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Comparative assessment of bio-agents in protected cucumbers against the two-spotted spider mite

İki noktalı kırmızı örümceğe karşı örtüaltı hıyar yetiştiriciliğinde biyo-ajanların karşılaştırmalı değerlendirilmesi

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A B S T R A C T

Protected cucumber production *Cucumis sativus* L. (Cucurbitaceae) faces significant threats from infestations by the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Globally, *T. urticae* has been effectively managed using biological control agents such as predatory mites — *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) — and predatory insects, including *Stethorus gilvifrons* (Mulsant) (Coleoptera: Coccinellidae), and *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae). This study aimed to assess and compare the reduction of *T. urticae* populations on two cucumber cultivars, Barcoda and Hisham, using these four bio-agents in comparison with the conventional acaricide bifenazate. Results showed that *P. persimilis* achieved a reduction of up to 96.24% on the Hisham cultivar, while *S. gilvifrons* provided a similar reduction of 94.89% on Barcoda. In contrast, bifenazate resulted in the lowest reduction rates, ranging from 60.23% to 74.96% across the two cultivars. All bio-agents significantly reduced *T. urticae* populations compared to untreated control. The other predatory species, *O. albidipennis* and *N. californicus*, also exhibited substantial, though slightly lower, levels of control. These findings underscore the potential of bio-agents as effective components of integrated pest management strategies for sustainable cucumber production. Further studies are recommended to optimize predator combinations and release rates.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a globally significant pest that infests a wide range of crops, including ornamentals, field crops, vegetables, and fruits (Dermauw et al. 2012). Reliance on chemical pesticides and acaricides for controlling *T. urticae* led to the development of resistance in the pest to several of chemical compounds (Gao et al., 2012, Gorman et al. 2002, Van Leeuwen et al. 2010).

For instance, bifenazate (Solo 24% SC) is widely used globally for *T. urticae* control due to its targeted mode of action (Van Leeuwen et al. 2015). According to the recommendations of the Egyptian Agricultural Pesticides Committee (APC-Egypt), the application rates of bifenazate is approximately 80 cm^3 per 100 liters of water for *T. urticae* infestations in conventional agriculture across various crops such as tomato, strawberry, grape, and apple (Anonymous, 2024). Despite its effectiveness, concerns have been raised regarding its persistence, rapid degradation, and suboptimal control (Abdelmaksoud et al., 2023, Ochiai et al. 2007).

In the traditional cucumber production areas at El-Beheira Governorate, the excessive use of chemicals has raised concerns about potential risks to environmental and human health (Barzman et al. 2015, Hassan et al. 2017). Consequently, there has been a growing need in protected cultivation areas to implement biological control strategies as an alternative to chemical pesticides.

Specialized predatory mites such as *Phytoseiulus persimilis* Athias-Henriot (Type I) (McMurtry et al. 2013) and *Neoseiulus californicus* (McGregor) (Type II) (McMurtry et al. 2013), have been extensively used in biological control programs targeting *T. urticae*. For example, *P. persimilis* is highly effective in suppressing spider mite populations due to its rapid reproduction rate, high prey consumption, and strong adaptation to greenhouse enviroments (Fraulo and Liburd 2007, Yanar et al. 2019). Similarly, *N. californicus* is favoured for its tolerance to variable environmental conditions and ability to survive on alternative prey, making it a valuable component of integrated pest management (IPM) systems (Akyazi and Liburd 2019, Muştu et al. 2016).

Additionally, predatory insects, such as *Stethorus gilvifrons* (Mulsant) (Coleoptera: Coccinellidae) and *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae), also play significant roles in biological control (Taghizadeh et al. 2018, 2020). The predatory beetle, *S. gilvifrons*, is well known for its voracious appetite for spider mites (Ebrahimifar et al. 2020). It is frequently released in greenhouses and field crops due to its efficiency in reducing mite populations (van Lenteren 2012). This species is recognized for its dispersal capability and lifecycle synchronization with its prey, ensuring consistent control (Jafari et al. 2022, Ragkou et al. 2004).

On the other hand, *O. albidipennis* targets a broad range of pests, including aphids, thrips, and spider mites. Its adaptability to diverse cropping systems and effectiveness at low prey densities make it a valuable biological control agent (Salehi et al. 2016, Taghizadeh et al. 2020).

Therefore, the present study aimed to evaluate the efficacy of the aforementioned predatory mite and insect species for controlling *T. urticae* on two cucumber cultivars under protected cultivation conditions in El-Beheira Governorate. Additionally, the study compared their reduction percentages with conventional chemical control using bifenazate. The overall goal was to identify the most effective biological control strategy for sustainable cucumber production and to provide an added value for protected cultivation growers by promoting a shift from chemical to biological pest control.

MATERIALS AND METHODS

Study area and plantation

The study was conducted in Badr centre, El Beheira Governorate. Two cucumber cultivars, Barcoda and Hisham were cultivated. The cultivation area was measured approximately 800 m^2 within a double unit high-plastic-net greenhouse, which was divided into 12 equal plots. Each plot was further subdivided into three separate replicates. Plots were completely isolated from each other using plastic sheets. All the experimental plots received uniform standard cultivation practices, including organic and mineral fertilization, drip irrigation, and mechanical weed control.

Predatory species

Stock cultures of *P. persimilis, N. californicus, S. gilvifrons* and *O. albidipennis* were obtained from a private company (Bio Kaha, Egypt). Each species was supplied in two forms:

i) cardboard bottles (four bottles), each containing 25.000 individuals (nymphs and newly-emerged adults);

and

ii) slow-release sachets (100 sachets), which contained 250 nymphs and adults (total number of each species was 125.000 individuals).

Acaricidal compound

Bifenazate (Solo 24% SC) was applied at a rate of 80 cm^3 per 100 liters of water. The product was manufactured and supplied by Shoura Company, Egypt.

Sampling procedure and experimental design

To evaluate the effects of the four predators and the acaricide treatments was laid out in a randomized complete block design (RCBD). Prior to release or spraying, 30 leaves were randomly collected from the three replicates of each treatment (10 leaves × 3 replicates).

The number of *T. urticae* eggs and active motile stages (nymphs and adults), as well as live and dead individuals, were counted and recorded weekly for each treatment. Weekly samples were also collected from untreated control plots adjacent to the experimental plots, starting from the day of application until the end of the experiment. Each sample was sealed in a paper bag and transported to the laboratory for examination under a stereomicroscope.

Data from predatory-treated and acaricide-treated plots were compared with untreated control plants for both cucumber cultivars. Predators were released once at an inundative rate of 1:10 (predator: prey) based on preliminary counts. Bifenazate was applied and replicated three times during the experimental period.

Statistical analysis

Reduction percentages were calculated using the Henderson and Tilton (1955) equation:

$$Reduction\ (\%) = \left(1 - \frac{n\ in\ Co\ before\ treatment \times n\ in\ T\ after\ treatment}{n\ in\ Co\ after\ treatment \times n\ in\ T\ before\ treatment}\right) \times 100$$

Where *n represents* the herbivore population, *T indicates* the treated plots, and *Co* indicates the control plots.

The mean numbers of *T. urticae* on the two cucumber cultivars following the application of predatory species were analyzed using one-way analysis of variance (ANOVA). Differences among treatment means were compared with Duncan's multiple range test at significance level of $P \leq 0.05$. All statistical analyses were performed using IBM SPSS computer software version 26 (SPSS, 2019).

RESULTS

Effect of predator release and acaricide spraying on T. urticae populations

Significant differences were observed in the mean population numbers of *T. urticae* across treatments in Barcoda and Hisham cultivars. In Barcoda, both bio-agent releases and acaricide application significantly reduced the active stages of *T. urticae* ($F = 40.84$; $df = (5, 135)$, $P = 0.000$) and egg stages ($F = 28.70$; $df = (5, 135)$, $P = 0.000$). Similarly, in Hisham cultivar, were the treatments significantly affected active stages ($F = 40.19$; $df = (5, 135)$, $P = 0.000$) and eggs ($F = 26.50$; $df = (5, 135)$, $P = 0.000$) (Table 1).

Releases of *P. persimilis* and *S. gilvifrons* resulted in the lowest *T. urticae* population levels, followed by *N. californicus* and *O. albidipennis*. In contrast, spraying with bifenazate yielded the highest *T. urticae* populations among all treatments (Table 1).

Slight but significant differences were also detected between cultivars. The number of *T. urticae* individuals per leaf was lower in Barcoda than in Hisham for both bio-agent releases and bifenazate treatments (Table 1).

Reduction percentages of T. urticae populations

Figures 1 and 2 show the reduction percentages of *T. urticae* in Barcoda and Hisham cultivars. Minor but statistically significant differences were observed in the effect of predator releases and chemical spraying against *T. urticae* eggs in Barcoda ($F = 8.03$; $df = (4, 110)$, $P = 0.000$) (Figure 1a) and Hisham ($F = 9.08$; $df = (4, 110)$, $P = 0.000$) (Figure 2a). Similarly, for motile stages, slight differences were recorded in the release and spray treatments against *T. urticae* moving stages in Barcoda ($F = 13.70$; $df = (4, 110)$, $P = 0.000$) (Figure 2b) and Hisham ($F = 14.51$; $df = (4, 110)$, $P = 0.000$) (Figure 2b).

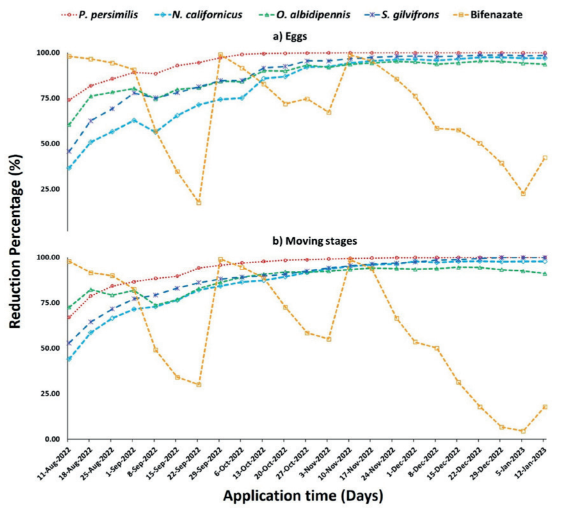

Figure 1. Reduction percentages of *Tetranychus urticae*, a) eggs and b) motile stages on the Barcoda cucumber cultivar following the release of predatory mite and insect species compared to bifenazate acaricide spraying

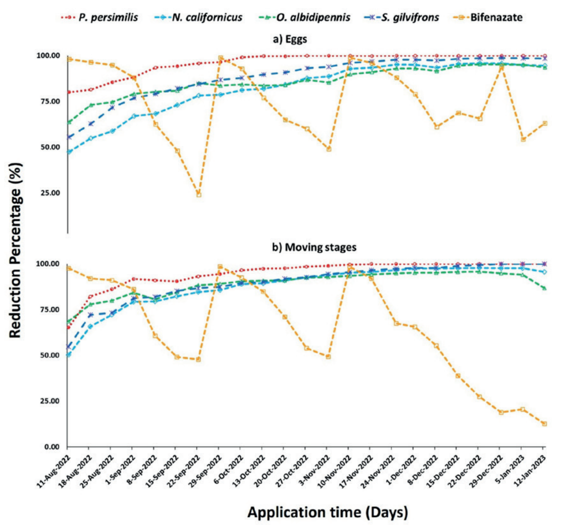

Figure 2. Reduction percentages of *Tetranychus urticae*, a) eggs and b) motile stages on the Hisham cucumber cultivar following the release of predatory mite and insect species comparing to bifenazate acaricide spraying

Table 1. Mean numbers of *Tetranychus urticae* eggs and active moving stages on two tested cucumber cultivars following the release of predatory mite and insect species and spraying of bifenazate

Cultivar	*T. urticae* stage	Treatment	Mean ± SE	$F_{df=(5, 138)}$	*Sig.*
Barcoda	Moving	*P. persimilis*	4.51 ± 0.96[c]	40.84**	0.000
		N. californicus	17.23 ± 0.94[b]		
		O. albidipennis	21.30 ± 1.55[b]		
		S. gilvifrons	13.12 ± 1.34[b]		
		Bifenazate	53.37 ± 10.06[b]		
		Control	271.11 ± 38.13[a]		
	Eggs	*P. persimilis*	1.68 ± 0.55[c]	28.70**	0.000
		N. californicus	13.08 ± 0.26[b]		
		O. albidipennis	15.80 ± 1.23[b]		
		S. gilvifrons	8.03 ± 0.28[c]		
		Bifenazate	26.46 ± 5.08[b]		
		Control	203.06 ± 35.35[a]		
Hisham	Moving	*P. persimilis*	6.48 ± 1.37[c]	40.19**	0.000
		N. californicus	21.91 ± 0.92[b]		
		O. albidipennis	26.47 ± 1.45[b]		
		S. gilvifrons	17.41 ± 1.80[b]		
		Bifenazate	56.39 ± 9.80[b]		
		Control	308.14 ± 43.84[a]		
	Eggs	*P. persimilis*	2.11 ± 0.72[c]	26.50**	0.000
		N. californicus	17.65 ± 0.62[b]		
		O. albidipennis	17.78 ± 1.39[b]		
		S. gilvifrons	9.53 ± 0.41[c]		
		Bifenazate	30.42 ± 5.58[b]		
		Control	194.23 ± 34.51[a]		

Different letters within the same column are statistically significant (Tukey test, P<0,05). SE: Standard error.

Within each cultivar, the specialist predatory mite, *P. persimilis* resulted in the highest reduction percentage of *T. urticae* eggs in Barcoda (95.73 ± 1.50) (Figure 1a) and Hisham (96.24 ± 1.31) (Figure 2a). As well as, for moving stages in Barcoda (94.55 ± 1.77) (Figure 1b) and Hisham (94.89 ± 1.70) cultivars (Figure 2b).

In the second place, the predatory insect species, *O. albidipennis* and *S. gilvifrons* almost resulted in similar reduction percentages against *T. urticae* eggs (87.35 ± 1.91 and 87.60 ± 2.93, respectively) and moving stages (88.13 ± 1.48 and 88.79 ± 2.59, respectively) in Barcoda (Figure 1a,b). In addition, in Hisham cultivar for eggs (85.93 ± 1.70 and 88.39 ± 2.52, respectively) and moving stages (89.17 ± 1.46 and 89.76 ± 2.34, respectively) (Figure 2a,b). Consequently, the predatory mite, *N. californicus* recorded lower reduction percentages than other species. However, *N. californicus* resulting percentages were not significantly different between the two tested cultivars (Figures 1, 2).

Contrary, spraying bifenazate recorded the lowest reduction percentages in *T. urticae* egg (69.69 ± 5.36) and moving stages (60.23 ± 6.65) in Barcoda (Figure 1a,b). While, it resulted slight significant difference in the case of Hisham for *T. urticae* eggs (74.96 ± 4.30) and active moving stages (64.05 ± 5.80) (Figure 2a,b).

DISCUSSION

The results confirmed the superior efficacy of biological control agents over chemical acaricides in managing *T. urticae* populations. The predatory mite *P. persimilis*, and the predatory insect, *S. gilvifrons*, were particularly effective due to their high predation rates and environmental adaptability. For example, Yanar et al. (2019) reported up to 95% in *T. urticae* populations in greenhouse cucumbers with *P. persimilis*, and Jafari et al. (2022) highlighted the over 90% reduction achieved by *S. gilvifrons* in field conditions. These results are consistent with earlier findings (El Arnaouty et al. 2018, 2020, Rhodes et al. 2006, Waheeb 2016) supporting the integration of predatory species into IPM programs.

Other studies also demonstrated the effectiveness of predatory mites in reducing spider mite populations in different crop systems. For instance, Fraulo and Liburd (2007) observed that the application of *N. californicus* on strawberry crops resulted in an 85% reduction of *T. urticae* populations within two weeks of release. This aligns closely with the moderate reduction percentages observed for *N. californicus* in our study, confirming its utility as a reliable biocontrol agent, especially under high-density pest conditions.

The predatory mite *P. persimilis* has consistently shown superior performance in greenhouse settings. Çakmak et al. (2005) reported a 96% reduction in carmine spider mite, *Tetranychus cinnabarinus* (Boisduval) (Acari: Tetranychidae) populations on protected strawberry crops in Türkiye, closely paralleling the 96.24% reduction in *T. urticae* observed in the Hisham cultivar in our study. This emphasizes the robust predation capacity of *P. persimilis,* particularly in controlled environments with abundant prey availability.

In addition to predatory mites, predatory insects have demonstrated remarkable success in pest control. Ragkou et al. (2004) recorded a predation rate of up to 94% for *Stethorus punctillum* Wiese in greenhouse-grown raspberries infested with spider mites. Similarly, the current findings showed that *S. gilvifrons* achieved a reduction percentage of 94.89% in the Barcoda cultivar. These results highlight the comparable efficacy of these predators across different crops and geographical regions, underscoring their adaptability and potential for integration into broader pest management programs.

In the current study, bifenazate's lower efficacy (60 – 75%) in both cultivars may be attributed to its rapid degradation and short residual activity compared to predatory bio-agents. Similar percentages were observed against *T. urticae* on strawberry (Massoud et al. 2018), and tomato (Allam et al. 2022), as well as against the red spider mite, *Oligonychus coffeae* Nietner (Acari: Tetranychidae) on tea (Kumari et al. 2012). According to Lewis et al. (2016) bifenazate ($C_{17}H_{20}N_2O_3$) is a neuronal inhibitor and a non-systemic compound with both contact and residual mode of action. This mode of action has lower mortality rates (LR_{50} g ha^{-1}) on beneficials; however, it has high mortality for *T. urticae* within 3-4 days after application (Abdelmaksoud et al. 2023, Tang et al. 2014).

It is assumed that the persistence percentage of bifenazate rapidly declines after application. According to Abdelmaksoud et al. (2023), bifenazate persistence decreased by up to 50% after 24 h, and the residue half-life value ($t_{½}$) was 0.99 days. Therefore, this rapid degradation, alongside the rapid increase in the *T. urticae* population, may explain the lower reduction percentages obtained in the current study.

Furthermore, research by Ochiai et al. (2007) highlighted its limited efficacy under conditions with high pest population densities, where rapid reproduction of *T. urticae* could outpace the chemical's action. These findings align with our observations of reduced control effectiveness, particularly in the Hisham cultivar, where the population reduction was limited to 74.96% for eggs and 64.05% for motile stages. Comparatively, bio-agents such as *P. persimilis* demonstrated a 96.24% reduction, underscoring the need to prioritize biological methods in integrated pest management strategies.

These limitations underscore the importance of integrating bio-agents into pest management strategies to reduce dependency on chemical controls and mitigate resistance development. For instance, studies like those by Çakmak et al. (2005) and Jafari et al. (2022) have demonstrated the success of *P. persimilis* and *S. gilvifrons* in mitigating pest infestations, achieving reduction percentages exceeding 90% in different crop systems. These examples highlight the proven effectiveness of bio-agents in sustainably reducing pest populations.

Releasing predatory species, particularly *P. persimilis* and *S. gilvifrons*, proved to be the most effective strategy for controlling *T. urticae* in protected cucumber production. The current study recommends employing these bio-agents as a sustainable and economic alternative to chemical acaricides for low-income farmers in El-Beheira Governorate.

Author's Contributions

Authors declare that each author's contribution is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Örtüaltı hıyar (*Cucumis sativus* L., Cucurbitaceae) üretimi, *Tetranychus urticae* Koch (Acari: Tetranychidae) adlı iki noktalı kırmızı örümceğin yoğun bulaşmaları nedeniyle önemli tehditlerle karşı karşıyadır. Dünya genelinde *T. urticae* ile mücadelede, predatör akarlar (*Phytoseiulus persimilis* Athias-Henriot ve *Neoseiulus californicus* (McGregor), Acari: Phytoseiidae) ile predator böcek türleri *Stethorus gilvifrons* (Mulsant), Coleoptera: Coccinellidae ve *Orius albidipennis* (Reuter), Hemiptera: Anthocoridae) gibi biyolojik mücadele etmenleri etkili şekilde kullanılmaktadır. Bu çalışma, Barcoda ve Hisham isimli iki hıyar çeşidinde, dört biyolojik etmenin *T. urticae* popülasyonlarını azaltma etkisini değerlendirmek ve bu etkileri geleneksel bir akarisit olan bifenazat ile karşılaştırmak amacıyla gerçekleştirilmiştir. Sonuçlara göre, *P. persimilis* Hisham çeşidinde %96.24'e varan bir azaltma sağlamış; *S. gilvifrons* ise Barcoda çeşidinde %94.89'luk benzer bir etki göstermiştir. Buna karşın, bifenazat her iki çeşitte de en düşük etkinliği göstermiş; azaltma oranları %60.23 ile %74.96 arasında değişmiştir. Tüm biyolojik etmenler, uygulama yapılmayan kontrol grubuna kıyasla T. urticae popülasyonlarında anlamlı azalmalar sağlamıştır. Diğer predatör türler olan *O. albidipennis* ve *N. californicus* da biraz daha düşük olmakla birlikte etkili düzeyde popülasyon baskılaması göstermiştir. Elde edilen bulgular, biyolojik etmenlerin sürdürülebilir hıyar üretimi için entegre zararlı yönetimi stratejilerinde etkili birer bileşen olarak kullanılabileceğini ortaya koymaktadır. Predatör tür kombinasyonlarının ve salım oranlarının optimize edilmesi amacıyla ileri düzey çalışmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: *Tetranychus urticae, Phytoseiulus persimilis, Neoseiulus californicus, Stethorus gilvifrons, Orius albidipennis,* bifenazate

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