

BASE LINE SUSCEPTIBILITY OF BEMISIA TABACI (HEMIPTERA: ALEYRODIDAE) TO PYRIPROXYFEN UNDER LAB CONDITIONS

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ABSTRACT

A systemic bioassay was carried out using cuttings of stem from cotton seedlings for baseline susceptibility of whitefly from district Bahawalpur, Punjab Pakistan. Bioassay studies were conducted by using a leaf dip bioassay method to determine the susceptibility of Bemisia tabaci to pyriproxyfen. Experiments were conducted for a susceptible laboratory colony and for 10 field collected populations during 2011 and 2012.. It was observed that all the B. tabaci populations were susceptible to pyriproxyfen. Baseline data of the susceptible colony showed a pooled LC₅₀ and RR₅₀ values of 0.039 and 2.316 mg ai L⁻¹, respectively. LC₅₀ values for field populations of B. tabaci ranged from 0.63 to 0.86 mg [AI] l⁻¹ during 2011 and from 0.46 to 2.08 mg [AI] l⁻¹ during 2012. No population had a RR₅₀ value over 3.5 in either year and the fiducially limits of the LC₅₀ values for the field and laboratory populations overlapped, indicating no differences among them. Pyriproxyfen is a promising tool in integrated pest management programs for B. tabaci, particularly where field populations have developed resistance to other insecticide groups. The laboratory and field baseline toxicity data generated in this study of field populations of B. tabaci confirmed their susceptibility to pyriproxyfen and would be useful in documenting any future changes in the susceptibility of the whitefly to the insecticide.

Keywords: *Baseline susceptibility, pyriproxyfen, Bemisia tabaci, cotton, Host plants*

INTRODUCTION

Pesticides are considered by many people to be essential to our everyday existence, or at least to our current standard of living. They have played a major role in humankind's efforts to grow and store enough food to feed it. Pesticides also have saved many human lives by reducing the impact of diseases that are transmitted by insects. For global agriculture, the use of pesticides is the most important and effective way to control pests; (Ahmed et al. 2010). Over the next 25 years, the increased world population will require an increase in food production of some 60% over current levels of production (Hall, 1999).

Furthermore, rising costs of crop production have forced farmers to intensify their efforts to achieve maximum yield per hectare of farm land. This situation has led to an increase in the use of synthetic pesticides (Curtis, 1995). Using pesticides is considered the most important factor in changing productivity patterns, either directly or indirectly. In maize, for example, there has been a 3-fold increase in yield since 1950 due to improved varieties, fertilizer, and crop management as use of pesticides (Ball et al., 1997). Pimentel et al. (1992) estimated that crop losses would increase by 10% (from current estimates of cost 30 %) if no pesticides were used at all, and for specific crops, losses might reach 100%.

Chlorantraniliprole (Rynaxypyr®, DPX-E2Y45, Coragen®, DuPont Crop Protection, Wilmington, Delaware) is a xylem systemic insecticide with a new mode of action in the new anthranilicdiamide chemical class (DuPont, 2008). Chlorantraniliprole has demonstrated efficacy in the field against biotype of *B. tabaci*, especially when applied to the root zone (Portillo et al., 2008; Schuster et al., 2008), and has been shown to be safe to non-target arthropods, including pollinators, numerous beneficial insects and predatory mites (Dinter et al., 2008; DuPont, 2008; Preetha et al., 2009; Brugger et al., 2010; Shaw & Wallis 2010; Gradish et al., 2010, 2011). Because of the potential for the development of resistance of *B. tabaci* to insecticides, a resistance management program was initiated in Florida in 2000 (Schuster & Thompson, 2001).

As new products are developed for use in managing *B. tabaci*, it is necessary to develop a base line susceptibility data base prior to the registration and commercial use of the product that can be used as reference for future resistance monitoring efforts. Nymph stages of white flies are affected more rapidly than the adults and the nymphs treated with spiromesifen did not molt properly and failed to reach adulthood (Nauen et al., 2005). To date active ingredient has not been reported to show cross resistance with any insecticide for which resistant mite or white fly field populations have been identified (Palumbo, 2004). Therefore, the objectives of the present investigation were to develop a bioassay in 2014 for estimating the susceptibility of *B. tabaci* to pyriproxyfen and to use the bioassay to establish the baseline susceptibility of field-collected populations in Punjab Pakistan.

MATERIALS AND METHODS

Host Plant and Susceptible Whitefly Colony

Cotton (*Gossypium hirsutum* L.) has already been used in bioassays (Schuster et al., 2010; Caballero et al., 2013), so in this study as host plant it was selected in the bioassays. For non-infested environment green house was used and seedlings were grown in pots. *B. tabaci* population was maintained on tomato plants (*Solanum lycopersicum* L.) under controlled conditions in laboratory without acquaintance to insecticides and without reintroduction of whiteflies from the field to compare the susceptibility of field populations to pyriproxyfen. In this experiment, adult whiteflies from the original susceptible colony were used to prepare a new colony on cotton plants.

Field Populations

The populations of *B. tabaci* were established by collecting nymph-infested foliage from tomato fields in district Bahawalpur. In 2011, five populations were collected and in spring 2012 additional six populations were collected and were maintained at 26°C, placed in cages (60 x 60 x 60cm) with non-infested cotton plants. F1 generations were obtained and maintained on host plants for the whole duration of experiments. For sufficient adult's population as to conduct experiments these populations were reared to F2-F4 generations.

Systemic Bioassay

Modified EARML method developed for the insect growth regulator Buprofezin and was also used to develop baseline susceptibility data for anthranilic diamides (Cahill et al., 1996c; Li et al., 2012; Caballero et al., 2013) was used for this bioassay. For bioassay 10 to 12 adults of whitefly were aspirated (unknown gender and age) and then transferred on the surface of leaf (abaxial) to lay eggs. Then after 24 hours eggs were counted under stereoscope. To avoid first instar migration from leaves and competition 20 eggs per leaf were adjusted. The height of 15 cm was standardized by cutting each stem from the growth point and its base, in order to ensure the uptake of insecticide homogeneously. Water treated samples were considered as control. Stem cuttings were placed in the solutions of insecticide (pyriproxyfen) in 12 mm diam × 60 mm vials then these vials were kept in the cages and were maintained at 26 °C for 2 weeks to monitoring the hatching of eggs up to the development of 2nd instar's nymphs. Eggs and 1st instar nymph's mortality was checked by subtracting the surviving 2nd instar nymph from initial eggs applied.

Statistical Analyses

Standard probate analysis was done to analyze the data as to estimate LC₅₀ values, fiducially limits (FL), slope and standard error (SE) of the regression line along with

the chi square value (χ^2) (SAS Institute, 1994). For each population the resistance ratio at 50 % mortality (RR_{50}) was calculated by dividing its LC_{50} value. Four replications for each experiment were done and the entire experiment was repeated 3 times on different dates over a period of a month with the susceptible colony to assess the consistency of the systemic bioassay. Fiducially limits of the LC_{50} values were compared to determine significant differences between field and laboratory colonies. The slopes of field populations and laboratory colonies were compared to check differences between both populations within years using covariance analyses (SAS Institute, 1994).

RESULTS

Pyriproxifen showed excellent toxicity to the field collected and susceptible laboratory *B. tabaci* nymphs in 2008 prior to the field use of Pyriproxifen in Pakistan. All whitefly populations had estimated LC_{50} values between 0.53 and 0.86 mg [AI] L^{-1} , with the lower value corresponding to the susceptible laboratory colony (Table 1). No colony had a RR_{50} (resistance ratio) value above 2.0 and the fiducial limits of the LC_{50} values for the laboratory and field populations overlapped, indicating no differences among them. The average LC_{50} and RR_{50} values for all of the field-collected populations were 0.74 mg [AI] L^{-1} and 1.4, respectively.

Bioassays conducted in 2012 (Table 2) confirmed susceptibility of *B. tabaci* to Pyriproxifen. The laboratory colony had an estimated LC_{50} value of 0.59 mg [AI] L^{-1} . LC_{50} values of field collected populations ranged from 0.56 to 2.08 mg [AI] L^{-1} . None of the colonies had RR_{50} values above 3.5. The average of the LC_{50} values of the field-collected *B. tabaci* populations of 2012 was slightly higher than the average LC_{50} value of 2011; however, as in 2012, fiducial limits of the LC_{50} values for the laboratory and field populations coincided, indicating minimum or zero differences among them. The average LC_{50} and RR_{50} values for 2012 were 1.06 and 1.79 mg [AI] L^{-1} , respectively

DISCUSSION

Baseline susceptibility bioassays in 2011 confirmed the full susceptibility of *B. tabaci* nymphs of the laboratory and field populations collected from 4 locations in Pakistan in the absence of Pyriproxifen exposure. The susceptibility was retained in 2012 when 3 of the tested populations were exposed to Pyriproxifen before collection of nymphs. The LC_{50} values for field populations over both 2011 and 2012 ranged from 0.46 to 2.08 mg [AI] L^{-1} , which was 4.5-fold difference in susceptibility.

This contrasts with results in Arizona and California where a 29-fold difference in susceptibility of field populations collected in 2005-2006 was observed (0.21 to 6.08 mg [AI] L⁻¹) (Prabhaker et al., 2008). No populations in the present study indicated RR₅₀ values over 3.5 in either 2011 or 2012. Although the average RR₅₀ value for the year 2012 was slightly higher than the RR₅₀ value during 2011, this may be attributed to the differences in persons who conducted the bioassays or to the variability in the field populations. Six of the 8 populations tested in 2006 against spiromesifen showed reduced susceptibility (RR₅₀ values \geq 10) to imidacloprid and thiamethoxam (Schuster et al., 2010), thus indicating the absence of any cross resistance between Pyriproxyfen and these neonicotinoids. Likewise, no cross resistance was detected in a laboratory strain selected for imidacloprid resistance (Prabhaker et al., 2008).

The RR₅₀ values obtained in the present study also were within the range of RR₅₀ values obtained elsewhere for *B. tabaci* biotypes B and Q, including neonicotinoid and pyriproxyfen resistant strains (Nauen et al., 2005). Although no resistance was observed to Pyriproxyfen under laboratory and field conditions, up to 15 fold resistance to spirotetramat (another lipid biosynthesis inhibitor) in *Tetranychus urticae* Koch under selection pressure has been demonstrated (Nauen and Konanz 2005). Pyriproxyfen has been reported to be safe to key beneficial arthropods (Kavitha et al., 2006; Lakshmi et al., 2006; Irigaray et al., 2007; Bielza et al., 2009). Toxicity of Pyriproxyfen to *B. tabaci* in the absence of cross resistance to neonicotinoids, spiromesifen and conventional insecticides suggest that Pyriproxyfen can be a valuable tool in management of insecticide resistance in *B. tabaci* in vegetable crops. The possibility of rapid development of resistance in *B. tabaci* to almost all classes of insecticides demands continuous resistance monitoring for Pyriproxyfen along with its judicious use in the field.

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Table 1: Susceptibility of whitefly nymphs from field populations to Pyriproxyfen during 2011 by using a leaf dips bioassay method

Site	Crop	<i>n</i>	χ^2	<i>P</i>	LC ₅₀ (mg [AI] L ⁻¹)	Fiducial	Limits	RR ₅₀
Lab colony	Cotton	302	0.34	0.79	0.50	0.28	0.61	-----
Mianwali	Cotton	273	2.83	0.63	0.69	0.59	1.06	1.44
Bahawalpur	Tomato	508	19.86	< 0.001	0.81	0.43	1.77	1.53
Layyah	Tomato	997	23.3	< 0.001	0.69	0.39	0.99	1.08
Multan	Cotton	413	10.8	0.09	0.68	0.47	1.24	1.39

Tables 2: Susceptibility of whitefly nymphs from field populations to Pyriproxyfen during 2012 by using a leaf dip bioassay method

Site	Crop	<i>n</i>	χ^2	<i>P</i>	LC ₅₀ (mg [AI] L ⁻¹)	Fiducial	Limits	RR ₅₀
Lab colony	Cotton	918	3.16	0.81	0.56	0.40	0.91	-----
Mianwali	Cotton	432	29.03	0.65	1.33	0.68	2.92	3.17
Bahawalpur	Tomato	1298	23.71	< 0.001	1.92	0.73	2.18	1.22
Layyah	Tomato	1170	9.13	< 0.001	0.79	0.33	1.77	0.99
Multan	Cotton	797	7.78	0.05	0.98	0.49	1.68	1.32