Chapter 4
Evaluation of Resistance and Toxicity of Different Insecticides on *Tuta absoluta* Meyrick Populations in Major Tomato Growing States of Nigeria

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Abstract The sudden invasion of the tomato leaf miner, *Tuta absoluta* Meyrick into tomato crops in Nigeria in 2015 has led to the extensive use of common insecticides, together with the introduction of a new insecticide referred to as "Tuta solution", as only control method. After two years the farmers in major tomato producing states of Nigeria (Kaduna, Kano and Katsina States) reported that most of the insecticides applied were no longer effective in controlling Tuta. This led to the investigation of the toxicities of common insecticides lambda-cyhalothrin (MoA group 3A) and deltamethrin (MoA group 3A), and of the newly introduced formulations of chlorantraniliprole + lambda-cyhalothrin (MoA group 28 + 3A) and spirotetramat + flubendiamide (MoA group 23 + 28). These insecticides were tested on larvae obtained from populations collected from major tomato-growing states (Bomo and Giwa in Kaduna State; Beriberi and Funtua in Katsina State; and Bagauda, Watari and Samawa in Kano State) and a susceptible laboratory population of the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. These populations were subjected to concentration mortality bioassays, according to susceptibility test method No. 022, devised by the Insecticide Resistance Action Committee (IRAC). Resistance to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin, and deltamethrin were observed in all the populations and compared with that of the susceptible NIHORT population. The resistance ratios obtained within the 7 populations ranged from 4.09 to 16.97 for chlorantraniliprole + lambda-cyhalothrin, 2.66 to 7.88 for lambda-cyhalothrin, and 3.23 to 6.24 for deltamethrin. However, resistance to spirotetramat + flubendiamide was not observed in all the 7 populations, with resistance ratio value of 1.05 as this combination was only introduced in 2017 for the control of *T. absoluta* in Nigeria and differently from the others, has not yet been abused. The sole dependence on, and indiscriminate usage of insecticides

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by farmers due to reckless recommendations, without regard for Integrated Pest Management, resulted in the widespread, higher levels of resistance observed in chlorantraniliprole + lambda-cyhalothrin, a product recently introduced into Nigeria in 2015.

Keywords Resistance Ratio Value · Insecticide Resistance Action Committee (IRAC) · Integrated Pest Management (IPM)

4.1 Introduction

In 2015 Nigeria experienced an unprecedented invasion by the tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) which completely ravaged tomato farms all over the nation, leaving farmers with almost zero yields. It crumbled the National tomato value chain, which is ranked the largest in Sub-Saharan Africa, leading to importation of tomato from neighbouring countries. *Tuta absoluta* is a very challenging pest to control because it attacks tomato plants in all developmental stages, damaging the stems, apices, flowers and fruits, as well as mining the leaves (Miranda et al. 1998). Since its invasion in Nigeria, excessive insecticides applications have been the main method of control. Guedes et al. (1994) reported that some of the compounds recommended for *T. absoluta* control are apparently not providing the desired effect. Excessive applications of the insecticides commonly applied to tomato crops during a single cultivation period (sometimes up to 36 sprays) could have led to the evolution of resistant pest populations, as well as the elimination of their natural enemies, thus leading to additional occupational hazards (Castelo and Franca 1992; Gonçalves et al. 1994; Picanço et al. 1995). The existence of resistance to organophosphates and pyrethroids in Chile (Salazar and Araya 1997, 2001) and to abamectin, cartap, methamidophos and permethrin in Brazil (Siqueira et al. 2000, 2001) have been reported. Bassi et al. (2012) stated that reliance on insecticides alone will not provide the flexibility and sustainability required for a rational insect resistance management scheme, as part of an integrated pest management (IPM) scenario: a reduction of the abuse of insecticides and the adoption in parallel of IPM principles will be mandatory, in order to mitigate directional selection of resistance in *T. absoluta* populations.

Chlorantraniliprole + lambda-cyhalothrin (Ampligo®[1]) was the first insecticide introduced, as a “Tuta solution”, and extensively used all over Nigeria for the control of *T. absoluta*. However, shortly after farmers realized that a solution had been found, they began to report that the insecticide had become inefficient. This compelled us, for the first time, to determine the resistance of *T. absoluta* to the most commonly applied insecticides, for its control in Nigeria.

[1] Registered trademark of Syngenta Group Company.

The objective of this work was, therefore, to evaluate the toxicity of chlorantraniliprole + lambda-cyhalothrin, spirotetramat + flubendiamide, lambda-cyhalothrin and deltamethrin to *T. absoluta* populations in the major tomato-producing locations in Nigeria. Main goal was to ascertain the claim of resistance development by farmers, which will guide us in designing resistance management strategies.

4.2 Materials and Methods

The *Tuta absoluta* populations used for analysis were collected from commercial tomato fields in March 2017, from 7 local government areas of the 3 major tomato-producing states in Nigeria. A population provided from the laboratory colonies of NIHORT, reared since 2016 without exposure to insecticide, was used as susceptible population (Table 4.1). Informations on insecticides used on each of the fields were obtained from farmers. Fourth instar larvae collected from each of the sampled farms were reared individually on tomato plants in cages, without insecticide exposure, in the laboratory. The emerged adults were reared on tomato plants in cages to obtain second instar larvae, which were used in the bioassays.

4.2.1 Insecticides

The insecticides used in this study were lambda-cyhalothrin (MoA group 3A, Karate®[1]) and deltamethrin (MoA group 3A, Decis®[2]), which have been mostly used for control of various pests on tomato for over 10 years, as well as chlorantraniliprole + lambda-cyhalothrin (MoA group 28 + 3A, Ampligo®[1]) and spirotetramat + flubendiamide (MoA group 23 + 28, Tihan®[2]), which were introduced for control of *T. absoluta* during its recent invasions into Nigeria in 2015 and 2017.

Table 4.1 The origin of the collected population of *Tuta absoluta*

Location of farm	Local government area	State
Bomo	Sabon gari	Kaduna
Giwa	Giwa	Kaduna
Beriberi	Faskari	Katsina
Funtua	Funtua	Katsina
Bagauda	Bebeji	Kano
Watari	Bagwai	Kano
Samawa	Garun Mallam	Kano
NIHORT	Ibadan North-West	Oyo

[2] Registered trademark of Bayer Crop Science.

4.2.2 Bioassays

The insecticide bioassays were conducted according to the susceptibility test method No. 022 formulated by the Insecticide Resistance Action Committee (IRAC). Young, tender, non-infested, untreated tomato leaves were collected and kept in sealed plastic bags to prevent wilting. For each bioassay, 7 different insecticide concentrations, including a control treatment of distilled water, were applied. Collected leaflets were dipped singly in the diluted concentrations for 3 s, with gentle agitation to ensure total submergence. The treated leaves were dried on wire net, with the upper leaf surface facing up. The dried treated leaflets were placed singly on moistened filter paper in Petri dishes (9 cm diam. × 1.5 cm height). A leaf square was cut around a 2nd instar larva with a scalpel and lifted with a brush on to the treated leaflet in the Petri dish and covered. The Petri dishes were arranged on a laboratory working bench at 29 ± 2 °C, under a photoperiod of 13:11 (L:D). Larval mortality was assessed after 72 h of treatment by prodding larvae with a hair brush. Larvae were considered dead if they were unable to move.

4.2.3 Data Analysis

Concentration mortality data were subjected to probit analysis (Proc Probit, SAS Institute 1997).

4.3 Results

There was a significant variation in the insect populations resistance to chlorantraniliprole + lambda-cyhalothrin. The lethal dose (LD_{50}) of the susceptible population (0.321) was significantly smaller than those from the other 7 population due to a failure of 95% confidence level (CL) to overlap (Table 4.2). The slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations, differed. Resistance to chlorantraniliprole + lambda-cyhalothrin was observed in all the 7 populations when compared with the susceptible NIHORT population. The resistance ratio ranged from 4.09 to 16.97 times, with populations from Beriberi having the highest resistance ratio (Table 4.2).

There was no variation in the resistance of the insect populations to spirotetramat + flubendiamide, as the 95% CL for all the 8 populations overlapped with relatively similar slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations (Table 4.3).

There was significant variation in the resistance of the insect populations to lambda-cyhalothrin.

Table 4.2 Comparative resistance of *T. absoluta* population to chlorantraniliprole + lambda-cyhalothrin (MoA group 28 + 3A)

Population	n	Slope ± SE	LD_{50} (95% CL)	Resistance ratio[a]
NIHORT	282	0.232 ± 0.05	0.321 (0.122–0.52)	
Bomo	279	0.180 ± 0.03	1.526 (1.311–1.741)	4.75
Giwa	280	0.178 ± 0.03	1.314(1.115–1.513)	4.09
Beriberi	328	0.614 ± 0.01	5.447 (4.708–6.186)	16.97
Funtua	289	0.246 ± 0.02	3.478 (3.048–3.908)	10.83
Bagauda	311	0.605 ± 0.01	5.086 (4.414–5.758)	15.84
Watari	321	0.393 ± 0.02	4.695 (4.092–5.298)	14.63
Samawa	290	0.243 ± 0.01	3.548 (3.108–3.988)	11.05

[a]Resistance ratio = LD_{50} field population/ LD_{50} NIHORT population

Table 4.3 Comparative resistance of *T. absoluta* population to spirotetramat + flubendiamide (MoA group 23 + 28)

Population	n	Slope ± SE	LD_{50} (95%CL)	Resistance ratio[a]
NIHORT	192	0.437 ± 0.02	0.947 (0.796–1.028)	
Bomo	258	0.424 ± 0.02	0.990 (0.879–1.058)	1.05
Giwa	203	0.422 ± 0.02	0.990 (0.878–1.058)	1.05
Beriberi	275	0.456 ± 0.02	0.999 (0.884–1.071)	1.05
Funtua	192	0.464 ± 0.02	0.999 (0.884–1.071)	1.05
Bagauda	223	0.482 ± 0.03	0.998 (0.884–1.071)	1.05
Watari	240	0.422 ± 0.02	0.998 (0.884–1.071)	1.05
Samawa	200	0.461 ± 0.02	0.998 (0.884–1.071)	1.05

[a]Resistance ratio = LD_{50} field population/ LD_{50} NIHORT population

The LD_{50} of the susceptible population (0.491) was significantly smaller than those from the other 7 population due to a failure of 95% CL to overlap (Table 4.4). The slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations, were relatively similar. Resistance to lambda-cyhalothrin was observed in all the 7 populations when compared with the susceptible population from NIHORT. The resistance ratio ranged from 2.66 to 7.88 times, with populations from Bagauda showing the highest ratio (Table 4.4).

There was significant variation in the resistance of the insect populations to deltamethrin. The LD_{50} of the susceptible population (0.307) was significantly smaller than those from the other 7 population due to a failure of 95% CL to overlap (Table 4.5). The slopes of the concentration mortality curve, showing the homogeneity of insect response to the 8 populations, were relatively similar. Resistance to deltamethrin was observed in all the 7 populations when compared with the susceptible population from NIHORT. The resistance ratio ranged from 2.23 to 6.24, with populations from Giwa having the highest resistance ratio (Table 4.5). Among all the 4 insecticides tested, the highest resistances of 15.84 and 16.97 were recorded in Bagauda and Beriberi populations, respectively, on chlorantraniliprole + lambda-cyhalothrin.

Table 4.4 Comparative resistance of *T. absoluta* population to lambda-cyhalothrin (MoA group 3A)

Population	n	Slope ± SE	LD_{50} (95%CL)	Resistance ratio[a]
NIHORT	192	0.104 ± 0.022	0.491 (0.037–0.061)	
Bomo	205	0.187 ± 0.012	1.976 (1.694–2.189)	4.02
Giwa	235	0.181 ± 0.012	2.044 (1.810–2.321)	4.16
Beriberi	205	0.192 ± 0.013	1.981 (1.607–2.255)	4.03
Funtua	192	0.133 ± 0.014	1.306 (1.058–1.576)	2.66
Bagauda	195	0.212 ± 0.010	3.868 (3.654–4.082)	7.88
Watari	195	0.207 ± 0.013	1.868 (1.627–2.100)	3.80
Samawa	192	0.195 ± 0.013	1.903 (1.672–2.153)	3.88

[a]Resistance ratio = LD_{50} field population/LD_{50} NIHORT population

Table 4.5 Comparative resistance of *T. absoluta* population to deltamethrin (MoA group 3A)

Population	n	Slope ± SE	LD_{50} (95%CL)	Resistance ratio[a]
NIHORT	180	0.199 ± 0.021	0.307 (0.473–0.651)	
Bomo	190	0.131 ± 0.013	1.386 (1.272–1.430)	4.51
Giwa	191	0.197 ± 0.013	1.916 (1.667–2.283)	6.24
Beriberi	195	0.147 ± 0.013	1.502 (1.320–1.745)	4.89
Funtua	195	0.186 ± 0.015	1.532 (1.434–1.632)	4.99
Bagauda	190	0.165 ± 0.013	1.821 (1.380–1.543)	5.93
Watari	188	0.150 ± 0.018	0.992 (1.298–1.521)	3.23
Samawa	191	0.144 ± 0.014	1.303 (1.201–1.427)	4.24

[a]Resistance ratio = LD_{50} field population/ LD_{50} NIHORT population

Table 4.6 Insecticides manufacturers' recommendations

Insecticide	Commercial name	Product recommendations/season
Lambda-cyhalothrin (MoA group 3A)	Karate	400 ml/ha. 3 applications between 7 and 10 days
Deltamethrin (MoA group 3A)	Decis	500 ml/ha. 3 applications between 7 and 10 days
Chlorantraniliprole + Lambda-cyhalothrin (MoA group 28 + 3A)	Ampligo	400ml/ ha. Apply at 1st sign of infestation of larvae in the leaves, or 1st signs of leaf damage. 3 applications at14–21 days interval
Cpirotetramat + flubendiamide (MoA group 23 + 28)	Tihan	400 ml/ha. Treat at the beginning of infestation. 3 applications within 14 days

The total number of insecticide applications between 2015, when the Tuta invasion was first recorded in Nigeria, and 2017 differed among the 7 populations, the 4 tested insecticides and from the manufacturers recommendations (Table 4.6).

Chlorantraniliprole + lambda-cyhalothrin recorded the highest application number range from 10–29, while spirotetramat + flubendiamide had the lowest application number range from 2–5 (Tables 4.7, 4.8, 4.9 and 4.10). Beriberi conse-

Table 4.7 Number of chlorantraniliprole + lambda-cyhalothrin applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	2	8	0	10
Giwa	3	8	0	11
Beriberi	5	19	5	29
Funtua	3	10	2	15
Bagauda	6	16	5	27
Watari	5	17	4	26
Samawa	3	12	2	17
Total	**27**	**90**	**18**	**135**
Mean	**3.9**	**12.9**	**2.6**	**19.3**

Table 4.8 Number of spirotetramat + flubendiamide applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	0	0	2	2
Giwa	0	0	2	2
Beriberi	0	0	2	2
Funtua	0	0	3	3
Bagauda	0	0	3	3
Watari	0	0	4	4
Samawa	0	0	5	5
Total	**0**	**0**	**21**	**21**
Mean	**0**	**0**	**3**	**3**

Table 4.9 Number of lambda-cyhalothrin applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	10	3	2	14
Giwa	12	5	3	17
Beriberi	10	3	2	15
Funtua	6	2	1	8
Bagauda	16	2	6	24
Watari	6	3	1	10
Samawa	6	3	1	10
Total	**66**	**21**	**16**	**98**
Mean	**9.4**	**3**	**2.3**	**14**

quently contributed the highest chlorantraniliprole + lambda-cyhalothrin application numbers of 29 (Table 4.7).

There was a significant linear relationship between the total number of applications and the resistance ratios of the insecticides to *T. absoluta* in the 7 populations (Figs. 4.1 and 4.2), except for spirotetramat + flubendiamide.

Table 4.10 Number of deltamethrin applied to the 7 populations within 3 planting seasons of 2015–2017

Population	Feb–Apr 2015	Feb–Apr 2016	Feb–Apr 2017	Subtotal application
Bomo	8	3	7	18
Giwa	13	6	8	27
Beriberi	8	5	6	19
Funtua	10	4	7	21
Bagauda	12	5	4	21
Watari	8	1	2	11
Samawa	8	3	5	16
Total	**67**	**27**	**39**	**133**
Mean	**9.6**	**3.9**	**5.6**	**19**

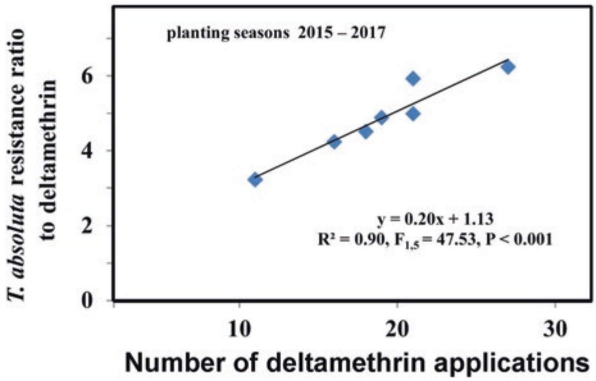

Fig. 4.1 Relationship between total number of chlorantraniliprole + lambda-cyhalothrin applications and resistance ratio of *Tuta absoluta* on the 7 populations within 3 planting seasons of 2015–2017

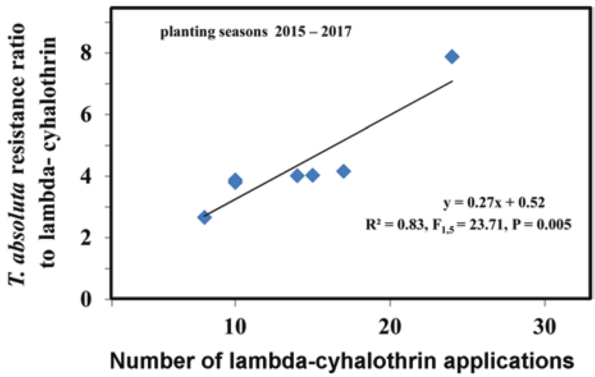

Fig. 4.2 Relationship between total number of lambda-cyhalothrin applications and resistance ratio of *T. absoluta* on the 7 populations within 3 planting seasons of 2015–2017

4.4 Discussion

This is the first time that a resistance study was reported on *T. absoluta* in Nigeria since the invasion in 2015. Immediately after its invasion, chlorantraniliprole + lambda-cyhalothrin was introduced recklessly as a solution for the insect control. As a result, sole and high dependence by farmers, led to consecutive and increased applications and abuse, despite manufacturers recommendations. As a consequence, on-farm inefficacy of chlorantraniliprole + lambda-cyhalothrin from farmers in Watari, Bagauda and Beriberi was reported. These locations had the highest numbers of applications of chlorantraniliprole + lambda-cyhalothrin, which resulted in the most resistant populations of *T. absoluta*.

The significant variations recorded among the populations in resistance to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin and deltamethrin revealed the presence of a genetic diversity underpinning *T. absoluta* resistance. Such variability in resistance levels indicates the occurrence of differential selection pressures, and/or a genetic diversity in the resistance mechanisms among the insect populations (Kerns and Gaylor 1992). The relative differences in the populations responses to chlorantraniliprole + lambda-cyhalothrin may be attributed to the significant variations in the application rates among the populations. Picanço et al. (1995) reported greater resistance levels for *T. absoluta* populations to abamectin and cartap, likely due to a higher selection pressure provided by the more intensive use of these insecticides in Brazil. Bassi et al. (2012) stated that, among the factors that could favour resistance to any insecticide MoA, the intensity of usage is the main parameter that has an overriding influence.

During the first year of invasion in 2015, when farmers were completely ignorant about *T. absoluta* infestation, lambda-cyhalothrin and deltamethrin were applied frequently because both insecticides were among the most commonly applied for the control of tomato major pests. The combination chlorantraniliprole + lambda-cyhalothrin was introduced during this time of invasion, with the lowest application rate, because it had not gained popularity among farmers and rotational application with lambda-cyhalothrin and deltamethrin. Nevertheless, the few farmers who used it reported its efficacy in all locations. In the subsequent planting year of 2016, its application rate became the highest due to the sole and high dependence on it, which was based on the efficacy report of previous year (2015). A drastic reduction in the application rate of chlorantraniliprole + lambda-cyhalothrin was recorded in 2017 due to its scarcity and high cost in the country, which was predicated by its high demand for the control in the country of another invasive pest, armyworm *Spodoptera frugiperda* (J.E. Smith) on maize. The few farmers that desperately purchased it, at high cost, expecting complete relief from the infestation were shockingly disappointed as chlorantraniliprole + lambda-cyhalothrin failed to reduce infestation due to the rapid development of resistance by *T. absoluta*, leading to a general outcry and reports of inefficacy. There was also a drastic reduction in the application rates of lambda-cyhalothrin and deltamethrin, and although there was scarcity of chlorantraniliprole + lambda-cyhalothrin, this reduction was the result of the introduction of an indigenous water + light Tuta trap tray (NIHORT-TTtray), designed by the first author to trap the adult *T. absoluta* in these locations. The NIHORT-TTtrays, which were set up every night, trapped between 2673 and 4872 adult *T. absoluta* daily across 48 farms in the 7 locations, thereby reducing the infestation massively.

The significant correlations between the rate of application and resistance ratio suggest that the variations in resistance of *T. absoluta* populations to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin and deltamethrin resulted from the variations in usage in the different locations. The unavailability of correlations between rate of application and resistance ratio of *T. absoluta* populations to spirotetramat + flubendiamide was reflected in the similarity of usage in the 7 locations and the fact that it had only been introduced in Nigeria against *T. absoluta* in 2017 , therefore it is yet to be abused like the others.

Lambda-cyhalothrin (MoA group 3A, Karate®[1]) and deltamethrin (MoA group 3A, Decis®[2]) should not have been used together because they both belong to the same mode of action, group 3A. The application of both insecticides together in the same seasons within 3 years increased the application of the active ingredient pyrethroids, which accelerated the development of resistance to both insecticides. Bassi et al. (2012) reported that the abuse of a single insecticidal mode of action (MoA) in commercial agriculture can lead to insect resistance in as little as 5 to 6 years, from the date of commercial introduction.

4.5 Conclusion

Populations of *T. absoluta* from the 7 major tomato-producing locations in Nigeria showed resistance to chlorantraniliprole + lambda-cyhalothrin, lambda-cyhalothrin and deltamethrin, confirming the farmers reports. The sole and high dependence and indiscriminate usage of insecticides by farmers due to reckless recommendations, without any adoption of IPM rules, have resulted in the widespread, higher levels of resistance observed in chlorantraniliprole + lambda-cyhalothrin, a product only introduced into Nigeria in 2015. Therefore, a rotational application, based on the manufacturer's recommendations, of insecticides that have different modes of action, utilised in combination with cultural and/or trapping methods and the preservation of natural enemies, must be adopted for the development of a sound IPM strategy to control *T. absoluta* on tomato.

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