect was caged in each oil palm a nylon trap (0.5 × 0.5 × 1.20 m). Treatments consisted of adding each insecticide at the calculated LC_{90} concentration and distilled water as the control with 4 replications per treatment. The LC_{90} concentration of each insecticide was applied directly on the galleries while distilled water was applied to the controls. Applications of 500 mL per gallery were made by a manual pump (Royal Condor, 10 L capacity). The application was evaluated after 5 days, when the insects build their galleries. Mortality of *S. aloeus* caused by insecticides was recorded every day during 15 days, and was corrected both in the laboratory and field bioassays according to Abbott (1925)

Statistics

The LC_{50-90}, LT_{50-99} and their confidence limits were determined by logistic regression based on the concentration probit-mortality (Finney 1964), with the program XLSTAT-PRO v.7.5 for Windows (XLSTAT 2004). Mortality data from the controlled field test were analyzed by one-way ANOVA. Mortality variables were summarized in percentages and the data were transformed by arcsine square root. Tukey's Honestly Significant Difference test (HSD) was used for comparison of the means at the 5% significance level (PROC ANOVA) (SAS 2002).

Results

Insecticide Susceptibility of *Strategus aloeus*

The highest mortalities were obtained with 50 and 100 µL mL^{-1} of the above mentioned insecticides. The 3 different lethal concentrations levels (LC_{50}, LC_{90} and LC_{99}) (Table 1) of each insecticide was estimated by Probit (χ^2; $P < 0.001$). The LC_{50-90} values indicated that fipronil (χ^2 = 37.81; df = 5), lambda-cyhalothrin (χ^2 = 42.52; df = 5) and thiamethoxam (χ^2 = 48.43; df = 5) were the most toxic compounds to the *S. aloeus* adults followed by thiacloprid (χ^2 = 27.84; df = 5). (LC_{50-90}) value was lower with imidacloprid (χ^2 = 13.07; df = 5) and spinosad (χ^2 = 12.02; df = 5). Mortality was always < 1% in the control.

LT_{50} values were recorded when the pest was exposed from 21 to 144 h (1-6 days) to the various insecticides each applied at 6.25, 12.5, 25, 50 and 100 µL mL^{-1} (Fig. 1). The elapsed times for LC_{50} values were recorded from 24 h to 144 h for the insecticide concentrations of 6.25 and 100 µL mL^{-1} The LT_{50} values recorded at the higher and lower concentrations were between 21.81 and 111.9 h for fipronil, 25.53 and 85.71 h for imidacloprid, 48.97 and 120.1 h for lambda-cyhalothrin, 72.01 and 144.1 h for thiamethoxam, 77.41 and 105.8 h for spinosad, and to 100.1 and 120.1 h for thiacloprid. The elapsed times for the LC_{99} values were recorded from 120 to 144 h for the insecticide concentrations of to 50 and 100 µL mL^{-1}. The LT_{99} values recorded at the higher and lower concentrations were 97.96 h to fipronil, 125.1 h to imidacloprid, 118.8 h to lambda-cyhalothrin and 142 h to thiamethoxam. It was not possible to estimate LT_{99} values for spinosad and thiacloprid at any of the exposure periods used.

Mortality in Controlled Field Tests

The mortality effects caused by the tested insecticides on the *S. aloeus* adults were statistically different in controlled field conditions using the previously estimated concentrations for the LC_{90} values ($F_{1,23}$ = 7.85; $P < 0.05$). Fipronil, lambda-cyhalothrin and thiamethoxam caused mortalities of 84.1 ± 9.5%, 88.3 ± 3.7% and 89.9 ± 12.4%, respectively, followed by mortalities by thiacloprid, imidacloprid and spinosad of 78.9 ± 8.9%, 73.9 ± 7.9% and 67.3 ± 7.3%, respectively Mortality never exceeded 3.14 ± 0.3% in the control (Fig. 2).

TABLE 1. LETHAL CONCENTRATIONS OF SIX INSECTICIDES ON *STRATEGUS ALOEUS* (COLEOPTERA: SCARABAEIDAE) ADULTS. DOSES OF INSECTICIDE WERE APPLIED TO THE SEMI-SOLID DIET. THE NUMBER OF DEAD INSECTS IN EACH CAGE WAS COUNTED AFTER EXPOSURE TO THE TREATED DIET AT INTERVALS OF 2 H OVER 6 DAYS.

Insecticide	[a]LC	[b]EV (µL mL^{-1})	[c]CI µL mL^{-1}	[d]χ^2
Thiamethoxam	50	1.092	0.98 - 1.21	48.43
	90	2.403	2.16 - 2.72	
	99	2.947	2.64 - 3.35	
Lambda-cyhalothrin	50	0.718	0.63 - 0.81	42.52
	90	1.841	1.64 - 2.11	
	99	2.306	2.04 - 2.66	
Fipronil	50	0.454	0.38 - 0.52	37.81
	90	1.409	1.25 - 1.62	
	99	1.805	1.59 - 2.09	
Thiacloprid	50	2.335	2.08 - 2.61	27.84
	90	5.754	5.15 - 6.54	
	99	7.167	6.39 - 8.19	
Imidacloprid	50	0.594	0.39 - 0.77	13.07
	90	3.392	2.96 - 3.98	
	99	4.552	3.96 - 5.38	
Spinosad	50	1.099	0.97 - 1.23	12.02
	90	2.701	2.42 - 3.06	
	99	3.365	3.01 - 3.83	

[a]$LC_{50, 90 \text{ and } 99}$ concentrations causing 50, 90 and 99% mortality; [b]EV, estimated value; [c]CI, confidence interval; [d]χ^2,chi-square value for lethal concentrations and fiducial limits based on a log scale with significance level at $P < 0.0001$.

DISCUSSION

The toxicity profiles for the 6 insecticides against the rhinoceros beetle, *S. aloeus* were determined from the bioassays in the laboratory and controlled field conditions. The insecticides fipronil, imidacloprid, lambda-cyhalothrin, spinosad, thiacloprid and thiamethoxam caused substantial mortality of the *S. aloeus* adults under laboratory conditions. The best results were obtained with concentrations of 50 and 100 µL mL^{-1}. The susceptibility of the Scarabaeids may vary with exposure in the different concentrations of insecticides (Villani et al. 1988; Cowles & Villani 1996; Gilbert & Gill 2010). Different studies show that *Macrodactylus subspinosus* (Fabricius), *Oryctes rhinoceros* (L.), and *Popillia japonica* (Newman) were susceptible to the insecticide concentration ranges applied in the food (Isaacs et al. 2004; Baumler & Potter 2007; Sreeletha & Geetha 2012).

The insects exposed to the neurotoxic insecticides: fipronil, thiacloprid and thiamethoxam displayed altered locomotion activity, and muscle contractions that were observed at high concentrations in $LC_{50\text{-}90}$ test. In contrast, the adults of *S. aloeus* gradually lost mobility when they were exposed to the lambda-cyhalothrin. In some individuals, the paralysis was constant with concentrations near the LC_{50} without recovery signs. The mode of action of the fipronil involves blocking the γ-aminobutyric acid (GABA)-gated chloride channel which is also the target for cyclodiene insecticides such as endosulfan and dieldrin (Cole et al. 1993; Hainzl & Casida 1996; Durham et al. 2002). Lambda-cyhalothrin and other pyrethroids are known to cause rapid paralysis in invertebrates by disrupting nerve conduction; their primary site of action are the voltage-gated sodium channels (Bloomquist & Miller 1986; Baumler & Potter 2007). Imidacloprid, thiacloprid and thiamethoxam appear to interfere with the nicotinic acetylcholine receptors located in the post-synaptic membrane, disrupting normal nerve function (Grewal et al. 2001; Matsuda 2001; Tan et al. 2008). Spinosad is a neurotoxin that targets the nicotinic acetylcholine receptor and the GABA receptors, and causes cessation of feeding followed later by paralysis and death (Salgado 1998; Crouse et al. 2001). In this study, the $LC_{50\text{-}90}$ values indicated that lethality of imidacloprid and spinosad were lower on *S. aloeus* using the evaluated concentrations. However, the lethalities of fipronil, lambda-cyhalothrin, thiacloprid and thiamethoxam on *S. aloeus* may have advantages by their mode of action on this insect and could be applied in rotation, under field conditions.

The insecticides evaluated required extended periods of time to achieve the mortality of *S. aloeus*. The LT_{50} varied from 21 to 144 h, whereas

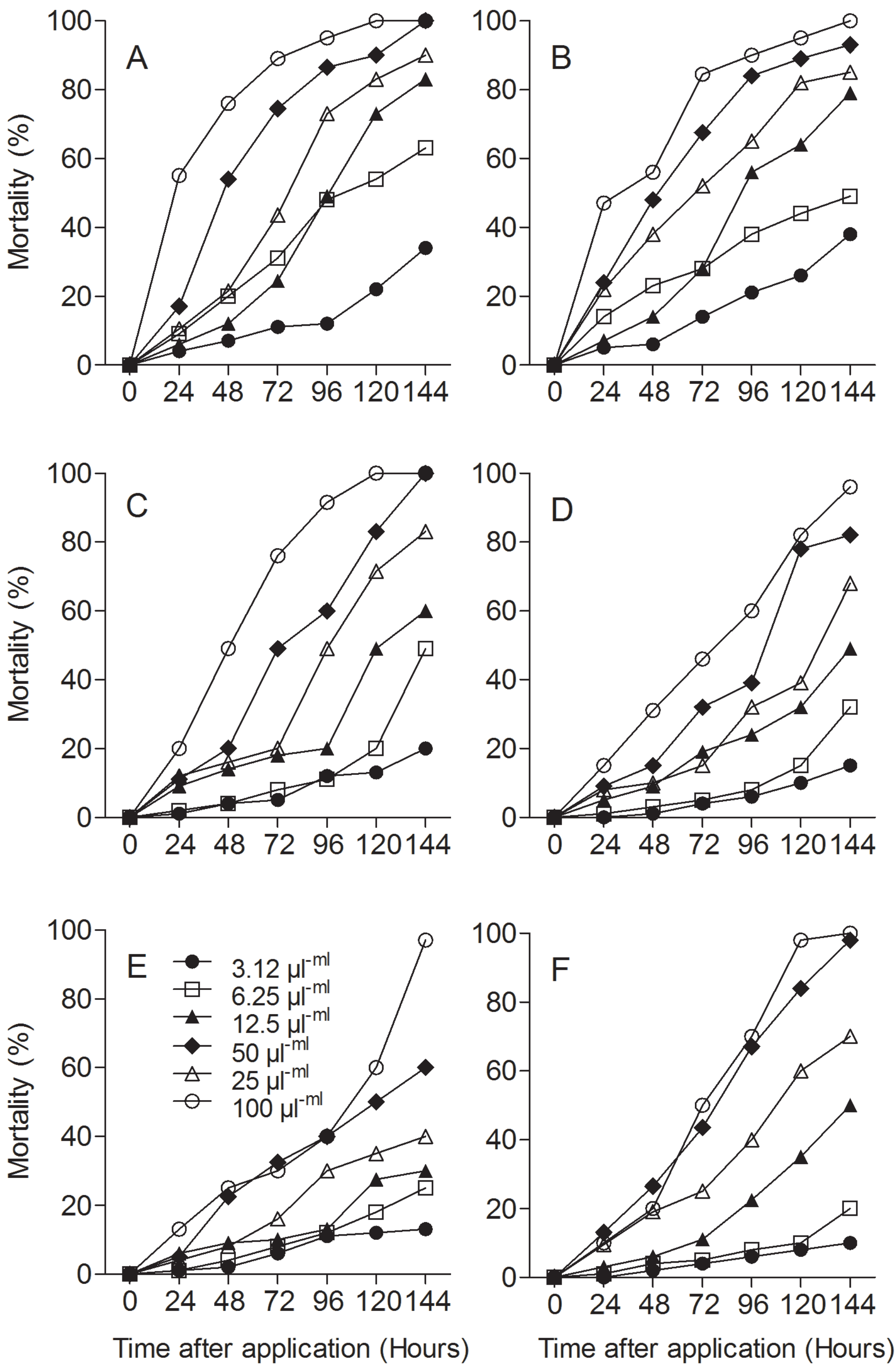

Fig. 1. Comparison of lethal times (LT_{50-99}) of various insecticides at the indicated concentration applied against *Strategus aloeus* (Coleoptera: Scarabaeidae) adults. The insecticides were fipronil (A), imidacloprid (B), lambda-cyhalothrin (C), spinosad (D), thiacloprid (E), and thiamethoxam (F). Mortality data collected during 6 days were used to calculate the LC_{50-90} values ($P < 0.0001$).

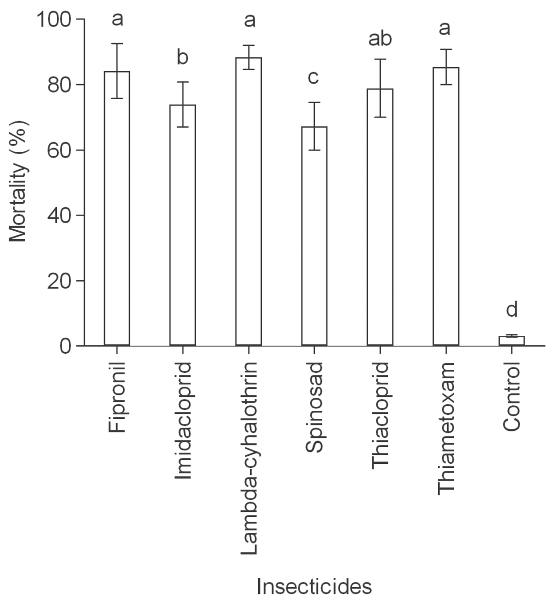

Fig. 2. Mortality in controlled field conditions of *Strategus aloeus* (Coleoptera: Scarabaeidae) adults by insecticides to level LC_{90} application. Treatments means (percent mortality ± SEM) differ significantly at $P < 0.05$ (Tukey's mean separation test).

the LT_{90} ranged from 120 and 144 h. The lethal effect of fipronil was observed quickly (in 21 h), followed by imidacloprid (25 h), lambda-cyhalothrin (48 h), thiamethoxam (72 h), while spinosad and thiacloprid showed lethal effects at 76 h and 100 h, respectively. In this study, the comparative effects on *S. aloeus* between the 6 insecticides were observed at various time points. Immediate lethal effect of insecticides are not essential to protect crops, unless the target insects vector viruses or other dangerous pathogens. Some insecticides cause cessation of feeding long before the target insects dies. However, a quick-acting insecticide is generally considered essential for impregnation of the palm stems; in this case, the beetles were able to feed, even if they died later. Neurotoxic insecticides can reduce injuries of commercial crops caused by the nocturnal beetles (Drinkwater 2003; Isaacs et al. 2004).

In the controlled field tests, the lethal effects of the insecticides on *S. aloeus* were consistent with those observed under laboratory conditions. Fipronil, lambda-cyhalothrin, thiacloprid and thiamethoxam showed strong lethal effects on *S. aloeus,* whereas imidacloprid and spinosad were less toxic. However, when the insecticides were applied to the insect in the field, the mortality levels were lower than those obtained under laboratory conditions. One possible reason for the efficacy of the insecticides under the field conditions could be contact exposure on the body. In this bioassay, it is difficult to accurately know the amount of the insecticide absorbed by the insect, but the lethal effects caused by these insecticides on *S. aloeus* showed continuity of that trend with the application of the LC_{90} concentration. The lethal effects of the insecticides and their effectiveness have also been studied in other pests under field conditions with fipronil being a potent control agent of *Diloboderus abderus* Sturm larvae, lambda-cyhalothrin and thiacloprid being a potent in controlling *P. japonica* larvae and adults, and thiamethoxam being a potent against *Heteronychus arator* Fabricius (Silva & Boss 2002; Drinkwater 2003; Baumler & Potter 2007).

This study showed the potential of 6 insecticides to control the *S. aloeus* adults in oil palm plantations. The toxicity of these insecticides with different modes of action may enable the control of the *S. aloeus* populations and may reduce injuries caused by this insect on *E. guineensis*. Fipronil, lambda-cyhalothrin, thiacloprid and thiamethoxam have lethal effects on *S. aloeus* with the potential to manage its field populations. In order to prevent or retard the development of insecticide resistance, insecticides with different modes of action should be used in rotation as indicated by Insecticide Resistance Action Committee (IRAC 2014). The IRAC mode of action groups to which the above insecticides belong are as follows: fipronil (2B, GABA-gated chloride channel antagonists), lambda-cyhalothrin (3A, Sodium channel modulators), imidacloprid, thiacloprid and thiamethoxam (4A, Nicotinic acetylcholine receptor [nAChR] agonists), and spinosad (5, Nicotinic acetylcholine receptor [nAChR] allosteric activators). Thus these insecticides belong to four IRAC mode of action groups, and their field applications should be rotated accordingly.

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