Thiacloprid + Deltamethrin Against the Cotton Aphid and the Parasitoid Wasp *Aphidius Colemani*: From Lethal to Sublethal Population Effects

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Abstract

The side effects of insecticides on non-target species are important to secure the intended effectiveness of pest management programs. The cotton aphid *Aphis gossypii* is a frequent problem in industrial greenhouse cultivation, and insecticide and parasitoids are often used for crop protection. Among insecticides, mixtures are playing increasing roles in pest management in greenhouse production system, as well as parasitoid use. The insecticide mixture thiacloprid + deltamethrin and the parasitoid wasp *Aphidius colemani* are the main agents used against the cotton aphid and are the focus of this study. Here, we report the effects of the thiacloprid + deltamethrin on *A. gossypii* and *Aphidius colemani*. The sublethal concentrations 2.80 mg ai/l (LC_{10}) and 7.28 mg ai/l (LC_{30}) compromised the demographic parameters of the aphid progeny and increased the development time at all juvenile stages. In addition, fecundity, longevity and population parameters (r , R_0 , and T) were significantly impaired by thiacloprid + deltamethrin, at both concentrations. The median lethal time estimate (LT_{50}) for *Aphidius colemani* was 18.73 h; the parasitoid mortality was significant up to 24 h after application, but decreased afterwards. Regardless, the parasitoid life table parameters were also compromised by insecticide exposure, similarly to the parasitoid population growth and parasitism efficacy. These results demonstrate the effect of the insecticide mixture against *A. gossypii*, as well as the increased risk of its adverse impacts on the parasitoid *Aphidius colemani*, which signals against their simultaneous application to control this pest species.

Introduction

Aphis gossypii (Glover) (Hemiptera: Aphididae) is a cosmopolitan and polyphagous pest that attacks a large number of plant species around the world. This insect pest species can cause severe damage to its host through feeding and disease transmission^{1,2}. The main management tactic used against *A. gossypii* (Glover) (Hemiptera: Aphididae) is the application of different chemical insecticide groups, including neonicotinoids, carbamate, pyrethroids and organophosphates³. However, the chemical management is costly, provides only a temporary control and promotes the rapid development of resistance⁴. Consequently, the application of insecticide mixtures has increased, as such resistance management tactic helps delaying problems related to insecticide resistance. For example, the use of thiacloprid + deltamethrin against sucking and chewing pests takes advantage of the systemic activity of the former compound, a neonicotinoid, and the contact activity of the latter mixture component, the pyrethroid deltamethrin⁵. Despite their potential problems, insecticide mixtures combined with suitable biocontrol agents have great potential impact for pest management^{6–8}.

Insecticide resistance and the side effects of these compounds on non-target organisms are among the biggest concerns for pest management in agro-ecosystems^{9,10}. A common (allegedly) cause of cotton aphid outbreaks is the presence of natural enemies, and, in some cases, the stimulation of aphid reproduction by pesticides, which reinforces the above stated concerns^{11,12}. The impact on biocontrol agents is particularly serious in contexts of frequent use, such as in greenhouse cultivation systems,

where insecticide application is also important. Thus, their compatibility is a matter of interest for effective pest management ^{13,14}.

The use of natural enemies is increasing in greenhouse pest management programs due to their efficacy and applicability ⁸. *Aphidius colemani* Viereck (Hymenoptera: Braconidae) is one of the parasitoids economically produced and globally distributed ^{15–17}. This parasitoid species is an important biological control agent, due to its mobility, high fecundity rate and short generation time, especially in industrial greenhouses. The performance of *Aphidius colemani* against aphid pest populations in greenhouses may reach efficacy levels as high as that of high-cost insecticides ¹⁸. In addition, the rearing of this parasitoid species is relatively easy, and the species is largely used against the cotton aphid ^{8,18}.

The combined use of biological control agents and insecticides requires awareness of the consequences of insecticides on the target insect, including natural enemies and pests ^{19,20}. Nonetheless, the use of insecticide mixtures is increasing, which aggravates the concerns regarding their association to biocontrol agents in greenhouse pest management. The impact of such mixtures goes beyond their potential lethal effects, as a range of sublethal effects frequently take place and are as important as or even more so than mortality, since they last longer and affect population dynamics ^{10,21,22}. Life-table (demographic) assessments are useful in that regard, as they encompass the impact of lethal and sublethal effects of insecticides ²³.

Results

Insecticide exposure

According to the results, the values 2.8, 7.2 and 14.1 mg ai/ l were estimated for LC₁₀, LC₃₀ and LC₅₀, respectively ($\chi^2 = 4.68$; P -value = 0.58).

Diagnostic exposure time for the parasitoid wasp

According to the results, the median lethal time for 50% of the wasp population was 24 h (Fig. 1). A quick decline in mortality was observed after such exposure time.

Insecticide effect on the aphid life-table

Thiacloprid + deltamethrin had a negative effect on the progeny development of treated adult aphids, which increased due to insecticide exposure (Table 1). Adult longevity also differs among the treatments. It is decreased by insecticide exposure, which was also observed for egg-laying and fecundity (Table 1).

Table 1

Life-history parameters (mean $\pm$ SE) of the cotton aphid *Aphis gossypii*, untreated and treated with the LC₁₀ and LC₃₀ of thiacloprid + deltamethrin

Parameters	stage	Treatment		
		Control	LC ₁₀	LC ₃₀
		Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Development time (d)	Nymph 1	1.24 $\pm$ 0.06 a	1.96 $\pm$ 0.04 b	3.64 $\pm$ 0.11 c
	Nymph 2	1.14 $\pm$ 0.05 a	1.48 $\pm$ 0.07 b	2.27 $\pm$ 0.08 c
	Nymph 3	1.08 $\pm$ 0.04 a	1.27 $\pm$ 0.07 b	1.92 $\pm$ 0.07 c
	Nymph 4	1.04 $\pm$ 0.03 a	1.49 $\pm$ 0.08 b	2.5 $\pm$ 0.13 c
Longevity of adult (d)		25.35 $\pm$ 0.67 a	19.21 $\pm$ 0.6 b	11.91 $\pm$ 0.61 c
Longevity of preadult (d)		4.51 $\pm$ 0.38 a	6.16 $\pm$ 0.09 b	10.38 $\pm$ 0.17 c
Longevity (d)		29.34 $\pm$ 0.83 a	22.38 $\pm$ 1.18 b	17.32 $\pm$ 1.15 c
Adult preovipositional period (d)		0.31 $\pm$ 0.07 a	0.91 $\pm$ 0.09 b	3.38 $\pm$ 0.11 c
Total preovipositional period (d)		4.82 $\pm$ 0.1 a	7.07 $\pm$ 0.13 b	13.76 $\pm$ 0.23 c
Oviposition period (d)		13.18 $\pm$ 0.25 a	11.6 $\pm$ 0.36 b	6.68 $\pm$ 0.51 c
Fecundity (offspring/adult)		80.24 $\pm$ 2.01 a	58.47 $\pm$ 2.4 b	25.88 $\pm$ 2.49 c
SE were estimated by using the bootstrap technique with 100.000 resampling. Difference was compared by the paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the three concentrations.				

The age-stage survival rate (s_{xj}) of each aphid age-stage, at different insecticide concentrations, was plotted in Fig. 2. It follows a concentration-dependent trend with the insecticide mixture, which significantly compromises survival through time. Such negative trends exhibited similar tendency for the age-specific maternity ($l_x m_x$) and the age-specific fecundity (m_x) (Fig. 2). The egg-laying starting age was delayed under insecticide exposure, and the peak of fecundity was 9.8, 6.6 and 4.2 nymphs/female at the control, LC₁₀ and LC₃₀, respectively.

The life expectancy (e_{xj}) for different stages of aphid is shown in Fig. 3. The higher the insecticide mixture concentration, the higher the trend, which was also observed for the age-stage-specific reproductive values (v_{xj}) (Fig. 3).

According to the results, the life-table parameters derived from the previous estimates for *A. gossypii* were also significantly affected (Table 2). The generation time increased and net reproductive rate decreased

as insecticide concentrations increased, which consequently caused a sharp decrease in the intrinsic rate of population growth (r) under insecticide mixture exposure.

Table 2

Population parameters (mean $\pm$ SE) of the cotton aphid *Aphis gossypii*, untreated and treated with the LC₁₀ and LC₃₀ of thiacloprid + deltamethrin

Parameter	Control	LC ₁₀	LC ₃₀
Intrinsic rate of population growth (r) (d ⁻¹)	0.4647 $\pm$ 0.006 a	0.3076 $\pm$ 0.007 b	0.1670 $\pm$ 0.008 c
Net reproductive rate (R_0)	78.64 $\pm$ 2.50 a	50.28 $\pm$ 3.52 b	17.6 $\pm$ 2.38 c
Generation time (T; d)	9.39 $\pm$ 0.11 a	12.73 $\pm$ 0.19 b	17.17 $\pm$ 0.32 c
SE were estimated by using the bootstrap technique with 100.000 resampling. Difference was compared by the paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the three concentrations.			

Life table of parasitoid wasp

Based on the results, life table parameters of *Aphidius colemani* were also significantly compromised by the insecticide mixture, following a concentration-dependent trend, including fecundity, development time and longevity (Table 3). Similarly to that observed for the cotton aphid, the thiacloprid + deltamethrin also compromised the parasitoid age-specific maternity ($l_x m_x$), age-specific fecundity (m_x) and age-specific survival rate (s_{xj}) (Fig. 4). Such trends were also similar to those for the parasitoid age-stage-specific reproductive values (v_{xj}) and life expectancy (e_{xj}) (Fig. 5). These trends significantly impacted the demographic parameters obtained for the parasitoid wasp under exposure to the insecticide mixture (Table 4). Insecticide exposure compromised the mean generation time and net reproductive rate by decreasing the former and increasing the latter, which reduced the intrinsic rate of population growth (r) (Table 4).

Table 3

Life-history parameters (mean $\pm$ SE) of the parasitoid wasp *Aphidius colemani*, untreated or treated with LC₁₀ and LC₃₀ of thiacloprid + deltamethrin

Stage	Treatment		
	Control	LC ₁₀	LC ₃₀
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
Longevity of preadult stages (d)	13.55 $\pm$ 0.14 a	14.53 $\pm$ 0.14 b	15.39 $\pm$ 0.11 c
Longevity of male (d)	17.91 $\pm$ 0.76 a	16.85 $\pm$ 0.94 a	12.88 $\pm$ 1.02 b
Longevity of female (d)	19.81 $\pm$ 0.32 a	18.07 $\pm$ 0.71 b	14.2 $\pm$ 0.86 c
Longevity (d)	32.16 $\pm$ 0.50 a	31.12 $\pm$ 0.75 a	28.16 $\pm$ 0.8 b
Adult preovipositional period (d)	0.48 $\pm$ 0.11 a	0.69 $\pm$ 0.11 a	1.56 $\pm$ 0.12 b
Total preovipositional period (d)	14.22 $\pm$ 0.18 a	15.46 $\pm$ 0.2 b	17.15 $\pm$ 0.19 c
Oviposition period (d)	13.26 $\pm$ 0.41 a	13.27 $\pm$ 0.4 a	9.56 $\pm$ 0.3 b
Fecundity (no. eggs/female)	101.19 $\pm$ 2.58 a	95 $\pm$ 4.28 a	58.43 $\pm$ 4.01 b
SE were estimated by using the bootstrap technique with 100.000 resampling. Difference was compared by the paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the three concentrations.			

Table 4

Population (demographic) parameters and parasitism rate (mean $\pm$ SE) of the parasitoid wasp *Aphidius colemani*, untreated or treated with LC₁₀ and LC₃₀ of thiacloprid + deltamethrin

Parameter	Control	LC ₁₀	LC ₃₀
Intrinsic rate of population growth (r) (d ⁻¹)	0.2081 $\pm$ 0.007 a	0.1929 $\pm$ 0.007 a	0.1643 $\pm$ 0.006 b
Net reproductive rate (R_0)	54.64 $\pm$ 7.24 a	51.3 $\pm$ 7.06 ab	35.06 $\pm$ 4.67 b
Generation time (T; d)	19.22 $\pm$ 0.21 a	20.41 $\pm$ 0.23 b	21.64 $\pm$ 0.16 c
C_0 (hosts parasitoid ⁻¹)	54.66 $\pm$ 7.23 a	51.30 $\pm$ 7.05 a	35.06 $\pm$ 4.66 b
Q_p (preys/offspring of predator)	$\cong 1$	$\cong 1$	$\cong 1$
ψ (hosts parasitoid ⁻¹)	0.33 $\pm$ 0.011	0.21 $\pm$ 0.009	0.18 $\pm$ 0.008
ω (hosts parasitoid ⁻¹ day ⁻¹)	0.41 $\pm$ 0.006	0.25 $\pm$ 0.006	0.21 $\pm$ 0.007
SE were estimated by using the bootstrap technique with 100.000 resampling. Difference was compared by the paired bootstrap test ($P < 0.05$). Lower case letters indicate significant differences between the three concentrations.			

Consumption rate

Table 4 presents the effect of the insecticide mixture at different concentrations on the parasitism rate of *Aphidius colemani*. According to the result, a significant decrease was observed in the finite (ω) and net parasitism rates (C_0). Figure 6 presents the effect of insecticide mixture at different concentrations on the age-specific net parasitism rate (q_x), age-specific parasitism rate (k_x) and parasitoid age-specific survival rate (l_x). Consequently, age-specific net parasitism rate (q_x) increased at first, but decreased as age increased. Also, both sublethal concentrations reduced the age-specific parasitism rate (k_x) and age-specific net parasitism rate (q_x) of *Aphidius colemani*.

Discussion

Pesticides are widely used in farms and quickly degraded after application; hence, target and non-target insects are often exposed to low (and largely sublethal) residues^{24–26}. The importance of such exposure is prevalent in the field and should not be neglected as it may affect the management of pest species^{26,27}. The intent of the present work was to evaluate the effect of such low insecticide concentrations on a pest species, *A. gossypii*, and one of its important natural enemies, the parasitoid wasp *Aphidius colemani*. The results obtained indicated that both organisms are significantly compromised by the insecticide mixture, following a concentration-dependent trend.

Previous studies have demonstrated that, if the population growth of cotton sucking pests is controlled, natural enemies will be able to control the population of these species²⁸. However, our results dispute such a notion and are consistent with those of Majidpour, et al.²⁹, who reported that a sublethal concentration (LC₁₀ and LC₃₀) of thiacloprid + deltamethrin significantly reduces the life table parameters of *A. gossypii* and *Aphidius flaviventris* (Hymenoptera: Aphelinidae). Furthermore, Shi, et al.³⁰ reported that fecundity and longevity of *A. gossypii* at LC₂₀ were significantly reduced by thiacloprid. Our results are also in line with Vojoudi and Saber³¹, who reported that a sublethal concentration of thiacloprid significantly increased pupal and larval development times and reduced adult longevity and fecundity of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). Similarly, Miao, et al.³² observed that sublethal concentrations of thiacloprid on *Sitobion avenae* (Fabricius) (Homoptera: Aphididae) reduced r , R_0 and increased generation time.

The significant effects of low concentrations of insecticide mixture affect not only the cotton aphid, but also other species. Their negative effects on the aphid parasitoid and biocontrol agent *Aphidius colemani* were also significant, especially on the aphid LC₃₀. The time-mortality results also indicated that LC₃₀ was highly lethal to parasitoid adults up to 24 hours after the application. Then, lethality dropped drastically. These findings are also consistent with those reported for other species. It has been reported that the pyrethroid cyhalothrin may reduce the population of natural enemies, which may favor the growth of *A. gossypii* population¹². Mardani, et al.³³ found that thiacloprid + deltamethrin presented greater negative impact than pirimicarb and pymetrozine on *Lysiphlebus fabarum* (Marshall) (Hymenoptera: Aphididae) and reduced its survival, adult emergence and fecundity.

Thiacloprid + deltamethrin also compromised the population parameters of *L. fabarum*, which is consistent with our findings for *A. colemani*. In another study, thiacloprid was tested on *Trichogramma evanescens* (Westwood) (Hymenoptera: Trichogrammatidae) and reduced adult emergence and parasitism by this species, although other species of this genus may not be as strongly affected. Mead-Briggs³⁴ found that the adult stages were significantly affected by thiacloprid, compared to the pre-imaginal stages in *Aphidius rhopalosiphii* (DeStefani-Perez) (Hymenoptera: Braconidae). These findings suggest that *Aphidius colemani* is more susceptible to the insecticide mixture thiacloprid + deltamethrin than other parasitoids.

Thiacloprid + deltamethrin presented high potential to control *A. gossypii*, even at sublethal concentrations. Given the results, thiacloprid + deltamethrin proved to be an effective insecticide for controlling *A. gossypii*. Nonetheless, the negative impact of insecticide mixture on life table parameters of *Aphidius colemani* is a concern. Thiacloprid + deltamethrin consists of a neonicotinoid and a pyrethroid, each with a different mode of action. Neonicotinoid insecticides affect acetylcholine receptors, while pyrethroids are sodium channel modulators, and both affect the insect nervous system. While affecting the nervous system, these compounds are also able to affect other physiological systems via indirect action, or via secondary action. Thus, the decreased fecundity and longevity in adult insects may result from failure in the neurosecretory system under the influence of these insecticides^{31,35,36}. Regardless, the

findings of the present study indicate that thiacloprid + deltamethrin is effective against *A. gossypii*, but the combined use of this pesticide with *Aphidius colemani* is not suitable for pest management programs.

Materials And Methods

Insecticide

The insecticide mixture thiacloprid + deltamethrin was obtained from Bayer Persian, Iran. This insecticide mixture contains an oil dispersion with 10 g ai/l of deltamethrin and 100 g ai/l thiacloprid.

Insects

The cotton aphid colony was reared on cucumber (*Cucumis sativus* L. var. Emperor) at $26 \pm 2^\circ\text{C}$, relative humidity (RH) of 60–70 %, and 16: 8 (L:D) photoperiod. *Aphis gossypii* were collected from a greenhouse of Yasouj University. Every week, uninfected plants were replaced by aphid-infected plants.

Aphidius colemani was purchased from Gyah Co. (Karaj, Iran). They were maintained on cotton aphids, which were themselves maintained on cucumber plants. The plants were removed from cages when most aphids were mummified, and the mummified aphids were transferred into 1-liter plastic jars.

Aphid concentration-mortality bioassay

The bioassay was performed on the 3rd instar nymphs (< 12 h old) of *A. gossypii* subjected to five insecticide mixture concentrations (3.3-52.25 mg ai/l), in addition to a (water-only treated) control on plastic Petri (6 cm in diameter). Distilled water containing 0.05% Tween-80 was used to prepare the insecticide solutions. Cucumber leaf discs were placed on soaked cotton and then, 20 similar-aged adult aphids (< 12 h old) were transferred to the discs. Next, 3 ml of either insecticide concentration were sprayed on aphids by Potter tower (Burkard Scientific, Uxbridge, UK). Mortality after 24 h was registered. Three replicates were used for each of the six concentrations tested.

Diagnostic exposure time for the aphid parasitoid knockdown

These bioassays were investigated with third-instar nymphs (< 12 h old) of the cotton aphid, using the LC_{30} of the insecticide mixture previously established. The treated nymphs were subsequently transferred to plastic jars for parasitoid release. Ten female parasitoids were released into the jars, after 0, 12, 24, 48, 72 h of the aphid nymph exposure to the insecticide mixture. Parasitoid mortality was assessed every 24 h after their release, and the bioassays were replicated five times, always under the same environmental conditions used for the aphid bioassays.

Aphid life-table bioassay

The aphid nymph progeny from the previously treated 3rd instar nymphs (LC₁₀ and LC₃₀, besides the untreated control) were transferred to a new Petri containing cucumber leaf disks free from insecticide residues, 24 h after birth, and maintained there until reaching the adult stage. Fifty similar-age newborn nymphs (< 12 h old) from these adults were transferred to leaf discs of cucumber in separate Petri dishes, and observed every 24 h, until the adult stage. Progeny production, mortality and survival were recorded daily.

Parasitoid Life table

Three concentrations of the insecticide mixture (LC₁₀ and LC₃₀, besides the control) obtained from the aphid bioassay were used to estimate the side effects of the mixture insecticide. For this purpose, 130 similar-age 3rd-instar aphid nymphs were transferred to Petri dishes, as previously described, and subjected to insecticide spraying again, as already reported. The aphid nymphs that survived after 24 h exposure were placed in the plastic jars. Then, in order to parasitize the aphids, the parasitoid wasp was released into the jars and removed after 24 h. Then, the parasitized aphids were placed in each Petri dish and were daily monitored until mummified aphids appeared. The parasitoids that appeared were paired, and the survival and fecundity of each couple were recorded until the couple died. The 3rd-instar aphid nymphs and a drop of honey were provided for the parasitoids during the observation period.

Concentration-mortality and survival analyses

The LC₁₀, LC₃₀ and LC₅₀ values were determined based on probit analysis, using the SPSS software system³⁷. The mortalities were corrected using Abbott's formula³⁸.

The time-mortality results of the parasitoid wasps using the LC₃₀ estimated for the cotton aphid nymphs were subjected to Cox regression, with right-censored data and surviving time as independent variables³⁷.

Life-table analyses

The life-table parameters of all individuals (aphids and parasitoids), such as development time of different stages, fecundity, longevity and population parameters, were analyzed by the computer program TWOSEX-MS Chart, based on the age-stage, two-sex life-table theory³⁹⁻⁴¹. The bootstrap technique with 100,000 bootstrap replicates was applied to calculate the standard errors of all population parameters⁴².

Predation parasitism rate

The CONSUME-MS Chart was used to estimate daily parasitism rates^{43,44}. The following parameters were calculated: age-specific net parasitism rate (q_x), net parasitism rate (C_0), finite predation rate (ω), stable parasitism rate (ψ), age-specific parasitism rate (k_x) and transformation rate (Q_p). The bootstrap technique with 100,000 bootstrap replicates was applied to calculate the standard errors of all parameters.

Declarations

Competing interests

The authors declare that there are no conflicts of interest.

Author contributions

MM and MGJ conceived and designed the experiments. MM performed the experiments. NM established and analyzed the bioassay data and life table. NM writing-original draft of manuscript. RNCG writing-review and editing the manuscript.

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Figures

Figure 1

Survival of *Aphidius colemani* exposed to its host aphid LC30 of thiacloprid + deltamethrin.

Figure 2

Age-stage survival rate (s_{xj}), age specific survival rate (l_x), fecundity (m_x) and net maternity ($l_x m_x$) of the cotton aphid *Aphis gossypii*, untreated and treated with LC10 and LC30 of thiacloprid + deltamethrin

Figure 3

Age-stage life expectancy (e_{xj}) and age-stage specific reproductive value (v_{xj}) of the cotton aphid *Aphis gossypii*, untreated and treated with LC10 and LC30 of thiacloprid + deltamethrin

Figure 4

Age-stage survival rate (s_{xj}), age specific survival rate (l_x), fecundity (m_x), and net maternity ($l_x m_x$) of the parasitoid *Aphidius colemani*, untreated and treated with the aphid LC₁₀ and LC₃₀ of thiacloprid + deltamethrin

Figure 5

Age-stage life expectancy (e_{xj}) and age-stage specific reproductive value (v_{xj}) of the parasitoid *Aphidius colemani*, untreated and treated with the aphid LC10 and LC30 of thiacloprid + deltamethrin

Figure 6

Age-specific survival rate (l_x), age-specific host feeding rate (k_x) and age-specific net host feeding rate (q_x) of the aphid parasitoid *Aphidius colemani* exposed to thiacloprid + deltamethrin.