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Can Sublethal Afidopyropen Exposure Be Enough Potent to Suppress the *Bemisia tabaci* Populations? – An Impromptu Study

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ABSTRACT

The tobacco whitefly, *Bemisia tabaci* biotypes comprise important crop pests, and among them, the cryptic species Asia I and Asia II-1 cause devastating damage to major cultivated crops in India. The pyropene compound afidopyropen targets insects' chordotonal organ TRPV channel and has translaminar activity.In this study, the lethal effects of seven commonly used insecticides, including the novel compound afidopyropen, on adult populations of *B. tabaci* Asia I was evaluated, and then the extent of key biological and physiological activities to sublethal concentrations wasdetermined.Among all tested insecticides, afidopyropen was the most toxic to whitefly adults with an LC_{50} of 2.56 mg L^{-1}. The sublethal effects of afidopyropen were observed by treating the *B. tabaci* adults with two different concentrations, LC_{25} (0.96 mg L^{-1}) and LC_{10} (0.24 mg L^{-1}), respectively. At LC_{25} concentration, afidopyropen prolonged the developmental duration except forthe third instar nymph and significantly decreased the survival rates of all the immature stages inthe F_1 generation. Unlike egg hatching rate, the oviposition duration, fecundity per female, and honeydew excretion by adults exposed to LC25 afidopyropen concentration were also significantly reduced.The present results indicate that sublethal concentrations of afidopyropen could reduce the survival, development and reproduction of *B. tabaci* that can help optimize the future IPM program.

Keywords : Honeydew; Pyropenes;Reproduction; Sublethal concentration; Toxicity; Whitefly

Hatching.

Introduction

The *Bemisia tabaci* Gennadius, often known as the cotton (sweet potato) whitefly, is one of the most detrimental and costly agricultural pests in the world (Oliveira *et al.,* 2001; Stansly and Naranjo, 2010). It also transmits more than 100 plant viruses, such as the Cotton Leaf Curl

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Virus (CLCV) and the Tomato Yellow Leaf Curl Virus (TYLCV), in addition to directly feeding on phloem sap (Hogenhout *et al.*, 2008). *B. tabaci* has developed resistance to a variety of active chemicals, including new classes like neonicotinoids, insect growth regulators (IGRs), and chordotonal organ modulators, in various crop systems as a result of the injudicious application of chemical insecticides (Wang *et al.*, 2021). Within the *B. tabaci* biotypes, the Asia I and Asia II-1 are the most prevalent cryptic species and have caused significant economic damage to different agri-horticultural crops in India (Ellango *et al.*, 2015; Naveen *et al.*, 2017 and Horowitz *et al.*, 2020). Concerns about the emergence of insecticide resistance to conventional molecules of an earlier generation have greatly stoked interest in the creation of innovative chemistries with distinctive modes of action (MoAs). However, due to the increasing requirements for toxicological safety and environmental sustainability, there are fewer novel chemicals available (Basit *et al.*, 2013). Pyropene molecules have been shown to control several hemipteran insect pest species worldwide (Chen *et al.*, 2018; Koch *et* al., 2020; Liu *et al.,* 2021; Zhang *et* al., 2021; Shi *et al.*, 2022). After the relative success of pymetrozine (9B), afidopyropen, which is listed in group 9D as chordotonal organ TRPV channel modulator in the IRAC MoA classification, has been demonstrated to cause cessation of feeding followed by starvation and mortality, thereby reduction of virus transmission capability of the target pests (Jeschke, 2021). In addition, it was also suggested that afidopyropen could be effective against pymetrozine resistant populations of *B. tabaci* with a lack of cross-resistance (Zhang, 2021). Being selective for insect pests, the relative negligible toxicity of afidopyropen to various natural enemy populations exhibited a desirable eco-toxicological profile of this insecticide (Koch *et al.,* 2020 and Kumar *et al.*, 2018). However, to understand the potential of afidopyropen as a promising insecticide against field populations of *B. tabaci*, it is important to confirm the sublethal effects of this molecule on *B. tabaci* Asia I which is the predominant strain of whitefly across India.

It can be assumed that the sublethal effects of an active ingredient on the physiological, reproductive and behavioral traits of a target pest should be considered to generate a comprehensive toxicological data set (Desneux *et al.,* 2007). In the current study, we assessed the toxicity of afidopyropen on adult *B. tabaci* Asia I in comparison with six other commercially formulated insecticides. In addition, the effects of sublethal concentrations of afidopyropen on developmental stages, survival rate, fecundity, oviposition, egg hatchability and feeding were also evaluated, respectively.

Materials and methods

Insects

A laboratory susceptible strain of *B. tabaci* Asia I (Lab-WB) was generated at Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India in 2016 by single pair cross technique as described previously (Roy, 2019). The insect population wassubsequently maintained on insecticide-free tomato plants (cv. NS-521) in the laboratory at 26 ± 2 p C, 60 ± 5% RH and a photoperiod of 16:8 h light: dark.All adults tested in bioassays were less than

7-days-old, and the sex ratio of both males and females was used to approximately 1:1.

Insecticides

Commercial formulations of afidopyropen 500 g a.i. L^{-1} (Sefina® 50 g/L DC, BASF India Ltd., India), pymetrozine 500 g a.i. KG^{-1} (Chess® 50 WG, Syngenta India Ltd., India), flonicamid 500 g a.i. KG^{-1} (Ulala® 50 WG, UPL Ltd., India), imidacloprid 200 g a.i. L^{-1} (Confidor® 200 SL, Bayer Crop Science, India), flupyradifurone 200 g a.i. L^{-1} (Sivanto® 200 SL, Bayer Crop Science, India), cyantraniliprole 102.6 g a.i. L^{-1} (Benevia® 10.26 OD, FMC, India), and spiromesifen 240 g a.i. L^{-1} (Oberon® 240 SC, Bayer Crop Science, India) were used for the bioassays.

Lethal effects of the tested insecticides on *B. tabaci* Asia I

The modified leaf-dip bioassay method was followed (Xie, 2014) for evaluating the toxicities of the seven insecticides to *B. tabaci* Asia I adults. The stock solutions (1000 mg L^{-1}) of each of the formulated molecules were prepared in acetone. Thereafter, required concentrations of the working solutions were made through serial dilution of the stock solution in double-distilled water dissolving 0.1g L^{-1} of Triton X-100. Bioassays consisted of four replicates per concentration with ten different concentrations (0.0, 0.01, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 50.0, and 100.0 mg L^{-1}) for each tested insecticide, including control.Cotton leaf discs (diameter 35 mm) were dipped in the serially diluted insecticide solutions for 20 s, or the diluent only for controls. Leaf discs were then placed with the adaxial surface downwards on an agar bed (10 g L^{-1}), held within a plastic Petri plate (diameter 40 mm), and air-dried. About 25-30 adults were then settled in each Petri plate and confined using a close-fitting perforated lid. The plates were maintained at 26 ± 2 p C, 60 ± 5% RH and a photoperiod of 16:8 h light: dark. Mortality was scored after 48 h and the immobile adults were assumed to be dead.

Sublethal effects of afidopyropen on *B. tabaci* Asia I

After the adult insects were exposed to sublethal concentrations (LC_{10} and LC_{25} values) of afidopyropen ("Results" section) for 48 h as described above, the following fitness parameters were monitored for the F_1 generation, respectively: developmental time and survival rate of different life stages in the subsequent generation; fecundity per adult female; oviposition duration; egg hatchability; and honeydew deposition. In brief, 15 insect-free cotton plants in total were equally placed in three individual insect-proof cages (two experimental cages for LC_{10} and LC_{25} concentrations, and one cage for the control) with five plants in each cage.Then, *B. tabaci* adults (n = 100)that were previously treated with afidopyropen (LC_{10} and LC_{25}) on cotton leaves, were inserted into each experimental cage for egg-laying. Besides, the same numbers of untreated adults were also confined within the control cage. After 12 h of oviposition, all the plants were removed from three cages, and two leaves in each plant (10 leaves from each cage) were randomly selected and tagged. Twenty eggs were retained on each selected cotton leaf with the aid of a microscope and confined in a small clip-cage (2.6 cm diameter).The position of each remaining egg was marked on the abaxial surface of these leaves using a black permanent marker pen which

helped us to track the fate of eggs until the adult emergence. Each plant was then kept in isolated climatic chambers at 26 ± 2 p C, 60 ± 5% RH and a photoperiod of 16:8 h light: dark. To record the fecundity of treated insects, freshly emerged adult whiteflies were transferred to new leaves and enclosed in clip cages until the death of all adults. Subsequently, the egg hatching rate was also determined.

To assess the sublethal effects of afidopyropen on honeydew excretion of *B. tabaci* Asia I, adult insects were exposed to LC_{10} and LC_{25} concentrations using the methodology described in the previous section with the exception that the Petri plates were placed upside down on filter paper treated in 0.1% bromocresol green (He *et al.*, 2013). The honeydew droplets excreted by whitefly adults were turned into blue spots when they came in contact with bromocresol green treated filter paper after 24 h of insecticide exposure (He *et al.*, 2012). The area marked with blue spots on the filter paper was measured on a millimetre squared (mm^2)graph paper sheet as the amount of feeding by *B. tabaci* adults. Four replicates of 3-day-old thirty adult females, previously starved for 2 h, for each tested concentration, was used for honeydew excretion.

Data analysis

Bioassay data were corrected for control mortality using Abbott's formulawhere required (Abbott, 1925). Data were analyzed using PoloPlus statistical software version 2.0 (LeOra Software Company, USA) for Probit analysis. The LC values and their 95% fiducial limits were calculated to compare changes in susceptibility(Robertson *et al.*, 2007). A one way ANOVA followed by Tukey's HSD test for multiple comparisons were done using SPSS software (version 18.0: Inc., Chicago, IL, USA) to analyze the significance of statistics in the survival rates and development time of *B. tabaci* among different treatments (afidopyropen LC_{10}, LC_{25}, and control), along with oviposition periods, fecundity, egg hatchability, and honeydew excretion.

Results and discussion

Toxicity of the tested insecticides on *B. tabaci* Asia I

The $LC_{50}$and LC_{90}values of seven insecticides against *B. tabaci* Asia I adults are listed in Table 1. Among the insecticides tested, the highest toxicity was observed with the afidopyropen (LC_{50} = 2.56 mg L^{-1}) treatment.As the counterparts, the LC_{50} values for spiromesifen (LC_{50} = 2.64 mg L^{-1}), flupyradifurone (LC_{50} = 2.71 mg L^{-1}), cyantraniliprole (LC_{50} = 2.82 mg L^{-1}), pymetrozine (LC_{50} = 3.15 mg L^{-1}), flonicamid (LC_{50} = 3.34 mg L^{-1}), and imidacloprid (LC_{50} = 5.49 mg L^{-1}) were also recorded in the adult populations, which were significantly lower than afidopyropen.

Sublethal effects of afidopyropen on *B. tabaci* Asia I

The bioassays of afidopyropen on *B. tabaci* Asia I adults showed that the LC_{90}, LC_{25}, and LC_{10} were at 11.53 mg L^{-1}, 0.96 mg L^{-1}, and 0.24 mg L^{-1} concentrations, respectively.Exposure of *B. tabaci* to the LC_{25} concentration of afidopyropen prolonged the duration of every growth stage (F = 15.538, df = 2,382, P = 0.003; F = 19.640, df = 2,346, P = 0.018; F = 28.436, df = 2,290, P = 0.007; F = 35.467, df = 2,416, P = 0.036), except the pseudopupae (F =

23.552, df = 2,420, *P* = 0.024), when compared to the control (Table 2). On the other hand, the LC_{10} concentration of afidopyropen only delayed the 2nd nymphal instar (10.22 days) and adult stage (20.42 days) in comparison with the control (9.94 and 20.25 days, respectively).Table 3 showed that the survival percentage of*B. tabaci*2nd (*F* = 5.824, df = 2,30, *P* = 0.004) and 3rd (*F* = 9.560, df = 2,30, *P* = 0.038) instar nymphs, and pseudopupae (*F* = 4.272, df = 2,30, *P* = 0.020) reduced significantly after the exposure to LC_{25} concentration as compared to the $LC_{10}$and control treatments. However, the survival rates of the 3rd nymphal instar and adults were significantly reduced with LC_{10} treatment in comparison with the unexposed group. Furthermore, exposure of *B. tabaci* Asia I to two sublethal concentrations (LC_{10} and LC_{25}) of afidopyropen significantly shortened the reproductive parameters such as oviposition duration (10.88 ± 0.08 and 9.12 ± 0.05 days, respectively) (Figure 1a) and fecundity per adult female (126.61 ± 1.24 and 109.54 ± 1.38 eggs per female, respectively) (Figure 1b) as compared with the control (12.65 ± 0.06 days and 138.24 ± 1.89 eggs per female, respectively) (*F* = 4.289, df = 2,54, *P* = 0.008; and *F* = 7.565, df = 2,54, *P* = 0.034, respectively). In contrast,no significant difference was observed between the exposed and unexposed conditions for egg hatching rate (Figure 1c) of *B. tabaci*. Besides, the honeydew excretion (Figure 1d)of the LC_{25} treated insects (16.19 ± 0.42 mm^2) was decreased significantly than the LC_{10} treated groups (23.74 ± 0.37 mm^2) and control (27.96 ± 0.58 mm^2), respectively (*F* = 5.862, df = 2,54, *P* = 0.011).

Considering that most of the insecticides could degrade gradually after their initial fieldapplication (Desneux *et al.*, 2007), turning lethal dosages into sublethal levels, we determined the effects of the sublethal concentrations of afidopyropen on *B. tabaci* Asia I.In the present study, it has been found that the afidopyropen registered the highest toxicity among the seven commonly used chemicals tested against *B. tabaci* Asia I, while spiromesifen, flupyradifurone, cyantraniliprole, pymetrozine, and flonicamid exhibited significantly higher LC_{50s}, and imidacloprid displayed low toxicity. Owing to the severity of insecticides resistance to widely used molecules such as flonicamid, pymetrozine, spirotetramat, flupyradifurone, cyantraniliprole and dinotefuran in *B. tabaci* Asia I, MED and MIAM1 in India, China, Pakistan and many other countries, respectively (Roy *et al.*, 2019; Wang *et al.*, 2020;Qiong *et al.*, 2012; Bielza *et al.*, 2019; Wang *et al.*, 2017; Dângelo *et al.*, 2018), identification of novel chemistry with a distinct mode of action is needed to accelerate the resistance management programs.The chordotonal organ TRPV channel modulator insecticides have been applied widely to control the populations of whiteflies, aphids, leaf and planthoppers and thrips. Even though the pyridine azomethine derivative, pymetrozine, exhibited remarkable efficacy against whitefly in the last decade,the phenomena of field-evolved resistance and cross-resistance with neonicotinoids in *B. tabaci* have been reported recently (Wang *et al.*, 2021 and Nauen *et al.*, 2013). The new generation pyropene compound afidopyropen is a promising alternative insecticide that could

be effectively used for the management of *B. tabaci* (Jeschke, 2021 and Kandasamy *et al.*, 2017). Combined with several studies reporting baseline susceptibility of *Diaphorina citri* (Chen *et* al., 2018), *Aphis glycines*)(Koch *et* al., 2020), *Myzuspersicae* (Liu *et al.*, 2021), *B. tabaci* MED (Zhou *et al.*, 2021) and *A. gossypii* (Shi *et al.*, 2022) in China and the United States, our study indicates a high susceptibility to afidopyropen in *B. tabaci* Asia I under controlled climatic conditions.Moreover, this molecule displayed high toxicity against pymetrozine-resistant *B. tabaci* MED and MEAM1 and no cross-resistance was detected (Zhang *et al.*, 2021), indicating that, unlike pymetrozine,a dissimilar metabolic mechanism might be responsible for the detoxification of afidopyropen in *B. tabaci*.

Apart from lethal concentrations, that directly kill insects, sublethal dosages of different insecticides also exerted a significant impact on the biological parameters of insect pests including a reduction in the fecundity, egg hatchability and oviposition duration (Qin *et al.*, 2013). In the present study, the LC_{25} concentration of afidopyropen significantly abated the reproductive components, except the egg hatching rate, and also prolonged the developmental duration of *B. tabaci* Asia I, in comparison with LC_{10} concentration and control. Similarly in other studies, sublethal concentrations of afidopyropen significantly decreased the time of egg and nymph stages of *A. gossypii* (Ma *et al.*, 2021) and *M. persicae* (Liu *et al.*, 2021). Moreover, from the present data, we observed that there was a smooth concentration-effect relationship, obviously, the LC_{25} exposure had a greater effect on *B. tabaci* than the LC_{10} concentration had. However, unlike our findings on egg hatchability of the Asia I genetic group, LC_{10} and LC_{25} concentrations of afidopyropen markedly reduced the rate of egg hatching, along with egg-laying duration and fecundity in the MED population of *B. tabaci.*[30]Comparing the reductions in the honeydew deposition by *B. tabaci* feeding on sublethal concentrations of afidopyropen treated leaves, it arises that honeydew excretion was significantly decreased at LC_{25} treatment. In a previous study, cyantraniliprole gave similar results since the *B. tabaci* Q biotype adults deposited significantly less honeydew when they fed on cotton leaves treated with sublethal concentrations of cyantraniliprole.[33]By contrast, in the present study, LC_{10} concentration of afidopyropen did not cause cessation in the honeydew excretion of adult whiteflies significantly. This phenomenon might be attributable to the typical symptoms of positive fitness effects by sublethal insecticides exposure in insect pests (Margus *et al.*, 2019). However, the results obtained for LC_{10} concentration of afidopyropen are following those of a previous study, in which, no significant impact on honeydew excretion was observed when *B. tabaci* B biotype fed on leaves treated with LC_{20} concentration of chlorpyrifos and carbosulfan (He *et al.*, 2013). On the contrary, sublethal exposure of insecticides occasionally could be beneficial for some insects, with most cases showing that sublethal dosages of beta-cypermethrin and cyantraniliprole could produce an increased population growth and higher fecundity in *Plutella xylostella (*Han *et al.*, 2011) and *Bactrocera dorsalis*

(Zhang *et al.*, 2015), respectively. However, no such cases for afidopyropen have been reported to date.

Sublethal concentrations of an active ingredient not only affect insect biology and physiology but also alter the biochemical composition and behavior of insects.Heterogeneously expressed TRPV channels were stimulated by afidopyropen in *Drosophila melanogaster* and *Acyrthosiphonpisum* (Kandasamy *et al.,* 2017). In another example, sublethal concentrations of afidopyropen reduced the inter-host (citrus) transmission of *Candidatus*Liberacter bacteria by *D. citri* (Chen *et al.*, 2018).Hence, it is likely that afidopyropen could change the feeding nature of *B. tabaci* that may reduce the whitefly-transmitted plant viruses. However, an Electrical Penetration Graph (EPG) study on the probing behavior of *B. tabaci* following exposure to the sublethal concentrations of afidopyropenremain to be investigated.

Conclusion

The results of the present study indicate that the LC_{50} values against *B. tabaci* Asia I adults were lower for afidopyropen than for six other commonly used insecticides. Although it has been found that various biophysical traits of *B. tabaci* are affected at sublethal concentrations of afidopyropen, the impact on other life-history parameters of this sap-sucking insect pest needs to be studied. Meanwhile, a long-term experiment should be conducted to evaluate the potential sublethal effects of afidopyropen on different key processes of natural enemies' effectiveness against *B. tabaci*in the field.Our study establishes a foundation for further investigation of afidopyropen on various toxicological aspects related to insecticide resistance in whiteflies.

Conflicts of Interest/Competing Interests: The authors have declared that no conflict of interest exists.

Ethics Approval : This article does not contain any studies with human or other animal subjects.

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