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# Can resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) be overcome with mixtures of neonicotinoids and insect growth regulators?

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ABSTRACT

Tobacco whitefly, *Bemisia tabaci* is an important polyphagous insect pest which has developed resistance to various insecticides worldwide. Mixtures of insecticides with different modes of action may delay the onset of resistance. Bioassays were performed to investigate the effects of various mixtures of neonicotinoid and insect growth regulator (IGR) insecticides against a susceptible and a resistant strain. The results of the study showed that potentiation ratio (PR) of all neonicotinoids + buprofezin or pyriproxyfen mixtures at 1:1, 10:1 and 20:1 ratios was greater than 1 suggesting synergistic interactions between insecticides. Maximum potentiation occurred at the 1:1 ratio (PR = 1.69–7.56). The PRs for mixture of acetamiprid, thiamethoxam, thiacloprid or nitenpyram with buprofezin or pyriproxyfen at 1:10 and 1:20 ratios were less than 1 indicating antagonistic interactions. Addition of synergists, S, S, S, tri-butyl phosphorotrithioate (DEF) or piperonyl butoxide (PBO) in the insecticide solutions largely overcame the resistance to all tested neonicotinoids, indicating that the resistance was associated with esterases or mono-oxygenases, respectively. Likewise, addition of both DEF and PBO in mixture with neonicotinoids and IGRs also suggested a similar mechanism of resistance in *B. tabaci* to the tested insecticide groups. The mechanism of synergism between neonicotinoids and IGRs is unclear. Implications of using mixtures to counteract pesticide resistance are discussed. Mixtures of neonicotinoids with buprofezin or pyriproxyfen at a 1:1 ratio could be used to restore the efficacy of these neonicotinoids against *B. tabaci*.



## 1. Introduction

The tobacco whitefly, *Bemisia tabaci* (Gennadius) is an economic agricultural pest world wide (Byrne et al., 1990). It is a highly adaptable and polyphagous insect pest that feeds on more than 700 plant species from 86 botanical families (Greathead, 1986). It directly causes injuries to crop plants by sucking phloem sap and indirectly by transmitting more than 100 plant viruses (Horowitz et al., 2003; Mugiira et al., 2008). The estimated loss caused by the whitefly is 3–4 millions of U.S. dollars each year in British Columbia, Canada (Moreau and Isman, 2012). In Pakistan, it caused the economic loss of US$5 billion from 1992 to 1997 due to transmission of cotton leaf curl virus disease (Briddon and Markham, 2000; Briddon, 2003; Basit et al., 2012). It has attained a major pest status because of its capabilities to replace existing biotypes, invading new geographical ranges and rapidly developing resistance to new pesticides including neonicotinoids and insect growth regulators (IGRs) (Byrne and Bellows, 1991; Horowitz and Ishaaya, 1996; Perring, 2001).

The usual response to the insecticide resistance problem is to increase the dosage and/or application frequencies and in some cases to substitute with new products. However, this has become expensive for both growers and industry. In the United States, cost of pesticide resistance has been estimated to be approximately $ 1.5 billion annually (Pimentel, 2005). Furthermore, availability of new products is limited because of the rising standards of environmental and toxicological safety. The most effective way to counteract the resistance problem is to reduce the selection pressure before resistance (Georghiou and Taylor, 1976; Ware, 2000) and to use resistance management strategies, which include use of insecticides in sequences, rotations and mixtures of two or more insecticides having different modes of action (Georghiou et al., 1983). Sequential use of insecticides has been discouraged due to the earlier development of resistance but the use of insecticides in rotations and mixtures merits consideration.

Two insecticides with different modes of action in a mixture may synergize each other and increase the efficacy consequently

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reducing the input cost (Martin et al., 2003; Attique et al., 2006). Synergism theory is based on the ability of one molecule to interfere with the metabolic detoxification of another molecule (Corbett, 1974). Synergism between organophosphates or carbamates and pyrethroids has already been reported in a number of insect pests (Martin et al., 2003; Corbel et al., 2006; Attique et al., 2006). However, there is no documented report of mixing neonicotinoids with IGRs, which are being widely used in Pakistan and elsewhere for the control of *B. tabaci*. Neonicotinoids act agonistically on postsynaptic nicotinic acetylcholine receptors (Elbert et al., 2007) and have been shown to have no cross-resistance to IGRs (Basit et al., 2012). The aim of the present study was to determine the effect of mixing a neonicotinoid (imidacloprid, acetamiprid, thiamethoxam, thiacloprid or nitenpyram) with an IGR (buprofezin or pyriproxyfen) against the laboratory susceptible (Lab-PK) and acetamiprid selected population (Aceta-SEL) of *B. tabaci*. The Aceta-SEL has been shown to have high level of resistance to neonicotinoids and cross-resistance to pyrethroid (bifenthrin) and endosulfan (Basit et al., 2011). To evaluate the mixtures of neonicotinoid insecticides and IGRs, a standard bioassay procedure was used to assess whether the mixture could yield additive, antagonistic or synergistic effects in the presence or absence of cytochrome P450 monooxigenase and an esterase inhibitor, pipronyl butoxide (PBO), and S, S, S, tri-butyl phosphorotrithioate (DEF), an esterase-specific inhibitor. Such studies could provide important information for devising resistance management strategies and so delay the development of resistance in *B. tabaci*.

## 2. Materials and methods

### 2.1. Insects

The resistant strain of *B. tabaci* was developed in the laboratory of Bahaudin Zakarya University (BZU), Multan, Pakistan from a field collected population by exposing to different selection pressures of the neonicotinoid insecticide, acetamiprid. The susceptible population was developed in 2009 by single pair crosses and was held in the laboratory for more than two years before the start of this study. Both populations were reared on cotton plants (*Gossypium hirsutum* L. var CIM-496) in the entomological laboratory of BZU, under photoperiod of 16 h at 26 ± 2 °C.

### 2.2. Insecticides and inhibitors

Commercial formulations used in the bioassays were: buprofezin 250 g a.i. $kg^{-1}$(FMC, Pakistan), pyriproxyfen 108 g a.i. $kg^{-1}$(Kanzo AG, Pakistan), acetamiprid 200 g a.i. $l^{-1}$ (Acelan®; FMC, Pakistan), imidacloprid 200 g a.i. $l^{-1}$ (Confidor®, Bayer Crop Sciences, France), thiamethoxam 250 g a.i. $kg^{-1}$ (Actara®, Syngenta, Berkshire UK), thiacloprid 480 g a.i. $l^{-1}$ (Talent®, Kanzo AG, Pakistan), nitenpyram 100 g a.i. $l^{-1}$ (Pyramid®, Kanzo AG, Pakistan), Piperonyl butoxide (PBO, Sigma Ltd, UK), S, S, S, tri-butylphosphorotrithioate (DEF, Sigma, Ltd).

### 2.3. Bioassays

The bioassays were carried out on whole cotton plants at two true leaf stages (20–22 days old) as described previously (Basit et al., 2011). Both leaves were washed, dried and dipped in the freshly prepared solutions for five to 10 s with slight agitation. Excessive liquid was allowed to drain off and the leaves were air dried for 1 h before confining the adults' *B. tabaci* in clip cages. There was one clip cage on each cotton plant and thus each plant was used as a single replicate. Each treatment (concentration) was replicated 3–4 times, including distilled water controls. Ten to fifteen whiteflies were briefly sedated with $CO_2$ before placing them on the lower side of cotton leaves in each clip cage (replicate) and thus a total of 30–40 adults were tested per concentration. The bioassays were kept at a temperature of 26 ± 2 °C, 65% relative humidity and 16:8 (light: dark) photoperiod. Mortality was assessed after 48 h.

### 2.4. Effect of inhibitors on insecticide toxicity

To evaluate the effect of inhibitors on the toxicity of insecticides alone and in mixture, PBO and DEF were used. Stock solution (1000 ppm) of PBO and DEF were prepared in acetone (analytical reagent grade, Fisher Scientific, Loughborogh, UK). To determine the dose at which mortality of *B. tabaci* was zero, a series of concentrations of DEF and PBO were tested. To test the effect of PBO and DEF on the toxicity of insecticides alone and in mixture, 100 ppm (zero % mortality) was added in each concentration tested. Mortality data were recorded after 48 h. The synergistic ratio was calculated by dividing the $LC_{50}$ of the population treated with the insecticide alone by the $LC_{50}$ of the population treated with insecticide plus synergist.

### 2.5. Evaluation of mixture

Each insecticide mixture could give greater (synergism) or less (antagonism) than the expected additive effect (summation). To determine which of these possibilities resulted, mixture of two insecticides were tested at ratios of 1:1, 1:10, 10:1, 1:20 and 20:1 using serial dilutions. Synergism or antagonism can be assessed using various methods and we used the method described by Hoel (1987). Potency ratios were calculated by dividing the estimated lethal concentration (LC) values of the mixtures, calculated for joint similar action by the experimental LC values observed in the bioassay. If PR = 1, the mixture was regarded ashaving additive action; if PR was <1, it showed an antagonistic action and if PR was >1, it exhibited a potentiating action. The estimated LC value of a mixture of A and B was computed as follows:

$$\text{Estimated LC}(A+B) = \frac{1}{\text{LC}(A) + \mu B/\text{LC}(B)}$$

where $\mu_A$ and $\mu_B$ represent the proportion of A and B in the mixture; $\mu_A + \mu_B = 1$.

### 2.6. Data analysis

Mortality data were analyzed and $LC_{50}$ values and their 95% fiducial limits were estimated using the POLO-PC computer based software (POLO, LeOra software, Menlo Park, California). Because of the inherent variability of bioassays, pairwise comparisons of $LC_{50}$ values were done at the 5% significance level (where individual 95% FLs for two treatments do not overlap) (Litchfield and Wilcoxon, 1949).

## 3. Results

### 3.1. Toxicity of tested insecticides alone and in combination to the Lab-PK and Aceta-SEL population

The toxicities of acetamiprid, imidacloprid, thiamethoxam, nitenpyram and thiacloprid were similar to one another and to the Lab-PK ($p < 0.05$, overlapping 95% fiducial limits). Similarly, $LC_{50}$ values of buprofezin and pyriproxyfen were also similar to each other for the Lab-PK (Table 1). However, mixtures of pyriproxyfen or buprofezin with acetamiprid, imidacloprid, thiamethoxam,