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Original article (Orijinal araştırma)

Impact of microemulsion formulations of selected botanical acaricides on the functional and numerical responses of *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae) feeding on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae)[1]

Bazı bitkisel akarisitlerin mikroemülsiyon formülasyonlarının *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) ile beslenen *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae)'nin fonksiyonel ve sayısal tepkilerine etkisi

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Abstract

This study investigated the functional and numerical responses of *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae) when preying on females of *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) under exposure to microemulsion formulations of a terpenoid blend, neem and orange oils compared to a microbial toxin acaricide, milbemectin. Acaricide applications were conducted on eggplant leaves, and their effects were assessed 168 h post-treatment under controlled laboratory conditions (70±5% RH, 27±1°C, and a 16 h light: 8 h dark) during the 2023-2024 experimental period in Bursa Uludag University and Adana Biological Control Research Institute. According to the logistic regression analysis, the control, orange oil, neem oil, and terpenoid blend treatments exhibited a Type II functional response, while milbemectin resulted in a Type III response. The attack rate and handling time of *A. swirskii* were significantly affected by treatment type. The control group exhibited the highest attack rate (1.03 h^{-1}) and the longest handling time (0.0272 h). Botanical acaricides, orange oil (0.78 h^{-1}), neem oil (0.63 h^{-1}), and the terpenoid blend (0.68 h^{-1}), reduced the attack rate and shortened handling time. Milbemectin had the most adverse effects, resulting in the lowest attack rate (0.29 h^{-1}). These findings suggest that microemulsion formulations of botanical acaricides are less disruptive to *A. swirskii* than milbemectin and may be better suited for incorporation into integrated pest management (IPM).

Keywords: Biological control, botanical acaricides, functional response, microemulsion formulation, Phytoseiidae

Öz

Bu çalışmada, *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) dişileriyle beslenen *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae)'nin, botanik kökenli akarisitlerin (portakal yağı, neem yağı ve terpenoid karışımı) mikroemülsiyon formülasyonlarına ve mikrobiyal toksin kökenli bir akarisit olan milbemectine maruz kalma durumunda gösterdiği işlevsel ve sayısal tepkileri araştırmıştır. Bu çalışma Bursa Uludağ Üniversitesi ve Adana Biyolojik Mücadele Araştırma Enstitüsünde 2023-2024 deneme döneminde akarisitler patlıcan yaprakları uygulandıktan 168 saat sonra kontrollü laboratuvar koşullarında (%70±5 bağıl nem, 27±1°C sıcaklık ve 16 s ışık ve 8 saat karanlık) değerlendirilmiştir. Lojistik regresyon analizine göre, kontrol, portakal yağı, neem yağı ve terpenoid karışımı uygulamaları Tip II fonksiyonel tepki sergilerken, milbemectin uygulaması Tip III işlevsel tepkiye neden olmuştur. *Amblyseius swirskii*'nin saldırı oranı (α) ve avı yakalama süresi (Th), akarisit uygulamasına bağlı olarak anlamlı şekilde etkilenmiştir. Kontrol grubunda en yüksek saldırı oranı (1.03 $sa.^{-1}$) ve en uzun yakalama süresi (0.0272 sa.) gözlenmiştir. Mikromülsiyon botanik akarisitlerden portakal yağı (0.78 $sa.^{-1}$), neem yağı (0.63±0.05 $sa.^{-1}$) ve terpenoid karışımı (0.68 $sa.^{-1}$), saldırı oranı azaltmış ve yakalama süresini kısaltmıştır. Milbemectin ise en olumsuz etkiyi göstererek en düşük saldırı oranına (0.29 $sa.^{-1}$) neden olmuştur. Bu bulgular, botanik akarisitlerin mikroemülsiyon formülasyonlarının *A. swirskii* üzerinde milbemectine kıyasla daha az zararlı olduğunu ve entegre zararlı yönetimi (IPM) programlarına daha uygun olabileceğini göstermektedir.

Anahtar sözcükler: Biyolojik mücadele, bitkisel akarisitler, işlevsel tepki, mikroemülsiyon formülasyon, Phytoseiidae

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Introduction

Phytoseiid mites (Acari: Mesostigmata: Phytoseiidae) are important biological control agents, with various species used worldwide to manage pest mites and small insects across many crops (McMurtry & Croft, 1997; McMurtry et al., 2013; Tixier, 2018). Among these pests, the two-spotted spider mite *Tetranychus urticae* Koch, 1836 (Acari: Trombidiformes: Tetranychidae), is a major agricultural pest that feeds on the leaves of various plants, leading to significant economic losses (Wang et al., 2004; Kazak & Kibritçi, 2008). The widespread use of synthetic acaricides against *T. urticae* has led to rapid resistance development, making it one of the most pesticide-resistant arthropods globally (Van Leeuwen et al., 2015). The extensive use of pesticides not only affects pest populations but also has negative consequences for natural enemies, such as predatory mites, leading to a disruption in biological balance (Sato et al., 2000). This disruption makes pest control more challenging and highlights the need for environmentally friendly alternative control methods.

The predatory mite *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Mesostigmata: Phytoseiidae) has been successfully used for the biological control of *T. urticae*, as well as other pests like thrips and whiteflies (Lee & Gillespie, 2011). As a highly adaptable and cost-effective Type III generalist predator, *A. swirskii* feeds on a wide range of pests and alternative food sources such as pollen, making it valuable in integrated pest management (IPM) programs (McMurtry et al., 2013). However, in high pest populations, *A. swirskii* may take longer to suppress *T. urticae* to economically acceptable levels, resulting in crop damage (Alzoubi & Çobanoğlu, 2007). Additionally, its preference for feeding on whiteflies may limit its effectiveness against *T. urticae* (Soleymani et al., 2016; Yari et al., 2023). To address this limitation, combining *A. swirskii* with acaricides or other biological control agents has been explored to enhance its efficacy in controlling *T. urticae* populations and reducing pest-related damage (Fernandez et al., 2017; Döker & Kazak, 2019; Satar et al., 2019).

Essential oils extracted from neem, mint, orange, rosemary, sage, and spearmint and thyme, along with terpenes, have shown strong effects by killing mites, repelling them, and reducing egg-laying (Choi et al., 2004; Miresmailli et al., 2006; Wu et al., 2017; Hassan et al., 2021). These plant-based compounds are gaining attention as eco-friendly alternatives to synthetic pesticides for controlling *T. urticae*. Such products, often targeting multiple pest sites, are less hazard to non-target organisms and help mitigate resistance development (Isman, 2000; Momen et al., 2018; Mossa et al., 2019; Balcı et al., 2020). They help manage *T. urticae* populations while offering ecological benefits such as rapid degradation in natural environments, which minimizes their residual impact. This rapid degradation can be advantageous for IPM programs, as it reduces the risk of pesticide residues harming beneficial organisms, including predatory mites (Kumar & Poehling, 2006). The compatibility of essential oils with effective biological control agents in IPM systems has been evaluated in several studies (Momen et al., 2001; Choi et al., 2004; Regnault-Roger et al., 2012; De Araújo et al., 2020). Essential oils generally have less harmful effects on *A. swirskii* than synthetic acaricides, especially at sublethal doses, whereas chemical acaricides significantly reduce survival and reproduction (Momen et al., 2001; Abo-Taka et al., 2014; Balcı et al., 2020; Demirtaş et al., 2022; Mousavi et al., 2022; Shirvani et al., 2023a).

Microemulsions and encapsulation techniques have improved the stability and efficacy of plant-based compounds, extending their effective duration (De Oliveira et al., 2014; Ahmadi et al., 2018a, b, 2020; Mossa et al., 2019; Balcı et al., 2020; Cheng et al., 2020; Hassanzadeh et al., 2021). Combining these products with biological control agents is essential for sustainable pest management (Castagnoli et al., 2002; Khanamani et al., 2014). Although these natural products are less toxic to non-target species, certain formulations may still negatively affect phytoseiid mites (Abo-Taka et al., 2014; Shirvani et al., 2023a). Evaluating both the direct and sublethal effects of these formulations on natural enemies is crucial to selecting safer alternatives and developing sustainable IPM strategies (Stark et al., 1995; Madanlar & Yoldaş, 1997; Galvan et al., 2005).

Understanding both the numerical and functional responses of predators is vital for optimizing biological control strategies (Kasap & Atlihan, 2011; Fathipour et al., 2017a, b, 2020). The functional response, which describes how predator consumption rates vary with prey density, and the numerical response, which reflects changes in predator populations in response to prey availability, are critical factors in determining the effectiveness of biological control agents such as *A. swirskii* (Escudero & Ferragut, 2005; Fathipour et al., 2017b, 2020; Sousa Neto et al., 2020). Several factors can influence the type of functional response and its parameters, including the prey species and developmental stage, predator age, host plant characteristics, and climatic conditions (Ahn et al., 2010; Farazmand et al., 2012; Döker et al., 2016; Song et al., 2016; Fathipour et al., 2017a, b). In recent years, interest has grown in examining the functional response of *A. swirskii* to *T. urticae* (Xiao et al., 2013; Fathipour et al., 2017b, 2020). A previous study found that microemulsion formulations of essential oils (orange oil, neem oil, and a terpenoid blend) did not adversely affect the development of *A. swirskii*, whereas milbemectin negatively impacted the predator's survival and fecundity (Mertoğlu Boz & Kumral, 2024). However, the effects of these microemulsion formulations on the numerical and functional responses of the efficient predatory mite *A. swirskii* have yet to be thoroughly investigated.

In this study, the functional and numerical responses of adult female *A. swirskii* feeding on *T. urticae* adults were evaluated 168 hours after treatment with orange oil, neem oil, a terpenoid blend, and milbemectin applied to eggplant leaves. A waiting period of 168 hours, previously found to be safe for essential oil formulations, was adopted in this study (Mertoğlu Boz & Kumral, 2024). This post-treatment interval was intended to minimize negative impacts on *A. swirskii*. The findings are expected to provide valuable insights for improving IPM strategies by combining natural predators with environmentally friendly pest management techniques that support the advancement of sustainable farming practices (Isman, 2000; Mossa et al., 2019; Balcı et al., 2020).

Materials and Methods

Acaricides

The commercial microemulsion formulations used in this study included neem oil (containing 0.35% azadirachtin), orange oil (60 g/L), and a blend of terpenoids consisting of 2% geraniol, 8% oregano oil, and 5% thymol. These botanical products were supplied by Nanomik Biotechnology (Istanbul, Türkiye) and are currently undergoing registration as plant protection products. They were applied at field rates of 0.5% for neem oil, 0.2% for orange oil, and 1% for the terpenoid mixture, in accordance with the manufacturer's recommendations. Milbemectin (Milbeknock EC), a natural acaricide provided by Sumi Agro Türkiye, was used as a positive control and applied at a field rate of 9.3 mg/L.

Plant rearing

The Turkish eggplant variety, *Solanum melongena* L. cv. Kemer, sourced from Bursa Tohum (Türkiye), was used in this study. The seeds were sown in a peat and perlite mixture (1:1 v:v) (Klasmann TS 1-Deilmann, Geeste, Germany). After eight weeks of growth, the seedlings were transplanted into 2.5-L pots filled with peat and placed in a growth chamber under controlled environmental conditions. A 16-hour light and 8-hour dark photoperiod was maintained in the chamber, with a temperature of 27±1°C and 70±5% relative humidity. The plants were watered with tap water every three days and were fertilized weekly with 100 mL of a water-soluble fertilizer containing nitrogen, phosphorus, potassium, iron, copper, sulfur, zinc, manganese, boron, and molybdenum, provided by Dr. Tarsa Company (Bursa, Türkiye). Five weeks after transplantation, five fully developed and uniform leaves were selected from each plant for the experiments.

Prey and predator colonies

The red form of *Tetranychus urticae* was collected from eggplants grown in a greenhouse in Adana province, Türkiye. According to Auger et al. (2013), the red form of *T. urticae* is considered a synonym of *Tetranychus cinnabarinus* (Boisduval, 1867) (Acari: Tetranychidae), based on morphological, biological, and molecular evidence. Species identification was confirmed following the relevant literature (Auger et al., 2013). For the infestation, ten to fifteen adult *T. urticae* individuals were transferred to five fully developed, uniform leaves of eggplant using a soft-bristle paintbrush. Colonies of *T. urticae* were subsequently established by rearing at least two generations on eggplant plants. The native population *of A. swirskii* was collected from an orange orchard in Adana, Türkiye, in 2020. This population was previously confirmed based on both molecular and morphological analyses, as reported by Mertoğlu Boz et al. (2024). The mites were kept in glass Petri dishes (15 cm diameter), with cotton placed at the bottom to maintain moisture (Overmeer, 1985). Female mites were provided with a plastic Petri dish or specially designed glass plate (12 cm) with cotton in the center, serving as oviposition sites. To prevent escape, a narrow strip of Tangle-trap was placed around the edge of the dishes or plates. The Petri dishes and cotton were kept moist at all times. Mites were reared on *Carpoglyphus lactis* (L., 1758) (Acari: Carpoglyphidae), which was brushed daily onto the upper surface of the Petri dish, supplemented with *Typha latifolia* L. (Tracheophyta: Typhaceae) pollen. New cultures were initiated by placing 15-20 freshly laid phytoseiid eggs in the Petri dishes. These colonies were mass-reared in climate-controlled growth chambers under the aforementioned conditions.

Functional response

The functional response of *A. swirskii* were evaluated on eggplant leaves treated with milbemectin, microemulsion formulations of a terpenoid blend, neem oil, and orange oil at recommended doses, in comparison to untreated controls. In the experiments, leaf samples were used 168 hours after the acaricide application, as this waiting period caused less than 25% toxicity on the predatory mite (Mertoğlu Boz & Kumral, 2025). The experiments were conducted in Plexiglas Munger cells (13 x 8 cm in size; 3 cm in diameter and 1 cm depth cylindrical cells) to assess the predator's responses to various pesticide treatments (Overmeer, 1985; Kolcu & Kumral, 2023). Potted eggplant leaves were separately treated with the acaricides at the recommended dose for *T. urticae*, using a hand sprayer to apply 2 mL of acaricide per leaf (Fernandez et al., 2017). To maintain experimental consistency, leaves of similar size and physiological age were selected for all treatments. The acaricide solution (2 ml per leaf) was applied using a hand-held sprayer at a fixed distance and angle to ensure even and uniform coverage of each leaf surface. For the control group, leaves were treated only with distilled water, which was also used in the preparation of the pesticide solution. The leaves were harvested 7 days after the treatment and placed in Munger cells for further observation. Adult female *T. urticae* were used as prey to assess the functional and numerical responses. Prior to the experiment, one gravid adult female *A. swirskii* was starved for 24 hours and then introduced into each Munger cell. Since the acaricides used are more effective against immature stages, adult stages were preferred in this experiment (Mertoğlu Boz & Kumral, 2025). Prey densities of 5, 10, 20, 40, and 80 *T. urticae* newly emerged females per leaf were provided, with a minimum of 15 repetitions for each trial. These prey densities were selected to cover a wide range from low to high prey availability, allowing us to observe the full functional response curve of the predator, as recommended in previous studies (Park et al., 2010; Kasap & Atlihan, 2011; Fathipour et al., 2020). The number of preys consumed was recorded 24 hours after the introduction of the predator (Park et al., 2010; Kasap & Atlihan, 2011).

Numerical response

In the numerical response experiment, the same general methodology as that used in the functional response assay was employed. However, in this case, the total number of eggs laid by each *A. swirskii* female was recorded over a 48-hour period at each prey density level. Importantly, no additional prey was provided during the second 24-hour interval. Prey consumption was assessed after the initial 24 hours.

Furthermore, the efficiency of conversion of ingested prey into egg biomass (E.C.I.) was calculated at each prey density using the equation proposed by Omkar & Pervez (2004) (Eq. 1):

$$E.C.I.=\frac{Number\ of\ egg\ laid}{Number\ of\ prey\ consumed}\cdot 100 \qquad \text{Eq. (1)}$$

Regression analysis was used to model the data on oviposition and ECI across different prey densities, in order to assess the relationship between (1) oviposition and prey density, and (2) the E.C.I. of female *A. swirskii* and prey density.

Statistical analysis

The functional response data were analyzed in two steps, based on Holling (1959)'s method. First, logistic regression was used to examine the relationship between the proportion of prey consumed and the initial prey density, helping determine the type of functional response curve for *A. swirskii* at various *T. urticae* adult densities (Eq. 2).

$$N_e/N_0\ =\frac{\exp\ (P_0+\ P_1\ N_0+P_2N_0^2+P_3N_0^3)}{1+\exp\ (\ (P_0+\ P_1\ N_0+P_2N_0^2+P_3N_0^3)} \qquad \text{Eq. (2)}$$

In this analysis, N_e refers to the number of prey consumed, N_0 to the initial prey density, and (N_e/N_0) to the probability of prey being consumed. The parameters P_0, P_1, P_2, and P_3 represent the intercept, and the linear, quadratic, and cubic terms, respectively, estimated using the maximum likelihood method. The type of functional response was determined based on the signs of P_1 and P_2. A negative linear coefficient ($P_1<0$) indicated a Type II functional response, where the proportion of prey consumed steadily decreased as the initial prey density increased (Equation 3.2).

In the following step, the handling time (*Th*) and attack rate (*α*) parameters for a Type II functional response were estimated using Rogers' random predator equation (Rogers, 1972) (Eq. 3).

$$N_e = N_0\ [1 - \exp\{-\alpha(T - T_hN_e)\}] \qquad \text{Eq. (3)}$$

When a positive density—dependent result for the proportion of prey consumed ($P_1>0$ and $P_2<0$) is obtained, the predator displays a Type III functional response (Juliano, 2001). If the functional response was identified as Type III, the attack rate (*α*) and handling time (*Th*) were estimated using the equation (Eq. 4) developed by Hassell et al. (1977).

$$N_e = N_0\ (N_0\ - N_e)[c\log\left\{\frac{N_0\ -N_e}{N_0}\right\} - bT_hN_e + bT] \qquad \text{Eq. (4)}$$

In these equations, N_0 stands for the initial prey density, N_e is the number of preys captured by the predator, *α* represents the attack rate, *T* indicates the trial duration (in days) within a given area, *Th* corresponds to the handling time (in days), and *b* and *c* are regression coefficients. Nonlinear regression (Proc NLIN, SAS Institute) was applied to estimate the attack rate and handling time values. The values of attack rate and handling time were estimated by fitting a functional response model to the pooled data from each treatment. As only one set of parameter estimates can be obtained per treatment, pseudo-replication was applied to allow for statistical comparisons. Specifically, parameter values were re-estimated multiple times by resampling subsets of the data (bootstrapping 10.000 times), and the resulting values were treated as replicates for the purposes of ANOVA. To evaluate the effects of prey density, acaricide type, and their interaction on prey consumption or egg laying, we used a generalized linear model (GLM) with a normal distribution. The effects of acaricides on the biological parameters of mites were statistically analyzed using one-way ANOVA, after verifying normality ($p<0.05$). Mean comparisons were performed using the Tukey test, with significant differences accepted at $p<0.05$ (IBM, 2015).

Results

Functional response

The logistic regression analysis results showed a significantly negative linear coefficient ($P_1<0$) for the control group, suggesting that the proportion of prey consumed by *A. swirskii* decreased as prey density increased. Similarly, positive quadratic coefficients ($P_2>0$) were observed for the orange oil, neem oil, and terpenoid blend treatments, while their linear coefficients were not significant, indicating a Type II functional response across all prey densities. In contrast, the milbemectin treatment exhibited a significantly positive linear coefficient ($P_1>0$) and a negative quadratic coefficient ($P_2<0$), suggesting a low proportion of prey consumption at low prey densities and a gradual increase with higher prey availability. Consequently, while the control, orange oil, neem oil, and terpenoid blend treatments resulted in a Type II functional response by *A. swirskii*, the milbemectin treatment induced a Type III functional response (Table 1).

Table 1. Maximum likelihood estimates and predation type classification were determined using logistic regression analysis to evaluate the functional response of adult female *Amblyseius swirskii* 168 hours after the application of recommended doses of, orange oil, neem oil, terpenoid blend and milbemectin on eggplant leaves across different prey (*Tetranychus urticae* adult) densities (N_0)

Treatments	Parameters	Estimates (P0, P1, P2, P3)	Standard error	t	*p*-value	Functional Response
Control	Intercept (P0)	0.4955	0.0900	5.50	< 0.01*	TYPE II (*P1*<0) and (*P2*>0)
	Linear (P1)	-0.0133	0.0044	-2.99	0.0033*	
	Quadratic (P2)	0.0011	0.0000	2.93	0.0039*	
	Cubic (P3)	-8.694e-6	0.0000	-0.79	0.4286	
Orange Oil	Intercept (P0)	0.3636	0.0870	4.17	< 0.01	TYPE II (*P1*<0) and (*P2*>0)
	Linear (P1)	-0.0070	0.0045	4.07	0.1220	
	Quadratic (P2)	0.0015269	0.0003	4.07	< 0.01	
	Cubic (P3)	-0.000022	9.538e-6	-2.31	0.0222	
Neem Oil	Intercept (P0)	0.2409526	0.08597	2.80	0.0058	TYPE II (*P1*>0) and (*P2*>0)
	Linear (P1)	0.0014297	0.004609	0.31	0.7569	
	Quadratic (P2)	0.0017193	0.000395	4.36	< 0.01	
	Cubic (P3)	-3.08e-5	9.402e-6	-3.28	0.0013	
Terpenoid Blend	Intercept (P0)	0.2724075	0.082559	3.30	0.0012	TYPE II (*P1*<0) and (*P2*>0)
	Linear (P1)	-0.000643	0.004421	-0.15	0.8846	
	Quadratic (P2)	0.0016612	0.000383	4.34	< 0.01	
	Cubic (P3)	-2.867e-5	9.025e-6	-3.08	0.0018	
Milbemectin	Intercept (P0)	0.2294074	0.080293	2.86	0.0049	TYPE III (*P1*>0) and (P2<0)
	Linear (P1)	0.0216901	0.000372	-0.14	0.5984	
	Quadratic (P2)	-5.157e-5	0.000372	-0.14	0.8900	
	Cubic (P3)	-3.942e-6	7.468e-6	-0.53	0.5984	

The acaricide treatment (control, orange oil, neem oil, terpenoid blend, and milbemectin), prey density (5, 10, 20, 40, and 80) and their interaction had a significant effect on the number of prey consumed (acaricide treatment $\chi^2=186.91$, $p<0.01$; prey density: $\chi^2=631.48$, $p<0.01$; interaction: $\chi^2=101.30$, $p<0.01$). At lower prey densities (5 and 10 preys), the control population exhibited the highest consumption, followed by the terpenoid blend and orange oil treatments. In contrast, the milbemectin treatment led to the lowest prey consumption, particularly at the lowest prey densities. At a density of 5 preys, the control consumed an average of 3.20 individuals, while the milbemectin treatment group only consumed 1.40, which is significantly lower ($p<0.01$). The control group's consumption decreased gradually as prey density increased, but it still maintained the highest consumption across all densities. At a prey density of 10, similar trends were observed, with the control group consuming the most (7.60 preys) and milbemectin again consuming the least (3.40 preys). Orange oil and neem oil populations showed intermediate consumption rates. These differences in prey consumption were statistically significant, indicating that the treatments had a clear impact on consumption rates at this prey density. As prey density increased to 20, 40, and 80 preys, the consumption rates for the control group remained relatively high compared to other treatments, though the proportion of prey consumed by all groups decreased. For example, at a prey density of 40, the control group consumed 22.33 individuals, while the milbemectin group consumed only 11.07. At a prey density of 80, the consumption for the control group was 33.87 preys, again significantly higher than in the milbemectin group, which consumed only 14.67 preys (Figure 1). Overall, as prey density increased, there was a general decline in the number of preys consumed across all treatments, with milbemectin showing the most pronounced reduction in prey consumption. The difference between the milbemectin groups was particularly notable, suggesting that milbemectin may not be as effective in sustaining prey consumption at higher densities.

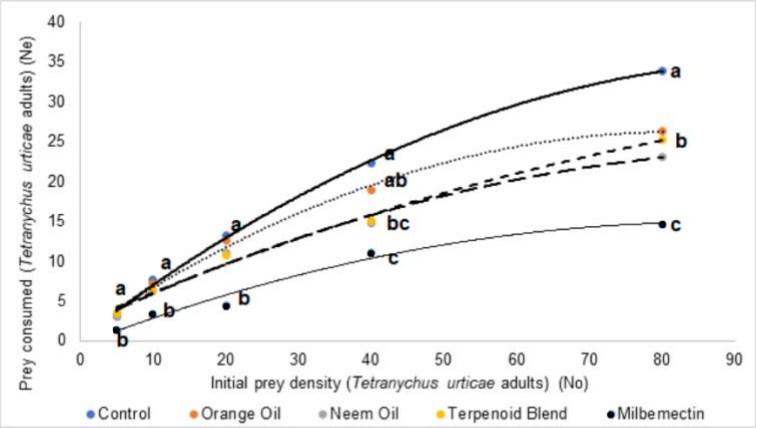

Figure 1. Functional response of *Amblyseius swirskii* released 168 hours after the application of the recommended rate of orange oil, neem oil, terpenoid blend microemulsion formulations, and milbemectin to eggplant leaves, at varying *Tetranychus urticae* adult densities (No). Different letters in the same prey density indicate statistically significant differences between means at the 5% significance level according to the Tukey test.

Attack rate and handling time

The attack rate (α) and handling time (Th) of *A. swirskii* were significantly influenced by the treatments ($p<0.01$). Predators in the control group exhibited the highest attack rate (1.03 h^{-1}) and the longest handling time (0.0272 h), both significantly different from the other treatments (Table 2).

Table 2. Attack rate and handling time of *Amblyseius swirskii* released 168 hours after the application of the recommended rates of orange oil, neem oil, terpenoid blend microemulsion formulations, and milbemectin to eggplant leaves, at varying *Tetranychus urticae* adult densities (Mean±SE)

Treatments	α* or b** (h^{-1})	Handling Time T_h (h)	Functional Response
Control	1.03±0.15a***	0.0272±0.0008a	Type II
Orange Oil	0.78±0.05ab	0.0203±0.0011b	Type II
Neem Oil	0.63±0.05b	0.0181±0.0011b	Type II
Terpenoid Blend	0.68±0.05b	0.0202±0.0013b	Type II
Milbemectin	0.29±0.03c	0.0111±0.0011c	Type III
Df; F; P	4,19; 11.91;	4,45; 30.21; <0.01	

* α=attack rate for Type II,

** b= parameter associated with the attack rate for Type III

*** Means followed by the same letter in a column are not significantly different (Tukey, $p<0.05$).

Among the botanical treatments, orange oil, neem oil, and the terpenoid blend showed moderately high attack rates, although lower than the control. All botanical treatments had shorter handling times than the control, suggesting that although predators captured prey less frequently than in the control, they processed prey more quickly. Milbemectin had the most pronounced negative effect, with the lowest attack rate (0.29 h^{-1})

while the shortest handling time (0.0111 h). This indicates a severe impairment in the predator's ability to find and consume prey efficiently under milbemectin exposure. Overall, while botanical acaricides slightly reduced the predators' attack rates compared to the control, they still allowed relatively efficient prey handling.

Numerical response

The number of eggs laid by *A. swirskii* on *T. urticae* adults varied across treatments and prey densities. The acaricide treatment (control, orange oil, neem oil, terpenoid blend, and milbemectin) had a significant effect on the number of egg laying (χ^2=12.06, *p*=0.017), whereas prey density (5, 10, 20, 40, and 80) and the interaction between acaricide and prey density were not significant (prey density: χ^2=5.36, *p*=0.25; interaction: χ^2=17.79, *p*=0.33). In the control, neem oil, and terpenoid blend treatments, oviposition increased steadily with the rising prey density. At the highest prey density (80 preys), the terpenoid blend showed the highest mean egg production (1.13 eggs), followed by the control (1.07 eggs) and neem oil (1.00 eggs). These treatments exhibited a clear positive numerical response, with egg production increasing proportionally as prey density rose. In contrast, the milbemectin treatment resulted in the lowest oviposition rates across all prey densities. Even at the highest prey level, egg production remained minimal (ranging from 0.13 to 0.27 eggs), indicating a strong suppression of the predator's reproductive capacity. The orange oil treatment had a moderately suppressive effect, with lower egg numbers compared to the control but not as severe as milbemectin. Overall, these results suggest that while the control, neem oil, and terpenoid blend treatments promoted a positive numerical response, milbemectin severely impaired reproduction, and orange oil exerted a moderate inhibitory effect (Figure 3). Statistical comparisons revealed that the egg production under milbemectin treatment was significantly lower than most other treatments, especially at higher prey densities. In contrast, the control, terpenoid blend, and neem oil treatments did not differ significantly from each other at high prey densities, suggesting similar levels of reproductive enhancement (Figure 2).

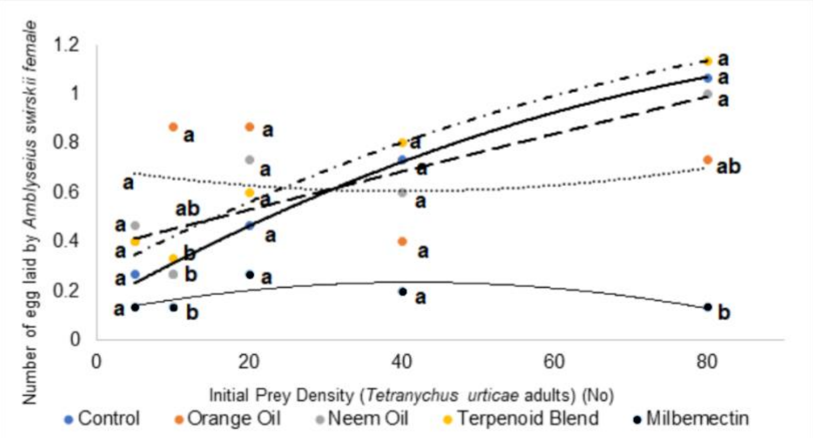

Figure 2. Number of eggs laid by *Amblyseius swirskii* released 168 hours after the application of the recommended rate of orange oil, neem oil, terpenoid blend microemulsion formulations, and milbemectin to eggplant leaves, at varying *Tetranychus urticae* adult densities (No). Different letters in the same prey density indicate statistically significant differences between means at the 5% significance level according to the Tukey test.

The efficiency of conversion of ingested food (ECI) by *A. swirskii* also varied with prey density and treatment. At the lowest prey density (5 preys), the highest E.C.I. values were observed under neem oil (19.11) and orange oil (16.89) treatments. Across all treatments, E.C.I. generally declined as prey density increased. For instance, under control conditions, E.C.I. decreased from 6.89 to 3.17, suggesting diminished reproductive efficiency at higher prey levels. Milbemectin consistently led to the lowest E.C.I. values, especially at the highest prey density (0.73 at 80 preys), highlighting its adverse effect on nutrient conversion. In contrast, botanical oils maintained relatively higher E.C.I. values at low prey densities (Figure 3). Interestingly, the E.C.I. under the terpenoid blend treatment followed a nonlinear trend, increasing at intermediate prey densities and then declining at the highest level, suggesting that optimal nutrient conversion occurred at moderate prey availability (Figure 3).

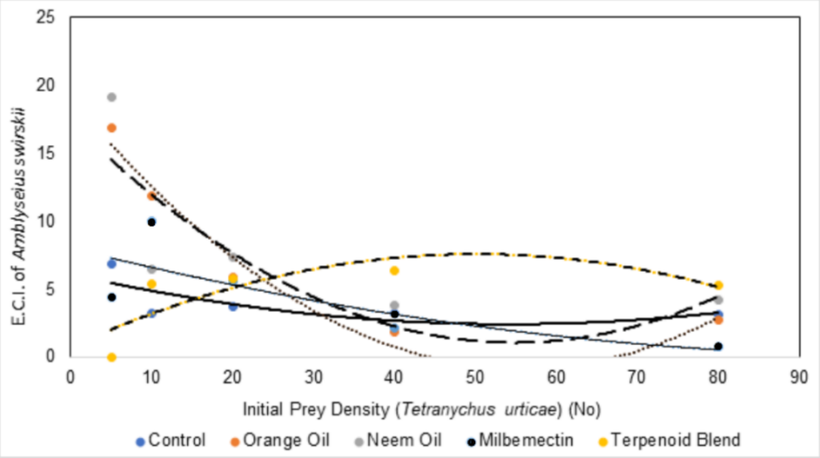

Figure 3. Efficiency of Conversion of Ingested Food (E.C.I.) of *Amblyseius swirskii* released 168 hours after the application of the recommended rate of orange oil, neem oil, terpenoid blend microemulsion formulations, and milbemectin to eggplant leaves, at varying *Tetranychus urticae* adult densities (No).

Regression analyses revealed distinct patterns in the relationship between prey density and E.C.I. across treatments (Table 3). The strongest relationship was observed under neem oil (*r*=0.9672), where E.C.I. declined sharply with increasing prey density. Control and milbemectin treatments also showed a moderate negative correlation (*r*=0.5034 and 0.5924, respectively), indicating reduced conversion efficiency at higher prey levels. Conversely, the terpenoid blend exhibited a nonlinear trend, with E.C.I. increasing at intermediate densities and declining thereafter (r=0.6208). The orange oil treatment showed the weakest correlation (*r*=0.1192), suggesting high variability in E.C.I. values across prey levels. These regression results support the visual patterns observed in Figure 3 and highlight the differential impact of treatments on predator nutrient conversion efficiency.

Table 3. Relationship between Efficiency of Conversion of Ingested Food (E.C.I.) of *Amblyseius swirskii* at different prey (*Tetranychus urticae* adult) densities (N_0) 168 hours after the application of recommended doses of, orange oil, neem oil, terpenoid blend and milbemectin on eggplant leaves

Treatments	Regresion equal	*r*
Control	$y=0.0013x^2 - 0.1369x + 6.1206$	0.5034
Orange Oil	$y=0.0064x^2 - 0.7143x + 19.069$	0.1192
Neem Oil	$y=0.0054x^2 - 0.5952x + 17.386$	0.9672
Terpenoid Blend	$y=-0.0027x^2 + 0.2741x + 0.7175$	0.6208
Milbemectin	$y=0.0007x^2 - 0.1505x + 8.0355$	0.5924

Discussion

Understanding the functional response of predatory mites is crucial for assessing their potential as biological control agents under suppressive acaricide treatments. In this study, *A. swirskii* demonstrated a Type II functional response under untreated conditions, indicating its efficiency in prey consumption at low prey densities. A Type II functional response is advantageous for biological control agents because it enables efficient prey consumption at low to moderate prey densities, contributing to effective pest suppression (Holling, 1959; Hassell, 1977). Our findings are consistent with previous studies showing similar functional response patterns in *A. swirskii* when preying on different developmental stages of spider mites and thrips (Fathipour et al., 2020; Shirvani et al., 2023a, b, 2024; Doğan & Kumral, 2025), as well as in other predatory species (Fernandez-Arhex & Corley, 2003; Badii et al., 2004; Xiao & Fadamiro, 2010). Supporting our results, Shirvani et al. (2024) demonstrated that *A. swirskii* exhibited a Type II functional response when fed on *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae) in the presence of essential oils derived from *Mentha piperita* L. (Tracheophyta: Lamiaceae) and *Laurus nobilis* L. (Tracheophyta: Lauraceae). Similarly, Shirvani et al. (2023a) observed a Type II response in *A. swirskii* feeding on western flower thrips larvae, which remained stable even when treated with *Zataria multiflora* essential oil. Consistent with our findings, the functional responses of *Neoseiulus baraki* Athias-Henriot, 1966 and *Typhlodromus ornatus* (Denmark & Muma, 1873) (Acari: Phytoseiidae), both predators of the coconut mite *Aceria guerreronis* Keifer, 1967 (Acari: Eriophyidae), were not affected by exposure to various acaricides (Lima et al., 2015) or coconut oil (Freitas et al., 2018).

This is in line with earlier studies indicating that some predators typically exhibit a Type II functional response under various conditions, although factors such as host plant species, prey stage, or pesticide exposure can influence this pattern (Gu, 1991; Li et al., 2006; Afshar & Latifi, 2017; Afza et al., 2021; Farazmand & Amir-Maafi, 2021). The results of this study revealed that exposure to milbemectin induced a shift in the functional response of *A. swirskii* from Type II to Type III, persisting even 168 hours after treatment. Similarly, Afza et al. (2021) observed a comparable shift in *Coccinella septempunctata* L., 1758 (Coleoptera: Coccinellidae) following sublethal exposure to imidacloprid, profenophos, and chlorpyrifos. Likewise, Gu (1991) reported that the parasitoid *Diaeretiella rapae* (McIntosh, 1855) (Hymenoptera: Braconidae) exhibited a Type III response following exposure to cypermethrin, dimethoate, and pirimicarb. In agreement, Li et al. (2006) documented a similar transition in *Scolothrips takahashii* Priesner, 1950 (Thysanoptera: Thripidae) after treatment with abamectin and fenpropathrin. This shift may be attributed to avermectins disrupting the insect nervous system, leading to disorientation and impaired predatory behavior. In a similar study, exposure to the recommended concentration of an abamectin and bifenazate blend acaricide shifted the functional response of a native *A. swirskii* population from type II to type III (Doğan & Kumral, 2025).

Attack rate (the time spent searching for prey) and handling time (the duration from prey encounter to completion of feeding) are critical parameters in evaluating predator effectiveness (Veeravel & Baskaran, 1997; Fathipour & Maleknia, 2016). Several factors can influence handling time, including predator speed, prey mobility, and the effort needed to subdue prey (Hassell, 1977). These are also related to the prey's behavioral and structural defense mechanisms (Ali et al., 2011). Our findings align with those of de Sousa Neto et al. (2020), who showed that acaricides such as abamectin, fenpyroximate, and azadirachtin increased search time, reduced prey consumption, and decreased oviposition in *Neoseiulus idaeus* Denmark & Muma, 1973 (Acari: Phytoseiidae). Similar negative effects have been reported in other studies, where sublethal pesticide exposure led to decreased attack rates and increased handling times in multiple predator species (Wang & Shen, 2002; Claver et al., 2003; Li et al., 2006; Doğan & Kumral, 2025). Exposure to essential oils moderately reduced prey consumption by *A. swirskii*, whereas milbemectin caused a substantial reduction across all prey densities. Although prey consumption increased with prey density, consumption efficiency declined. These results suggest that essential oils are less disruptive than milbemectin, which severely impaired the predatory capacity of *A. swirskii*. Similar results were observed by Shirvani et al. (2024) following essential oil treatments.

The numerical response of *A. swirskii* was significantly influenced by the treatments, with the highest oviposition observed under terpenoid blend, neem oil, and control treatments at higher prey densities. These findings are consistent with prior studies showing that sublethal botanical compounds can enhance reproductive output in phytoseiid mites (Momen et al., 2001; Shirvani et al., 2023a, b). In contrast, milbemectin severely suppressed oviposition at all prey densities, suggesting persistent sublethal toxicity even 168 hours after application. Orange oil had a moderate effect, reducing egg production compared to the control, though less severely than milbemectin. The efficiency of conversion of ingested food (E.C.I.) by *A. swirskii* varied with prey density and treatment. The highest E.C.I. values were recorded at the lowest prey density under neem oil and orange oil treatments. While orange oil was less harmful than milbemectin, its impact on predator performance was inconsistent. The weak correlation between prey density and E.C.I. under orange oil treatment suggests variable physiological responses, possibly due to fluctuating sublethal effects. Botanical oils promoted higher E.C.I. at low prey densities, indicating better reproductive efficiency under limited prey conditions. These results support earlier findings that prey density and treatment influence energy allocation and reproduction in predatory mites (Omkar & Pervez, 2004; Sabaghi et al., 2011).

Conclusion

In conclusion, this study demonstrated that essential oil treatments had a moderate impact on the functional and numerical responses of *A. swirskii*, whereas milbemectin significantly reduced predatory performance, even after a 168-hour waiting period. Following essential oil exposure, *A. swirskii* largely maintained a Type II functional response, consistent with previous studies, while milbemectin induced a shift to a Type III response, reflecting substantial disruption of its predatory behavior. Changes in attack rate and handling time further highlight the high sensitivity of *A. swirskii* to chemical treatments, with milbemectin showing more severe negative effects than botanical oils. These results highlight that while botanical acaricides can affect the foraging and reproductive behavior of *A. swirskii*, they tend to preserve key ecological traits such as numerical response and conversion efficiency. In contrast, milbemectin disrupts both functional and numerical responses, potentially undermining the efficacy of *A. swirskii* as a biological control agent. Therefore, careful selection and timing of acaricide applications are essential when incorporating such predators into integrated pest management (IPM) strategies. Furthermore, our findings emphasize the importance of evaluating sublethal pesticide effects beyond mortality, focusing also on behavioral and functional traits. The observed shift from a Type II to a Type III response under milbemectin exposure underscores potential ecological consequences in pest-predator dynamics and reinforces the need for IPM approaches that minimize harm to beneficial arthropods. These findings provide important insights into the ecological effects of acaricides on biological control agents and support the relative compatibility of essential oils with predatory mites in IPM programs. While laboratory-based studies are valuable for understanding predator-prey dynamics, they may not fully represent the complexity of natural systems. The moderate impact of essential oils suggests their potential inclusion in IPM with minimal disruption to natural enemies, whereas synthetic acaricides like milbemectin should be used with greater caution. Future research should prioritize semi-field and field studies to validate these results under realistic conditions and explore the long-term compatibility of essential oils with predatory mites in sustainable pest management strategies.

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