International Journal of Research in Agricultural Sciences
Volume 5, Issue 2, ISSN (Online): 2348 – 3997

Development of Multiple and Cross-Resistance in Cotton Whitefly (*Bemisia Tabaci Gennadius*) Population in Gezira, Sudan

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Date of publication (dd/mm/yyyy): 22/03/2018

Abstract **– The effects of endosulfan, deltamethrin and chlorpyrifos on the control of whitefly and development of cross and multiple resistances was investigated in laboratory bioassay with five sprays in the field with three insecticides, the laboratory bioassay test was done to the survival from field spray, each insecticides whitefly population exposed to other two insecticide, the results reveal that the endosulfan population developed high level of cross and multiple resistance after 3rd and 4th to deltamethrin and chlorpyrifos, respectively. The results reveal that when endosulfan used to control whitefly the chemical can be alternate with deltamethrin up to second spray, because after the 3rd spray the RR increase to 10 fold that means the deltamethrin cannot be use after two sprays with endosulfan to control whitefly due the development of cross resistance. In case of chlorpyrifos same scenario will happen but after the 4th spray the RR increase to 10 fold means chlorpyrifos cannot be use after three sprays with endosulfan to control whitefly due to the development of multiple resistances.**

Keywords **– Whitefly *Bemisia Tabaci*, Cross Resistance, Multiple Resistance, Endosulfan, Deltamethrin, Chlorpyrifos, Resistance Ratio.**

I. Introduction

Cotton whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), a key pest of many crops throughout subtropical and tropical regions of the world, causes also significant problems in protected agricultural systems in temperate regions (Naranjo, 2001). The impact of direct feeding and honeydew excreta that favors sooty mold production is factors that affect crop yield in both quantitative and qualitative terms (Oliveira *et al.,* 2001). However, the most economically significant losses are due to virus transmission, especially in tomatoes (Robledo-Camacho *et al.,* 2009). Chemical management is costly and, at best, provides only partial control because of the rapid development of resistance, a worldwide problem (Cahill *et al.,* 1996 a, b; Vinuela, 1998; Kumar *et al.,* 2008). The cotton whitefly is one of the most important pests of cotton, okra, and other horticultural; ornamental crops. It causes damage by direct feeding and production of large quantities of honeydew, although it is now perhaps the most feared as a vector of up to 60 Gemini-viruses (Bedford *et al..* 1992).

Insecticide resistance has long been seen by many as the greatest threat to chemical means of controlling noxious organisms including insect-pests of agricultural crops. Some believe that chemical methods will be severely curtailed as a result of resistance. But under field situations most failures are caused by faulty equipment, insecticide of inferior quality, inadequate or untimely application. However, there are times when pests survive even after repeated correct applications of recommended insecticides and hence, there are grounds of suspecting the control failure due to resistance.

Due to indiscriminate use of insecticides in cotton especially for the control of bollworms, populations of whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae) developed resistance to most of the commonly used insecticides. Sharma and Batra (1995) have reported a varied level of resistance in *B. tabaci* to 15 different insecticides in Haryana, whereas, Prabhaker *et al.* (1985) reported 20 to 54 fold resistance to sulprofos and methyl parathion in field populations from California.

Due to indiscriminate use of insecticides and development of resistance, this pest has changed its status from incidental to primary pest in field and vegetable crops (Cahill *et al.,* 1995).

Among the various methods for its management, chemical control is the primary method. But the use of chemicals to manage *B. tabaci* field populations has been inadequate for the past several years. Two major factors that contribute to the control failure of whitefly populations and resurgence on cotton are: development of resistance to various groups of insecticides used for its control (Prabhaker *et al.,* 1985) and ineffective coverage of aerially applied insecticides on the lower surface of cotton leaves, where large number of nymphs are present and cause substantial amount of plant damage by larval feeding and honeydew excretion (Johnson *et al.,* 1982).

The rate at which resistance develops in a population depends upon; the frequency of resistance genes present in the population; the nature of resistance genes (either single or multiple, dominant or recessive); the intensity of selection pressure *i.e.* magnitude of population exposed to the chemical and the proportion killed and the rate at which species breeds (number of generations per season) (Singh and Jaglan 2005). The last two factors are the most important wherein selective breeding from resistant survivors tends to give resistant progeny (Perry *et al.,* 1998). The gene/s that allow an individual pest to survive already may exist in a pest population or may arise through mutation. Therefore, the following generations of pest will have higher proportion of resistant individuals. Resistant populations may persist for many generations in the field even after the selection pressure of insecticide has been removed (Singh and Jaglan 2005).

Pesticide application has occurred in Sudan since the mid-1930s. DDT (dichlorodiphenyltrichloroethane) was used in the Gezira scheme from the mid-1940s until 1982, when its use was replaced by organophosphates and carbamates. The Gezira Scheme in central Sudan is the largest and oldest agricultural project in the country, with around 0.8 million hectares of land under irrigation. Traditionally, cotton was the main cash crop, but wheat, sorghum, groundnuts, and vegetables have been introduced. Extensive aerial and ground spraying is carried out to protect these crops, as a consequence of wide spread pesticides application resistance of *B. tabaci* was demonstrated for the first time in the 1981/1982 season, and has been monitored since then. The over-reliance on broad-spectrum insecticides may account for the serious resistance problem in *B. tabaci* (Dittrich and Ernst, 1983). Insecticide resistance in *B. tabaci* was first reported in early 1980's for populations infesting Sudanese cotton (Dittrich and Ernst 1983). The highest resistance was reported for dimethoate and monocrotophos (OPs), which were used in large quantities (Dittrich and Ernst, 1983; Dittrich *et al.,* 1985 and 1986; Ahmed *et al.,* 1987; Abdeldaffie *et al.,* 1987; Yassin *et al.,* 1989 and Assad *et al.,* 2006).

An increase in LD_{50} value by a factor of 4 or 5 may render control uneconomical or increase toxicological hazards and dangers of environmental contamination because of tendency of farmers to increase either dose of the insecticide or frequency of application to achieve control (Assad *et al.,* 1999).

The present work focus on examines the effects of different chemical group with different mode of action deltamethrin (pyrethroids), endosulfan (organochlorine) and chlorpyrifos (organophosphate) use consecutively to control whitefly in development of cross and multiple resistance.

II. Materials and Methods

The field trials were conducted at the University of Gezira farm at Neshishiba, Wad Medani (14º 24'N 33º 29'E, 407m above sea level), Sudan. The experimental area reserved for the trials was 0.63 hectare (hectare =10000m^2). From this only 0.32 hectare, was sown with cotton and the neighboring plots (i.e. the rest of the area) were left bare to minimize the migration of pest from one plot to another. The area was sown in mid-August with cotton, *Gossypium barbadense*. Spacing was 80cm between ridges and 50cm within ridges. Five seeds were planted per hole, but seedlings were eventually thinned to three per hole. The entire experimental area was treated with the pre-emergence herbicide, stomp (pendimethalin) (50% EC) applied by tractor-mounted sprayers at the rate of 1905g a.i. per ha. All plots received nitrogen fertilizer at the rate of 2N (190Kgs) per hectare, in the form of urea immediately after thinning. Supportive hand weeding was done twice and irrigation was conducted fortnightly.

Three insecticide treatments and an untreated control were compared in a randomized complete block design (RCBD) with three replications [plot size was 16 x 21m (i.e. 0.034 hectare)].

A. *Insecticides Tested and Method of Application*

Field population of whitefly was placed under selection pressure with deltamethrin, chlorpyrifos and endosulfan at the recommended dose. The insecticides tested were (a) chlorpyrifos (Dow chemical Ltd., England) 4.8% EC at 1143g a.i. per hectare (*i.e.* 67.5ml/plot), (b) deltamethrin (Dow chemical Ltd., England) 2.5% EC at 15g a.i. per hectare (*i.e.* 20.8ml/plot) and (c) endosulfan 50% EC at 964 g a.i. per hectare (*i.e.* 65ml/plot). All the chemicals were commercial formulations applied at the dosage recommended by the Agricultural Research Corporation (ARC) of the Sudan.

Insecticides were dissolved in water and administered by pressurized knapsack sprayers at a spray volume of 57 L/ hectare (2 L per plot). The pressure was calibrated at 2-3 bar. The first insecticide application was delayed to allow the build-up of the whitefly population. The field was sprayed with insecticides in calendar spray each two weeks as insecticides pressure.

After each field spray the survivals were left to rose, during this time whitefly were collected for laboratory bioassay test, to check the development of cross and multiple resistance.

B. *Laboratory Bioassay*

After 24hrs from field sprays with three insecticides, early in the morning the adult whitefly survive the insecticides used in the spray were randomly collected from the plots. The population of the collected adults' whitefly was subjected to the susceptibility bioassay test in the laboratory each insecticide survive population were exposed to the other two insecticides which are from different groups.

C. *Insecticides Preparation*

Three insecticides were used, 5 concentrations for each, freshly prepare aqueous solutions 100ml each time.

D. *Bioassay for Checking Multiple and Cross Resistance Procedure*

Bioassay for obtaining concentration response line was described by Dittrich *et al.,* (1985 and 1990) and Prabhaker *et al.,* (1985) with some modification. Cotton leaf disc were prepare 5cm in diameter, the cotton leaves disc were dipped in 100ml of an aqueous solution of the desired concentration of each insecticides (i.e. deltamethrin, chlorpyrifos and endosulfan) for 15 seconds, then allowed to dry. Treated leaf disc were placed on plastic Petri dish lid with a thin layer of agar, then 25-50 adults whitefly were transferred into each of Petri-dish by an aspirator, actual number is recorded. Mortality of adults was recorded 24hrs after, at least five concentrations were tested for each insecticide, and three replicate for each concentration, and each bioassay test for each insecticides repeated three times after each field spray with different insecticide. Results were expressed as percentage mortalities correcting for untreated (control) mortality using Abbott's formula (Abbott, 1925). Data were analyzed by probit according to Finney (1971). LC_{50} and LC_{90} calculated after each field spray to check the development of multiple and cross resistance after each spray (1st, 2nd, 3rd. 4th and 5th).

III. Results and Discussion

Data presented in Table. 1 and 2 shows the development of resistance and the change in the response of *Bemisia tabaci* adult toward the deltamethrin, endosulfan and chlorpyrifos used in the field. When the whitefly survive the three insecticides used in the field and the survive population of each insecticides exposed to the other two insecticides in the laboratory bioassay test, the LC_{50} and LC_{90} values increased steadily after 1st, 2nd, 3rd, 4th and 5th field spray for example endosulfan selected population exposed to the deltamethrin show the increase in LC_{50} as follow 1.14, 1.86, 10.93, 31.14 and 38.45 ppm respectively, same trend found in LC_{90} the value increased after 1st, 2nd, 3rd, 4th and 5th field spray by 13.05, 31.62, 478.07, 1584.89 and 10000 ppm respectively. This means the rate of reduction in potency of deltamethrin increased by the continuous stress after each field spray by endosulfan until it reached the high reduction after 5th field spray and if divided the LC_{50} of the 5th field spray by the LC_{50} 1st field spray we got RR (resistant ratio) 34 fold, mean the selection pressure caused by endosulfan develop cross resistance to deltamethrin and increase by the number of sprays in the field (particularly if the insect have many generation per season such as whitefly which has about 19 generation per season). These results come in line with study done by Assad *et al.,* (1999) who found that the RR increase with the increase in number of spray in the field.

Regarding chlorpyrifos the data presented in table (1 and 2) shows the development of resistance and the change in the response of *Bemisia tabaci* adult endosulfan selected population exposed to the chlorpyrifos show increase in LC_{50} after 1st, 2nd, 3rd, 4th and 5th field spray as follow 11.25, 13.62, 26.50, 112.74 and 174.02ppm respectively, same trend found in LC_{90} the value increased after 1st, 2nd, 3rd, 4th and 5th field spray by 139.73, 122.90, 277.86, 2428.94 and 1595.91ppm respectively. This means the rate of reduction in potency of chlorpyrifos increased by the continuous stress after each field spray (LC_{90} after 1st field spray 139.73 ppm jump up and reached the high reduction after 5th endosulfan field spray LC_{90} 1595.91ppm), if divided the LC_{50} of the 5th field spray by the LC_{50} 1st field spray we got RR (resistant ratio) 15 fold. This reflect that the selection pressure done by endosulfan the survive whitefly population also resistance to chlorpyrifos, and since this chemical have different mode of action than endosulfan can say whitefly population reflects multiple resistance compared to deltamethrin, chlorpyrifos seem more effective than deltamethrin if compare RR of deltamethrin 34 fold with 15 fold of chlorpyrifos. The whitefly population highly affected by endosulfan selection which develops deltamethrin cross resistance and chlorpyrifos multiple resistances. However, is reported that the selecting agent (including endosulfan increase resistance to organophosphate significantly (Farnham and Sawicki, 1976).

From these results, it is clear that selection pressure of field population of whitefly with pyrethroid deltamethrin and organophosphate chlorpyrifos resulted in the development of resistance to these insecticides. This may be explained by the fact that the resistant genes responsible for resistance to pyrethroids and organophosphate exist within the population and the whitefly would be expected to have a head start in developing resistance to pyrethroids and organophosphates and both insecticide play as tool for selection pressure, this result agree with study results by Assad *et al.,* (2006) the population of the whitefly increase whenever increase the number of field spray with the same insecticides. With history of pyrethroid and organophosphates, the Ops been used on cotton to control cotton pest complex (whitefly, Jassid, aphid, leaf worm and cotton bollworm) for long time during cotton growing season.

When pesticides are the sole or predominant method of pest control, resistance is commonly managed through pesticide rotation. This involves alternating among pesticide classes with different modes of action to delay the onset of or mitigate existing pest resistance. In this study the results reveal that when endosulfan used to control whitefly the chemical can be alternate with deltamethrin up to second spray, because after the 3rd spray the RR increase to 10 fold that means the deltamethrin cannot be use after two sprays with endosulfan to control whitefly due the development of cross resistance. In case of chlorpyrifos same scenario will happen but after the 4th spray the RR increase to 10 fold means chlorpyrifos cannot be use after three sprays with endosulfan to control whitefly due to the development of multiple resistances.

Insecticide resistance is the adaptation of pest population targeted by insecticide resulting in decreased susceptibility to that chemical. In other words, pests develop a resistance to a chemical through natural selection: the most resistant organisms are the ones to survive and pass on their genetic traits to their offspring (PBS, 2001).

In summary it assumes that number of generations or length of time between uses of any of the three insecticides is sufficient to allow resistance to increase. Also, factors determining the selection of resistance to insecticides can be classified genetically relating to the chemical itself and how applied.

It is concluded that deltamethrin cannot be used to control whitefly after endosulfan in the same season after two sprays with endosulfan but chlorpyrifos can be used to control whitefly after two sprays but not after 4th spray due to the development of multiple resistances.

Table I. Toxicity (LC_{50}) of three insecticides in the laboratory test to adult stage of cotton whitefly after five sprays in the field with three insecticides tested

Insecticide use in the field	Insecticides use in the lab,	LC_{50} (ppm) after each field spray					RR after 5th spray
		1st	2nd	3rd	4th	5th	
endosulfan	deltamethrin	1.14	1.86	10.93	31.14	38.45	34
	chlorpyrifos	11.25	13.62	26.50	112.74	174.02	15

Insecticide use in the field	Insecticides use in the lab,	LC_{50} (ppm) after each field spray					RR after 5th spray
		1st	2nd	3rd	4th	5th	
deltamethrin	endosulfan	1.65	1.93	4.12	14.11	18.74	11
	chlorpyrifos	14.13	15.08	44.81	125.54	134.71	10
chlorpyrifos	endosulfan	1.11	2.18	4.14	8.90	21.74	20
	deltamethrin	1.06	1.52	6.85	14.33	48.44	46

RR = resistant ratio

Table II. Toxicity (LC_{90}) of three insecticides in the laboratory test to adult stage of cotton whitefly after five sprays in the field with three insecticides tested

Insecticide use in the field	Insecticides use in the lab,	LC_{90} (ppm) after each field spray				
		1st	2nd	3rd	4th	5th
endosulfan	deltamethrin	13.05	31.62	478.07	1584.89	10000.00
	chlorpyrifos	139.73	122.90	277.86	2428.94	1595.91
deltamethrin	endosulfan	15.15	11.67	88.70	221.71	214.08
	chlorpyrifos	115.95	148.10	669.40	4774.34	4318.11
chlorpyrifos	endosulfan	9.51	23.49	32.13	105.98	669.24
	deltamethrin	32.48	22.15	388.19	1488.10	11364.64

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