

Article

Precise Control and Prevention Methods for Whitefly in Greenhouse Vegetables

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Abstract: *Trialeurodes vaporariorum* Westwood (greenhouse whitefly) are worldwide polyphagous pests of economic importance that damage solanaceous vegetables. Neonicotinoid pesticides and parasitoid *Encarsia formosa* Gahan are the main management strategies applied worldwide, but precise control methods in greenhouse vegetables need to be developed to reduce the application amounts of pesticides and improve the suppression of whitefly populations. Therefore, we assessed the indoor acute toxicities and risk assessment of neonicotinoids for *T. vaporariorum* and *E. formosa* adults and pupae and compared the control effects of *E. formosa* and neonicotinoid acetamiprid. According to the acute toxicities results, most neonicotinoid insecticides were more toxic to *E. formosa* than *T. vaporariorum*, and pupae were much less susceptible than adults of both species. Moreover, acetamiprid had a low risk effect on *E. formosa*. Sole application of *E. formosa* and acetamiprid could effectively control *T. vaporariorum*, but their combined application resulted in antagonistic effects on the control of *T. vaporariorum*. The results showed that a combined application or sole use of *E. formosa* could reduce the use of insecticides, slow down the development of insecticide resistance in whiteflies, and improve the efficiency of controlling the growth of whitefly populations in greenhouse vegetable production.

Keywords: *Trialeurodes vaporariorum*; greenhouse vegetable; *Encarsia formosa*; neonicotinoid; integrated strategy



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1. Introduction

Pest management has always been an important scientific issue in food production and agricultural ecology, as 20–40% of crop production is lost to pests annually [1,2]. Whiteflies, including the tobacco whitefly (*Bemisia tabaci* Gennadius) and the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood, Hemiptera: Aleyrodidae), are the most economically important agricultural pests in the world, infesting many agricultural and ornamental plants grown under greenhouse and field conditions [3]. In particular, solanaceous vegetables, such as tomatoes, cucumbers, eggplants, and pepper, which accounted for 32% of global total vegetable production in 2020 [4], are mainly infested by whiteflies. Whiteflies cause devastating damage by direct phloem feeding and honeydew excretion, and indirectly acting as a medium for sooty mold fungi and vectors of plant pathogens [5,6].

Although many novel control strategies such as semiochemicals, endosymbionts, RNA interference, and genetic modifications of plants for the expression of anti-whitefly proteins [7–9], chemical pesticides and parasitoid *Encarsia* spp. are still the main management strategies applied worldwide [10].

It is a fact that the use of chemical pesticides has negative effects on environmental and ecological safety and food security [11,12]. Furthermore, the excessive and continuous application of pesticides has led to the development of resistance in whiteflies [13–17]. Thus, strictly controlling the types and amounts of insecticides used is crucial for managing pesticide resistance and minimizing ecological side effects. Neonicotinoid insecticides are most commonly used to control sucking pests; they had a 25% share of the global insecticide market in 2014 [18]. They are broad spectrum insecticides with high target specificities and toxicities and long persistence times [19,20]. However, whiteflies have developed a high level of resistance to neonicotinoids imidacloprid and thiamethoxam [21], and the European Union prohibited the outdoor use of three neonicotinoids (clothianidin, imidacloprid, and thiamethoxam) in 2018 with concern about their effects on pollinators [22–24]. However, other neonicotinoids like acetamiprid and thiacloprid are friendly to pollinators [25,26], and using low doses of insecticides in combination with natural enemies can be an effective strategy for controlling pests such as *Myzus persicae* (green peach aphid) and *Frankliniella occidentalis* (western flower thrips) [27,28]. Whether low-dose neonicotinoids combined with *Encarsia* spp. can effectively control whiteflies needs further investigation.

Encarsia formosa Gahan, which can feed on first and second instar nymphs and oviposit in second to fourth instar nymphs of whiteflies [29–31], is the most important and successful biological control agent for the control of whiteflies under protected cultivation [32]. Both inundative and seasonal inoculative release methods are applied to control greenhouse whiteflies with *E. formosa*. When managing whiteflies, it is important to consider the host plant species and the development stage of the whiteflies to determine the most effective control methods [33]. However, the developmental rate and daily parasitism of *E. formosa* are influenced by the temperature of the environment, and are disadvantaged at lower temperatures [34]. Under these conditions, it is better to utilize pesticides to control whiteflies. Although it is known that *E. formosa* adults are extremely sensitive to the neonicotinoids imidacloprid, thiamethoxam, nitenpyram, and acetamiprid [35], it is still necessary to test the toxicity of neonicotinoid insecticides to the different developmental stages of *E. formosa* and evaluate the possibility of combining neonicotinoids and *E. formosa*.

In this study, we estimated the susceptibility of *T. vaporariorum* and *E. formosa* to neonicotinoid pesticides and compared the control effects of *E. formosa* and neonicotinoid pesticides applied separately or combined in low doses. The main aim was to develop precise whitefly control methods in greenhouse vegetables, reduce the application amounts of pesticides, and improve the suppression of whitefly populations.

2. Materials and Methods

2.1. Insects and Insecticides

Laboratory colonies of *T. vaporariorum* and *E. formosa* were collected from tomato fields in Jinan, Shandong Province, China (36.40° N, 117.00° E). The *T. vaporariorum* were reared on tobacco seedlings, and the *E. formosa* were reared on tobacco seedlings infected with *T. vaporariorum* nymphs growing on tobacco leaves. All laboratory experiments were conducted in an artificial climate chamber (YCRC-300C, Jiangnan Instrument Factory, Ningbo, China) at 26 ± 1 °C, relative humidity of $70 \pm 5\%$ RH, and photoperiod of 14 L:10 D, in the Institute of Plant Protection, Shandong Academy of Agricultural Sciences.

Eight neonicotinoid insecticides were tested in this study, including imidacloprid WG (70%, Bayer Crop Science, Leverkusen, Germany), nitenpyram AS (10%, Zhejiang Shijia Technology Co. Ltd., Huzhou, China), acetamiprid SP (20%, Jiangsu Longdeng Chemical Co. Ltd., Changzhou, China), thiacloprid SC (2%, Shandong Guorun Biological Pesticide Co. Ltd., Taian, China), thiamethoxam WG (25%, Syngenta Crop Protection, Basel, Switzerland), clothianidin SC (20%, FMC Corporation, Philadelphia, PA, USA), dinotefuran

SG (20%, Mitsui Chemicals AGRO, Tokyo, Japan), and flupyradifurone SL (17%, Bayer Crop Science, Monsanto, St. Louis, MO, USA). All pesticide stock solutions were prepared with distilled water (without a carrier solvent) immediately prior to use.

2.2. Acute Toxicity Bioassays

Leaf-dip bioassay [36] was used to detect the toxicity of neonicotinoid insecticides on whitefly adults. Leaf discs (20 mm in diameter) collected from tomato leaves were dipped into distilled water or different concentrations of insecticides (Table 1) for 10 s and dried with filter paper in a ventilated hood for 20 min. Subsequently, the leaf discs were placed on agar beds (2 mL, 20 g/L) in glass tubes with their adaxial surface downwards. About 20 adult whiteflies were added into each tube and the tube was covered with a black cloth. The mortality was scored after 48 h.

Table 1. Neonicotinoid insecticides and concentrations used in this study.

Insects	Test Concentration (mg a.i. L ⁻¹)							
	Imidacloprid	Nitenpyram	Acetamiprid	Thiacloprid	Thiamethoxam	Clothianidin	Dinotefuran	Flupyradifurone
Adult of <i>T. vaporariorum</i>	2.5	2.5	7.6	20	5	2.5	6.1	4.76
	5	5	12	40	10	5	9.766	8.57
	10	10	19	80	20	10	15.625	15.43
	20	20	33.33	160	40	20	25	27.78
	40	40	50	320	80	40	40	50
Nymph of <i>T. vaporariorum</i>	3.5	11.11	1	22.22	1.25	93.75	10	62.5
	35	33.33	10	66.67	12.5	375	20	125
	350	100	100	200	125	1500	40	250
	3500	300	1000	600	1250	6000	80	500
	35,000	900	10,000	1800	12,500	24,000	160	1000
Adult of <i>E. formosa</i>	10,937.5	0.1	0.105	0.002	0.001	0.02	0.02	0.1953
	21,875	0.5	0.420	0.02	0.006	0.2	0.2	0.7813
	43,750	2.5	1.680	0.2	0.036	2	2	3.125
	87,500	12.5	6.720	2	0.216	20	20	12.5
	175,000	26.5	26.880	20	1.296	200	200	50
Pupae of <i>E. formosa</i>	0.012	0.02	0.032	0.8	0.005	1.28	0.16	0.39
	0.12	0.2	0.16	4	0.05	3.2	0.64	1.5625
	1.2	2	0.8	20	0.5	8	1.6	6.25
	12	20	4	100	5	20	4	25
	120	200	20	500	50	50	10	100

Larval-dip bioassay following Elbert and Nauen [37] with minor modifications was carried out to detect the whitefly nymphs. Tomato plants with four leaves were prepared for adult whiteflies to lay eggs for 24 h. When the 3rd instar nymphs appeared on the infested tomato seedlings, the leaves with about 40 3rd nymphs were picked and dipped into distilled water or different concentrations of insecticides (Table 1) for 10 s. The bottom stems of leaves were immersed in water after the solution of insecticides on the leaves dried. The number of total treated nymphs and emerged adults were counted after adult emergence (about 15 days post-treatment).

The adult *E. formosa* bioassay was conducted following the dry film residual contact method [38]. Different concentrations of insecticides (Table 1) or distilled water were added into a glass tubes (3 cm in diameter, 12 cm in height), respectively, and the glass tubes were rolled for approximately 2 h to generate a homogenous film on the surface of the vials. About 50 ± 10 newly emerged adults (after 24 h) in glass tubes were allowed to crawl to each treated tube in the light. Small streaks of 10% honey solution scribed with cotton swabs were added as food sources. The glass tubes were sealed with black cloth. Mortalities were recorded after 24 h. The adults were considered dead if their feet failed to move upon a light touch.

For *E. formosa* pupae, pupae-dip bioassay was conducted following Simmonds et al. [39] with minor modifications. Pupae sticking to cards were dipped into distilled water or different concentrations of insecticides (Table 1) for 10 s when they turned black for 3 days. The cards were then placed in glass tubes after they were dried. The number of treated pupae and emerged adults were counted after 15 days of treatment.

Five concentration gradients were set for each insecticide, and each treatment had three biological replications. All treatments were kept at 25 ± 2 °C, $70 \pm 5\%$ RH, and a photoperiod of 16 L:8 D (h). The obtained data were corrected by the mortality of control (which was never larger than 10%) using Abbott's formula [13] prior to analysis. The slope, 50% lethal concentration (LC₅₀), 95% confidence interval, and the application rate for 50% mortality (LR₅₀) were estimated using SPSS 18.0 (Chicago, IL, USA).

2.3. Risk Assessment

The risk assessment for *E. formosa* was based on the environmental risk assessment guidelines for non-target arthropods, which was described by Lin et al. (2020) [27]. The in-field predicted exposure rate ($PER_{in-field}$) = the application rate \times the multiple application factor (MAF) and the hazard quotient ($HQ_{in-field}$) = $PER_{in-field}$ / the application rate for 50% mortality (LR₅₀). $HQ_{in-field} < 2$ indicated low risk; higher tier tests were needed if $HQ_{in-field} \geq 2$ [27].

2.4. Greenhouse Efficacy Trial

The greenhouse efficacy trials were conducted in a greenhouse from 12 January to 17 April 2020. Eight cages ($90 \times 90 \times 90$ cm³) were set in a greenhouse and 20 tomato seedlings (15–20 cm in height) were in each cage. On 12 January, about 100 adult whiteflies were released into each cage and the number of whiteflies on each seedling was surveyed on 18 January. The following four treatments were established: (I) control group: 10 mL water sprayed only; (II) biological group: 20 *E. formosa* pupae stuck on a paper card were introduced to each seedling; (III) chemical group: 10 mL acetamiprid solution with recommended concentration (64 mg a.i./L) was sprayed; (IV) integrated group: 10 mL acetamiprid (10.31 mg a.i./L, the LC₂₀ for adult *T. vaporariorum*) was sprayed and 10 *E. formosa* pupae stuck on a paper card were introduced to each seedling. Each group contained three repetitions. In all groups, water and acetamiprid solution was sprayed evenly on the back and top sides of the leaves. Acetamiprid or water was sprayed on 21 January and 31 January, and the *E. formosa* pupae were introduced on 22 January, 1 February, and 11 February. The number of nymphs and adults of *T. vaporariorum* and black pupae of *T. vaporariorum* that were parasitized with *E. formosa* in each treatment were counted every 10 days from January 18. The control effect (%) was computed per Lin et al. (2021) [28].

2.5. Statistical Analysis

The population reduction rate for *T. vaporariorum* and the control effect of each treatment were calculated. A two-way ANOVA was used to analyze the control efficiency of *T. vaporariorum* under different treatments. The Student–Newman–Keuls test was used as a post hoc test ($p < 0.05$) when significant differences were detected among different treatments on the same day.

3. Results

3.1. Acute Toxicity of Neonicotinoids on *T. vaporariorum* and *E. formosa*

The acute toxicities of the eight neonicotinoid insecticides to adults and nymphs of *T. vaporariorum* and *E. formosa* are shown in Tables 2 and 3, respectively. The LC₅₀ values of the neonicotinoids on *T. vaporariorum* adults ranged from 6.82 mg a.i. \cdot L⁻¹ for nitenpyram to 59.22 mg a.i. \cdot L⁻¹ for thiacloprid, which were much higher than the LC₅₀ values of most neonicotinoids on adult *E. formosa* ranging from 0.043 mg a.i. \cdot L⁻¹ for thiamethoxam to 3.28 mg a.i. \cdot L⁻¹ for flupyradifurone, except for the confusingly high LC₅₀ value of imidacloprid on *E. formosa* adults of 140,034.5 mg a.i. \cdot L⁻¹ (Table 2). The *T. vaporariorum* nymphs were much less sensitive to neonicotinoids than the *E. formosa* pupae. The LC₅₀ values of *T. vaporariorum* ranged from 32.71 mg a.i. \cdot L⁻¹ for nitenpyram to 377,488.75 mg a.i. \cdot L⁻¹ for acetamiprid, while LC₅₀ values of *E. formosa* ranged from 1.99 mg a.i. \cdot L⁻¹ for dinotefuran to 22.19 mg a.i. \cdot L⁻¹ for thiacloprid (Table 3). In addition, the *T. vaporariorum*

nymphs and *E. formosa* pupae were less sensitive to neonicotinoids than the adults, except for imidacloprid.

Table 2. Acute toxicity data of eight neonicotinoid pesticides tested on adult whiteflies *T. vaporariorum* and parasitoid *E. formosa*.

Pesticides	Insects	Acute Toxicity Regression Equation	LC ₂₀ (mg a.i.·L ⁻¹)	95% Confidence Interval (mg a.i.·L ⁻¹)	LC ₅₀ (mg a.i.·L ⁻¹)	95% Confidence Interval (mg a.i.·L ⁻¹)	Correlation Coefficients (r ²)
Imidacloprid	<i>T. vaporariorum</i>	Y = 1.03 X + 3.84	2.05	1.04–3.39	13.32	8.87–19.38	0.972
	<i>E. formosa</i>	Y = 1.74 X – 3.95	45,930.46	36,155.17–58,812.76	140,034.5	100,940.1–234,800.8	0.921
Nitenpyram	<i>T. vaporariorum</i>	Y = 2.79 X + 2.68	3.40	2.55–4.21	6.82	5.72–7.92	0.915
	<i>E. formosa</i>	Y = 0.67 X + 4.97	0.06	0.015–0.16	1.11	0.47–3.35	0.915
Acetamiprid	<i>T. vaporariorum</i>	Y = 2.92 X + 1.20	10.31	8.03–12.26	20.01	17.64–22.46	0.947
	<i>E. formosa</i>	Y = 0.81 X + 4.62	0.27	0.17–0.39	2.98	2.23–4.08	0.990
Thiacloprid	<i>T. vaporariorum</i>	Y = 1.97 X + 1.51	22.13	14.80–29.34	59.22	47.77–71.36	0.952
	<i>E. formosa</i>	Y = 0.49 X + 5.62	0.001	0.0001–0.005	0.06	0.02–0.19	0.940
Thiamethoxam	<i>T. vaporariorum</i>	Y = 3.12 X + 0.80	11.97	9.88–13.96	22.28	19.40–25.78	0.911
	<i>E. formosa</i>	Y = 0.93 X + 6.27	0.005	0.002–0.01	0.043	0.02–0.08	0.968
Clothianidin	<i>T. vaporariorum</i>	Y = 2.33 X + 2.53	5.01	3.94–6.04	11.51	9.78–13.68	0.973
	<i>E. formosa</i>	Y = 0.57 X + 4.90	0.05	0.003–0.23	1.47	0.33–9.58	0.934
Dinotefuran	<i>T. vaporariorum</i>	Y = 3.09 X + 1.31	8.33	6.848–9.668	15.59	13.87–17.54	0.957
	<i>E. formosa</i>	Y = 0.95 X + 4.75	0.24	0.078–0.558	1.86	0.80–5.91	0.904
Flupyradifurone	<i>T. vaporariorum</i>	Y = 2.75 X + 1.88	6.73	5.28–8.07	13.59	11.77–15.57	0.957
	<i>E. formosa</i>	Y = 1.74 X + 4.10	1.07	0.51–1.75	3.28	2.05–5.30	0.948

Table 3. Acute toxicity data of eight neonicotinoid pesticides tested on *T. vaporariorum* nymphs and *E. formosa* pupae.

Pesticides	Insects	Acute Toxicity Regression Equation	LC ₂₀ (mg a.i.·L ⁻¹)	95% Confidence Interval (mg a.i.·L ⁻¹)	LC ₅₀ (mg a.i.·L ⁻¹)	95% Confidence Interval (mg a.i.·L ⁻¹)	Correlation Coefficients (r ²)
Imidacloprid	<i>T. vaporariorum</i>	Y = 0.55 X + 3.13	71.09	19.51–184.10	2359.85	890.51–9409.28	0.98
	<i>E. formosa</i>	Y = 0.79 X + 4.27	0.73	0.26–1.56	8.44	4.12–19.73	0.99
Nitenpyram	<i>T. vaporariorum</i>	Y = 2.38 X + 1.39	14.51	10.25–19.00	32.71	25.91–39.48	0.99
	<i>E. formosa</i>	Y = 0.37 X + 4.65	0.05	0.001–0.24	8.79	2.37–68.20	0.95
Acetamiprid	<i>T. vaporariorum</i>	Y = 0.25 X + 3.61	159.70	53.31–538.63	377,488.75	38,965.09–29,408,114.02	0.92
	<i>E. formosa</i>	Y = 0.67 X + 4.73	0.14	0.09–0.19	2.52	1.87–3.53	0.99
Thiacloprid	<i>T. vaporariorum</i>	Y = 1.37 X + 1.94	41.65	30.08–53.67	171.48	138.59–218.17	0.99
	<i>E. formosa</i>	Y = 1.58 X + 2.88	6.50	4.24–9.07	22.19	16.61–29.90	0.98
Thiamethoxam	<i>T. vaporariorum</i>	Y = 0.47 X + 2.83	640.40	317.67–1588.31	38,856.53	10,848.28–288,236.84	0.92
	<i>E. formosa</i>	Y = 1.06 X + 4.16	0.10	0.46–1.96	6.16	3.05–17.12	0.94
Clothianidin	<i>T. vaporariorum</i>	Y = 1.33 X + 1.08	207.25	138.47–286.82	892.03	692.60–1125.47	0.99
	<i>E. formosa</i>	Y = 1.91 X + 3.06	3.74	3.32–4.17	10.32	9.43–11.34	0.99
Dinotefuran	<i>T. vaporariorum</i>	Y = 1.69 X + 2.38	11.23	8.42–14.02	35.26	30.25–40.83	0.99
	<i>E. formosa</i>	Y = 0.79 X + 4.76	0.17	0.05–0.34	1.99	1.29–3.32	0.97
Flupyradifurone	<i>T. vaporariorum</i>	Y = 1.94 X + 0.70	60.90	45.40–76.39	165.72	140.91–191.11	0.97
	<i>E. formosa</i>	Y = 1.31 X + 4.00	1.32	0.60–2.21	5.78	3.71–8.87	0.97

3.2. Risk Assessments of the Pesticides to *E. formosa* in Field

We conducted an in-field risk assessment since the application of *E. formosa* was limited to the greenhouse. As seen in Table 4, the HQs of imidacloprid and acetamiprid for adult *E. formosa* were less than 2, and the two neonicotinoids were considered low-risk insecticides. HQs of other neonicotinoids ranging from 2.97 to 192.63 indicated that the risks of these neonicotinoids were high risk without further higher tier tests. As for *E. Formosa* pupae, only the HQs of flupyradifurone was higher than 2, indicating a high risk. Other neonicotinoids were low risk to *E. Formosa* pupae since all HQs of these neonicotinoids were less than 2 (Table 4). Thus, only imidacloprid and acetamiprid were low risk to both *E. Formosa* adults and pupae. However, considering the high resistance to imidacloprid in whiteflies and side effects on pollinators, we selected acetamiprid for further research.

Table 4. Risk assessment of eight neonicotinoid pesticides to *E. formosa* adults and pupae based on acute toxicity data and field exposure levels (DT₅₀—50% of pesticide degradation time (usually the default is 10d). MAF—multiple application factor. PER—predicted exposure rate. LR₅₀—application rate for 50% mortality. HQ—hazard quotient).

Pesticides	Period	DT ₅₀ (Days)	Number of Applications	Application Interval (Days)	Recommended Application Rates (g a.i.·ha ⁻¹)	MAF	PER in-Field (g a.i.·ha ⁻¹)	LR ₅₀ (g a.i.·ha ⁻¹)	HQ In-Field	Risk
Imidacloprid	adult pupa	10	2	7	63.06	1.62	101.88	1,537,998.91 92.67	6.62 × 10 ⁻⁵ 1.10	low risk low risk
Nitenpyram	adult pupa	10	3	10	29.99	1.75	52.48	12.18 96.56	4.31 0.54	high risk low risk
Acetamiprid	adult pupa	10	1	365	29.99	1.00	29.99	32.69 27.63	0.92 1.09	low risk low risk
Thiacloprid	adult pupa	10	2	7	9.00	1.62	14.54	0.60 243.69	24.07 0.60	high risk low risk
Thiamethoxam	adult pupa	10	2	7	56.31	1.62	90.97	0.47 67.71	192.63 1.34	high risk low risk
Clothianidin	adult pupa	10	1	365	48.00	1.00	48.00	16.17 113.33	2.97 0.42	high risk low risk
Dinotefuran	adult pupa	10	2	7	120.12	1.62	194.06	20.40 21.84	9.52 1.99	high risk low risk
Flupyradifurone	adult pupa	10	2	7	102.00	1.62	164.79	35.97 2.60	4.58 5.78	high risk high risk

3.3. Greenhouse Efficacy Trial

The population of *T. vaporariorum* kept increasing throughout the entire investigation period, with the total population number increasing from 10 to 795.75 per plant (Figure 1). The number of *T. vaporariorum* adults in the biological group was always below 17.23 per plant, while the highest number of *T. vaporariorum* pupae was 97.33, observed on 27 February. The highest population number of *T. vaporariorum* in the chemical group was observed on 17 April, with 29.78 adults and 39.5 nymphs per plant. The total population of *T. vaporariorum* in the integrated group was 3.65 per plant on 7 February, and began to increase after 17 February, reaching 316.48 per plant on 17 April (Figure 1). The control effects of *E. Formosa* were always over 90%, except on 31 January (−2.21%) and 27 February (56.84%). In contrast, the control effects of the chemical group were all above 92.5% during the investigation (Table 5). The integrated group demonstrated the highest control effect of 97.74% on 7 February, followed by a decreased control effect of 72.2% on 7 April. Among these three groups, the control effects of the chemical group were significantly higher than those of the biological group and the integrated group from 31 January to 8 March. However, the control effect of the biological group became the highest (Table 5).

Table 5. Control effect of *T. vaporariorum* populations under different treatments compared with the untreated control on tomato seedlings set up in field cages of 90 × 90 × 90 cm within a greenhouse. Assigned lowercase letters to each group of data (same day) indicate the results from Student–Newman–Keuls multiple comparison tests; the same letters in the same column indicate that values are not significantly different.

Control Measures	Control Effect during the Experiment (%)							
	31 Jan.	7 Feb.	17 Feb.	27 Feb.	8 Mar.	18 Mar.	28 Mar.	7 Apr.
<i>E. formosa</i>	−2.21 ± 9.71 c	93.94 ± 0.05 c	78.26 ± 4.85 b	56.84 ± 6.93 b	90.40 ± 1.27 b	98.00 ± 0.01 a	98.98 ± 0.02 a	99.34 ± 0.02 a
Acetamiprid	98.63 ± 0.28 a	99.94 ± 0.00 a	98.54 ± 0.78 a	97.96 ± 0.21 a	98.00 ± 0.03 a	97.53 ± 0.32 a	95.91 ± 0.07 a	92.50 ± 0.08 b
Acetamiprid + <i>E. formosa</i>	38.66 ± 20.94 bc	97.74 ± 0.18 b	88.91 ± 0.37 ab	89.65 ± 0.66 a	89.65 ± 0.33 b	86.65 ± 1.40 b	77.11 ± 1.41 b	72.20 ± 0.74 c

The number of *E. Formosa* pupae was investigated in both the biological and the integrated group (Figure 2). In the biological group, *E. Formosa* pupae only emerged between 27 February and 18 March, with the population number increasing from 27.28 to

82.4 per plant. On the other hand, the population numbers in the integrated group were significantly lower, ranging from 0.53 to 0.8 *E. Formosa* pupae per plant from 8 March to 18 March.

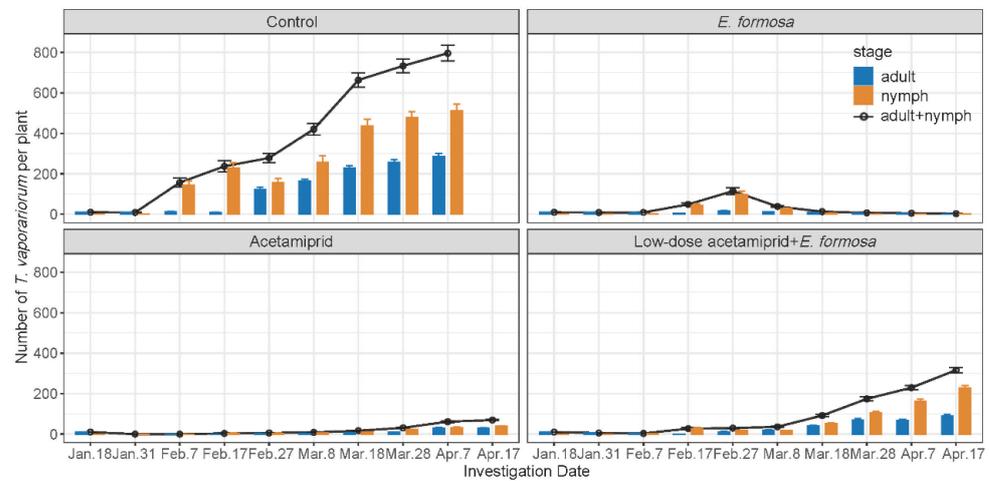


Figure 1. Number of *T. vaporariorum* (mean \pm SE) under different treatments on tomato seedlings grown in field cages of $90 \times 90 \times 90$ cm set up within a greenhouse.

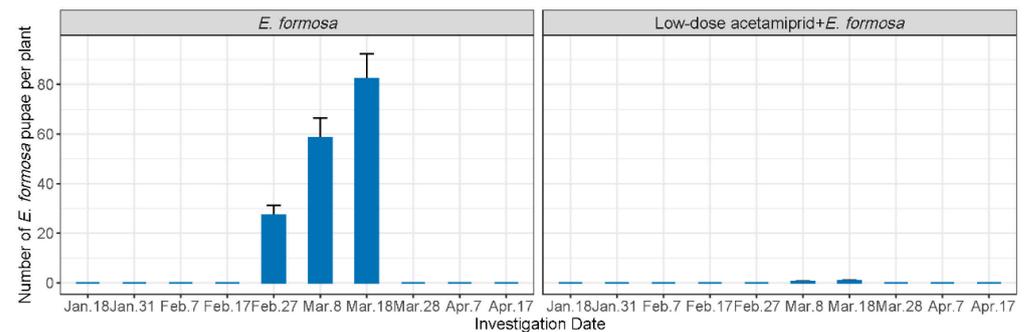


Figure 2. Number of *E. Formosa* pupae (mean \pm SE) in each treatment.

4. Discussion

Whiteflies are economically important pests that induce havoc on a wide range of crop plants. They have a high reproduction rate and can achieve an exceptionally high population size within a few generations [10]. The management of whiteflies is difficult due to the tiny body size, covert nymph, and population explosion. In this research, we found that the sensitivity of third instar nymphs of whiteflies to neonicotinoids is lower than that of adults, and the LC_{50} values of some neonicotinoids (imidacloprid, acetamiprid, thiamethoxam, and clothianidin) for nymphs far exceed the recommended dosage. Field trial data also show that the continuous application of neonicotinoid acetamiprid twice in the early stage of whitefly occurrence effectively controls the population of whiteflies for 3 months. We also found that the sensitivity of *E. Formosa* pupae to neonicotinoids is lower than adults, and the use of low doses of acetamiprid and *E. Formosa* can effectively control the number of whiteflies for 45 days. In the early stages of whitefly occurrence, the release of *E. Formosa* can continuously control the number of adult whiteflies at a lower level.

The continuous and effective control of whiteflies with neonicotinoid acetamiprid in this research was due to the sensitive population of whiteflies, and the application of acetamiprid during the period when only adults and young nymphs were present. Here, we found that neonicotinoid toxicity toward adults was high, but was relatively low toward third instar *T. vaporariorum* nymphs. Furthermore, first and second instar whitefly nymphs are more sensitive to neonicotinoids than adults, while third instar nymphs are the least sensitive [15,40,41]. Thus, application of acetamiprid significantly reduces the number of

adults and early aged nymphs. However, whiteflies have tremendous potential to develop resistance to insecticide [13,14,21], and increasing the resistance levels to neonicotinoids also increases the LC₅₀s for first instar nymph in whitefly field strains [38]. Therefore, in order to slow down the development rate of resistance to insecticides and keep the high control effects of insecticides, it is necessary to control the application time of insecticides and reduce the application amount. In addition, the population size of whiteflies began to increase after March 28, and would develop into an outbreak without taking measures. This means that the effectiveness of applying insecticides to control whiteflies can persist for 3 months at the most, and other methods need to be considered afterwards.

In this research, the application of *E. Formosa* could continuously control the number of adult whiteflies at a lower level, while the number of *T. vaporariorum* nymphs increased to 97.3 per plant on 27 February. We believe that these nymphs may have been parasitized due to the subsequent decrease in the population of whiteflies and the increase in *E. Formosa* pupae. However, in the last month of the experiment, both populations of whiteflies and *E. Formosa* decreased to the lowest level, meaning the population growth could not be controlled by *E. Formosa* after new whiteflies moved in. Furthermore, most greenhouse vegetables grown in winter are infested by *T. vaporariorum*, while control by *E. formosa* is delayed due to the significantly prolonged development duration at the low winter temperatures (<15 °C) [42,43]. At these times, applying insecticides or increasing the amount of *E. formosa* may be helpful for improving control effects on whiteflies. In order to not weaken the control effects of *E. formosa* when applying insecticides during the use of *E. formosa*, a feasible approach is to improve the insecticide resistance in *E. formosa*. The development of insecticide resistance in parasitoids could be affected either directly by exposure to the spray or indirectly by factors such as the insecticide penetrating the body of host insects and exposure to plants when host insects feed on them [44]. The resistance of parasitoids *Tetrastichus brontispae* and *Asecodes hispinarum* to acetamiprid has been increased by using the resistance found in *Brontispa longissimi* [45]. In this research, *E. formosa* adults showed a high level of resistance to imidacloprid. Therefore, it may be feasible to develop resistant populations of *E. formosa* to reduce the negative effect of neonicotinoid insecticides.

In this research, the control effects of a combination of low dose acetamiprid and *E. formosa* could last for 45 days. The early control of the number of whitefly adults and nymphs may have been caused by low doses of insecticide acetamiprid, affecting the number and fertility of *T. vaporariorum* adults. Another possibility is that *E. formosa* fed on the *T. vaporariorum* nymphs. Previous research has shown that *E. formosa* feeds on all stages of *T. vaporariorum*, especially more frequently on second and late fourth instars than on first and third instars [46], while preferring third and early fourth instars for oviposition [47]. Previous studies have focused on combining insecticides with biocontrol agents, applied as low-dose insecticides and predators, achieving good control effects [24,25,48,49]. However, in this research, the number of *E. formosa* pupae never exceeded one per plant, and the whitefly population was gradually out of control after March 18. Sublethal insecticide applications induce adverse effects, such as prolonged development duration, reduced emergence, decreased reproduction, and weakened parasitic behavior [50,51], therefore, in order to achieve persistent integrated control effect, it is better to delay the release timing of *E. formosa* and increase the release frequency one or two more times. Whether this advice can improve the control effect of combination of low dose acetamiprid and *E. formosa* needs further validation.

5. Conclusions

This research analyzed the advantages and disadvantages of neonicotinoids and *E. formosa* in controlling whiteflies based on indoor toxicity testing and semi-field experiments, and preliminarily explored the effectiveness of using low-dose insecticides and *E. formosa* in combination to control whiteflies. In order to reduce the use of insecticides, slow down the development of insecticide resistance in whiteflies, and improve the efficiency of controlling the growth of whitefly populations in greenhouse veg-

etable production, further studies based on precise application timing and *E. formosa* resistance development are needed to improve the efficiency of combined *E. formosa* and acetamiprid treatments.

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