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Article

Selectivity of Insecticides Used for the Management of Cocoa Pests on Non-Target Predatory Ant Species

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Abstract

Although information on insecticide toxicity on pests abounds, this is limited on non-target organisms like ants in cocoa production systems. This study determined the toxicity of insecticides (containing acetamiprid 40 g L^{-1} EW, acetamiprid 64 g L^{-1} + emamectin benzoate 48 g L^{-1} EC, acetamiprid 20 g L^{-1} + lambda-cyhalothrin 15 g L^{-1} SC, emamectin benzoate 5% WDG and pyrethrins 50 g L^{-1} EW) used against cocoa pests on non-target predatory ants (*Crematogaster africana*, *Oecophylla longinoda* and *Pheidole megacephala*) under laboratory (using filter-paper method) and field conditions to identify less harmful products for use in the cropping system. Ant knockdown and mortality varied significantly among insecticides at their recommended rates, with emamectin benzoate being the least toxic and acetamiprid + lambda-cyhalothrin the most toxic. LC_{95}s varied significantly, with emamectin benzoate having the highest predator safety index, while pyrethrins had the lowest. Generally, selectivity towards *O. longinoda* was higher than that towards the other ants. In the field, emamectin benzoate had the least acute adverse effect on ant abundance. Ant population generally rebounded to pre-treatment levels at 1 month after the last insecticide application. The study indicates differential toxicity and identifies ant-compatible insecticide options contingent on the autochthonous ant composition.

Keywords: ants; toxicity; mortality; knockdown; cocoa; insecticides

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1. Introduction

Existential threats to crop production, such as cocoa, include insect pests and diseases. In many parts of the world, pesticides are used to address these threats [1–3], and cocoa production is no exception [1]. Ideally, pesticides should be selective, thus toxic to pests and non-toxic to non-target organisms. However, in many instances, pesticides such as insecticides are more toxic to non-target insects compared to pests [4–7]. This can negatively affect the activities of non-target organisms in the agro-ecosystem, thereby depriving the agro-ecosystem of the benefits they provide. Hence, the selectivity of an insecticide and its impact on non-target organisms, such as natural enemies, are important factors to consider before integrating it into pest management packages [6–8]. Since the judicious use of insecticides is a major constituent of Integrated Pest Management (IPM), its compatibility

with other IPM components, such as the conservation of natural enemies, is of major concern. Hence, there is an increasing interest in insecticide lethality towards natural enemies and other non-target arthropods, and in the compatibility of insecticide use with other pest management methods.

Arthropod predators are important in crop production systems due to their effect on phytophagous pests. Ants such as *Oecophylla longinoda*, *Crematogaster africana*, and *Pheidole megacephala* are predators of many pests including insect pests of cocoa [1,9,10]. Although these ants are usually generalists, they are able to significantly suppress pest populations by adapting to different prey and cropping systems [9,11–14] including cocoa. These attributes enhance their potential for success as biological components of IPM across different agroecologies.

Cocoa, a major cash crop in West Africa, is an important source of foreign revenue for the two leading global producers, Ghana and Cote d'Ivoire. Despite efforts to enhance production, diverse biotic and abiotic factors challenge productivity. Naturally, the cocoa ecosystem hosts numerous arthropods and comparatively few are pests [1]. These pests, however, account for substantial yield losses [1,10]. Mirids such as *Sahlbergella singularis* and *Distantiella theobroma* are among the key pests of cocoa in the sub-region, in addition to other sap suckers, borers and defoliators [1,10,15]. Using insecticides is a historical method of controlling these pests [1,16], and over the decades, active ingredients with high efficacy and relatively low environmental impact have been developed for use by farmers. However, insecticides should be compatible with other control methods so that the potential benefits from the various options are not traded off. Compatibility of pest-efficacious products within IPM is enhanced when they are selective, less toxic to natural enemies and have minimal environmental footprints [5–8].

Insecticides used for cocoa pest control have varying effects on target organisms and similar effects may also occur in non-target organisms [5–8], as both are exposed to chemicals in the agro-ecosystem, although the extent of exposure may vary. Hence, biological control agents could be disadvantaged by chemical control if the insecticides are toxic to them. Research on predatory ant species on cocoa have mostly centred on their diversity and community structure as well as their role as predators [17–22], although insecticide toxicity and spraying techniques have also been studied. In Owusu-Manu's study [23], the effects of insecticides on non-target organisms including predatory ants were investigated. Majer [24] also evaluated the effect of different spraying strategies, blanket or selective, on predatory ant species including *O. longinoda* and *Crematogaster* spp. using two insecticides. These studies provided insights on the impact of insecticides on predatory ant populations in the cocoa agro-ecosystem. However, despite widespread insecticide use, empirical studies on their toxicity and selectivity towards predatory ants in cocoa agro-ecosystems are scarce. Although a recent study [25] assessed the toxic effects of fipronil and bifenthrin on predatory ants in the cocoa agro-ecosystem, broader comparative assessments of commonly used insecticides are limited. This is particularly important since some previously evaluated products are no longer approved for use on cocoa. Focusing on ecologically important species such as *O. longinoda*, *C. africana* and *P. megacephala* [1,18] provides insights into species-specific sensitivity and selectivity toward insecticides, thereby helping to identify safer compounds for the ants in cocoa production. Although situated in the context of cocoa production, it will bridge the knowledge gap on optimising and integrating chemical and biological control approaches with potential application in tropical cropping systems [9,13] similar to the cocoa agro-ecosystem. Knowledge of the differential toxicity of insecticides to target and non-target organisms, such as predatory ants, guides pest management decisions based on the local predator guild and its selectivity for insecticides [25]. As a result, this informs growers'

choice of compatible insecticides to reap the dual benefits of chemical and biological control of noxious insects. Hence, this study assessed the toxicity of insecticides used for insect pest control on cocoa on three predatory ants to identify their selectivity for insect pest management through natural enemy compatibility.

2. Materials and Methods

2.1. Insect Species

Three non-target ant species (*Crematogaster africana*, *Oecophylla longinoda* and *Pheidole megacephala*), which are important natural enemies (predators) of insect pests in the cocoa agro-ecosystem [1,10,18], were used in the toxicity tests in the laboratory. The ants were collected from experimental fields (4× insecticide treatments per year and insecticide-free for at least 5 months before ant collection) at the Cocoa Research Institute of Ghana (CRIG), New Tafo-Akim, Ghana. Ant nests were gently removed into collection containers (plastic containers of top diameter = 22 cm, bottom diameter = 19 cm, height = 25 cm with a diameter of 14 cm cut in the top lid and fitted with muslin net) containing pieces of cocoa chupons and leaves. Individual ants were also brushed into separate collection containers of the same dimensions as above. Transporting ants to the insectary took < 10 min. The ants were acclimated at the insectary (26 ± 3 °C, 80 ± 5% relative humidity and 12 h light: 12 h dark photoperiod) for 2–4 h before testing to remove dead and weak ants. Collections from 4 colonies were used for the testing.

2.2. Insecticides

Five representative insecticides (Table 1) currently used for the management of mirids and other cocoa insect pests in Ghana were used for the toxicity tests. These insecticides are approved for use in Ghana and are among the most commonly used products in cocoa production [10]. Two bioassays were conducted; the recommended field rate (RFR) for cocoa insect pest control and half of the RFR (50% RFR) were used for Bioassay 1, while a series of insecticide concentrations to obtain a concentration–mortality response effect was used for Bioassay 2.

Table 1. Information on insecticides used in the study.

∞ Insecticide	Formulation	Active Ingredient (Concentration)	Class	* IRAC Group	† Rate per ha
BSAC	EW	Acetamiprid (40 g L^{-1})	Neonicotinoids	4A	490 mL
ESAE	EC	Acetamiprid (64 g L^{-1}) + Emamectin benzoate (48 g L^{-1})	Neonicotinoids + Avermectins	4A + 6	300 mL
TSAL	SC	Acetamiprid (20 g L^{-1}) + Lambda-cyhalothrin (15 g L^{-1})	Neonicotinoids + Pyrethroids	4A + 3A	550 mL
ATEM	WDG	Emamectin benzoate (5%)	Avermectins	6	880 g
AGPY	EW	Pyrethrins (50 g L^{-1})	Pyrethrins	3A	400 mL

∞: Codes have been used to represent the names of the insecticide products; * IRAC: Insecticide Resistance Action Committee; †: Recommended field rate of formulated product per hectare (=2.5 × recommended field rate per acre); EW: Emulsion, oil in water; EC: Emulsifiable concentrate; SC: Suspension concentrate; WDG: Water-dispersible granules.

2.3. Bioassay 1: Toxicity Test at Recommended Field Rate (RFR) and 50% RFR

The RFR and 50% RFR were selected to assess ant mortality when exposed to these products. The 50% RFR was used to observe mortality when non-target organisms are exposed to sub-lethal concentrations of an insecticide due to a degradation of the chemical. The toxicity test was done using the Petri dish method containing an impregnated filter paper [15,26] as follows. A Whatman No. 10 filter paper was impregnated with an insecticide concentration (1 mL), allowed to dry and used to line the base of the Petri dish

(110 mm in diameter) (Cytiva, Maidstone, UK). Ten worker ants (*O. longinoda*: 8–10 mm, *C. africana*: 4–6 mm; *P. megacephala*: 2.0–2.5 mm in size) were placed on the filter paper and the Petri dish was closed. This was replicated 4 times for each concentration and the control. Whatman No. 10 filter paper impregnated with distilled water (1 mL) was used as the control to estimate natural mortality. Ant knockdown was recorded during the first 60 min of exposure. Ants that could not right themselves and those that only moved when probed thrice with a camel-hair brush were considered knocked down. The experimental set-ups were maintained at the following laboratory conditions: 26 ± 3 °C, 80 ± 5% relative humidity and 12 h light: 12 h dark photoperiod. Mortality was recorded after 24 h by probing the ants with a camel-hair brush and those that did not display any sign of movement were considered dead.

2.4. Bioassay 2: Concentration–Mortality Response Test

A series of concentrations (1% RFR-125% RFR) of the insecticides was prepared with distilled water for the test. The concentrations used were: 0.000356, 0.00178, 0.0089, 0.0178, 0.0356 and 0.0445% active ingredient (a.i.) (acetamiprid) [BSAC], 0.00061, 0.00305, 0.01525, 0.0305, 0.061 and 0.07625% a.i. (acetamiprid + emamectin benzoate) [ESAE], 0.00035, 0.00175, 0.00875, 0.0175 and 0.035% a.i. (acetamiprid + lambda-cyhalothrin) [TSAL], 0.0008, 0.004, 0.02, 0.04, 0.08 and 0.1% a.i. (emamectin benzoate) [ATEM] and 0.00036, 0.0018, 0.009, 0.018, 0.036 and 0.045% a.i. (pyrethrins) [AGPY].

The ant species were exposed to all the concentrations of the insecticides except BSAC, ESAE and AGPY. The highest concentrations of these insecticides (BSAC, ESAE and AGPY) were tested only on *O. longinoda* because the other concentrations did not induce very high mortality in the ants for probit analysis. The control, insecticide exposure method, replicates and laboratory conditions were the same as described for Bioassay 1.

2.5. Field Experiment: Insecticidal Effect on Ant Abundance

This was conducted on CRIG's experimental plots at New Tafo-Akim, Ghana from May 2023 to December 2023. The cocoa trees were mixed hybrids with a spacing of 3 m × 3 m and aged 6 years. The experimental site is situated within the semi-deciduous forest zone of Ghana. Five treatments comprising 4 insecticides [(ESAE: acetamiprid + emamectin benzoate), (TSAL: acetamiprid + lambda-cyhalothrin), (ATEM: emamectin benzoate) and (AGPY: pyrethrins)] and a control (no insecticide application) were used in a randomised complete block design with 3 replicates. Thus, there were 3 blocks and each block contained all the treatments which were randomly assigned to the plots within the block. Each treatment plot was 0.5 acres with a buffer of 20 m between plots. For each treatment, the recommended field rate was applied using a motorised mistblower (Solo Port 423) with the restrictor knob on the 2nd position (Solo Kleinmotoren GmBH, Sindelfingen, Germany). The treatments were applied once in August and September 2023 at 28 days interval [10], although sampling commenced (3 May 2023) prior to these months. The abundance of ants (using their nests) was sampled on a monthly basis for 4 months before insecticide application, just before insecticide application, 24 h after the 1st insecticide application, 28 days after the 1st insecticide application (1 month post treatment), 28 days after the 2nd insecticide application (2 months post 1st treatment/1 month post 2nd treatment), 56 days after the 2nd insecticide application (2 months post 2nd treatment) and 84 days after the 2nd insecticide application (3 months post 2nd treatment). On each plot, 50 trees were randomly sampled for ant nests up to a height of 2 m using the method of Collingwood [27] from 6:30 am to 11:00 am. Meteorological data were obtained from the local meteorological station at CRIG.

2.6. Data Analysis

Bioassay 1: Ant knockdown at 60 min was computed as a percentage of the number of knocked-down ants relative to the total number of exposed ants for each insecticide concentration or control. Percentage mortality was also computed relative to the total number of exposed ants for each concentration or control. Mortality when exposed to insecticide concentration was adjusted using the formula of Abbott [28] when mortality in the control was 5–20%. Mortality data were arcsine-transformed and subjected to a 2-way analysis of variance (ANOVA) at $p < 0.05$ to observe the effect of treatment and ant species on mortality for each insecticide. Toxicity ranking of the insecticides was done using the International Organisation for Biological Control (IOBC) categorisation [29].

Bioassay 2: Corrected mortality data from the concentration–mortality response bioassay were subjected to probit analysis using Insecticide Resistance Monitoring Application (IRMA)-QCal (Version 1, March 2024) [30] to compute the lethal concentration to 95% of the exposed insects (LC_{95}), 95% Confidence Interval (CI), slope and intercept. Non-overlapping 95% CIs indicate that their respective LC_{95} values are significantly different. The Predator Safety Index (PSI) was computed by comparing the LC_{95} for the pest (mirid) and each natural enemy (ant) species, thus

$$\text{PSI} = \frac{\text{LC}_{95} \text{ of predator (ant)}}{\text{LC}_{95} \text{ of pest}}$$

An insecticide was considered selective when the LC_{95} for mirid was lower than that of the natural enemy and non-selective when their LC_{95}s were similar. However, an insecticide was considered harmful when the LC_{95} value of the natural enemy was lower than that of the pest. A PSI value of <1 indicates that an insecticide is more toxic to the predator compared to the pest while a value of 1 indicates that an insecticide has the same toxic effect on both the pest and the predator. A PSI value > 1 indicates that an insecticide is less toxic (safer) to the predator compared to the pest. Hence, a PSI value of $\leq$1 indicates non-selectivity, while >1 indicates selectivity; the higher the value, the more selective it is.

Ant abundance on the field: Data on ant abundance (nests) were logarithm transformed before performing 2-way ANOVA at $p < 0.05$ with treatment and ant species as independent factors. A heatmap of the mean count of ant abundance was generated using PAST version 5.2 [31]. Reduction in ant abundance (RAA) after treatment was determined as follows:

$$\text{RAA (\%)} = 100 \times \frac{\text{Abundance at previous sampling day} - \text{Abundance at current sampling day}}{\text{Abundance at previous sampling day}}$$

3. Results

3.1. Insecticidal Activity on Ants at RFR and 50% RFR

When applied at the RFR, acetamiprid + lambda-cyhalothrin (TSAL) induced the highest knockdown (KD) on ants (80–100%), followed by pyrethrins (7.5–100%), acetamiprid + emamectin benzoate (17.5–65%), acetamiprid (22.5–67.5%) and emamectin benzoate (2.5–10%) (Table 2). The highest knockdown to each insecticide was 10% to emamectin benzoate (*C. africana*), 65% to acetamiprid + emamectin benzoate, 67.5% to acetamiprid and 100% to pyrethrins (*P. megacephala*) and 100% to acetamiprid + lambda-cyhalothrin (*C. africana* and *P. megacephala*).

All the insecticides except emamectin benzoate induced a mortality of 90–100% on at least one ant species at 50% RFR (Table 3 and Figure S1). At the RFR, all the insecticides except emamectin benzoate also caused 100% mortality on two ant species. At this rate, acetamiprid, acetamiprid + emamectin benzoate and pyrethrins were less toxic to *O.*

longinoda while acetamiprid + lambda-cyhalothrin and emamectin benzoate were less toxic to *C. africana* and *P. megacephala*, respectively. Emamectin benzoate was harmless or slightly harmful to all the ants. Except acetamiprid + lambda-cyhalothrin, the insecticides were harmless or slightly harmful to *O. longinoda* based on the IOBC toxicity categorization (Table 3 and Figure S1).

Table 2. Knockdown of ants at the recommended field rate of each insecticide.

Insecticide (Active Ingredient)	Knockdown (%) After 60 min Exposure		
	C. africana	*O. longinoda*	*P. megacephala*
BSAC (acetamiprid)	22.5	25	67.5
ESAE (acetamiprid + emamectin benzoate)	20	17.5	65
TSAL (acetamiprid + lambda-cyhalothrin)	100	80	100
ATEM (emamectin benzoate)	10	7.5	2.5
AGPY (pyrethrins)	7.5	60	100

Knockdown in the respective controls was 0%.

Table 3. Mortality of ants after 24 h of exposure to the recommended and 50% recommended field rates of insecticides.

Insecticide (Active Ingredient)	Ant	Mortality (%)		IOBC Toxicity Category *
		50% RFR	RFR	
BSAC (acetamiprid)	*C. africana*	85.7 [cm]	100.0 [cm]	4
	O. longinoda	30.0	47.5	2
	P. megacephala	97.4 [cm]	100.0 [cm]	4
ESAE (acetamiprid + emamectin benzoate)	*C. africana*	100	100.0	4
	O. longinoda	20.0	25.0	1
	P. megacephala	93.9 [cm]	100.0 [cm]	4
TSAL (acetamiprid + lambda-cyhalothrin)	*C. africana*	88.6 [cm]	88.6 [cm]	3
	O. longinoda	58.8 [cm]	100.0 [cm]	4
	P. megacephala	100 [cm]	100.0 [cm]	4
ATEM (emamectin benzoate)	*C. africana*	32.5	40.0	2
	O. longinoda	12.5	15.0	1
	P. megacephala	0.0	12.5	1
AGPY (pyrethrins)	*C. africana*	100 [cm]	100.0 [cm]	4
	O. longinoda	42.5	70.0	2
	P. megacephala	100 [cm]	100.0 [cm]	4

RFR: Recommended field rate; [cm]: Corrected mortality (adjusted mortality when control mortality was 5–20%); IOBC: International Organisation for Biological Control; *: Categorisation was based on IOBC standards [21, [1: <30% mortality (Harmless); 2: 30–79% mortality (Slightly harmful); 3: 80–99% mortality (Moderately harmful); 4: >99% mortality (Harmful)]].

There were significant treatment and species effects on ant mortality (Table S1). Treatment × ant species interaction effect was also significant. Similarly, when considering treatment type as the two insecticide rates (thus, RFR and 50% RFR), there was a significant treatment, ant species (except acetamiprid + emamectin benzoate exposure) and treatment × ant species interaction effect for all the insecticides except the interaction effect for emamectin benzoate and acetamiprid + emamectin benzoate (Table S1).

3.2. Concentration–Mortality Response Effect on Ants

The LC_{95} values were significantly different and varied based on the ant species and insecticide (Table 4). *Pheidole megacephala* had the lowest LC_{95} value when exposed to acetamiprid and pyrethrins. When exposed to emamectin benzoate, acetamiprid + emamectin

benzoate and acetamiprid + lambda-cyhalothrin, *C. africana* had the lowest LC_{95} value. *Oecophylla longinoda* had the highest safety index when exposed to the insecticides.

Table 4. Lethality of insecticides to ant species after 24 h of residual contact exposure under laboratory conditions.

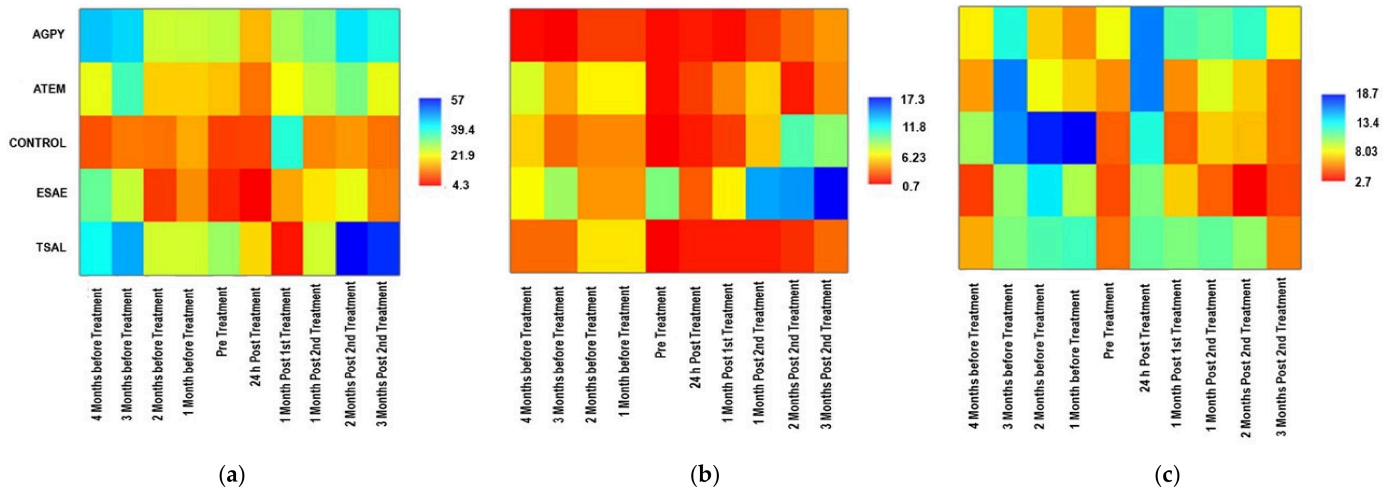
Insecticide (Active Ingredient)	Ant	LC_{95} (%)	95% CI (%)	Slope ± SE	Intercept ± SE	PSI
BSAC (acetamiprid) (LC_{95} for mirid = 0.0356% a.i.)	*C. africana*	0.0366 [c,d]	0.0280–0.0478	1.9999 ± 0.1917	9.5610 ± 0.8775	1.03
	O. longinoda	1.2866 [g]	0.4798–3.4494	0.8257 ± 0.0949	2.7364 ± 0.3923	36.14
	P. megacephala	0.0217 [b]	0.0169–0.0280	1.9939 ± 0.1679	10.5789 ± 0.8567	0.61
ASAE (acetamiprid + emamectin benzoate) (LC_{95} for mirid = 0.061% a.i.)	*C. africana*	0.0139 [a,b]	0.0078–0.0248	0.8655 ± 0.1051	6.6471 ± 0.6755	0.28
	O. longinoda	2.8639 [g,h]	0.7950–10.3171	0.9855 ± 0.1500	1.9075 ± 0.4901	46.95
	P. megacephala [‡]	-	-	-	-	-
TSAL (acetamiprid + lambda-cyhalothrin) (LC_{95} for mirid = 0.035% a.i.)	*C. africana*	0.0604 [d,e]	0.0422–0.0863	1.4614 ± 0.1304	7.0474 ± 0.6136	1.73
	O. longinoda	0.2127 [f]	0.1136–0.3984	0.9531 ± 0.0912	4.4196 ± 0.4398	6.08
	P. megacephala	0.1518 [e,f]	0.0608–0.3791	0.5907 ± 0.0656	4.0578 ± 0.3927	4.34
ATEM (emamectin benzoate) (LC_{95} for mirid = 0.080% a.i.)	*C. africana*	6.0056 [g,h]	1.7133–21.0521	0.741 ± 0.0929	1.6160 ± 0.3136	75.07
	O. longinoda	116.2334 [h]	4.4598–3029.6428	0.6124 ± 0.1287	0.0319 ± 0.4202	1452.92
	P. megacephala [†]	-	-	-	-	-
AGPY (pyrethrins) (LC_{95} for mirid = 0.036% a.i.)	*C. africana*	0.0131 [a,b,c]	0.0057–0.0299	0.5887 ± 0.0935	5.4976 ± 0.6234	0.36
	O. longinoda	0.2180 [f]	0.1325–0.3587	1.0808 ± 0.0966	4.5909 ± 0.4149	6.06
	P. megacephala	0.0095 [a]	0.0070–0.0129	1.9955 ± 0.1856	12.2345 ± 1.1387	0.26

LC_{95}: 95% Lethal Concentration; CI: Confidence Interval; SE: Standard Error; PSI: Predator Safety Index (LC_{95} of ant/LC_{95} of mirid); [‡]: Analysis aborted by software because algorithm was diverging; [†]: Analysis aborted by software because maximum likelihood estimates may not exist; -: LC_{95} and associated parameters could not be computed. LC_{95} values with the same alphabet are not significantly different based on their overlapping 95% CIs. LC_{95} of each insecticide for mirid was determined prior to its registration for use on cocoa and this served as the recommended field rate. A PSI of <1, 1 and >1 means harmful to the predator (more toxic to the predator compared to target organism), non-selective and safe (less toxic) to the predator, respectively. The higher the PSI, the safer it is for the predator.

3.3. Insecticidal Effect on Ant Abundance on the Field

Table S2 presents the meteorological data while Figure 1 presents the abundance of the ant species prior to, during and post treatment application. Apart from the control, *O. longinoda* population under all the treatments reduced at 24 h after the first treatment application. *Crematogaster africana* population only reduced on acetamiprid + emamectin benzoate plots while *P. megacephala* abundance increased on all the treatment plots. Among the insecticides, the highest increase in *C. africana* and *P. megacephala* abundance was observed on emamectin benzoate-treated plots (Table 5). At 1 month after the second insecticide application, the population of *O. longinoda* was higher than the pre-treatment level for all the treatments except acetamiprid + lambda-cyhalothrin (Table 5 and Figure S2). However, the population of *C. africana* and *P. megacephala* increased for all the treatments. The highest increase in *O. longinoda*, *C. africana* and *P. megacephala* among the insecticide treatments was on acetamiprid + emamectin benzoate-, emamectin benzoate- and acetamiprid + lambda-cyhalothrin-treated plots, respectively (Table 5 and Figure S2).

The residual effect of the treatments at 3 months after the second insecticide application showed higher *O. longinoda* populations for all the treatments compared to their pre-treatment levels, with the highest increase of 90% on acetamiprid + emamectin benzoate-treated plots (Table 5 and Figure S2). A similar effect was observed in *C. africana* with the control having the highest increase among the treatments while acetamiprid + lambda-cyhalothrin had the highest among the insecticides. With *P. megacephala*, there was a 6% increase on the acetamiprid + lambda-cyhalothrin-treated plots, no change on the control and acetamiprid + emamectin benzoate-treated plots and a decrease on the other insecticide plots (Table 5 and Figure S2). Compared to the pre-treatment levels, the highest increase in *O. longinoda* and *C. africana* abundance was observed on acetamiprid + emamectin benzoate- and acetamiprid + lambda-cyhalothrin-treated plots, respectively (Figure 1 and Table 5).



(**a**) (**b**) (**c**)

Figure 1. Mean count of *Oecophylla longinoda* (**a**), *Crematogaster africana* (**b**) and *Pheidole megacephala* (**c**) nests. AGPY (pyrethrins); ATEM (emamectin benzoate); Control (no insecticide treatment); ESAE (acetamiprid + emamectin benzoate); TSAL (acetamiprid + lambda-cyhalothrin).

Table 5. Reduction in ant nests after insecticide application at different time periods.

Ant	Treatment	% Reduction in Nests				
		24 h P1	1 MP1	1 MP2	2 MP2	3 MP2
O. longinoda	ESAE	38.6	−124.3	−190.0	−232.9	−90.0
	TSAL	32.8	80.1	12.9	−98.6	−87.1
	ATEM	30.5	−26.0	−50.9	−75.1	−31.6
	AGPY	35.4	−6.5	−16.7	−55.9	−40.7
	Control	−3.5	−321.8	−57.5	−72.4	−41.4
C. africana	ESAE	70.0	33.3	−52.2	−55.6	−92.2
	TSAL	−85.7	−85.7	−85.7	−142.9	−328.6
	ATEM	−100.0	−270.0	−430.0	−30.0	−270.0
	AGPY	−30.0	0	−100.0	−200.0	−300.0
	Control	−85.7	−185.7	−614.3	−1328.6	−1142.9
P. megacephala	ESAE	−148.8	−62.8	−9.3	37.2	0
	TSAL	−126.0	−114.0	−126.0	−106.0	−6.0
	ATEM	−180.7	0	−52.6	−22.8	17.5
	AGPY	−92.8	−41.0	−36.1	−48.2	7.2
	Control	−170.2	0	−48.9	−42.6	0

P1: Post 1st insecticide application; MP1: Month Post 1st insecticide application; MP2: Months Post 2nd insecticide application. Negative numbers represent an increase in the number of nests.

Treatment had a significant effect on *O. longinoda* ($F_{(4,60)}$ = 2.90, p = 0.029) and *C. africana* ($F_{(4,60)}$ = 3.16, p = 0.020) abundance while sampling day had a significant effect on *C. africana* ($F_{(5,60)}$ = 2.83, p = 0.023) and *P. megacephala* ($F_{(5,60)}$ = 3.27, p = 0.011). There was no significant treatment × sampling day interaction effect on all the ant species.

4. Discussion

4.1. Toxicity of Different Insecticide Rates on Ants

Insecticidal impact and selectivity on non-target organisms are essential considerations for integration into pest management packages that include insecticides. This ensures compatibility and safeguards non-target organisms, particularly those that provide critical ecosystem services. Although a common pest management practice in cocoa production zones of West Africa [32], information on insecticidal impact on non-target organisms such as predatory ants is limited. In this study, the toxicity of insecticides used to manage cocoa

insect pests on non-target ant species was assessed. Ant knockdown varied depending on the insecticide and ant species. Knockdown toxicity was in the order emamectin benzoate < acetamiprid + emamectin benzoate < acetamiprid < pyrethrins < acetamiprid + lambda-cyhalothrin. *Pheidole megacephala* was the most easily knocked-down species, being the most susceptible to the insecticides except emamectin benzoate. In terms of size, it was also the smallest among the ant species. Generally, knockdown by emamectin benzoate was very low (2.5–10%) and for each ant species was the lowest compared to the other insecticides, except for pyrethrins-exposed *C. africana*. Knockdown momentarily terminates ant activity, impairing their foraging abilities. This, in turn, can affect the delivery of ecosystem services such as prey searching and soil engineering. Being knocked down also makes ants prone to predation and desiccation, which can contribute to their mortality. Insecticides from the pyrethroids and pyrethrins group are known for their knockdown abilities due to their activity on the voltage-gated sodium channel. This is evident in this study, as both the binary and sole insecticides containing active ingredients (acetamiprid + lambda-cyhalothrin and pyrethrins, respectively) from this class produced the highest knockdowns.

Half of the recommended rates of the insecticides except emamectin benzoate were lethal to *C. africana* and *P. megacephala*. The high mortality is similar to the findings of Bacci et al. [5] where some natural enemies exposed to half of the field rates of insecticides had mortalities as high as 100%. In that study, half of the field rate of acetamiprid was more toxic to two natural enemies (*Lasiochilus* sp. and *Encarsia* sp.) of *Bemisia tabaci* compared to the target pest. It was however less toxic to two other natural enemies (*Acanthinus* sp. and *Discodon* sp.) [5]. This is similar to the contrasting effect on the ant species in our study where mortality due to acetamiprid was >85% on *C. africana* and *P. megacephala* and 30% on *O. megacephala*. While acetamiprid + lambda-cyhalothrin induced 100% knockdown of *C. africana*, mortality after 24 h was slightly lower, indicating that some of the knocked-down individuals recovered. This shows that not all knockdowns lead to mortality and hence, knockdown is dynamic within the continuum of recovery and mortality [33].

Combining active ingredients from different insecticide classes may yield synergistic effects on insect pests. This synergy could also harm non-target organisms. While acetamiprid was harmful to *C. africana* and *P. megacephala*, acetamiprid + lambda-cyhalothrin was harmful to all the ant species. Emamectin benzoate was the safest, while emamectin benzoate combined with acetamiprid was safer towards *O. longinoda* compared to acetamiprid alone. Probably, the inclusion of emamectin benzoate as a component of a binary insecticide could attenuate the toxicity of the binary product and increase its selectivity to non-target organisms while retaining its efficacy against target pests.

4.2. Lethal Concentration of Insecticides on Ants

Insecticides pose differential toxicity to natural enemies [34] and this is reflected in the concentration–mortality response in our study. For each insecticide, the highest LC_{95} was observed on *O. longinoda* while the lowest was on *P. megacephala* (acetamiprid and pyrethrins) and *C. africana* (acetamiprid + lambda-cyhalothrin, acetamiprid + emamectin benzoate and emamectin benzoate). Overall, the lowest and highest LC_{95} values were recorded for pyrethrins-exposed *P. megacephala* and emamectin-benzoate-exposed *O. longinoda*, respectively. Consistently, the LC_{95} of *O. longinoda* exposed to all the insecticides was higher than the recommended field rate, indicating the selectivity of these insecticides for *O. longinoda*. Among the insecticides with a single active ingredient, emamectin benzoate had the highest LC_{95} while pyrethrins had the lowest. With the binary insecticides, acetamiprid + emamectin benzoate had the highest (on *O. longinoda*) and lowest (on *C. africana*) LC_{95} values.

Acetamiprid was safer to only *O. longinoda* and harmful to *P. megacephala*. This is similar to the toxicity of acetamiprid to non-target natural predators as reported by Cheng et al. [7] in *Harmonia axyridis*. The combination of acetamiprid with emamectin benzoate was highly safer to *O. longinoda* compared to acetamiprid with lambda-cyhalothrin in our study. However, while acetamiprid with emamectin benzoate was harmful to *C. africana*, the acetamiprid-lambda-cyhalothrin combination was relatively safer to *C. africana*, indicating varying responses to the binary insecticides. Emamectin benzoate is safer to several natural enemies such as predators and parasitoids [35] while lambda-cyhalothrin, either as a single active ingredient or in combination (with thiamethoxam), is toxic to parasitoids such as *Trichogramma exiguum*, *Trichogramma evanescens* and *Telenomus podisi* [36–38]. It was also the most toxic among a group of insecticides evaluated on a coccinellid predator (*Pullus mediterraneus*) [8].

In a recent study [7], acetamiprid posed a higher risk to the predator *H. axyridis* whereas emamectin benzoate posed a lower risk. Hence the relatively lower risk due to emamectin benzoate compared to acetamiprid and lambda-cyhalothrin [7,35–37] might have had an ameliorating effect on *O. longinoda* in our study when combined with acetamiprid compared to the acetamiprid-lambda-cyhalothrin combination. Safety to *O. longinoda* was in the order emamectin benzoate > acetamiprid + emamectin benzoate > acetamiprid > acetamiprid + lambda-cyhalothrin > pyrethrins highlighting that emamectin benzoate was safer. With the limited selective chemical compounds for pest management, natural enemy-compatible chemical combinations integrating emamectin benzoate with more toxic and non-selective active ingredients could be explored to expand the chemical control toolbox. Although usually deemed to be safer, organic active ingredients may be toxic to non-target organisms. In our study, pyrethrins was more toxic to the ants compared to acetamiprid, contrasting with the work of Roubos et al. [6] on *Orius insidiosus*, *Hippodamia convergens* and *Chrysoperla rufilabris*. However, in the same study [6], pyrethrins induced similar mortalities as acetamiprid after a 24 h exposure to insecticide media which had been aged for 0 and 3 days. The findings of our study also agree with the work of Jansen et al. [39] who observed pyrethrin toxicity on adult parasitic wasps and ladybird larvae. Although pyrethrins are biodegradable and photoliable, acute toxicity to ants could ensue due to the contact uptake by ants. However, its residual toxicity in the long term could be lower [6] due to its short persistence.

Selectivity of the insecticides varied in our study, and this can occur between natural enemies and the target pests. This could be due to differences in physiological selectivity [40,41], whereby differential physiological toxicity occurs between target and non-target insects. This could also occur within the same species as observed in different *Trichogramma pretiosum* strains [42]. Since insecticide usage is widespread, depending on the predatory ant composition in the agroecology, selective insecticides and natural enemies can be combined for effective pest management [43]. Even though some insecticides were not physiologically selective, they could be rendered ecologically selective through various means such as their mode of delivery. These could result in differential access in terms of contact with organisms and making the insecticides more toxic to the target pest and hence selective, since selectivity is a combination of toxicity and probability of contact [44]. The biology and behaviour of the ant species may also reduce their likelihood of contact with the insecticides and hence enhance their selectivity.

As observed in our study, the higher selectivity towards *O. longinoda* could account for the dominance of *O. longinoda* in cocoa agro-ecosystems despite the reliance on insecticides [45]. For insecticides that were less selective physiologically, selectivity could be ecological, accounting for the presence and population rebound of ants in the agroecosystem after insecticide application [25]. Despite the fact that spatio-temporal dynamics

of insect response to insecticides could vary and their behavioural patterns could affect the concentration that insects come into contact with, ex situ studies [5,6,34,35] like our study provide a uniform deposit surface [46] to compare toxicity under the same conditions. It is also an essential first step in the sequential process of determining insecticide toxicity on non-target organisms [29].

4.3. Effect of Insecticides on Ant Abundance on the Field

Oecophylla longinoda abundance was reduced a day after insecticide application, an indication of the acute toxic effect on the species. This, however, contrasted with the effects on *C. africana* and *P. megacephala*, demonstrating variability in species responses to the insecticides. In the laboratory, the ants had a direct and prolonged contact with the insecticides due to their confinement in the assay enclosure for the entire 24 h exposure period. However, in the field, they may avoid direct contact with insecticides through several means, such as staying away or escaping from treated areas and surfaces and sheltering in nests (physical barriers), which may account for the lower adverse effects compared to laboratory studies. Unlike the filter paper, insecticide distribution on exposure surfaces in the field such as trunks, pods and leaves may be patchy and uneven affecting the concentration that the ants are exposed to [47].

A month after the last insecticide application, *O. longinoda* abundance on all the treatments except acetamiprid + lambda-cyhalothrin increased denoting a rebound of the ant population. The insecticide treatment with the highest percentage increase was acetamiprid + emamectin benzoate. For *C. africana* and *P. megacephala*, their levels exceeded the pre-treatment level for all the treatments with emamectin benzoate and acetamiprid + lambda-cyhalothrin displaying the highest percentage increase among the insecticides, respectively. On the field, environmental factors like rain, sunlight (ultra-violet), temperature and wind could affect insecticide toxicity [47], thereby impacting the ant species. The sole effect of biotic factors like microbial activity, predators of the test species, ant caste and foraging and grooming behaviours, as well as their interaction with the insecticides, could also affect ant abundance. These biotic and abiotic factors, as well as the degradation of the insecticides, could impact the residual effect of the insecticides on the ants, resulting in a population rebound. At 3 months after the last insecticide application, *O. longinoda* abundance increased for all the treatments compared to the pre-treatment levels, with the highest increase on acetamiprid + emamectin benzoate-treated plots. Similarly, *C. africana* population increased on all the treatment plots, with the controls having the highest increase among the treatments while acetamiprid + lambda-cyhalothrin had the highest among the insecticide treatments. There was a marginal increase in *P. megacephala* abundance on only acetamiprid + lambda-cyhalothrin plots while the other treatments witnessed a decrease in abundance. Since these are formulated insecticides, the co-formulants may also contribute to the activity of the insecticides. Insecticides with repellent and deterrent effects can also affect the abundance of ants. With the findings in this study, there is the need to investigate the selectivity of other insecticide molecules used in cocoa production in Ghana. Also, non-selectivity is not limited to insecticides, as demonstrated with the herbicide glyphosate [48] and fungicides fenbuconazole [49] and metalaxy + copper (I) oxide [50]; hence, further studies are required to assess the selectivity of other pesticides for their compatibility in a combined pesticide–natural enemy conserved cropping system.

5. Conclusions

This study assessed the toxic effects of insecticides used for pest management in cocoa against three non-target predatory ant species. The recommended field rate of TSAL (containing acetamiprid + lambda-cyhalothrin) was the most toxic to the ants while

ATEM (containing emamectin benzoate) was the least toxic under laboratory settings. The concentration–mortality response effect confirmed the selectivity and safety of emamectin benzoate. Although ant mortality responses varied, the insecticides were more selective to *O. longinoda*. In the field, *O. longinoda* abundance reduced at 24 h after insecticide application; however, its population rebounded afterwards. This study provides valuable information to guide the regulation and integration of insect pest management practices and the protection of beneficial ant species. Integrating selective insecticides which have low ant toxicity leverages on the potential benefits of both pest control strategies (chemical and biological) without dispensing with or substantially minimising either benefit.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agrochemicals5020028/s1, Figure S1: Ant mortality after 24 h exposure to the various treatments; Figure S2: Mean number of ant nests on treatment plots; Table S1: Results of analysis of variance of mortality response at recommended field rate due to treatment and ant species; Table S2: Weather data of the location (New Tafo-Akim, CRIG) during the study period in 2023.

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