African Journal of Biotechnology Vol. 8 (8), pp. 1528-1535, 20 April, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Evaluation of the toxicity of Actara 25 WG for the control of the cocoa mirid *Sahlbergella singularis* Hagl. (Hemiptera: Miridae) in Nigeria

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Accepted 23 January, 2009

In Nigeria, the brown cocoa mirid *Sahlbergella singularis* is responsible for over 30% yield loss in cocoa. Insecticides still remain a very important component among the strategies for effective control of major insect pests of cocoa in Nigeria. The Cocoa Research Institute of Nigeria (CRIN) has the "National Mandate" to evaluate and recommend new insecticides for use on cocoa in Nigeria. The mortality rates of mirids on exposure to the various concentrations (0.01, 0.013, 0.015 and 0.02%) of Actara 25 WG increased with period of exposure of the mirids in the laboratory. Both Actara and the Standard Miricide were similar at 0.020% concentration as both gave a 100% kill of mirids at the 90th min. No mortalities were recorded in control cages throughout the exposure period. The insecticide did not produce any adverse side effects on cocoa seedlings and mature plants sprayed with the various concentrations over the years. The relative toxicity of the insecticide at the four different concentrations to adult mirids, 24 h after the first field treatment application, was 93.3, 82.4, 81.8 and 100% adult mortality, respectively. In the case of the nymphal mirids, percentage mortality was 89.6, 81.5, 86.8 and 94.3% for the various concentrations, respectively. The population of both the adult and nymphal mirids crashed to almost zero (0) in most cases suggesting about 100% mortality 24 h after the second spraying on the residual mirid populations. Mirid numbers remained so low until the third spray application after which it was unnecessary to spray the sub-plots, thereafter. Spraying of mature and fruiting cocoa farms at application rate of 0.015% was found adequate and recommended taking into consideration the impact of the product on the environment and for economic reasons.

Key words: Mortality, concentration, insecticide, spraying, miricide, exposure.

INTRODUCTION

Over 75% of the world's cocoa is grown in West Africa. Nigeria is currently the 5th world producer of cocoa with an estimated production figure of 165,000 metric tons in 2006/07 (ICCO, 2007; FAO, 2007). Mirid damage is one of the three most important scourges affecting cocoa production in West African cocoa growing countries. The other two scourges are the cocoa swollen shoot virus disease transmitted by mealybugs, and the black pod disease caused by *Phytophthora palmivora* and the more virulent *Phytophthora megakarya.*

The feeding activities of mirids are characterized by dark markings (lesions) on both pods and shoots, which result from the collapse of plant tissue caused by the toxic saliva of mirids. Secondary damages (canker and dieback) occur when the feeding lesions are invaded by parasitic fungi notably *Calonectria rigidiuscula* Berk. and Br. (Sacc.) and *Fusarium decemcllulare* (Entwistle, 1972). The combined activities of mirids and the pathogenic fungi usually result in over 30% yield loss in cocoa annually.

Insecticides still remain a very important component among the strategies for effective control of mirids and other major insect pests of cocoa in Nigeria. The Cocoa Research Institute of Nigeria (CRIN) has the national mandate to evaluate and recommend novel insecticides from various groups that fall within the EU standards so that replacement could be made easily in a situation where they become unavailable in the local market or to

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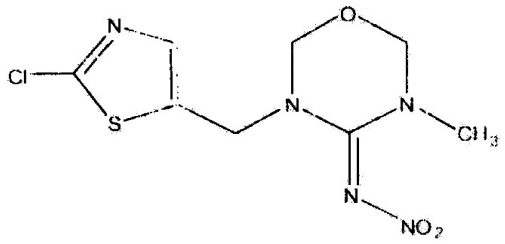
combat resistance problems that may arise as a result of over-dependence on a particular class of insecticides. Such insecticides usually undergo rigorous testing to determine and recommend the correct dose necessary to produce toxic reactions to the targeted pest without any adverse effects on the environment and non-target organisms.

An intensive use of a given insecticide for protection of perennial tree crops such as cocoa against economic pests sometimes result in the development of resistance of these pests to such insecticides (e.g. the cocoa mirid *Sahlbergella singularis* developed resistance to Gammalin 20 EC in some of the cocoa producing areas of Nigeria from the mid sixties). Several insecticides were screened from early seventies to combat the resistance problem until Unden 20 EC (Propoxur), Mipcin 75WP and Elocron 50 WP (Dioxacarb), all carbamates were found to be efficacious and recommended as suitable alternatives to Gammalin 20 EC in late seventies and early eighties, respectively. Also recommended were Dursban 48EC (Chlorpyrifos) an Organophosphate (O.P.) and Thiodan 35 EC (Endosulfan) (a Cyclic Sulphurous ester). Basudin 600 EC (Diazinon) and Decis-Dan/Cracker 282 EC, a mixture of Endosulfan and pyrethroid, (Deltamethrin) was later identified and recommended in the mid-eighties as suitable alternatives to replace the earlier insecticides. New and existing insecticides are usually evaluated periodically by CRIN, for their efficacy against the cocoa mirid in order to forestall epidemic mirid outbreaks, which normally accompany the development of resistance. Hence, the evaluation of the efficacy of Actara 25 WG (Thiamethoxam), a Neonicotinoid insecticide for routine protection of cocoa farms against the brown cocoa mirid was undertaken.

The evaluation of this insecticide, which started with laboratory and large-scale field trials, was completed in 2007, with quantitative determination of the residues of the active ingredient (Thiamethoxam) of this insecticide in cocoa beans obtained from farms, which had been routinely sprayed with the insecticide. Syngenta Crop Protection AG sponsored and financed this project. The detailed results of the various aspects of the evaluation and other technical information on the insecticide (NRA, 2001), which are pertinent to the conclusion and recommendations made here, are given in this report.

TECHNICAL INFORMATION ON ACTARA 25 WG

Actara 25 WG was manufactured in Syngenta Crop Protection AG, Switzerland. Its code number is CGA293343; design code, A9584C; Chemical Abstracts Service (CAS)-Registry number, 153719-23-4; chemical name (*IUPAC*), 3-(2-chloro-thiazol-5-ylmethyl)-5-methyl-(1,3,5) oxadiazinan-4-ylidene-N-nitroamine. It has a colour that changes from beige to brown, with musty odour and pH of 7 – 11 at 1% w/v. Actara 25 WG molecular weight is 291.7, having $C_8H_{10}ClN_5O_3S$ as its empirical formula, while is structural formula is given below:



It belongs to the Neonicotinoid insecticide group, and it has as its component the major active ingredient, Thiamethoxam (98%). It is made up of wettable granules/water-dispersible granules; its water solubility is miscible. It is a broad spectrum, non-systemic insecticide, and highly degradable. In WHO hazard rating, it is rated as third class, while in dust explosion class as first class. Its acute oral toxicity is LD50 Rat, > 5,000 mg/kg; acute dermal toxicity, LD50 Rat, > 5,000 mg/kg; acute inhalation toxicity LC50 Rat, > 5,290 mg/m^3, 4 h on rats.

It is a highly diluted miscible suspension product with water, and so it should be applied to canopy and trunk of cocoa trees with common portable knapsack sprayers. Seeking medical help immediately is advised if poisoning is suspected. If ingested, vomiting should not be induced, while contaminated eyes or body should be flushed/ washed with large quantity of clean water. If swallowed, 200 ml of liquid paraffin should be administered followed by gastric lavage with approximately four litres of water.

METHODOLOGY

Laboratory tests

The efficacy of Actara 25 WG under laboratory conditions of temperature and relative humidity 27±3°C and 60±10%, respectively was tested at four concentrations of 0.01, 0.013, 0.015 and 0.020%. A mixture of nymphs (3rd-5th instars) and adults of *Sahlbergella singularis* collected from cocoa plots at CRIN Headquarters, Ibadan, were exposed to filter papers impregnated with various concentrations of the insecticide inside micro-cages of transparent plastic petri-dishes with perforated lids. Ten mirids were placed in each Petri-dish. A standard miricide was used for comparison while distilled water was used as control. The experimental design was a Completely Randomized Design with 10 replications per treatment. Mortality of the mirids was recorded at ten minutes intervals for 2 h, which was the maximum time period it took to achieve 100% mortality in over 60% of the cages.

Phytotoxicity tests

For three consecutive years (2003 to 2005), one thousand (1,000) cocoa seedlings in CRIN nursery were sprayed with Actara 25 WG at 0.013, 0.015 and 0.020% concentrations. Three applications were made each year at 35 days intervals. The test was extended to same number of mature cocoa trees in the plots (1 ha) within the same time period. The treated plants were routinely observed for possible adverse side effects of the insecticides such as wilting,

Table 1. Laboratory toxicity of Actara 25 WG to the brown cocoa mirid, *Sahlbergella singularis* in Nigeria.

Conc. (%)	Exposure periods (min)											
	10	20	30	40	50	60	70	80	90	100	110	120
0.010%	0.0*	0.0	0.0	0.0	0.0	6.7	6.7	13.3	13.3	13.3	33.3	33.3
0.013%	0.0	0.0	6.7	6.7	6.7	6.7	6.7	20.0	26.7	40.0	53.3	60.0
0.015%	0.0	6.7	13.3	20.0	33.3	33.3	33.3	46.7	60.0	86.7	86.7	100
0.020%	13.3	20.0	33.3	33.3	46.7	46.7	60.0	86.7	100	100	100	100
Std. Miricide	13.3	33.3	33.3	46.7	60.0	86.7	86.7	100	100	100	100	100
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Mirid mortality (%). Each value represents mean of ten replicates.

scorching, necrosis as well as outbreak of any unusual insect pests in the nursery and in the treated cocoa plots.

Field tests

The field evaluation of the miricidal toxicity of Actara 25 WG (Thiamethoxam) was carried out during the mirid seasons for three consecutive years. The trial was conducted using Randomized Complete Block Design. The treatment plots contained five trees each per replicate, which was separated by three rows of trees. The insecticide was tested at four concentrations 0.01% (4 g/10L.H_2O), 0.013% (5.2 g/10L.H_2O), 0.015% (6 g/10L.H_2O) and 0.020% (8 g/10L.H_2O). Each concentration was replicated four times. A standard miricide was used for comparison, while the control plot was not sprayed. The insecticide water miscible suspension was applied with a lever-operated knapsack-spraying pump. Field applications were carried out between 7 and 9 a.m. when the weather was still calm and the mirids were not too mobile. Three applications were made during the season at 35 days interval. A pre-count of the number of adults and nymphal mirids found on each tree was taken before application of the various insecticide treatments. This was followed by a post-treatment count of the number of the insect still found on each tree 24 h after treatment. Percentage mortality was computed to determine the relative toxicity of the four concentrations of Actara 25 WG applied. These were compared with percentage mortality in unsprayed plots and plots sprayed with the standard miricide.

Residue analysis

The trial was conducted at three cocoa agro-ecological zones; Ibadan (Oyo State), Owena (Ondo State) and Ikom (Cross-River State) using Randomized Complete Block Design. The treatment plots contained five trees each per replicate, which were separated by three rows of trees. The insecticide was tested at three concentrations 0.013, 0.015 and 0.020%. Each concentration was replicated four times. A standard miricide was used for comparison, while the control plot was not sprayed. The insecticide/water suspension was applied with a lever-operated knapsack spraying pump. Three applications were made each year (between January and April) at 35 days intervals. Mature and ripened cocoa pods were harvested separately from the plots treated with the various concentrations two months after the previous year's spraying. The pods were broken, beans extracted, fermented and sun-dried. The beans from each plot were kept separately in sealed thick brown envelopes and stored. The coded cocoa bean samples were forwarded to a standard analytical laboratory for residue analysis to determine the levels of the residue of the active ingredient (Thiamethoxam) of Actara 25 WG present in the cocoa bean samples.

RESULTS

Laboratory test

The effect of Actara 25 WG on mirid mortality at all the four concentrations tested in the laboratory bioassay is given in Table 1. Mortality increased with period of exposure. The mortality rates of Actara 25 WG and the standard miricide were similar at 0.020% concentration as both Actara 25 WG and the Standard miricide gave a 100% kill of mirids in the laboratory at the 90th min. No mortalities were recorded in control cages throughout the exposure period.

Phytotoxicity test

The insecticide (Actara 25 WG) did not produce any of the usual adverse side effects such as wilting, scorching, necrosis as well as outbreak of any unusual insect pests on cocoa seedlings and mature plants sprayed with the various concentrations over the years. A zero percent toxicity was recorded on both the cocoa seedlings and mature trees in the plots after treatment (Table 2).

First year field evaluation

Figures 1 and 2 show the relative toxicity of the insecticides at different concentrations to adult and nymphal mirids, respectively. Twenty four hours after the first treatment application, adult mortalities of 93.3, 82.4, 81.8 and 100% were recorded for 0.01, 0.013, 0.015 and 0.02% concentrations, respectively. In the case of the nymphal mirids, percentage mortality was 89.6. 81.5, 92.1 and 94.3% for the various concentrations. Twenty-four hours after the second spraying of the residual mirid populations, the population of both the adult and nymphal mirids crashed to almost zero (0) in some cases suggesting about 100% mortality. Since both the adult and nymphal mirids were virtually eliminated after the second spray application in this trial, the third spray application could be considered as unnecessary. Therefore two applications per season at 0.013 to 0.02%

Table 2. Field phytotoxicity of Actara 25 WG to cocoa seedlings in the nursery and mature trees.

Actara 25WG	1st Year Application			2nd Year Application			3rd Year Application		
	Total No.	Infested No.	% Toxicity	Total No.	Infested No.	% Toxicity	Total No.	Infested No.	% Toxicity
Cocoa seedlings									
0.013%	1000	0	0%	1000	0	0%	1000	0	0%
0.015%	1000	0	0%	1000	0	0%	1000	0	0%
0.020%	1000	0	0%	1000	0	0%	1000	0	0%
Mature cocoa trees									
0.013%	1000	0	0%	1000	0	0%	1000	0	0%
0.015%	1000	0	0%	1000	0	0%	1000	0	0%
0.020%	1000	0	0%	1000	0	0%	1000	0	0%
Std.	1000	0	0%	1000	0	0%	1000	0	0%
Control	1000	0	0%	1000	0	0%	1000	0	0%

Location: CRIN Headquarters, Ibadan.

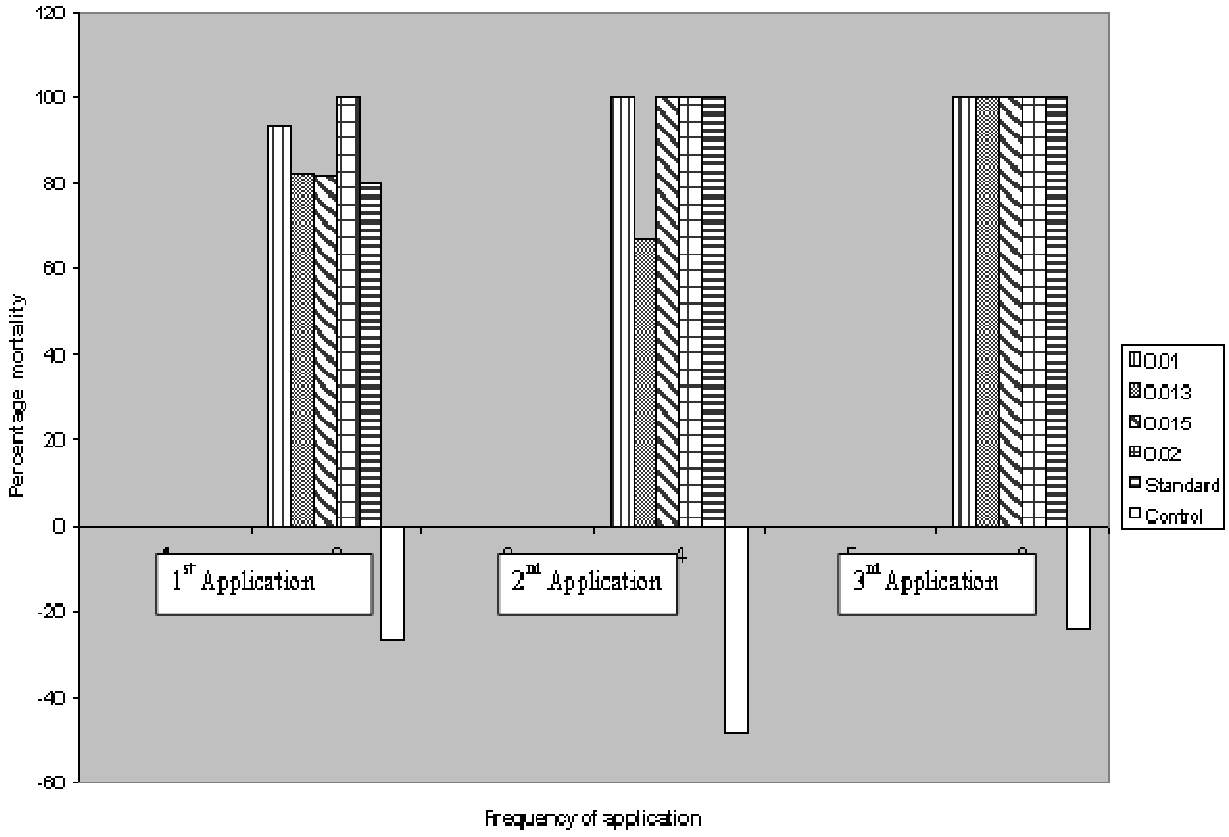


Figure 1. First year field toxicity of Actara 25 WG to adult mirid. Each value represents mean of four replicates. Negative values indicate infestation resulting from either further breeding of resident mirid colonies or mirid influx from neighbouring unsprayed plantations.

concentrations are considered adequate to keep the cocoa mirid populations below economic threshold. The negative values indicate mirid influx from neighbouring unsprayed plantations.

Second year field evaluation

Figures 3 and 4 showed the relative toxicity of the insecticide at various concentrations to adult and nym-

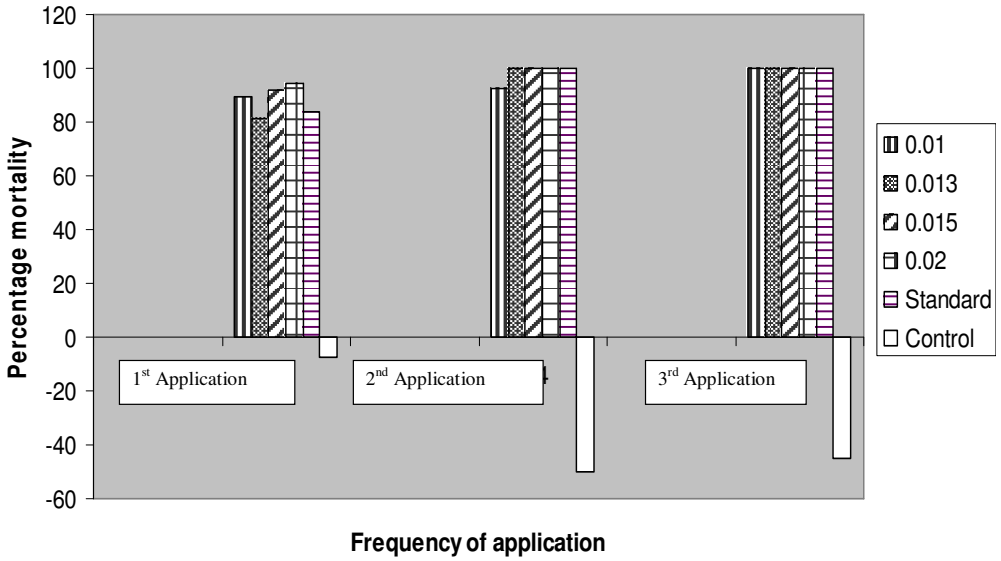


Figure 2. First year field toxicity of Actara 25 WG to nymphal mirid. Each value represents mean of four replicates. Negative values indicate infestation resulting from either further breeding of resident mirid colonies or mirid influx from neighbouring unsprayed plantations.

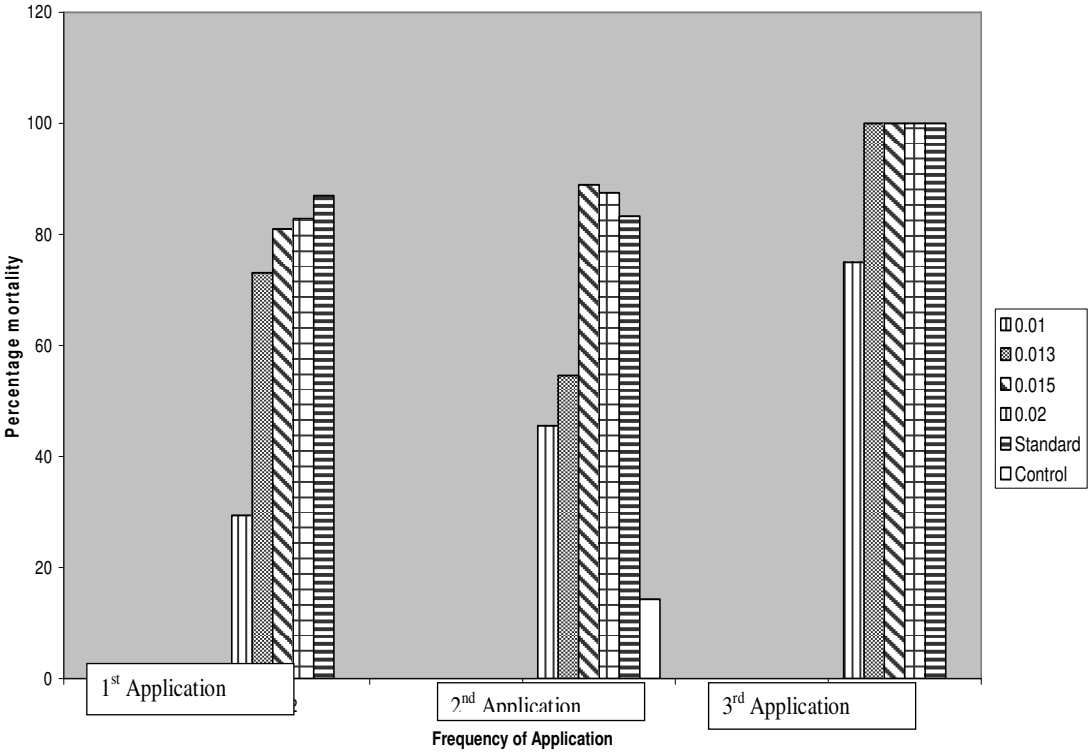


Figure 3. Second year field toxicity of Actara 25 WG to adult cocoa mirid. Each value represents mean of four replicates.

phal mirids, respectively. Twenty-four hours after the first treatment applications, 29.4, 73.1, 81.0 and 82.8% adult mortality were recorded for 0.01, 0.013, 0.015 and 0.02% concentrations, respectively. Percentage nymphal mortality recorded after the first spray application was 42.9, 51.1, 59.1 and 66.7% for 0.01, 0.013, 0.015 and 0.02%

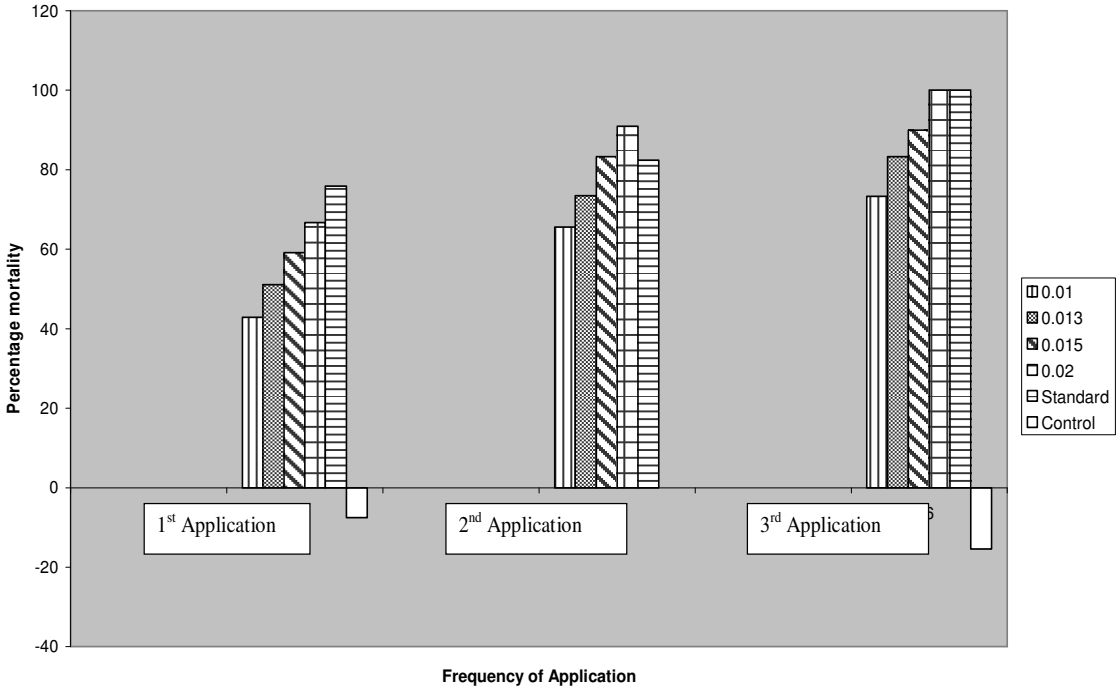


Figure 4. Second year field toxicity of Actara 25 WG to nymphal mirid population. Each value represents mean of four replicates. Negative values indicate infestation resulting from either further breeding of resident mirid colonies or mirid influx from neighbouring unsprayed plantations.

concentrations, respectively. The trend was also same on the residual mirid populations following the second field application.

Third year field evaluation

Figures 5 and 6 summarized the relative toxicity of the insecticides at the four different concentrations tested. An adult mirid mortality of 40.9, 64.7, 73.5 and 84.9% was recorded twenty-four hours after the first treatment application for the 0.01, 0.013, 0.015 and 0.02% treatment concentrations respectively, while the nymphal mirid population was reduced by 59.3, 70.4, 85.2 and 86.6% at the same treatment levels. Relative increase in mortality was also observed when the residual populations were applied with the second insecticide applications. The population of the adult mirid crashed to zero on plots treated with 0.013, 0.015 and 0.020% concentrations of the insecticide.

Similar trends were observed in the efficacy of the various concentrations of the insecticide on nymphal mirids where mortality rate increased from the first application to the third. Higher dosages of 0.015 and 0.020% gave better kill of the adult mirids recording high percentage mortality of 73.5 and 84.6%, respectively, after the first spray application. The nymphal mirid population observed crashed to near zero after the second spray on plots treated with the highest dosage.

In summary of the three years field evaluation, the negative values recorded for the control plots (Figures 1, 2, and 4) indicate mirid infestation resulting from either further breeding of resident mirid colonies or mirid influx from neighbouring unsprayed cocoa plantations. Also the minimal mirid mortality recorded in the control plots (Figures 3 and 5) could be as a result of any or conbination of the following:

- ✓ Natural control agents (natural enemies) killing the mirids.
- ✓ Some of the dead mirids could have reached the peak of their life span as at the time of the study and died naturally.
- ✓ Minimal drift from the sprayed plots could have killed some of them.
- ✓ The dead mirids could have also migrated from the sprayed plots in their bid to survive.

However, these factors were not taken into consideration, since the mirids were not confined to a particular place as in the case of laboratory bioassay or cage spray methods. This study depended solely on natural field populations of the mirids.

Residue analysis

The report of the residue analysis of Thiamethoxam in cocoa beans as carried out by ADME BIOANALYSES

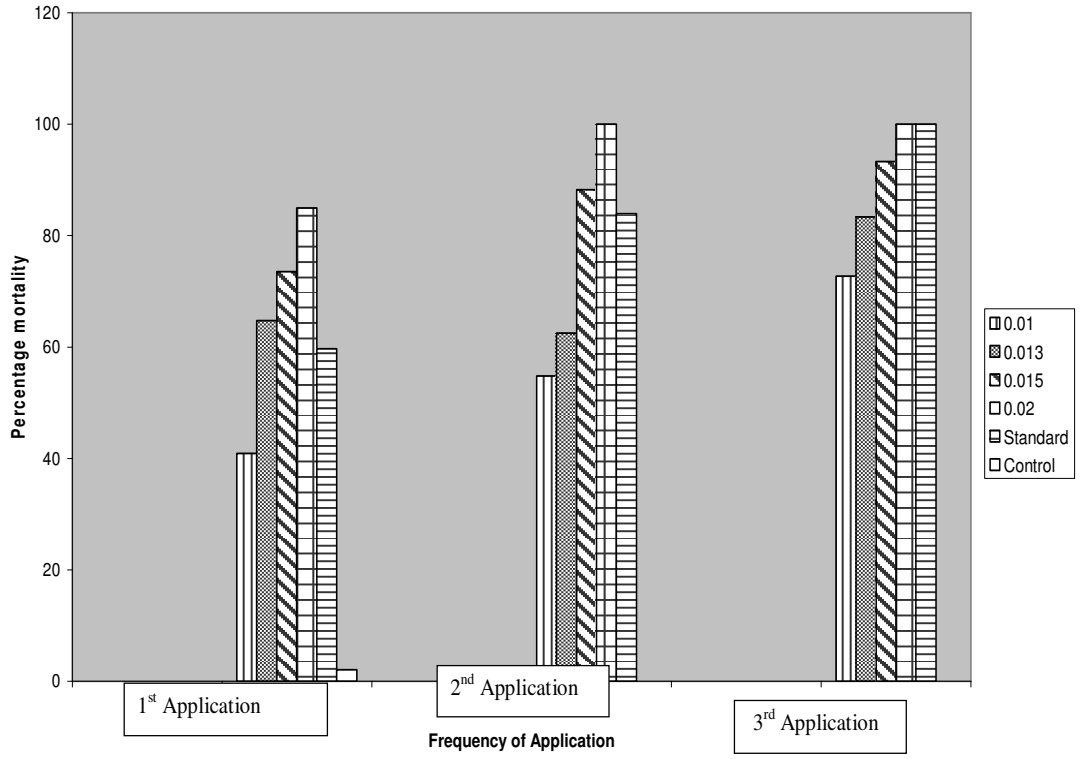


Figure 5. Third year field toxicity of Actara 25 WG to adult mirid. Each value represents mean of four replicates

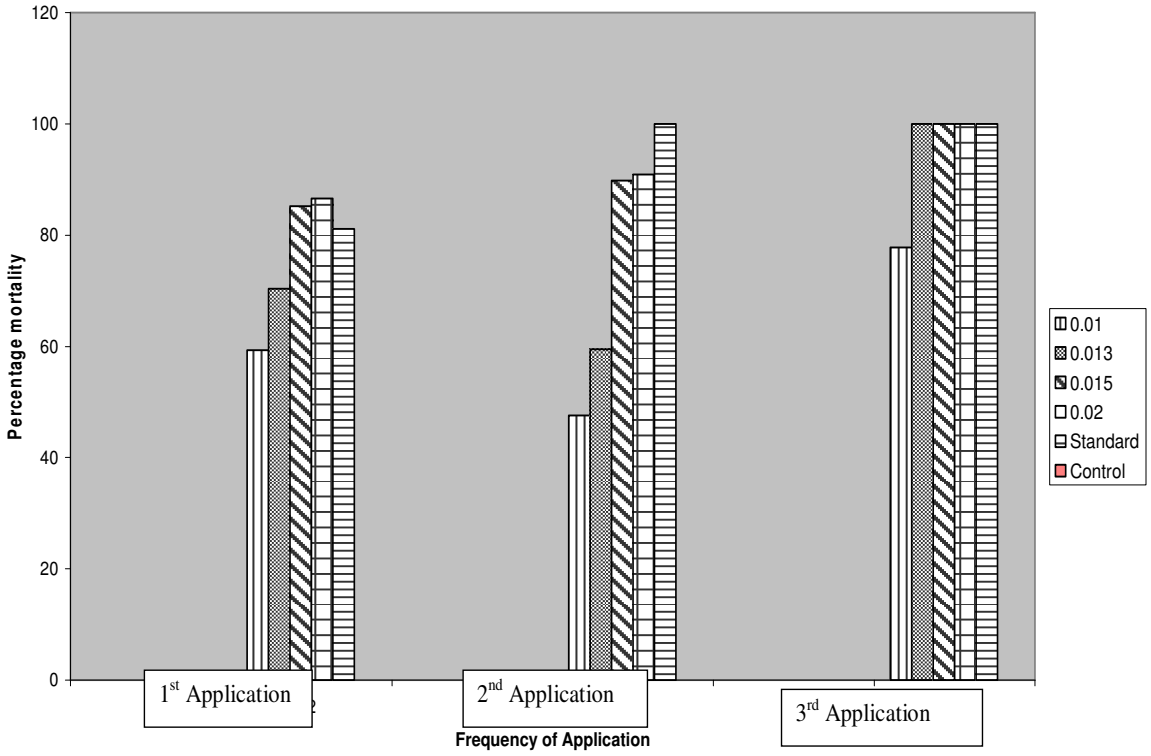


Figure 6. Third year field toxicity of Actara 25 WG to nymphal mirid population. Each value represents mean of four replicates.

LABORATORY through Syngenta Nigeria Limited gave a residue limit of 0.02 mg/kg, which is lower than and conforms to the European Union standard (NRA, 1996; COPAL, 2008). However, regular monitoring of this product and further residue analyses will be undertaken locally by CRIN through an independent analyst in order to confirm that the residue left on cocoa is within acceptable limits.

DISCUSSION

Actara 25 WG has proven to be a consistent efficacious insecticide over the years for the control of the cocoa mirid. Comparatively, the toxicity of the insecticide is more consistent on the adult mirids, whose population crashed to zero much earlier than that of nymphal mirids. The active constituent of this insecticide, Thiamethoxam is known to act on the nicotinic acetylcholine receptor of insects where it mimics the messenger chemical acetylcholine and binds to the receptor site, irreparably damaging the insect nervous system and eventually leading to the insect death (NRA, 2001). The high level performance of the insecticide at each stage was a prerequisite for its recommendation to proceed to the next stage of the screening process. The screening of such novel insecticide could be stopped at the laboratory or preliminary field trials if any undue adverse effects are noticed.

Actara 25 WG when routinely sprayed to protect cocoa farms against the cocoa mirid, at dosages of 0.015% was found to be as efficacious as other standard insecticides approved for routine protection of cocoa in Nigeria. Although the potency of the product increased with increasing concentration under laboratory condition, this superiority at higher concentration, virtually disappeared under field conditions. It is suggested therefore that for economic reasons and impact of the product on the environment, routine spraying of mature and fruiting cocoa farms at application rate of 0.015% is adequate and this is therefore recommended. Meanwhile the performance of this insecticide will be monitored periodically for any sign of insect resistance development, high toxicity/phytotoxicity to the crop or the environment as well as high chemical residues on cocoa beans. If any of these signs are observed, this recommendation may be withdrawn.

Insecticides undergo rigorous testing to determine the dose necessary to produce a toxic reaction. “LD 50” is a term often used to rank insecticides by their toxic levels- the lower the LD 50, the more toxic the insecticide. For example, nicotine, considered a natural insecticide, is extremely toxic with an oral LD 50 of 55 (Hillock and Bolin, 2004). With acute oral and dermal toxicity of LD50 Rat, > 5,000 mg/kg and acute inhalation toxicity of LC50 Rat, > 5,290 mg/m^3, 4 h on rats (NRA, 2001), Actara 25 WG is considered safe for use. The screening process of Actara 25 WG at CRIN depended largely on the field populations of mirids and that accounts for the number of years taken to achieve the completion of the process.

ACKNOWLEDGEMENTS

The authors wish to thank the staff of Entomology Section and the management of the Cocoa Research Institute of Nigeria for their support.

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