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Monitoring Of Insecticide Resistance Against Cotton Whitefly (*Bemesia Tabaci*) Under Laboratory Conditions

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Abstract: Development of insecticide resistance mainly depends on the management techniques for the control of whitefly, *Bemesia tabaci*. Seven insecticides were tested against filed collected and laboratory reared whitefly population during the years of 2016 to 2018 to evaluate their resistance level against field population of adult whitefly using leaf dip method. Very low level of resistance was found in whitefly against Pyriproxyfen and Spirotetramat, whereas, high level of resistance was observed against other tested insecticides. Gradual resistance was observed against Diafenthiuron. It is concluded that for the management of whitefly repetition of same insecticide should be avoided. In the present studies Imidachloprid and Acetamiprid showed high level of resistance. The use of these insecticides may be reduced to overcome resistance against whitefly.

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Key words: *Bemesia tabaci,* Insecticide resistance, Resistance Ratio**,** Pyriproxyfen, Spirotetramat

1. Introduction

Cotton plays vital role of backbone in the economy of Asian countries. Due to its worldwide economic importance, this cash and fiber crop (Amjad *et al*., 2009) is known as 'silver fiber' (Sajjad *et al.* 2015) and 'white gold' in Pakistan. It is also known as queen of the fibre plants (Rashid *et al.* 2016). Pakistan is the fourth biggest producer of cotton after USA, China and India. It is the source of big amount of foreign exchange and contributed about 2.9 % of GDP and about 11.7 % of value added in agriculture. It also shares about 69.5 % of contribution in national oil production (Aslam *et al., 2004*). But relative contribution of this crop to send out profit is per over 68 % which shows that per hectare national average yield is low (Sahito *et al*., 2017).

In Pakistan, 30% reduction in cotton yield is caused by the attack of 145 species of insect pests (Rashid *et al*., 2012). A wide range of sucking insect pests (up to 96 %), particularly whitefly (*Bemesia tabaci*), jassid (*Amrasca biguttula*), mealybug (*Phenacoccus solenopsis*) andaphids (*Aphis gossypii*), attack different growth stages of crop due to enrich of greenish leaves (Sahito *et al*., 2017). Up to 50 to 60 percent decrease in cotton production is caused by sucking insect pests and cotton boll worms (Rajput *et al*., 2017). Among these pests, *B. tabaci* is the most notorious and major pest (Amjad *et al*., 2009). History of cotton whitefly infestation is very old i.e earlier than the introduction of modern insecticides. Many agricultural crops are infested by this polyphagous insect. It is cosmopolitan in distribution and along with the direct damage to crop, it constrains photosynthetic activity and impairs quality of cotton fiber. It also carries vector of various well-known viral diseases of several economic crops (Razaq *et al*., 2003).

Use of chemicals is necessary and unavoidable part of IPM (Integrated pest management) in crop protection. Even, the technologically progressive countries expended about 3 % of market value of agriculture on toxic pesticides and their application. In Pakistan, more than ten billion worth chemicals are imported, out of which about 70 to 80 percent are used against cotton insect pests (Aslam *et al*., 2004). Chemicals are the main intend to control massive infestation and to control sudden outbreak of insect pests. 1n 1950, the pesticides were used to combat insect invasion in Pakistan very interestingly (Sahito *et al*., 2017). Many researchers in the past have evaluated different insecticides to test their comparative toxicity against this insect pest under different environmental conditions (Razaq *et al*., 2003).

Among other issues, whitefly outbreaks in recent years have been triggered on large scale by the misuse of insecticides for the control of whitefly and other co-existing insect pests, which caused large scale decrease of its natural enemies and resistance development to most of the commonly used insecticides (Ahmad *et al*., 2002, Ishayaa and Horowitz 1995). Therefore, environment friendly insecticides with novel mode of action are required for the management of *B. tabaci* (Peng *et al*., 2017). Insecticide resistance is currently considered as a major threat to effective whitefly control (Abou-Yousef *et al*., 2010, Ahmad *et al*., 2002). Keeping in consideration the above mentioned facts, different insecticides were examined in this study to test their resistance level against adult cotton whitefly (*Bemesia tabaci*).

2. Materials and Methods

The experiment was conducted at Toxicology Laboratory Entomological Research Institute, Ayub Agricultural Research Institute, Faisalabad in the years of 2016-2018 to test the resistance of seven different insecticides against cotton whitefly namely Confidor 200 SL, Confidor 70 WS, Rani 20 SL, Dimogreen 4EC, Polo 500 SC, Priority 10.8 AS and Movento 240 SC against cotton white fly under laboratory conditions. The experiment was laid out in completely randomized design including control. The insecticides used in the experiment were purchased from local market and laboratory doses (ppm) were calculated from field recommended doses using formula given below (1). There were 8 treatments including control, having 5 repeats each. The insecticides were tested using leaf dip bioassay IRAC method. Serial dilutions of each dose of insecticide were made and leaves cut with leaf disc cutter according to the size of small plastic petri dishes (5 cm), were dipped in the insecticide solution. Treated leaves were then air dried at ambient room temperature. 25 adults of white fly were released per treatment i.e 5 insects per leaf. The control leaves were dipped in water only. To study the LC50, insect mortality was recorded after 24 hours. Insects showing no movement on pressing them with needle were considered as dead. Corrected mortality was calculated by Abbott's formula (2). Values of LC50 were calculated using polo pc software. To evaluate the resistance factor, baseline values (LC50 of susceptible/laboratory reared strain) were obtained from susceptible strain of *B. tabaci*. Resistance factor was calculated by the formula given below (3).

1. $\mu l = \frac{\text{Required ppm} \times \text{water in ml}}{\%F \times 10}$
2. $\text{Corrected Mortality} = \frac{\text{No.of Insects in Control} - \text{No.of Insects Treated}}{\text{No.of Insects in Control}}$
3. Resistance Factor: LC_{50} of field strain / Baseline value

3. Results and Discussion

Confidor possesses imidacloprid as its active ingredient which works as neurotoxin and interferes with the transmission of the stimuli within insect nervous system. Rani is a brand name of acetamiprid which is a neonicotinoid and causes interruption in brain signals throughout the insect body. Dimogreen contains dimethoate as an active ingredient and it also disrupts the normal functioning of nervous system. Polo is globally used insecticide having diafenthiuron as its active ingredient and it paralyses the target insect when it comes in contact. Priority (Pyriproxyfen) is an insect growth regulator and mimics natural insect hormones which stop young ones to mature into adults. Movento is a new chemistry insecticide containing spirotetramat as an active ingredient and it inhibits the ability to produce lipids, develops symptoms of poisoning leading to insect mortality.

Insecticides were tested against field collected and lab reared whitefly population. During first year of the study (2016), it has been revealed that movento (Spirotetramat) showed lowest LC_{50} value and proved to be the most effective insecticide among tested insecticides which are being widely used to control whitefly (Table 1). It also showed lowest base line value for lab reared population. On the other hand, maximum LC_{50} (514.07 and 8.17) for field collected and lab reared populations were shown by confidor having 200 SL formulation followed by Rani (acetamiprid), Dimogreen (Dimethoate), Polo (Diafenthiuron), Priority (Pyriproxyfen) and Movento (Spirotetramat) respectively. But, very high resistance was only shown in case of Confidor 70 WS. Whitefly had high resistance against Confidor 200 SL, Rani, Dimogreen and Polo.

Table 1. Lethal effects of insecticides on whitefly in first year (2016)

Insecticides	LC_{50}	Base Line Values	Resistance Ratio	Resistance Level
Confidor 200 SL	514.07	8.17	62.92	High
Confidor 70 WS	490.51	4.91	99.90	Very High
Rani 20 SL	316.14	4.03	78.45	High
Dimogreen 40 EC	35.18	0.74	47.54	High
Polo 500 SC	88.78	4.09	21.71	High
Priorty 10.8 AS	4.10	0.81	5.06	Very Low
Movento 240 SC	3.56	1.07	3.33	Very Low
Resistance scale	Very low <10, low >11-20, Moderate>21-50, high>51-100, very high>100			

In second year of testing (2017), maximum LC_{50} was shown by Confidor 200 SL followed by Confidor 70 WS, Rani, Polo, Dimogreen, Priority and Movento respectively. Highest resistance factor was witnessed in case of Confidor 70 WS (101.67) followed by Rani (75.83), Confidor (71.96) and Dimogreen (53.76). Whitefly showed low to very low level of resistance to other insecticides.

Table 2. Lethal effects of insecticides on whitefly in second year (2017)

Insecticides	LC_{50}	Base Line Values	Resistance Ratio	Resistance Level
Confidor 200 SL	587.97	8.17	71.96	High
Confidor 70 WS	499.21	4.91	101.67	Very High
Rani 20 SL	305.62	4.03	75.83	High
Dimogreen 40 EC	39.78	0.74	53.76	High
Polo 500 SC	84.14	4.09	20.57	Low
Priorty 10.8 AS	3.90	0.81	4.81	Very Low
Movento 240 SC	3.12	1.07	2.94	Very Low
Resistance scale	Very low <10, low >11-20, Moderate>21-50, high>51-100, very high>100			

During the Last year of study (2018), whitefly showed very high level of resistance against confidor 70 WS followed by Rani, Confidor 200 SL, Dimogreen, and Polo respectively. Whitefly showed low to very low level of resistance to other tested insecticides.

Table 3. Lethal effects of insecticides on whitefly in third year (2018)

Insecticides	LC_{50}	Base Line Values	Resistance Ratio	Resistance Level
Confidor 200 SL	615.64	8.17	75.35	High
Confidor 70 WS	745.21	4.91	151.77	Very High
Rani 20 SL	329.34	4.03	81.72	High
Dimogreen 40 EC	41.99	0.74	67.55	High
Polo 500 SC	92.65	4.09	22.65	High
Priorty 10.8 AS	6.71	0.81	8.28	Very Low
Movento 240 SC	6.15	1.07	5.74	Very Low
Resistance scale	Very low <10, low >11-20, Moderate>21-50, high>51-100, very high>100			

All the results were combined and it was revealed that whitefly showed very high resistance to confidor 70 WS followed by Rani 20 SL, Confidor 200 SL, Dimogreen 40 EC, Polo 500 SC, Priority 10.8 AS and Movento 240 SC respectively (Fig 1).

Table 4 represents that as per resistance rating scale, whitefly has very low cumulative resistance to Movento 240 SC and Priority 10.8 AS having resistance factor below than 10. Whitefly has moderate resistance to Polo 500 SC while having resistance factor more than 20 but below 50. But, whitefly showed high level of resistance to Dimogreen 40 EC, Rani 20 SL and Confidor 200 SL as all these insecticides had resistance factor in between 51-100. Whitefly showed very high level of resistance in case of only one insecticide Confidor 70 WS as it showed resistance factor more than 100.

Table 4. Cumulative Resistance Ratio of Insecticides

Insecticide	2016	2017	2018	Cumulative	Resistance Level
Confidor 200 SL	62.92	71.96	75.35	70.07	High
Confidor 70 WS	99.9	101.67	151.77	117.78	Very High
Rani 20 SL	78.45	75.83	81.72	78.67	High
Dimogreen 40 EC	47.54	53.76	67.55	56.28	High
Polo 500 SC	21.71	20.57	22.65	21.64	Moderate
Priority 10.8 AS	5.06	4.81	8.28	6.05	Very Low
Movento 240 SC	3.33	2.94	5.74	2.89	Very Low
Resistance Scale	Very low <10, low >11-20, Moderate>21-50 high>51-100, very high>100				

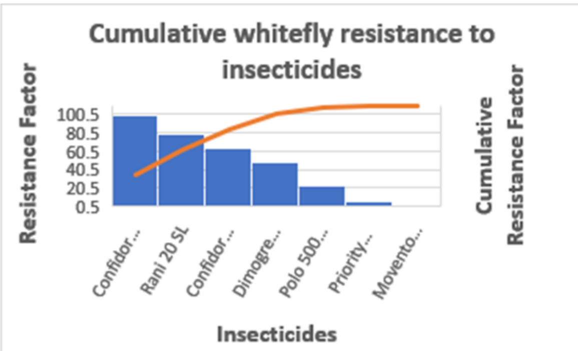

Fig. 1. Cumulative Whitefly resistance to insecticides

Abou-Yousef *et al.* (2010) evaluated different insecticides for their resistance to whitefly. They selected lambda-cyhalothrin resistant population and results revealed that confidor 20% SL showed resistance ratio, 14.46 which is quite low as compared to the cumulative resistance ratio found during the present study. This difference may occur due to variation in formulation. Confidor 200 SL was evaluated against different susceptible and resistant strains of whitefly (Nauen *et al.* 2008) and different resistance ratios were found. Maximum resistance ratio was 580 (Mexico strain) followed by 70 (Bayer crop science strain). Strain from Bayer crop science had same level of resistance as found during the present study.

Abou-Yousef *et al.* (2010) evaluated different insecticides for their resistance to whitefly. They selected lambda-cyhalothrin resistant population and results revealed that acetamiprid showed resistance ratio, 6.06 and 0.69 after 6 and 13 generations bearing election pressure of lambda cyhalothrin. During the present study, high cumulative resistance ratio was witnessed. This difference might be due to the selection pressure with lambda cyhalothrin. A pressure selection was made and resistant population was referred as Aceta-SEL population (Basit *et al.* 2011). This population was tested for their resistance to acetamiprid which gave resistance ratio, 37 which falls in moderate resistance category while ratio witnessed in present study has high level of resistance. This different might be caused due to number of generations and selection pressure.

Dimethoate showed varying response to the different strains of whitefly. Maximum resistance ratio was 782 while minimum was 1 (Ahmad *et al.* 2002). During the present study, a resistance ratio of 56.28 (Table 4) was witnessed. This difference might have been caused due to the variation in site of population collection. This is very crucial factor as farmers belonging to various localities use different insecticides for the management of whitefly.

Whitefly strain selected with pressure of pyriproxyfen was evaluated for its resistance to diafenthiuron and pyriproxyfen. This population showed resistance ratio, 65 for pyriproxyfen and 0.7 for diafenthiuron. During the present study, diafenthiuron expressed low level of resistance which is much higher than the very low level resistance found by (Ishaaya and Horowitz, 1995). They also witnessed high level of resistance in case of pyriproxyfen as compared to the very low resistance level witnessed during the present study.

Two whitefly strains were evaluated for their resistance to pyriproxyfen and resistance ratio of 1 and 89.71 were found (Ma *et al.* 2010). One strain showed very low resistance level to pyriproxyfen as witnessed in our findings but the other strain expressed high level of resistance. This variation might be due to the difference in locality and previously used insect management techniques for the management of whitefly.

Different strains of whitefly were collected for five years and year wise resistance ratios were calculated. It was revealed that whitefly resistance to spirotetramat was increasing gradually and maximum resistance ratio was 79.24 in the last year of study (Peng *et al*. 2017). They witnessed moderate level of resistance in only one whitefly strain. In the present study, very low level of resistance was observed during three years of study. This difference might have been caused due to difference in use of this chemical for managing whitefly population.

4. Conclusion

It has been observed that whitefly management techniques play an important role in development of resistance to insecticides. Whitefly population was found susceptible to Pyriproxyfen and Spirotetramat, while it found to be resistant to other insecticides. Diafenthiuron is developing resistance in whitefly gradually. Therefore, Insect Pest Management (IPM) strategies should include pyriproxyfen and spirotetramat as an alternative to each other to control whitefly population.

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