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ORIGINAL ARTICLES

Control of *Bemisia tabaci* in Greenhouse Tomato using Systemic Insecticide-treated Trap Plant

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Abstract This study aimed to develop a new control method of *Bemisia tabaci* using a tobacco plant treated with systemic insecticide in tomato greenhouse cultivation. In the preference tests, it was demonstrated that tobacco is a better *B. tabaci* attractant than the seven tested solanaceous plants. Most importantly, *B. tabaci* prefers tobacco (65%) to the main host plant, tomato (35%). Among 6 systemic insecticides, dinotefuran WG was highly effective (mortality > 97.3%) against *B. tabaci* and exhibited less or non-disruptive tendency on insect behavior on dinotefuran-treated tobacco plants. The dinotefuran concentrations in tobacco leaves obtained using modified QuEChERS method with liquid tandem mass spectrometry rapidly increased over time, and mortality of *B. tabaci* populations recovered from tobacco leaves was over 90% within 24 hrs after insecticide treatment. The mortalities were consistently over 90 to 100% until 35 d. The upper leaves of tobacco showed slightly higher concentration of insecticide, including the resultant *B. tabaci* mortalities than the lower leaves. Compared with that of greenhouse with no tobacco plants (100%), the population of *B. tabaci* in greenhouses with two and three insecticide-treated tobacco plants increased by 53%-63% and 22% respectively. These results confirmed that a dinotefuran-treated tobacco plant can be an effective trap plant for the control of *B. tabaci* in tomato cultivation greenhouses.

Key words *Bemisia tabaci*, systemic insecticide, trap plant, greenhouse tomato

Introduction

Bemisia tabaci (whiteflies) causes considerable damage and economic losses to vulnerable crops, particularly solanaceous plants such as tomato, eggplant, and tobacco (Choi et al., 2016). The pest has an extremely wide range of host plants and capacity to vector more than 110 plant pathogenic viruses (Fortes et al., 2020). Both nymphs and the adults suck sap and while feeding, they emit sugary excreta that promote ‘sooty mold’ on the foliage and fruits, resulting in negative effects on crop productivity. In particular, *B. tabaci* is one of the most difficult pests to control in Korean tomato greenhouses. Since it was firstly found in Tongyoung, Gyungsang province in 1998, the pest has continued to significantly affect tomato productivity to date (Guo et al., 2022; Lee et al., 2000). Various pest management methods including chemicals have been performed but most of them have limitations. In general, agricultural chemicals are preferred for the control of *B. tabaci* due to their availability, convenience, and efficiency in Korea. However, the reckless use of these chemicals can result in development of insecticide resistance in the target pests as well as pollute soil and water or disturb the ecosystem (Kim et al., 2021; Lee et al., 2010; Wang et al., 2020; Wang et al., 2018). Therefore, a robust, cost-effective, and eco-friendly control method of *B. tabaci* is urgently required.

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Due to the prevalent development of insecticide resistance in various populations, various environmentally-friendly control strategies (Lee et al., 2013) including predatory mites to prey on the eggs of *B. tabaci* (Kim et al., 2008), entomopathogenic fungi isolated from *B. tabaci* (Park et al., 2014), trap plants (Choi et al., 2016; Seo et al., 2020), and vegetable oriented oil (Choi and Kim, 2004) have been investigated. However, in practice, they have often failed to reach management goals. One of these strategies, trap cropping, i.e., the use of alternative host plants to reduce pest damage on a focal cash crop or other managed plant can be sustainable strategy for pest control. Trap plants are used for attracting the pests away from the main crop in the field. Previous studies have demonstrated that some plants such as eggplants or herbs are potential trap plants. Lemon balm, rose geranium, and apple geranium have also been investigated as trap plants in tomato cultivation (Seo et al., 2020). Among them, lemon balm had a high potential to attract whiteflies, while rose and apple geraniums showed repellent to whiteflies. In addition, previous studies have demonstrated the possibility of using insecticide-treated-eggplants as trap plants (bait) to lure and control *B. tabaci* (Choi et al., 2014; Choi et al., 2016). Carbamates, organophosphates, pyrethroids and neonicotinoids are touted to be effective in the control of *B. tabaci* through trap plant treatment (Horowitz et al., 2020; Zhou et al., 2022). In particular, systemic insecticides tend to be absorbed by and transported through plants, hence, rendering the plant toxic to insects that feed on plant tissue, including *B. tabaci*. Therefore, the use of systemic insecticides in trap plants rather than the main crop is a potential eco-friendly control method for the notorious *B. tabaci*. Therefore, the current study aimed to select the most appropriate trap plant out of 8 solanaceous plants and to select the most effective systemic insecticide for the control of *B. tabaci*.

Materials and Methods

Trap plant selection

Trap plant preference tests were conducted as follows: The 8 selected solanaceous plants were divided into two groups of four plants each (4 choice test). Tobacco, black nightshade, petunia, and Goji berry constituted group 1 while chili pepper, eggplant, capsicum, and jimsonweed were tested in group 2. The leaves of each plant (from one month old plants) were wrapped with wet cotton and the

Fig. 1. The design of four choice test. A. Cotton-wrapped leaves were placed in an acryl round container; B. Whiteflies on chili pepper leaf.

four plants of the group were placed in an acryl round (0.22 m diameter × 0.17 m) container at a spacing of 10 cm apart (n=10) (Fig. 1A). The container was self-designed and built in our laboratory. Fifty insects were then released into each container (n=10). The number of whiteflies settled on each leaf was counted after 48 hrs of releasing for comparing host preference among 4 plants. The final choice test was conducted with the most preferred plant along with tomato following the same procedure. Tobacco (the most preferred trap plant) and tomato plants of similar size were placed in an acryl round container and 50 whiteflies were released into the container.

Selection of systemic insecticides and mortality of *B. tabaci* in tobacco trap plant

Six systemic insecticides selected based on the Xerces Society's database (2021) were tested to determine the appropriate insecticide for use with a trap plant (Table 1). Each insecticide (100 mg/kg) was prepared by diluting it in distilled water. The diluted insecticides were subsequently added to 0.2 g of instant bed soil (Seoul Bio Inc., Korea) in each pot (0.08 m × 0.1 m) of the targeted plant. Fifty adults were released into the cage and the dead and live insects were counted at 3 d after the pesticide treatment. Mortality rates were compared among the 6 tested insecticides, and the best performing insecticide was selected for further experimentation. Finally, the selected insecticide was tested on both tomato and tobacco plant following the same procedure as described above. Fifty adults were released in cage and monitored.

Table 1. Six systemic insecticides used in this study

Chemicals	Formulation[a)]	AI[b)] (%)	IRAC[c)] Class
Cyantraniliprole	EC	5	Diamides, 28
Dinotefuran	WG	20	Neonicotinoids, 4A
Emamectin benzoate	EC	2.15	Avermectin, 6
Flonicamid	WG	10	Flonicamid, 29
Imidacloprid	SC	8	Neonicotinoids, 4A
Sulfoxaflor	SC	7	Sulfoximine, 4C

[a)] EC, emulsifiable concentrate; WG, wettable granule; SC, suspension concentrate
[b)] AI, active ingredient [c)]IRAC, Insecticide Resistance Action Committee

Insecticide concentration and mortality in insecticide-treated tobacco leaves

Dinotefuran analysis in tobacco leaves

Analytical grade dinotefuran (98.5% purity) was provided by Dr. Ehrenstöfer (London, UK). Extraction packets containing 4 g $MgSO_4$, 1 g sodium chloride, 1 g sodium citrate, 0.5 g disodium citrate sesquihydrate and dispersive solid phase extraction (d-SPE) containing (150 mg $MgSO_4$/25 mg primary secondary amine (PSA) and (150 mg $MgSO_4$/ 25 mg PSA/25 mg graphitized carbon black (GCB)) were obtained from Agilent (Agilent Technologies, USA).

A liquid chromatograph (Shimazu LC) equipped with tandem mass spectrometry (MS/MS) (Shimazu-8060) was operated for the analysis. The analyte was separated on a non-polar column (Agilent Eclipse Plus C18 2.1 mm i.d. × 100 mm L, 1.8 μm particle size, Waters, MA, USA) using mobile phase A (0.1% formic acid, 5 mM ammonium formate in DW) and mobile phase B (0.1% formic acid, 5 mM ammonium formate in MeOH). Injection volume was 5 μL and flow rate was 0.2 mL. In the multiple reaction monitoring mode, qualitative ion and confirmation ion (m/z) were 203.15 > 114.15 and 203.15 > 87.00 in positive electrospray ionization mode, respectively. The MS conditions were as follows: ion spray voltage of 5,500 V, nebulizer gas of 50 psi, curtain gas of 25 psi, drying gas of 50 psi, collision gas of 10 psi, and drying gas temperature of 500°C.

Two or three tobacco leaves from upper, middle, and lower parts of each plant were collected (n=3) 2, 4, 6, 9, 12 hrs, 1, 2, 3, 5, 7, 9, 14, 21, 28, 35, and 42 d after treatment. Each sample was pulverized with dry ice using a blender. The target compound in tobacco leaf samples was extracted using the modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method (Chamkasem et al., 2013). Final

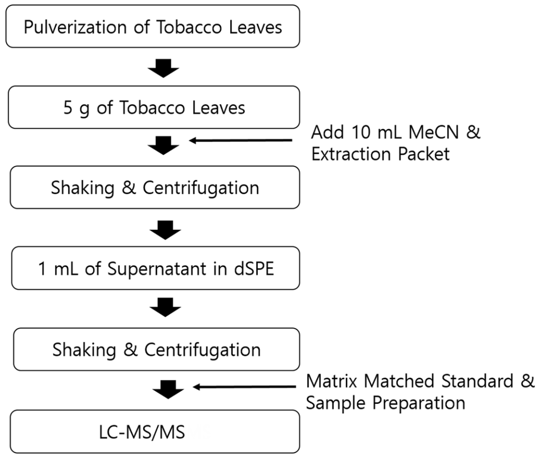

Fig. 2. Analytical method flow chart. MeCN, acetonitrile; dSPE, dispersive solid phase extraction; LC-MS/MS, liquid tandem mass spectrometry.

sample preparation for the instrumental analysis is displayed in Fig. 2. At two fortification levels of 0.01 and 0.1 mg/kg, recovery rates (%) with relative standard deviation (RSD, %) were achieved by plotting matrix-matched standards for the analysis of LC-MS/MS. For quantitative analysis, matrix-matched standards were prepared by mixing solvent standard solution with blank matrix extracts (1:9) at the levels of 1, 2.5, 5, 10, 25, 50, and 100 ng/mL. Limit of quantitation (LOQ) and linearity were evaluated by plotting each matrix-matched calibration curve. LOQ was < 0.01 mg/kg and the correlation coefficients (r^2) of matrix-matched standards were > 0.99.

Insecticide treatment and mortality test

Tobacco seeds were planted and grown for about 45 d in greenhouse conditions for systemic insecticide treatment. Dinotefuran WG (20%) (200 mg/kg) was diluted in distilled water and was subsequently added to the bed soil (1.4 kg) in each pot (0.25 m × 0.25 m) of tobacco plant. Two or three leaves collected from upper, middle, and lower parts

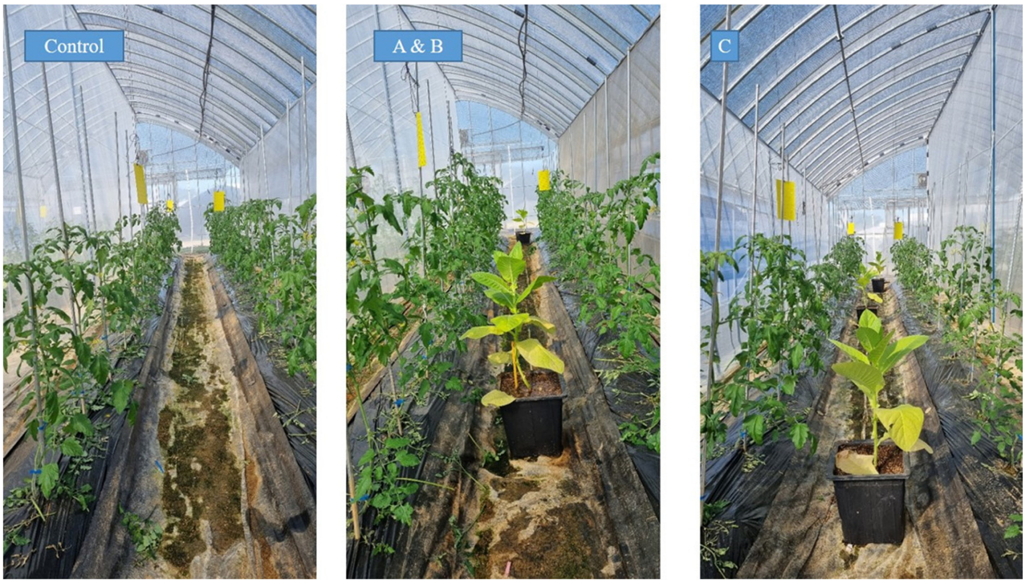

Fig. 3. Field experimentation. Control greenhouse where no tobacco plants were placed. A & B. 2 tobacco plants placed at 10 m distance; C. 3 tobacco plants placed at 5 m distance in furrow between two tomato planted lines.

of each plant were collected at 2, 4, 6, 9, and 12 hrs and 1, 2, 3, 6, 9, and 35 d after the treatment (n=3). Each leaf was cut into a disk of about 3 cm in diameter before being transferred into a petri dish. A portion of each trimmed leaf was kept in a deep freezer (-80°C) for further analysis of dinotefuran residue. Thirty adult *B. tabaci* were introduced into the petri dish. The number of dead and live *B. tabaci* were checked at 3 d after inoculation (n=3).

Greenhouse experimentation

Tomato seedlings were planted at two lines in four separate greenhouses (Control, A, B, and C) located in the National Institute of Agricultural Sciences, Wanju, Jollabuk do on the 16th of May in 2023 (Fig. 3). On 23rd of May, 10 L of dinotefuran (200 mg/kg) was prepared and then, 1 L was added to soil (20 kg) of each tobacco plant. One week after, the same amount of dinotefuran was applied to each tobacco plant. No tobacco plant was placed in the control greenhouse (18 m × 8 m). In greenhouse A and B, two dinotefuran-treated potted tobacco plants were placed at 10 m distance between the two lines. In greenhouse C, 3 tobacco plants were instead used but the spacing distance was reduced to 5 m apart. A total of 100 whiteflies were released into each greenhouse and three density yellow traps (Greenagrotech Inc., Gyeongsan, Korea) were installed at 3 m distance. The number of insects in each yellow trap was recorded on a weekly basis for 8 weeks from one week after the introduction of tobacco plants.

Statistical analysis

Variance analysis using Duncan's Multiple Rage Test (DMRT) and Tukey's test were performed for the determination of statistical differences among samples in the preference test and the mean mortalities of *B. tabaci* in systemic insecticide treatment using SPSS v.13 software (IBM Analytics, Armonk, NY).

Results and Discussion

Selection of a trap plant

Out of the 8 solanaceous plants, tobacco was the most attractive trap plant, and was selected for further experimentation. Figure 4 shows the preference test results. In the first group test, tobacco (36%) and black nightshade (35%) were preferred to petunia and Goji berry (Fig. A). Eggplant (41%) and belly pepper (29%) were the most preferred plants in the second group (Fig. 4B). Through the results of two preference tests, four candidate plants were selected. In the final test, tobacco (40%) and eggplant (32%) were the most preferred plants for *B. tabaci* (Fig. 4C). The DMRT test showed that they were not significantly different, which is consistent with the previous study showing that *B. tabaci* preferred eggplants, tobacco, and cucumber to cotton and tomato (Tian et al., 2020). However, tobacco was selected as a trap plant in this study because its leaves have a large surface area and therefore can attract and harbor more whiteflies, and the plant is easy to be managed as a trap.

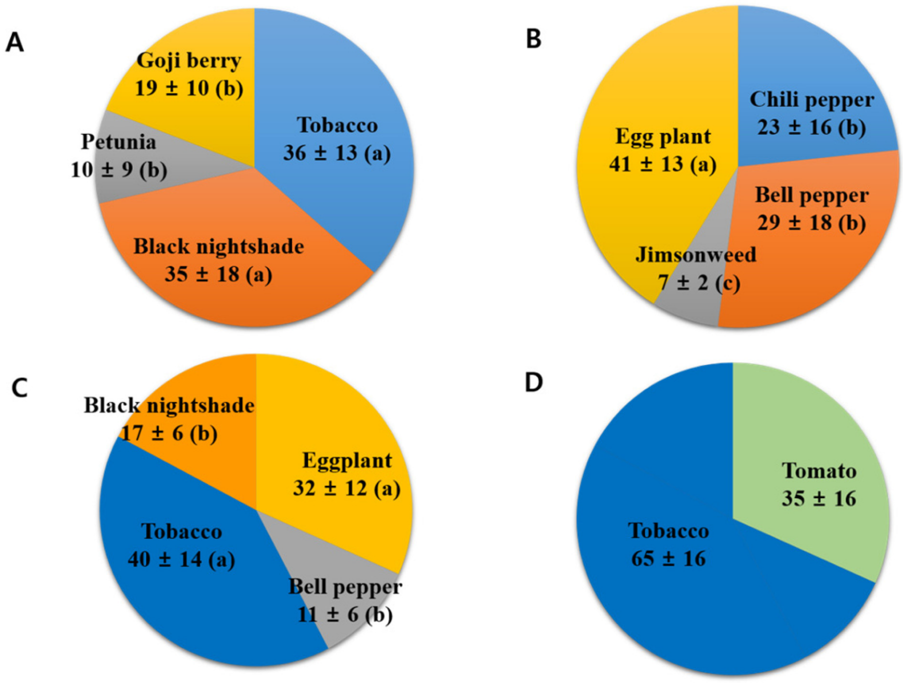

Fig. 4. Choice test results of *B. tabaci.* A. attraction rates (%) of tobacco, black nightshade, petunia, and Goji berry; B. attraction rates of eggplant, bell pepper, chili pepper, and jimsonweed; C. attraction rates (%) of the four candidate plants selected, D. attraction rates of tobacco and tomato. The different letters (lowercase) in the attraction rates indicate significant differences in the 95% DMRT test.

This has already been demonstrated in previous studies. For instance, eggplant was used as a trap plant due to its easy to manage attributes compared to cucumber even though eggplant (82.3%) and cucumber (82.5%) yielded similar attraction rates (Choi et al., 2016). It was also hypothesized that *B. tabaci* adults were more sensitive to visual cues than to odor and the pest preferred the eggplant and cucumber to horseweed. In the final test, the attraction rates of the tobacco plant to *B. tabaci* adults were 65%, compared to that of tomato (35%) (Fig. 4D), indicating that tobacco has high a potential as a trap plant to lure *B. tabaci* adults in tomato greenhouse cultivation.

Selection of systemic insecticides and their efficacy against *B. tabaci* in tobacco trap plant

Dinotefuran was finally selected as the best compound for systemic treatment in the tobacco plant and the mortality test results are shown in Fig. 5. Out of six systemic insecticides, cyantraniliprole EC (96.7% ± 1.2%), dinotefuran WG (97.3% ± 4.6%), and sulfoxaflor SC (98.7% ± 1.2%) showed high mortalities in our study. The DMRT tests showed that they are not significantly different. Insect mortalities recorded in treatments with emamectin benzoate, imidacloprid, and flonicamide were 28.0% ± 6.9%, 52% ± 16.0%, and 67.3% ± 4.2%, respectively. Even though imidacloprid is known to be effective for control of *B. tabaci*, it showed a low mortality of 52% ± 16%, which is consistent with several other published literature (Kumar et al., 2016).

Additional experiment was conducted to check whether the three best performing insecticides (cyantraniliprole EC, dinotefuran WG, and sulfoxaflor SC) can effectively control *B. tabaci* in the presence of tomato plants (Fig. 6). Dinotefuran exhibited less or non-disruptive tendency on insect behavior and highest mortality rate of 66% was recorded in tobacco leaves treated with dinotefuran. Mortality rates in treatments with cyantraniliprole EC and sulfoxaflor SC were 58% and 48%, respectively. These results indicated that treatment of tobacco plants with these systemic insecticides have a limited potential to reduce the density of *B. tabaci* in the tobacco greenhouse cultivation.

In the presence of tomato plants, the mortalities of *B. tabaci* were reduced compared with no tomato plants. These results may be due to the feeding behavior disorder or repelling effect of these insecticides. Previous studies have shown that cyantraniliprole has a negative effect (repellent effect) on the feeding behavior of *B. tabaci* (Kwon et al., 2008), and dinotefuran plays a repellent role against green peach aphid (Seo et al., 2009). In the case of

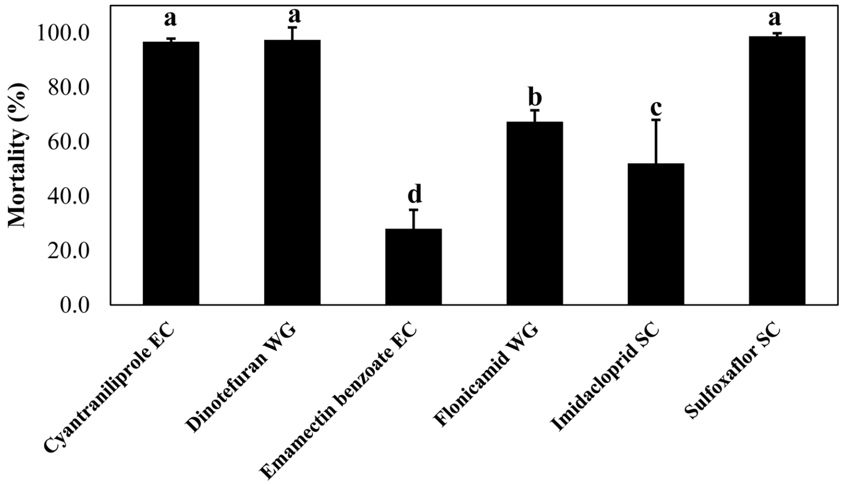

Fig. 5. Mortality (%) of *B. tabaci* on tobacco plants treated with systemic insecticides. The different letters on bars indicate significant differences in the 95% DMRT test.

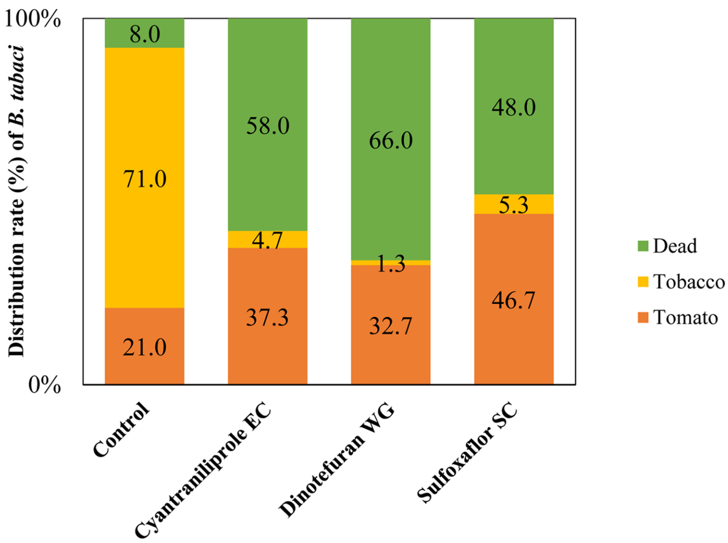

Fig. 6. *B. tabaci* distribution between tomato and insecticide-treated tobacco. Distribution rate (%) of *B. tabaci* found on tomato plants (yellow color) and the insecticide-treated tobacco plant (orange color) at 3 d after being released into the cage. Green color indicates the percentage of dead *B. tabaci*.

dinotefuran, 67.3% (66.0% of mortality and 1.3% of live individuals) of *B. tabaci* preferred dinotefuran-treated tobacco plants, indicating that the insecticide did not greatly influence the preference of *B. tabaci*. Compared with the control (8%), dinotefuran-treated tobacco plants gave higher mortality of *B. tabaci*. Previous study also indicated that dinotefuran, as the third generation of neonicotinoids is one of the most powerful insecticides against *B. tabaci* (Qu et al., 2017). It has also been used as a systemic insecticide in eggplant trap-plants for *B. tabaci* (Choi et al., 2016).

Contents of dinotefuran in tobacco leaves

Analytical method optimization and validation

This study applied the modified QuEChERS method to analyze dinotefuran in tobacco leaves because it is very simple but robust. Recovery rates from both clean-up methods were not significantly different (Table 2). At two spiking levels, the recoveries of Clean-up 1 ranged from 93.2% to 93.7% and the recoveries of Clean-up 2 ranged from 87.6% to 92.8%, demonstrating that the analytical method was effective. Relative standard deviation for both methods was less than 7.0%, indicating both methods are

Table 2. Recovery rates obtained from two clean-up procedures

Spiking level (mg/kg)	Recovery rates (%) ± RSD[a)]	
	Clean-up 1	Clean-up 2
10	93.2 ± 6.2	87.6 ± 2.4
100	93.9 ± 4.7	92.8 ± 6.5

[a)] RSD, relative standard deviation

accurate. However, d-SPE containing GCB (Clean-up 1) with $MgSO_4$ and PSA was selected for real sample analysis in this study. GCB in the d-SPE clean-up procedure is known to remove high contents of chlorophyll derived from green vegetables (Kim et al., 2019; Lee et al., 2017). $MgSO_4$ and PSA are common sorbents for adsorbing strong acids or polyacidic materials mostly derived from sample matrices in the QuEChERS clean-up. However, it has been reported that GCB retains planar pesticides (Kim et al., 2019) but in this study, the recoveries indicated that no dinotefuran was retained by GCB in the clean-up procedure. It can be hypothesized that the use of GCB can reduce the chromatographic problems that interfere with sensitive analysis or escalate the maintenance cost of the instrument by removing the tobacco-derived chlorophyll.

Dinotefuran concentrations in tobacco leaves and mortality of *B. tabaci*

The dinotefuran concentrations in tobacco leaf location (upper, middle, and lower) were obtained after its application to the soil in greenhouse conditions (Fig. 7). The results showed that the dinotefuran concentrations in upper leaves were generally higher than those in middle and lower leaves (Fig. 5), Within 12 hours, the dinotefuran concentration reached 4 mg/kg on the upper leaves. The peak concentration was recorded at 20 days after treatment. After 20 days, the insecticide concentration of upper side leaves slowly decreased but steadily increased in middle and lower leaves. Dinotefuran has relative stability in soil, and its half-life has been reported to be 50 to 100 days in soil (Liu et al., 2021; Morrissey et al., 2015). This data demonstrated that the insecticide quickly moves through the plants, and can persist in the leaves for more than 3 months. Therefore, dinotefuran-treated plants can potentially sustain a control effect on *B. tabaci* populations over an extended period.

The mortalities of *B. tabaci* recovered from tobacco leaves were 73-94% at 12 hrs and 72-100% at 1, 2, 3, 6, 9 and 35 d after treatment (Fig. 8). Slightly higher mortality was recorded in the upper leaves. Additionally, there was a steady increase in mortality rates over time. Mortality steadily increased from 12-20% at 2 hrs to 24 hrs after the introduction of dinotefuran-treated tobacco plants in greenhouse. This data indicated that *B. tabaci* can be controlled at one day after dinotefuran-treated tobacco plants are placed in the greenhouse.

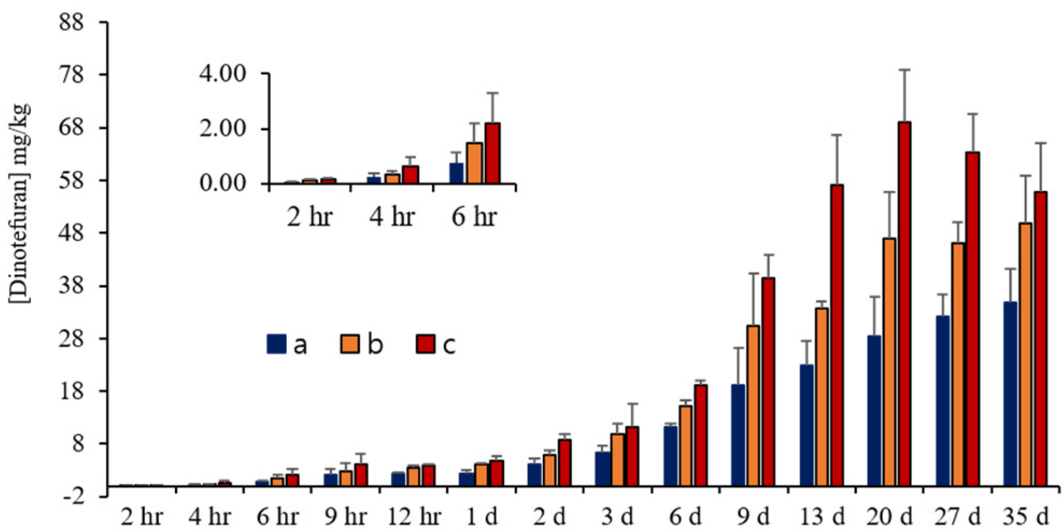

Fig. 7. The concentrations of dinotefuran in tobacco leaves by leaf location (lower leaves (a), middle (b), and upper leaves (c)).

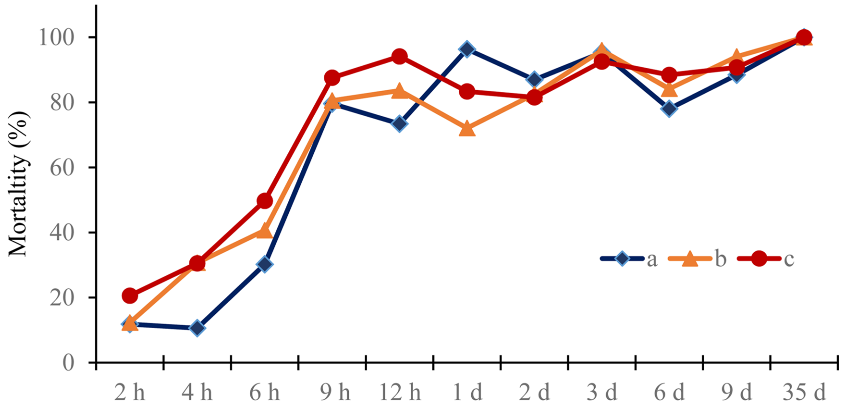

Fig. 8. The mortality of *B. tabaci* in tobacco leaves treated with dinotefuran (lower leaves (a), middle (b), and upper leaves (c)).

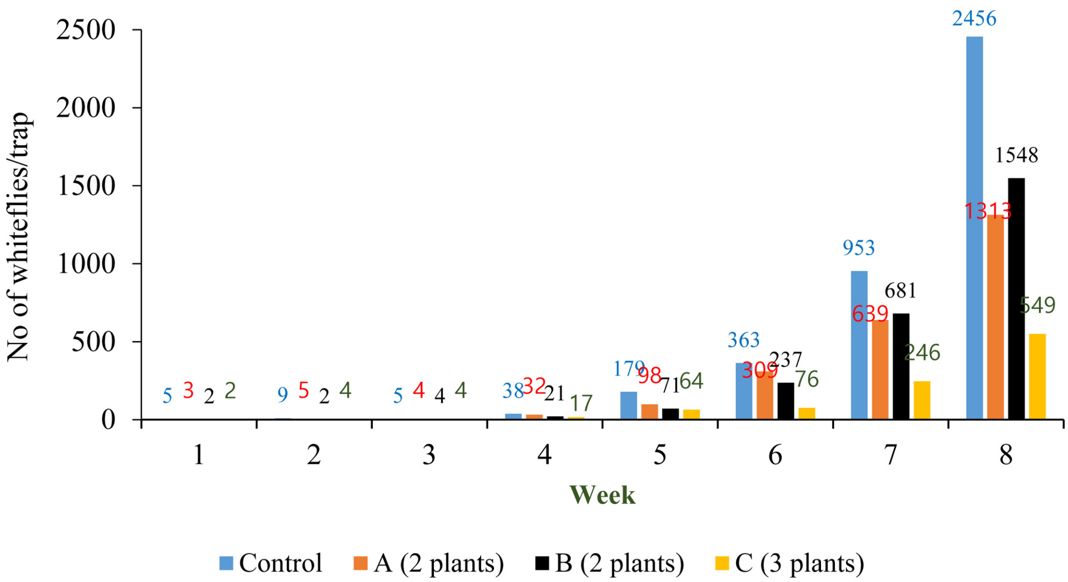

Fig. 9. The number of *B. tabaci* per trap. (A) two tobacco plants placed in the greenhouse located nearby control greenhouse, (B) two tobacco plants, and (C) three tobacco plants.

Greenhouse experimentation

The results (Fig. 9) showed that the insecticide-treated tobacco plants significantly controlled *B. tabaci* populations in the greenhouse. The number of *B. tabaci* in the control greenhouse skyrocketed eight weeks after the release of the initial *B. tabaci* population (Fig. 9). Compared with that of control greenhouse (100%), the population of *B. tabaci* in greenhouses A and B increased by 53% and 63% and the population in greenhouse C increased by 22%. The results in greenhouse C highlight the importance of selecting the appropriate spacing of trap plants to maximize efficiency in attracting and controlling the targeted pest (placed at 5 m distance). Correct spacing can be effective in controlling *B. tabaci* populations in greenhouse. The control effect of dinotefuran lasted for more than two months in this study. This research demonstrated that tobacco plants can play an effective role in controlling the rapid increase of *B. tabaci* in the tomato greenhouse condition.

It also proved that the method using dinotefuran-treated tobacco plant can be a robust, reliable, and cost-effective strategy in reduction of the *B. tabaci* population in tomato greenhouse cultivation. High concentration of dinotefuran was shown to persist in the tobacco leaves for 35 d, and *B. tabaci* population was controlled for more than 2 months with dinotefuran treated tobacco plants in the tomato greenhouses. However, considering the possibility of insecticide resistance development in the targeted pest, it is advisable to develop a treatment regime including different systemic pesticides such as cyantraniliprole EC or sulfoxaflor SC, which are equally effective as demonstrated in the current study during the screening phase. Tobacco plants have many advantages such as easy management and can be grown for more than 3 months, and thus, new tobacco plants treated with different kinds of insecticides can easily be placed in the same greenhouse.

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

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