Phyton-
International Journal of
Experimental Botany

Tech Science Press

http://doi.org/10.32604/phyton.2026.075836

ARTICLE

Tritrophic Interactions Underpinning Cucumber Protection: Evaluating the Impact of *Encarsia formosa*, *Orius laevigatus*, and *Amblyseius swirskii* on Pest Suppression

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Received: 10 November 2025; Accepted: 19 March 2026; Published: 30 July 2026

ABSTRACT: The research examined the effects of predators and parasitoids on *Trialeurodes vaporariorum* (TV) and *Frankliniella occidentalis* (FO) through their introduction into the cucumber pest management. Parasitoids and predators from the genus *Encarsia*, *Orius*, and *Amblyseius* were used to manage TV and FO. The study was performed at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, from August to October 2023. The research investigates the influence of zoophagous species on TV and FO, utilizing data collected on six distinct dates throughout the cucumber growing season. The findings indicate that biocontrol agents positively influence flight and negatively affect the pest. Chemically treated variants exhibited an average of 4.09 adults per plant, whereas biologically treated variants (zoophagous use) displayed an average of 0.24 adults per plant. The research demonstrated that chemical treatment exhibited a significantly reduced leaf count per plant than zoophagus species plots. This reduction is plausibly associated with elevated pest populations and the phytotoxic stress induced by chemical applications, which may adversely affect plant growth and development. The study concludes that predators and parasitoids demonstrate greater efficacy than chemical treatments and entail lower costs, rendering them an effective and economical method for pest control in greenhouses. Over the long term, employing biocontrol agents constitutes an effective and economical strategy for pest management in greenhouses.

KEYWORDS: *Frankliniella occidentalis*; greenhouses; insects; parasitoids; predators; pest management; *Trialeurodes vaporariorum*

1 Introduction

Greenhouse horticultural crops cultivated globally are susceptible to numerous pest infestations, as controlled environments create optimal conditions for pest proliferation [1]. In particular, cucumbers are known for their sensitivity to whiteflies, spider mites, and thrips [2]. Cucumber is among the most widely consumed vegetable crops globally [3], and the use of pesticides could affect the physiology of the plant [4]. Currently, there is a prevailing tendency towards consuming organic food. Consumers seek fresh produce cultivated in environmentally sustainable conditions with little pesticide use. Farmers adjust to customer demands by seeking solutions that meet consumer needs and reduce production costs. This shift not only involves moving towards organic production but also emphasizes reducing pesticide use through approaches like Integrated Pest Management (IPM) [5], which allows for the limited application of pesticides to support biological control methods [6,7]. Pesticides leave residues in the soil [8], contaminating air [9] and water [10]. Therefore, solutions that help farmers meet customers' demand for organic products will be the future of sustainable agriculture. Various strategies were evaluated to mitigate whiteflies in controlled environments, including yellow panels [11], but these appear insufficient to control *Trialeurodes vaporariorum* Westwood (TV) populations. The solution to pest insect problems is the use of biocontrol agents. In 1602, Aldrovandi discovered for the first time insect parasitism. The initial misconception led to the accurate interpretation of this host-parasitoid relationship, perhaps becoming the first to document the presence of parasitoids [12]. TV has a lot of natural enemies, including insects from the *Encarsia* genus [13], *Orius* genus [14], and the mite *Amblyseius swirskii* Athias-Henriot [15]. *Encarsia* genus parasitoids (Hymenoptera: Aphelinidae) serve as significant biological control agents for whiteflies [13]. Particular species within this genus not only parasitize their hosts but also terminate them via host feeding [16]. *A. swirskii* is a recognized predator employed for the management of spider mites and greenhouse whiteflies, TV [17]. *Orius laevigatus* Fieber is a generalist predator commonly employed in augmentative strategies to manage key pests, including *Frankliniella occidentalis* Pergande (FO), and is also recognized for its ability to control TV populations [18]. The objective of the research was to understand the impact of predators and parasitoids on the pests TV and FO by introducing them into the cucumber crop. Another objective was to monitor the progression of whitefly and thrips population densities in response to the treatment administered during the cucumber plants' vegetative growth.

2 Materials and Methods

2.1 Biological Control Agents

The parasitoids and predators utilized in the study included *Amblyseius swirskii* Athias-Henriot, *Orius leavigatus* Fieber, and *Encarsia formosa* Gahan. These biological control agents were procured from a Spanish company specializing in the production of insects for ecological and biological pest management. Of the ten rows of cucumbers, four rows remained with chemical treatment, while six rows were segregated for the release of parasitoids and predators. The six rows were insulated with two layers of microporous foil 23 GR/MP-7.5 × 25 m. Two launches occurred at different times.

2.2 Experimental Design and Treatments

Observations were conducted over six weeks, from August 31 to October 13, with sampling at regular intervals on August 31, September 6, September 13, September 20, September 28, and October 13. The study was performed in a greenhouse at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania (46°45′39.12059″ N, 23°34′4.77737″ E).

The experimental plot was planted on August 15, using the Spino hybrid. Ten rows were planted at a distance of 90 cm between them and 40–45 cm between plants per row. Each row had 30 plants initially. The greenhouse environmental conditions were maintained at a temperature of 23–25°C and relative humidity of 56–58%. The total area of the plot was 150 sq m, of which 90 sq m were isolated in order to launch the above-mentioned parasitoids and predators (Fig. S1). Observations were conducted on all plants, counting the number of leaves and individuals per leaf to determine the total number of individuals per plant.

The hybrid Spino, an F_1 semi-long cucumber hybrid provided by USAMV Cluj-Napoca (*Cucumis sativus* L.), is primarily cultivated for fresh consumption. This hybrid is characterized by uniform fruit, high productivity, and good adaptability to diverse growing conditions, making it a preferred choice for protected cultivation systems.

Fertilization was applied based on the following scheme. On August 28, a foliar fertilization was applied in the cucumber greenhouse using 60 g per plot of Kristalon Maro, a water-soluble fertilizer containing macroelements (N–P–K: nitrogen, phos-phorus, and potassium) and a balanced range of microelements (iron, manganese, zinc, copper, boron, and molybdenum), combined with 50 mL per plot of Synergizer, a com-plex foliar fertilizer formulated with both macro- and micronutrients designed to op-timize the plant's nutritional balance and enhance yield. On September 6, a subsequent application was made using 50 g per plot of Green K, a potassium-rich fertilizer containing soluble K_2O and trace elements that support fruit development and stress tolerance, along with 30 mL per plot of Philgreen, a biostimulant derived from marine algae (*Ascophyllum nodosum*) containing natural phytohormones, amino acids, and polysaccharides that promote root growth and improve plant vigor.

During each observation, detailed counts of pest individuals were conducted on every leaf of the sampled plants to ensure precise quantification of population density and its temporal variation. A total of 1824 individual readings were performed during the experimental period, of which 772 were obtained from plants treated with chemical control products, and 1052 from variants involving the introduction or presence of zoophagous (predatory or parasitoid) species. The differentiation between chemically treated and biologically protected variants can provide a robust framework for evaluating the relative efficiency of integrated pest management strategies.

The initial release of biological control agents was conducted on 31 August 2023, followed by a second release on 20 September 2023. At the first application on 31 August 2022, cucumbers were at BBCH stage 22, while by September 20 they had reached BBCH stage 73. During each assessment period, every experimental plant was carefully examined leaf by leaf, and the populations of greenhouse whitefly TV and western flower thrips FO were quantified by counting the number of individuals present on each leaf. This monitoring procedure enabled an accurate estimation of pest population densities and their temporal dynamics within the crop canopy.

The biological control program employed three zoophagous species released in both adult and larval stages: the predatory mite (*A. swirskii*; 50 individuals m^{-2}), the predatory bug (*O. laevigatus*; 3 individuals m^{-2}), and the parasitoid wasp (*E. formosa*; 3 individuals m^{-2}). These species were selected for their complementary trophic roles and feeding strategies, targeting different developmental stages of the principal pest species. The simultaneous presence of adults and larvae ensured immediate predatory activity and the subsequent establishment and persistence of the populations over time. Releases were synchronized with the crop's phenological stages and with observed increases in pest density, thereby optimizing the potential efficacy of the biological control agents under greenhouse conditions.

In the conventional treatment variant, pest control was achieved chemically, using Flonicamid (50%) and Pyridaben (100 g L^{-1}) as active substances. Two chemical treatments were administered concurrently with the release dates of the biocontrol agents to ensure experimental comparability between management strategies. This integrated experimental design enabled a direct, quantitative evaluation of pest suppression efficacy under biological versus chemical control regimes.

2.3 Statistical Analysis

Statistica version 12 and Polifact software were used to analyze data on the population of adult pests on leaves and plants under two conditions: conventional treatment and biological control. The Duncan test and ANOVA were employed to analyze the results. Each row was treated as a separate replication, resulting in four replications for the chemical treatment variant and six for the biological control variant.

3 Results

3.1 For Control of Trialeurodes vaporariorum

The data below were collected and enumerated from TV on six distinct dates throughout the cucumber growing season. The damage inflicted by greenhouse whitefly was assessed using two indicators: the adult count per leaf and the adult count per plant. The data are illustrated in the graphs 1–5. The first reading focused on assessing the pest population levels across all ten rows studied. It was observed that pest distribution was uniform, with an average of 1 to 6 adults per plant and 0.1 to 0.8 adults per leaf. Additionally, on August 31, protective foil was installed to facilitate the launch of parasitoids and predators.

The introduction of biocontrol agents had a positive impact on flight and an adverse effect on the examined pest, with adult counts per plant ranging from 1.83 to 2.24, averaging 2.03. In comparison to the variants subjected to zoophagous species release, the variants treated with chemical methods (conventional) exhibited an average of 4.09 adults, with a confidence interval ranging from 3.6 to 4.6, with a minimum of 3 and a maximum of 6. The maximum values observed in the zoophagous variant did not surpass the minimum values in the conventional treatment variants. This assertion is further corroborated by the median values for the two variants. The conventional variants exhibited a median of 2 (adults/plant), whereas the biological control variant had a median of 0, indicating that over 50% of observations for this variant were 0 (Fig. 1). The second indicator assesses the precise level of TV pest infestation by measuring at the leaf level. Analysis of Fig. 2 reveals that, even for this indicator, significant differences are evident.

The biocontrol variants exhibited an average of 0.14 adults per leaf, with a maxi-mum of 0.2 and a minimum of 0.08. The conventionally treated variants recorded a mean of 0.24 adults per plant, with a minimum of 0.14 and a maximum of 0.32, excluding the extremes. The situation is corroborated by the median observations, which indicate that over 50% of the total observations recorded at variants where zoophagous species were introduced had a value of 0, with the median also reflecting this value.

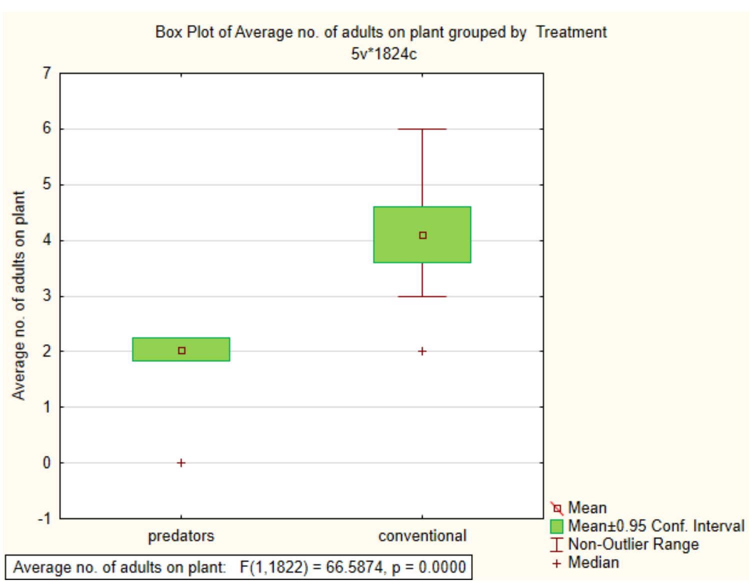


Figure 1: The influence of the applied treatment on the average number of adults per plant. Predators: biological control by use of *A. swirskii*, *O. laevigatus* and *E. formosa*; Conventional: chemical control.

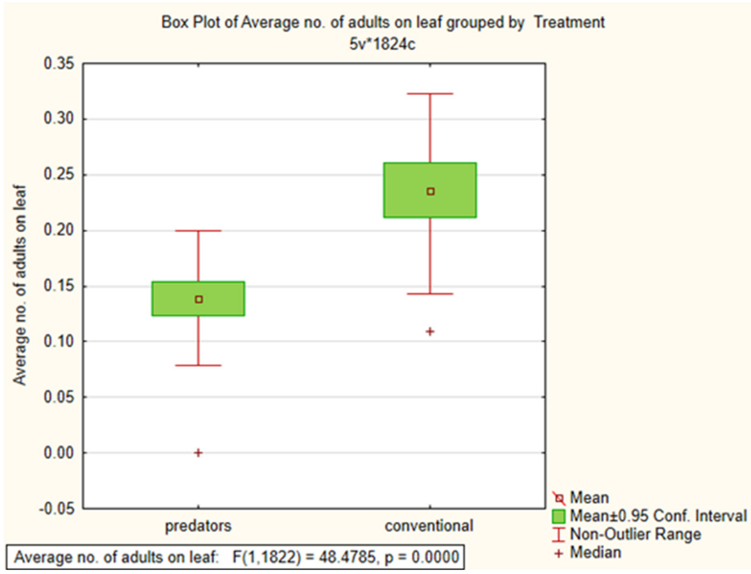


Figure 2: The influence of the applied treatment on the average number of adults per leaf. Predators: biological control by use of *A. swirskii*, *O. laevigatus*, and *E. formosa*; Conventional: chemical control.

The introduction of biocontrol agents for managing the greenhouse whitefly has demonstrated significantly positive effects on cucurbitaceous crops. An intriguing observation is that chemically treated varieties exhibit a substantially lower leaf count per plant than zoophagous-infested varieties. This may be attributed to the increased pest populations and the potential stress-inducing effects of chemical products on plant development. This fact is effectively illustrated in Fig. 3, which depicts leaf per plant and highlights the differences observed. The two treatment variants indicated a significant difference, with an average of 10 more leaves per plant in the zoophagous species-utilizing variant (36.37 with biocontrol agents and 25.21 with conventional methods). The identical two indicators depicted in Figs. 4 and 5 were considered.

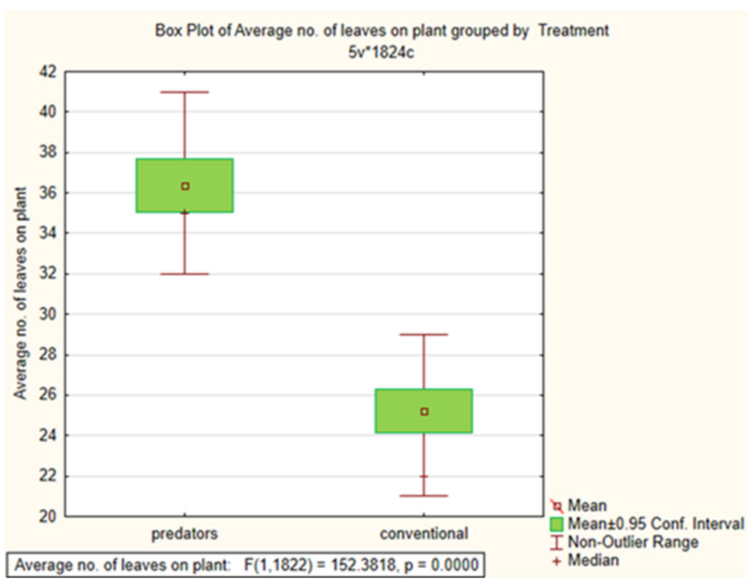
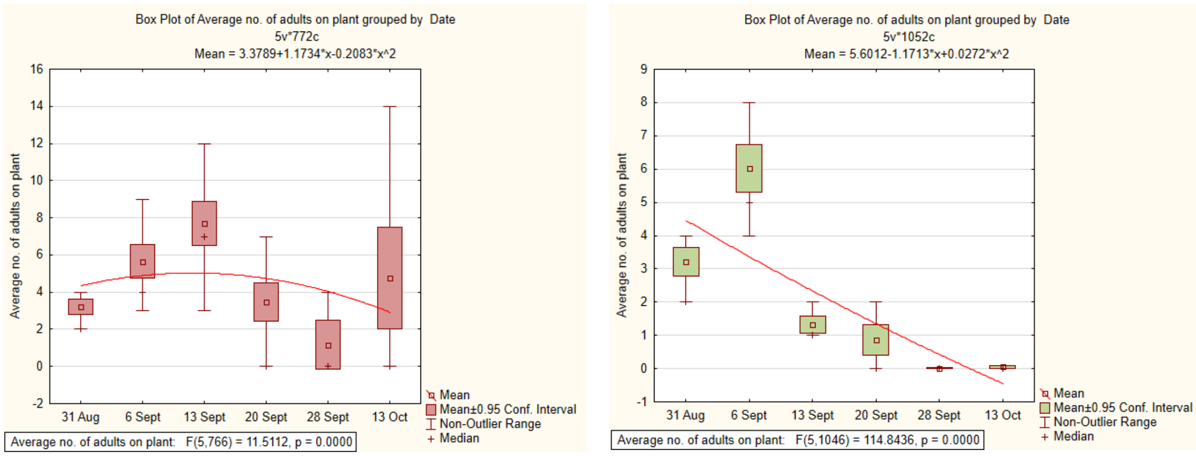
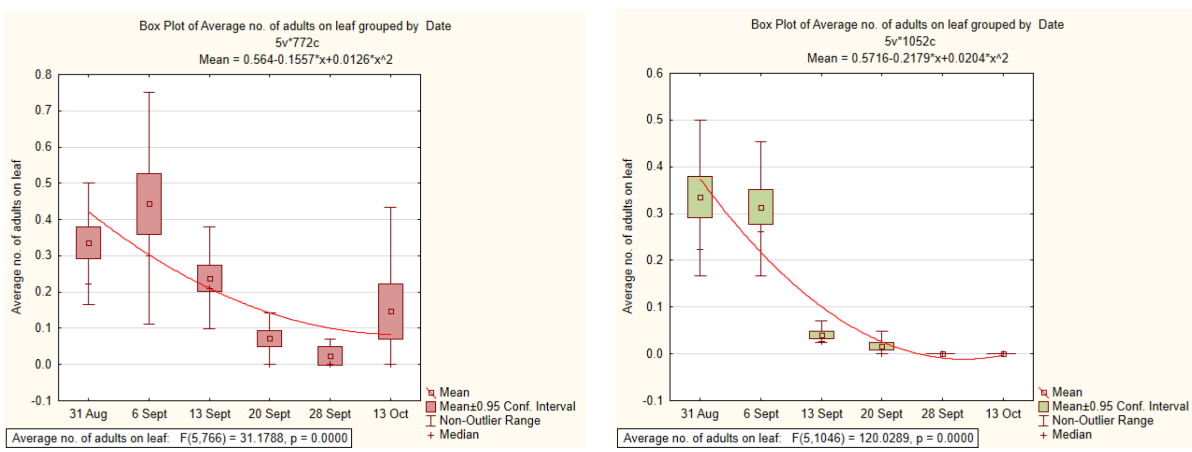


Figure 3: The numerical density of leaves under conventional treatment and under the release of zoophagous species. Predators: biological control by use of *A. swirskii*, *O. laevigatus*, and *E. formosa*; Conventional: chemical control.



(**A**) (**B**)

Figure 4: Comparative outcomes of population density and its temporal variation in *Trialeurodes vaporariorum* adult counts per plant between the conventional treatment and the release of the parasitoid. (**A**) Conventional: chemical control; (**B**) biological control by use of *A. swirskii*, *O. laevigatus*, and *E. formosa*.



(**A**) (**B**)

Figure 5: Comparative outcomes of population density and its temporal variation in *Trialeurodes vaporariorum* adult counts per leaf between the conventional treatment and the release of the parasitoid. (**A**) Conventional: chemical control; (**B**) biological control by use of *A. swirskii*, *O. laevigatus*, and *E. formosa*.

Upon examining the adult population per plant, it is evident that on the initial observation date, approximately 1 week post-treatment application, the population per plant increased in both scenarios; however, in the chemical variants, it was 5.66, whereas in the zoophagous species variants, it was 6.03. The distinctions between the two are insignificant and negligible. In the second week, a significant transformation occurs, as observed on 13.09.2024, where the population level in the chemical variants increases to 7.67 adults per plant, whereas in the variants subjected to zoophagous species, the population level decrease plummets to 1.32 adults per plant. The differences were statistically significant both between the chemical version and the zoophagous species version, as well as between the first observation and the September 13 observations. In week 3, the effect of the chemical treatment is evident, as the second treatment was applied. The population level drops below 4%, and the chemical variant approaches the level observed at the start of the observations, with the difference insignificant. Compared to the variants with predators, in the 3rd week, values below one adult per plant were recorded for the first time (0.86). Starting from week 3, it is found that in all experimental variants, more than 50% of the observations had the value 0, both in the chemical variants and in the variants with biocontrol agents. This fact is demonstrated by the biocontrol agent values shown in Fig. 4. From week 4, things become different for the two tested treatment variants. If in the plants where the zoophagous species were released, the values were close to 0, in the conventional versions the number of adults dropped to 1.67 in the first phase, following that in the last reading date it exceeded 4.75% exceeding even the initial value, from the first reading, the differences compared to the first reading being significant (Tables 1–3). There are notable differences in the number of adults on the plants based on the type of treatment, particularly starting from the third reading date of September 13 and continuing through September 20 (Table 3). However, by September 28, the differences between the two treatments become less pronounced. The observations recorded on October 13 show a significantly lower number of adults in the biological treatment variant, suggesting that the chemical treatment's effect is diminishing.

The second indicator demonstrates that both treatment options exhibit a declining trend in pest population levels (Fig. 5). Before treatment, 0.34 specimens per leaf were recorded. At the second assessment, approximately one week post-chemical treatment or predator and parasitoid release, the outcomes varied. In the variants with predator release, the population level decreased slightly, with negligible differences. Conversely, in the chemically treated variants, the number of adults per leaf rose to 0.44, demonstrating significant differences. After two weeks, the most substantial decline is observed, with the population level decreasing to 0.04 adults per leaf in the predator variants and 0.24 in the chemically treated variants. On September 13, the second release occurred simultaneously with the administration of the second treatment, resulting in the population levels of the variants with predators nearly reaching zero, ranging between 0.02 and 0.00. Conversely, the chemically treated variants exhibit a different trend; although the adult population on the leaves initially diminishes to a minimum of 0.02 on September 28, it subsequently rises to 1.15 by October 13. The changes in the number of adults per leaf between the chemical and biological treatments differ from the initial reading, with significant differences maintained until September 13 (Table 2). After this date, the distinction between the two treatments becomes less evident, and no significant difference is observed between them. By the last reading, however, the differences are re-established and become significant once again. This observation highlights the short-term effectiveness of the chemical treatment.

Table 1: Analysis of variance of *Trialeurodes vaporariorum* Westwood.

	Analysis of Variance Marked Effects are Significant at $p < 0.05000$							
	SS Effect	**df Effect**	**MS Effect**	**SS Error**	**df Error**	**MS Error**	**F**	***p***
Average no. of leaves on plant	679,564.4	11	61,778.58	38,816.79	1812	21.42207	2883.876	0.00
Average no. of adults on plant	9071.3	11	824.67	44,442.28	1812	24.52664	33.623	0.00
Average no. of adults on leaf	44.2	11	4.02	117.78	1812	0.06500	61.867	0.00

Table 2: Average no. of adults on leaf of *Trialeurodes vaporariorum* Westwood.

Treatment Date	**Duncan Test; Variable: Average no. of Adults on Leaf Marked Differences are Significant at $p < 0.05000$** {1} M = 0.33523	{2} M = 0.31406	{3} M = 0.04044	{4} M = 0.01605	{5} M = 0.00018	{6} M = 0.00079	{7} M = 0.33523	{8} M = 0.44367	{9} M = 0.23756	{10} M = 0.07218	{11} M = 0.02398	{12} M = 0.14720
predators 31 Aug {1}												
predators 6 Sept {2}	0.493546											
predators 13 Sept {3}	0.000004	0.000004										
predators 20 Sept {4}	0.000005	0.000004	0.461322									
predators 28 Sept {5}	0.000001	0.000001	0.253702	0.632367								
predators 13 Oct {6}	0.000001	0.000005	0.247597	0.621588	0.984266							
conventional 31 Aug {1}	1.000000	0.522965	0.000004	0.000001	0.000001	0.000001						
conventional 6 Sept {2}	0.000660	0.000055	0.000005	0.000001	0.000002	0.000001	0.000456					
conventional 13 Sept {3}	0.002279	0.013442	0.000003	0.000004	0.000005	0.000004	0.002900	0.000004				
conventional 20 Sept {4}	0.000004	0.000003	0.304514	0.097858	0.039450	0.037506	0.000004	0.000004	0.000011			
conventional 28 Sept {5}	0.000004	0.000004	0.594481	0.797496	0.490880	0.483760	0.000005	0.000001	0.000004	0.141821		
conventional 13 Oct {6}	0.000003	0.000011	0.000806	0.000057	0.000010	0.000010	0.000004	0.000004	0.003435	0.015227	0.000138	

Green boxes highlight significant differences between treatment methods on the same monitoring date.

Table 3: Average no. of adults on plant of *Trialeurodes vaporariorum* Westwood.

Treatment Date	**Duncan Test; Variable: Average no. of Adults on Plant Marked Differences are Significant at $p < 0.05000$** {1} M = 3.2107	{2} M = 6.0316	{3} M = 1.3228	{4} M = 0.86076	{5} M = 0.01274	{6} M = 0.05000	{7} M = 3.2107	{8} M = 5.6602	{9} M = 7.6863	{10} M = 3.4804	{11} M = 1.1667	{12} M = 4.7549
predators 31 Aug {1}												
predators 6 Sept {2}	0.000010											
predators 13 Sept {3}	0.002377	0.000004										
predators 20 Sept {4}	0.000218	0.000001	0.472532									
predators 28 Sept {5}	0.000005	0.000001	0.050298	0.184199								
predators 13 Oct {6}	0.000004	0.000001	0.051529	0.176927	0.950523							
conventional 31 Aug {1}	1.000000	0.000011	0.001669	0.000183	0.000004	0.000005						
conventional 6 Sept {2}	0.000089	0.536158	0.000004	0.000005	0.000001	0.000001	0.000111					
conventional 13 Sept {3}	0.000004	0.005872	0.000005	0.000001	0.000002	0.000001	0.000004	0.001073				
conventional 20 Sept {4}	0.653350	0.000044	0.000623	0.000040	0.000005	0.000004	0.675652	0.000419	0.000004			
conventional 28 Sept {5}	0.001240	0.000005	0.794875	0.610420	0.079005	0.078337	0.000964	0.000004	0.000001	0.000278		
conventional 13 Oct {6}	0.013763	0.043212	0.000004	0.000004	0.000001	0.000005	0.016829	0.131631	0.000005	0.033788	0.000004	

Green boxes highlight significant differences between treatment methods on the same monitoring date.

3.2 For Control of Frankliniella occidentalis

Regarding the surveillance of FO populations, the initial assessment shows a notably high population, averaging approximately 55 specimens per plant. Following the use of conventional treatment and the introduction of the predator *O. laevigatus*, in combination with the other two (*A. swirskii* and *E. formosa*) there is a noticeable decrease in thrips population in both experimental treatments. It is critical to recall that there are significant differences in efficiency between the two types of treatments. There were 25.2 individuals per plant in the chemically treated variants, while in the predator-treated variants, there were 7.56 individuals per plant. From the third observation onwards, there is a substantial decrease in population levels, dropping to 2 individuals per plant with a predator species present and 7 individuals per plant with chemical treatment (Fig. 6).

On September 20, a fourth observation was conducted at the same time as the second treatment and launch, showing that in the predator variant, the thrips population remains at 0 for the duration of the experiment. While the chemical treatments were initially effective, the second round did not reduce the thrips population, which stayed at 4–5 insects per plant and showed a slight growth trend at the end of the observing period.

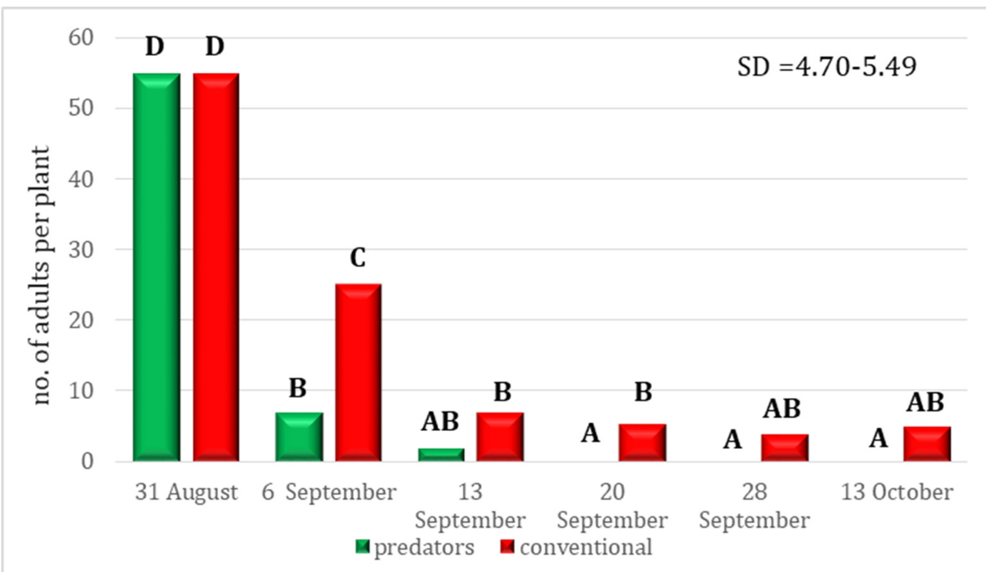


Figure 6: Numerical density of thrips after the introduction of the predators. Predators: biological control by use of *A. swirskii*, *O. laevigatus*, and *E. formosa*; Conventional: chemical control. A–D denote the significance groups identified by Duncan's multiple range test; groups labeled with different letters differ significantly.

4 Discussion

Results similar to those presented presently have been documented in various countries across Europe [19,20], Asia [21,22] and Africa [22]. The efficacy of the biocontrol agents was primarily observed in the protected environments, as the results pertain to these areas.

Our studies complement previous ones which demonstrate that *A. swirskii*, *O. laevigatus*, and *E. formosa* all demonstrate effective pest suppression in greenhouse crops, with *A. swirskii* providing strong whitefly and thrips control at appropriate release rates [23], *E. formosa* achieving 60–97% parasitism of whiteflies under favorable temperature conditions [24,25], and *O. laevigatus* contributing to thrips management [19] particularly in combination with other agents [26], though success across all three depends critically on release timing, environmental conditions, and strategic agent combinations that minimize intraguild predation.

A study conducted in Egypt during 2022–2023, by Adly and Sanad [22], demonstrated the efficacy of biocontrol agents against mites, greenhouse whiteflies, and thrips. Both predators and parasitoids are more effective than chemical treatments, as pests are developing resistance to insecticides [27] and show lesser declines in population after chemical application. Comparable findings were reported by Stenseth and Aase [28], who showed that a group of 5 adults of *E. formosa* can manage a population density of up to 100 adults of TV. In India [29], it was demonstrated that *E. formosa* parasitizes all larval stages of the greenhouse whitefly, with the parasitoid inducing a mortality rate of 52% and a parasitism rate of 50%. Deeksha [13] indicates that the cucumber crop ranks as the third most suitable host plant for Encarsia, with a parasitism rate of 31.6% in third-instar larvae of the greenhouse whitefly. According to Calvo [30], *A. swirskii* can potentially kill and significantly reduce whitefly population with a release of 75 mites/m^2. Although there is no data in the current work about the separate action of the predator *A. swirskii*, according to the literature, Weintraub [31] discovered that the reserve of thrips is significantly diminished when *A. swirskii* is combined with *O. laevigatus* [26] or with other species [23]. Other studies show the effectiveness of combining biological treatments with predators with those based on essential oils such as neem oil [23], which is part of the integrated pest control strategy IPM. Messelink [32] found that *A. swirskii* can reduce pest populations, particularly in the presence of both pests FO and TV, indicating the beneficial effects of mixed diets on predator efficacy and pest management.

5 Conclusions

The data indicate that the introduction of predators and parasitoids consistently exerts a detrimental impact on TV and FO, with population levels declining from the initial release and continuing to decrease, particularly following the second release of *O. laevigatus*, *A. swirskii*, and *E. formosa.* The efficacy of these biocontrol agents increases as the pest population diminishes, effectively regulating even subsequent generations of the pest. Conversely, the efficacy of chemical treatments is described as a variable; they can regulate the pest's populations temporarily but lack effectiveness in subsequent generations. The number of leaves increases in the predators and parasitoids treatment. By deploying all three biocontrol agents, the population is not only substantially diminished, but the values approach zero for both the greenhouse whitefly and thrips, indicating that these predators and parasitoids can be reliably used in integrated pest management systems.

Acknowledgement: Not applicable.

Funding Statement: The authors received no specific funding for this study.

Author Contributions: Conceptualization, Diana Dragan and Teodora Florian; methodology, Alexandru Apahidean, Teodora Florian and Rodica Sima; software, Vasile Florian; validation, Horia Bunescu and Vasile Florian; formal analysis, Vasile Florian and Teodora Florian; investigation, Diana Dragan, Mihaela Chertes and Ionut-Bogdan Hulujan; resources, Teodora Florian and Mihaela Chertes; data curation, Diana Dragan and Vasile Florian; writing—original draft preparation, Diana Dragan; writing—review and editing, Vasile Florian and Teodora Florian; visualization, Ximena Rau and Arnilva Mara; supervision, Teodora Florian; project administration, Diana Dragan and Teodora Florian; funding acquisition, Mihaela Chertes. All authors reviewed and approved the final version of the manuscript.

Availability of Data and Materials: Not applicable.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

Supplementary Materials: The supplementary material is available online at https://www.techscience.com/doi/10.32604/phyton.2026.075836/s1.

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