

# Short communication. Evaluation of the efficiency of imidacloprid and *Encarsia inaron* Walker (Hymenoptera: Aphelinidae) integration to control the whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), under greenhouse conditions

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## Abstract

The integrated whitefly control systems are based on the contribution of chemical and biological control fostered by conservation of natural enemies. This study attempts to establish the integrated impact of the parasitoid *Encarsia inaron* Walker (Hymenoptera: Aphelinidae) in junction with the biorational imidacloprid against the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae), feeding on bean plants cv. Contender under greenhouse condition. Experiments were carried out to establish the individual and combined effects of the parasitoid and insecticide to control the greenhouse whitefly. A synergy effect was observed in the simultaneous use of *E. inaron* and imidacloprid causing 97.2% mortality in the population of immature whiteflies. There was no significant difference between the application of imidacloprid and the introduction of *E. inaron* alone which caused 90.1% and 78.7% whitefly mortality, respectively. The application of imidacloprid significantly reduced the percentage of the adult emergence and parasitism of *E. inaron* to 18% and 25% respectively, compared to control with 47% adult emergence and 57.9% parasitism. The current results suggest that despite some negative impacts of imidacloprid on *E. inaron*, the combination of these two factors works more efficiently than the use of them separately against the greenhouse whitefly.

**Additional key words:** biorational; control; greenhouse whitefly; integrated pest management; interaction; parasitoid.

## Resumen

### Comunicación corta. Evaluación de la eficacia de combinar imidacloprid y *Encarsia inaron* Walker (Hymenoptera: Aphelinidae) en el control de la mosca blanca *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae) bajo condiciones de invernadero

El control integrado de la mosca blanca utiliza un control químico complementado con un control biológico que fomenta la conservación de sus enemigos naturales. Este estudio intenta establecer el impacto de la utilización conjunta del parasitoide *Encarsia inaron* Walker (Hymenoptera: Aphelinidae) con el insecticida biorracional imidacloprid, en la lucha contra la mosca blanca *Trialeurodes vaporariorum*, al colonizar plantas de judía cv. Contender bajo condiciones de invernadero. Se llevaron a cabo experimentos para establecer los efectos individuales y combinados del parasitoide e insecticida sobre la mosca blanca, observándose un efecto de sinergia que causó un 97,2% de mortalidad en la población de moscas inmaduras. No hubo diferencias significativas entre la aplicación de imidacloprid y la introducción de *E. inaron* por separado, que causaron un 90,1% y 78,7% de mortalidad, respectivamente. La aplicación de imidacloprid redujo significativamente el porcentaje de la emergencia de adultos y el parasitismo de *E. inaron* al 18% y 25% respectivamente, comparado con el control (47% de emergencia de adultos y el 57,9% de parasitismo). Estos resultados sugieren que a pesar de algunos efectos negativos de imidacloprid sobre *E. inaron*, la combinación de los dos factores es más eficiente que el uso de ellos por separado contra la mosca blanca en invernadero.

**Palabras clave adicionales:** gestión integrada de plagas; insecticida biorracional; interacción; mosca blanca de invernadero; parasitoides.

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Abbreviations used: IPM (integrated pest management).

Chemical and biological control agents are two important tools in the control programs of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, a major pest of numerous greenhouse vegetables as well as ornamentals worldwide. Current whitefly control measures include the use of biorational insecticides solely or combined with natural enemies through integrated pest management (IPM) programs. There is ample evidence that the integration of beneficial natural enemies with insecticides for IPM relies heavily upon the validity of the available information on the impacts of insecticides on the natural enemies (Hull and Beers, 1985; Hassan, 1992, 1994). There is also substantial evidence of whitefly parasitoids activity post application of some insecticides (Simmons and Jackson, 2000; González-Zamora *et al.*, 2004; Naranjo and Ellsworth, 2009). To establish such a notion, some scholars have employed the combination of more environmentally compatible insecticides and parasitoids to control whitefly populations (Birnie and Denholm, 1992; Devine *et al.*, 2000; Van Driesche *et al.*, 2001; González-Zamora *et al.*, 2004; Hoseini and Pourmirza, 2010).

It has been determined that many predators, parasitoids and fungi attack whiteflies in a number of agricultural systems (Breene *et al.*, 1994; Gerling *et al.*, 2001), and some evidence suggests generalist predators and aphelinid parasitoids act as key factors in the population dynamics of this pest in several crops in the absence of insecticides (Naranjo and Ellsworth, 2005; Asiimwe *et al.*, 2006; Karut and Naranjo, 2009). We chose *Encarsia inaron* Walker, a well-known biological control agent of different whiteflies. This parasitoid has been used successfully to control the ash whitefly, *Siphoninus phillyreae* (Halliday) in North America (Gould *et al.*, 1992; Pickett and Pitcairn, 1999; Pickett and Wall, 2003; Perlman *et al.*, 2006). It is also used to control *T. vaporariorum* in Russian greenhouses (Slobodyanyuk *et al.*, 1993; De Oliveira *et al.*, 2003). Despite such reports on the performance of *E. inaron* in different whitefly control programs, rare compiled information is available on the use of this parasitoid alongside pesticides through IPM and consequences of a chemical approach on its survival. In the present study, we provided an experiment to examine the integration of a selective insecticide with *E. inaron* for the suppression of a *T. vaporariorum* population. We used the biorational, imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylidenamine] as well as a conventional insecticide in Iran for the management of different populations of whiteflies.

In this experiment, imidacloprid was tested to determine its effect on survival and parasitism capability of *E. inaron* in the control of *T. vaporariorum*, using a greenhouse cage evaluation. A colony of *T. vaporariorum* was initiated from adults collected in the West Azerbaijan province (northwest of Iran), from tomato (*Solanum lycopersicon* L.) and ornamental (*Tropaeolum majus* L. and *Euphorbia pulcherrima* Willd.) plants, and kept in a rearing room, on tobacco (*Nicotiana tabacum* L.) plants. *E. inaron* was collected in this region too, from parasitized nymphs of *Aleyrodes singularis* Danzig (Homoptera: Aleyrodidae) on *Lactuca* sp., and was reared on tobacco plants, infested with *T. vaporariorum* nymphs. The rearing room was kept at  $26 \pm 2^\circ\text{C}$  with a photoperiod of 16:8 (Light: Dark) during the experiment.

The experimental design studied two levels (presence and absence) of two factors (parasitoid and insecticide). Therefore, there were four treatments: (T1) application of imidacloprid, (T2) application of imidacloprid + introduction of *E. inaron*, (T3) introduction of *E. inaron*, and (T4) no application of imidacloprid and no introduction of *E. inaron* (control). Each treatment was replicated eight times, using one two-leaf stage bean plant cv. Contender per treatment and replicate, making a total of 32 plants. The plants were infested with *T. vaporariorum* adults in the rearing room, allowed to lay eggs for 3 days on leaves and then removed. The plants were kept in the rearing room and, after 10 days, first and second instar whitefly nymphs were present on the leaves. Then,  $180 \pm 5$  nymphs per leaf were allotted and each plant was maintained in the greenhouse inside a transparent construct covered with organdy for the remainder of the experiment.

Imidacloprid (Confidor® SC 35%, Bayer CropScience AG, Germany) was applied in T1 and T2, using a trigger-operated hand sprayer, at a rate of  $0.003 \text{ g a.i. plant}^{-1}$  (equivalent to  $90 \text{ g a.i. ha}^{-1}$ , with  $30,000 \text{ plants ha}^{-1}$ ). It was applied 12 days after the plants had been put in the transparent constructs, when the insects were in the pupal stage.

In T2 and T3, *E. inaron* was introduced in cages at a total rate of 18 females per plant, following the recommended ratio proposed by Jones *et al.* (1999). The parasitoid was introduced two times, 1 and 7 days after the plants had been put in the transparent constructs.

Plants were evaluated every 3-4 days during the first week and then weekly until all adults of *T. vaporariorum* and *E. inaron* emerged. The number of living whitefly nymphs and pupae, the number of parasitized whitefly nymphs and parasitoid pupae, and the number

**Table 1.** Arcsine  $\sqrt{X}$  average mortality of immature stages of *T. vaporariorum*, in homogeneous subsets, related to the four treatments of the experiment on the last day of testing

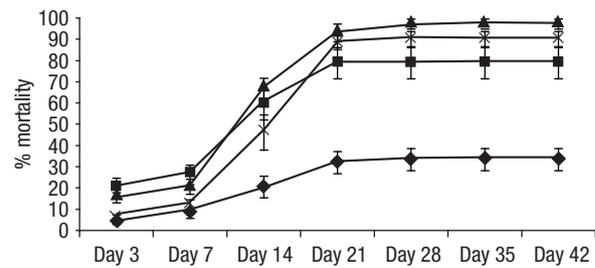
Treatment	N	Subset for $\alpha = 0.05$		
		1	2	3
Imidacloprid (T1)	8		73.97	
<i>E. inaron</i> + Imidacloprid (T2)	8			84.32
<i>E. inaron</i> (T3)	8		63.85	
Control (T4)	8	35.08		
Significance		1.000	0.380	0.361

of pupal cases from where an adult (whitefly or parasitoid) had emerged were counted. This experiment included only one generation of the species.

Analysis of variance was performed on mortality with the transformation of  $z = \arcsine \sqrt{X}$ ; where  $X$  is mortality. A  $2 \times 2$  factorial analysis was applied to analyze total mortality data at the last day of the experiment. If treatments were significant at  $p < 0.05$ , then differences between means were determined using the Turkey's HSD test at 95% confidence level. Omega-square formula was used to compare magnitude of simple effects and interaction. The T-test was performed to compare the means of emergence and parasitism.

The mortality percentage of *T. vaporariorum* nymphs treated with imidacloprid was significantly different from untreated nymphs ( $F = 47.375$ ;  $d.f. = 1, 28$ ;  $p < 0.0001$ ). The introduction of *E. inaron* also produced a significant effect on the mortality of *T. vaporariorum* ( $F = 20.653$ ;  $d.f. = 1, 28$ ;  $p < 0.0001$ ). A marginal interaction was observed between the two factors studied ( $F = 4.754$ ;  $d.f. = 1, 28$ ;  $p = 0.045$ ).

Omega-square values for insecticide, parasitoid and interaction were calculated as 0.0468, 0.0198 and 0.0037 respectively.

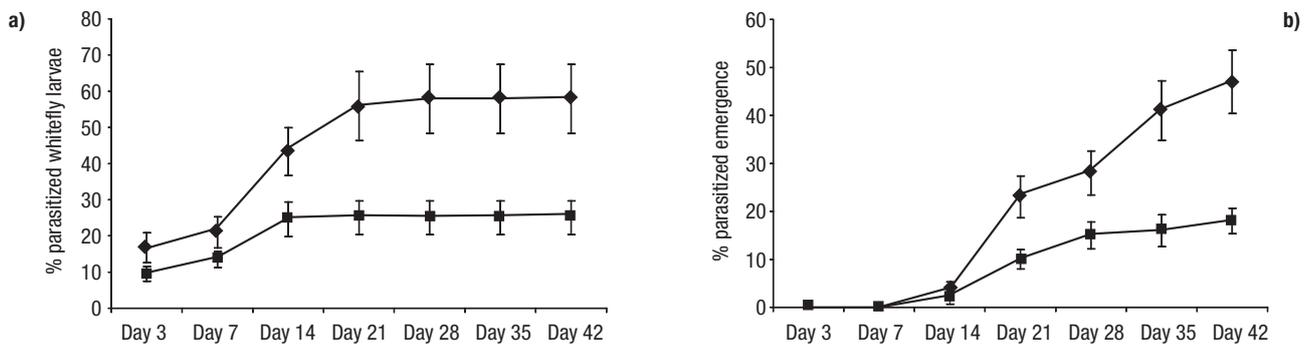


**Figure 1.** Percentage of accumulated mortality of *Trialeurodes vaporariorum* larvae in the experiment, considering the four treatments: imidacloprid (x), imidacloprid plus *Encarsia inaron* (▲), *E. inaron* (■) and control (◆). Vertical bars indicate the standard error of the mean.

Table 1 shows Tukey's HSD test, related to the arcsine  $\sqrt{X}$  transformation data of the average mortality percentages of *T. vaporariorum* in the four treatments. The whitefly mortality in application of imidacloprid combined with *E. inaron* was significantly higher than the amount of control cohort and the use of the biological control agent solely. Whitefly mortality in both parasitoid and insecticide treatments was also significantly higher than control group, but these two treatments were not significantly different from each other.

The results of whitefly mortality in the four treatments are shown in Figure 1. The whitefly mortality percentage was 97.2% in performance of *E. inaron* in conjunction with imidacloprid. Application of either insecticide or *E. inaron* alone caused 90.1% and 78.7% mortality, respectively. The whitefly mortality in control was 33.4% under test conditions.

The number of immature whiteflies with signs of parasitization was also recorded in the two treatments where *E. inaron* was present (Fig. 2a). Total parasitism percentage in T2 (application of imidacloprid plus introduction of *E. inaron*) was significantly reduced.



**Figure 2.** Effect of imidacloprid on *Encarsia inaron*, parasitizing *Trialeurodes vaporariorum* nymphs. (a) Percentage of accumulated parasitism of *E. inaron*. (b) Percentage of accumulated emergence of *E. inaron* adults from *T. vaporariorum* nymphs, considering the two treatments: imidacloprid plus *E. inaron* (■) and *E. inaron* (◆). Vertical bars indicate the standard error of the mean.

ced compared to T3 (only introduction of *E. inaron*) ( $T = -4.89$ ;  $d.f. = 7$ ;  $p = 0.008$ ). Eventually, the rate of parasitoids that emerged at the end of the experiment from whitefly pupal cases (Fig. 2b), was significantly higher in plants not treated with imidacloprid ( $T = -7.17$ ;  $d.f. = 7$ ;  $p = 0.000$ ).

In general, the results showed: (i) that *E. inaron* can control *T. vaporariorum* populations, (ii) there was an interaction between *E. inaron* and imidacloprid and (iii) there was a negative impact exerted by imidacloprid on the parasitism and emergence rate of *E. inaron*.

The results revealed that both imidacloprid application and introduction of the parasitoid wasp caused a significant effect on the control of *T. vaporariorum*. Whitefly mortality data alone cannot show the fate of the population dynamism, because the experiment only included one generation of the whitefly and parasitoid. To obtain a comprehensive profile, several authors (Birnie and Denholm, 1992; Goolsby *et al.*, 1998; Heinz and Parrella, 1998) have attempted to use the parasitoids for more than one generation.

The whitefly control rate by *E. inaron* was not significantly different from control by imidacloprid, a well-known insecticide for the management of different species of whiteflies (Talebi Jahromi, 2008; Sheikhi *et al.*, 2009). The high whitefly mortality exerted by *E. inaron* is caused by active parasitism and the feeding activity of the adult parasitoids. This last component can be very important, as Heinz and Parrella (1998) showed for different adult parasitoids, including *E. inaron*. The whitefly population reached low numbers, significantly lower than the control in which no parasitoid was added. Furthermore, a synergy effect between the two factors (parasitoid and insecticide), measured in terms of whitefly mortality, was observed and enhancing the whitefly control level in their combination was also discerned.

A similar pattern has been found with a selective insecticide applied alongside *E. inaron* to control the greenhouse whitefly. Hoseini and Pourmirza (2010) revealed that the insect growth regulator (IGR) pyriproxyfen and *E. inaron* combination works more effectively than using them separately. In the same way, Ahmadzade and Hatami (2006) used imidacloprid and the predator *Chrysoperla carnea* (Stephens), separately and together, against *T. vaporariorum*; they found that the best way to control this whitefly is the combination of *C. carnea* and imidacloprid. They also specified that the use of this predator alone resulted in little control on the whitefly population. The combination of an

insecticide and a whitefly parasitoid was also studied by Gonzalez-Zamora *et al.* (2004) using *Eretmocerus mundus* and oxamyl at the same time and separately to control *Bemisia tabaci* Gennadius in the same manner as in this study; according to their findings, no interaction was detected between *E. mundus* and oxamyl.

There were insufficient data to absolutely conclude that the parasitoid and pesticide combination would work adequately in a greenhouse situation where spatial issues and overlapping generations of whiteflies would occur. However, more through studies on the interaction and possible synergism between imidacloprid and *E. inaron* with combination of different rates in an IPM program is warranted.

On the other hand, significant difference between parasitism percentage and emergence rate of the adult parasitoids in presence and absence of imidacloprid in the current study shows that this insecticide is a limiting factor to *E. inaron* and also affected its survival and parasitism. In the field, Bethke and Redak (1997) in application of a flowable formulation of imidacloprid on *B. tabaci* B-biotype (= *Bemisia argentifolii* Bellows and Perring) showed that parasitism by *E. formosa* occurred at low levels (<10%) on this whitefly. Rebek and Sadof (2003) also showed that imidacloprid decreased parasitism of euonymus scale, *Unaspis euonymi* (Comstock) by *Encarsia citrina* and failed to control this pest and agree with the data herein. However, imidacloprid cannot be assessed as a non-retractable insecticide with biological control agents because, although some limits, the significant interaction and the whitefly control enhancement in current study indicate that this insecticide has some positive features to improve the whitefly IPM by the contribution of *E. inaron*.

The primary point that can be gleaned from this discussion is that the results of the current research were in line with findings of recently mentioned studies. The enhanced control level can always be considered a valuable tool in the framework of IPM programs preventing the pest outbreak, enhancing the effectiveness of other management strategies, reducing doses and costs for their application. According to the omega squared values, imidacloprid clearly accounted for the greatest portion of the variance (mean square = 4,335.335) in the whitefly mortality, but *E. inaron* also accounted for a considerable effect (mean square = 1,889.957). Although the control level of *T. vaporariorum* population obtained in the assay solely with

*E. inaron* treatment could not be acceptable by growers, the results of the current study indicate that the integration of *E. inaron* and imidacloprid is a more efficient approach to enhancing the whitefly control level.

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