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Article

Evaluate the efficiency of releasing two predatory species at their optimal temperature for controlling *Tetranychus urticae* (Acari: Tetranychidae) in a croton greenhouse

Dalia Adly 

Biological Control Research Department, Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt; E-mail: dalia.adly@arc.sci.eg

ABSTRACT

Croton, *Codiaeum variegatum* L., is a beautiful tropical plant. The two-spotted spider mite, *Tetranychus urticae* Koch, causes economic damage to croton. The present study aimed to control *T. urticae* by releasing two predatory species, *Phytoseiulus persimilis* Athias-Henriot and *Stethorus punctillum* Weise, at the optimal temperature and humidity for each predator. The experiments were carried out in commercial plastic greenhouses for two seasons. Once the *T. urticae* infestation was detected, the predators were released. *Phytoseiulus persimilis* was released three times, with a rate of 9/m². Its population increased when the temperature and humidity ranged from 25.5–29 °C and 72.8–86.4% RH, respectively, while it disappeared when the temperature increased above 30 °C. There was a negative correlation between *P. persimilis* and temperature and a positive correlation with relative humidity. *Stethorus punctillum* was released twice, at a rate of 0.5/m². Its population increased when the temperature and humidity ranged from 31.8–35 °C and 65–77% RH, respectively. There was a positive correlation between *S. punctillum* and temperature and a negative correlation with relative humidity. In the two seasons, the population of *T. urticae* decreased gradually to nil/croton leaf in the biological greenhouse while increasing to 59.47 ± 6.34 and 85.63 ± 10.45 mites/croton leaf in the control greenhouse in 2019 and 2020, respectively, at the end of the season. The combination of the two predators was able to achieve effective control of *T. urticae* when used according to the optimal temperature for each predator in croton greenhouses.

KEYWORDS: Biological control, mites, *Phytoseiulus persimilis*, *Stethorus punctillum*, temperature.

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INTRODUCTION

Croton, *Codiaeum variegatum* L. (Euphorbiaceae), is one of the most beautiful indoor and outdoor plants that needs agricultural care. Moreover, it is a small tree or shrub grown in the garden as an ornamental or pot plant. Croton leaves come in a variety of sizes, shapes, and colors. Croton leaf extracts have a wide range of medicinal properties, including purgative, sedative, anti-cancer and antiviral effects (Deshmukh and Borle 1975; Kupchan *et al.* 1976).

Croton is also a rich source of alkaloids, and flavonoid secondary metabolites (De Marino *et al.* 2008). Cuttings, grafting, seeds, and air layering are all used to grow croton. Each year, 20 plants can be grown from the shooting tips of one mother/stock plant (Nasib *et al.* 2008). The agricultural

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strategy in Egypt is now more focused on the production of "ornamental plants" for local consumption and exportation (Sana *et al.* 2012).

Codiaeum variegatum is severely harmed by temperatures below 50 °F (10 °C). Crotons have one of the highest transpiration levels of frequently cultivated leaf plants and frequent watering is required (Stamps and Osborne 2003). They grow well in humid environments (Nasib *et al.* 2008).

The two-spotted spider mite, *Tetranychus urticae* Koch, is a major pest of many greenhouse plants, ornamental nurseries, and field crops. It can cause webbing, fine stippling, leaf yellowing, leaf dropping, and even plant death (Helle and Sabelis 1985). Also, it causes significant economic losses to many crops (Cashion *et al.* 1994; Stamps and Osborne 2003).

In Egypt, millions of tons of pesticides are applied yearly to control pests. According to FAO (2023), Egypt used 1757.57, 4515.36, and 5079.56 tons of herbicides, insecticides, and together (fungicides and bactericides), respectively, for a total of 11352.49 tons in 2020. Pesticides are frequently used for controlling greenhouse pests, and the risk of developing resistance has been demonstrated by various studies (Carbonaro *et al.* 1986, Campos *et al.* 2014).

Several successful biological control programs have demonstrated that greenhouses are suitable for using programs of biological control agents because they are closed systems with barriers against natural enemy dispersal (Perdikis *et al.* 2008). Accordingly, several native parasitoids and predators in agro-ecosystems can be used to control pests in greenhouses (Nicoli and Burgio 1997).

The biological control of spider mites on crops and ornamentals using predatory mites has been the subject of numerous studies (Gerson and Weintraub 2007; Weintraub and Palevsky 2008). Studies of several target pest species reported that there is growing evidence that using a single species as a biocontrol agent is no longer the most effective method of pest control (Ehler 1992; Macrae and Croft 1993, 1997; Lucas *et al.* 1998; Roy *et al.* 1999; Rott and Ponsonby 2000).

Phytoseiulus persimilis Athias-Henriot (Phytoseiidae) has been the most popular predator for the biological control of spider mites for decades. It only eats spider mites, mainly those of the genus *Tetranychus* (McMurtry and Croft 1997). Environmental factors like humidity and temperature have an impact on *P. persimilis* ability to perform and reproduce (Zhang 2003).

It is ineffective at controlling spider mites in climate-controlled growth chambers with low humidity and high temperatures; it is also unable to persist and survive in a plant environment with low densities of spider mites (Stenseth 1979; Schausberger and Walzer 2001; Blümel and Walzer 2002).

The adults and larvae of *Stethorus* species are specialist predators of spider mites (Bailey and Caon 1986; Aksit *et al.* 2007; Biddinger *et al.* 2009). This species was suggested as a potential biological control agent for this pest in crops (Hull *et al.* 1976, 1977).

This species is small and exceptionally well adapted for living and searching for prey. Also, they are winged predators that can quickly reach spider mite hotspots, which is a benefit that can be used in biological control programs (Rott and Ponsonby 2000).

Stethorus punctillum Weise (Coccinellidae) successfully develops to the adult stage between 14–34 °C, but survival is low at both ends of the temperature range and its activity increases significantly as temperatures increase to 30 °C. The optimal temperature for fast development of *S. punctillum* ranges from 34–36 °C. Furthermore, fecundity and ovipositional period increases as temperature increases up to 30 °C (Rott and Ponsonby 2000; Roy *et al.* 2002).

According to previous reports, the optimal temperature of the two mentioned predators is different. The optimal temperature of *P. persimilis* is 27 °C. It is sensitive to temperatures above 30 °C and will stop feeding at about 35 °C. On the other hand, *S. punctillum* activity increases up to 30 °C.

Therefore, the aim of this study is to evaluate the efficiency of releasing two predatory species, *P. persimilis* and *S. punctillum* at their optimal temperatures for controlling the target pest *T. urticae* on croton under greenhouse conditions.

MATERIALS AND METHODS

Sources of the predators

The two predators used in this study were *P. persimilis* and *S. punctillum*. *Phytoseiulus persimilis* was obtained from the Plant Protection Research Institute, Agricultural Research Center, Giza, Egypt. *Stethorus punctillum* was reared in an eggplant greenhouse of 240 m² (30 m × 8 m) infested with the two-spotted mite, *T. urticae*. Adults of the predator were collected from leaves and prepared for release.

Greenhouses

The experiments were carried out at Egypt Green Farm, Mansouria, Giza, Egypt, in 2019 and 2020. The farm grows many ornamental plants in greenhouses, including croton, which is infested with mites most of the year. The plastic greenhouses used in the experiment were 540 m² (60 m × 9 m each) and planted with 200 croton plants, *C. variegatum* cv. Petra. The infestation of the target pest occurred naturally. Fertilization and regular irrigation were done following all recommended agricultural practices. *Phytoseiulus persimilis* was released from March to May at a rate of 9 individuals/m², and *S. punctillum* was released from June to August at a rate of 0.5 individuals/m² (Fig. 1). Each predator was mixed with bran and released in hotspots. The treatment was replicated three times in a randomized complete block design.

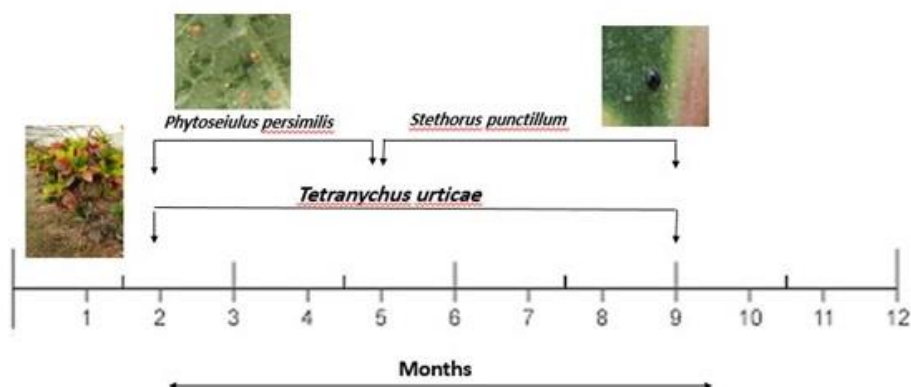


Figure 1. A timeline representing the release of predatory species *P. persimilis* and *S. punctillum* to control the two-spotted mite *T. urticae* in a croton greenhouse.

Sampling technique

The sampling started on March 7th, 2019 and February 13th, 2020, the dates of the pre-treatment count, and continued for 28 weeks each year. The application of predators started after *T. urticae* was identified. The sampling started on March 7th, 2019 and February 13th, 2020, the dates of the pre-treatment count, and continued for 28 weeks each year. The application of predators began after *T. urticae* was identified. A hand-held 10× magnifier was used to count all mobile stages of *T. urticae* at 3 levels (top, middle, and lower) of the plant weekly and also to count all mobile stages of the two predators on the whole plant weekly (25 plants/replicate).

Climatic conditions

The temperature and humidity were recorded daily using a mini digital air temperature and humidity meter sensor (UNI-T UT333) installed in the greenhouse. The mean daily temperature ranged from 26.54 to 31.62 °C during the experiment period. Minimum and maximum daily temperatures ranged between 25.2–27.5 °C and 29.45–34.7 °C, respectively. Minimum and maximum

daily relative humidity ranged between 58.6–54.10% and 88.20–96.30%, respectively, during the experiment period.

Statistical analysis

Data were coded and entered using the statistical package SPSS version 22. Data were statistically described in terms of mean and standard error for quantitative variables. Analysis of variance (ANOVA) was applied using the Holm-Sidak method, $P < 0.05$. The data on the population was correlated with biotic and abiotic factors. The data thus obtained was correlated with weather parameters by computing a simple correlation coefficient (r).

RESULTS

The population of the two-spotted spider mite, T. urticae and the two predators

In 2019, the population of *T. urticae* started on March 7th with 1.2 ± 0.28 mites/croton leaf in the predators' greenhouse (Fig. 2). Once the mite infestation was detected, *P. persimilis* was released three times: on March 7th, 14th and April 4th. After the three releases, the population of *P. persimilis* increased to a peak of 10.2 ± 0.52 predators/croton plant on April 18th. The increase of *P. persimilis* led to a decrease in the population of *T. urticae* to 2.3 ± 0.33 mites/croton leaf on May 23rd (Fig. 2). After that, the population of *P. persimilis* decreased gradually to reach zero on May 23rd which gave *T. urticae* a chance to increase again.

The temperature and humidity values from March 7th to May 9th, 2019, ranged between 25–29.8 °C and 65–86.4% RH, respectively, which were suitable for *P. persimilis* activity. The predator disappeared on May 23rd when the temperature reached 34.65 °C, which wasn't suitable for the predator's activity.

On June 27th, the population of *T. urticae* reached 15.4 ± 0.5 mites/croton leaf in the predators' greenhouse, which was controlled by the release of *S. punctillum* twice, on June 27th and July 4th. The population of *S. punctillum* increased to a peak of 7.2 ± 0.48 predators/croton plant on July 18th. At the end of the season, the population of *T. urticae* decreased gradually to nil/croton leaf in the predators' greenhouse (Fig. 2). From July 4th to August 3rd, the temperature and humidity ranged from 33.7–35.8 °C and 59.7–72.3% RH, respectively, which was suitable for the predator *S. punctillum*'s activity.

In the control greenhouse, the population of *T. urticae* started with 4.2 ± 0.51 mites/croton leaf on March 7th and increased through the season to reach 70.5 ± 6.34 /croton leaf at the end of the season (Fig. 3). The population of *T. urticae* decreased significantly ($P < 0.001$) in the predators' greenhouse and increased significantly ($P < 0.001$) in the control greenhouse. There was a significant interaction between the predator and control greenhouses ($F = 5322.9$, $df = 1$, $p < 0.001$). There was also a significant interaction between the inspection date and treatments ($p < 0.001$).

In 2020, the infestation of *T. urticae* started on February 20th, with 4.63 ± 2.03 mites/croton leaf in the predators' greenhouse (Fig. 2). The population of *T. urticae* increased to 20.23 ± 3.3 /croton leaf on March 5th. *Phytoseiulus persimilis* was released three times on March 5th, 12th and 19th. After the three releases, the population of *P. persimilis* increased to a peak of 12.5 ± 0.4 predators/croton plant on March 26th. The increase of *P. persimilis* led to a decrease in the population of *T. urticae* to 3.7 ± 0.3 mites/croton leaf on May 7th (Fig. 2). At the same time, the population in the control greenhouse increased to 50.27 ± 3.24 mites/croton leaf (Fig. 3).

The population density of *P. persimilis* increased from February 27th to April 23rd, when the temperature and humidity ranged between 25.5–29 °C and 72.8–86.4% RH, respectively, while it disappeared when the temperature increased above 30 °C and the humidity decreased.

About two months after the last release of *P. persimilis*, the mite *T. urticae* population appeared with 10.2 ± 2.56 mites/croton leaf in the predators' greenhouse, which was controlled by the release of *S. punctillum* twice on May 28th and July 4th. The population of *S. punctillum* increased to a peak

of 9.9 ± 0.5 predators/croton plant on June 25th (Fig. 2). At the end of the season, the population of *T. urticae* decreased gradually to nil/croton leaf in the predators' greenhouse (Fig. 2) and the population increased in the control greenhouse to 85.63 ± 10.45 mites/croton leaf (Fig. 3).

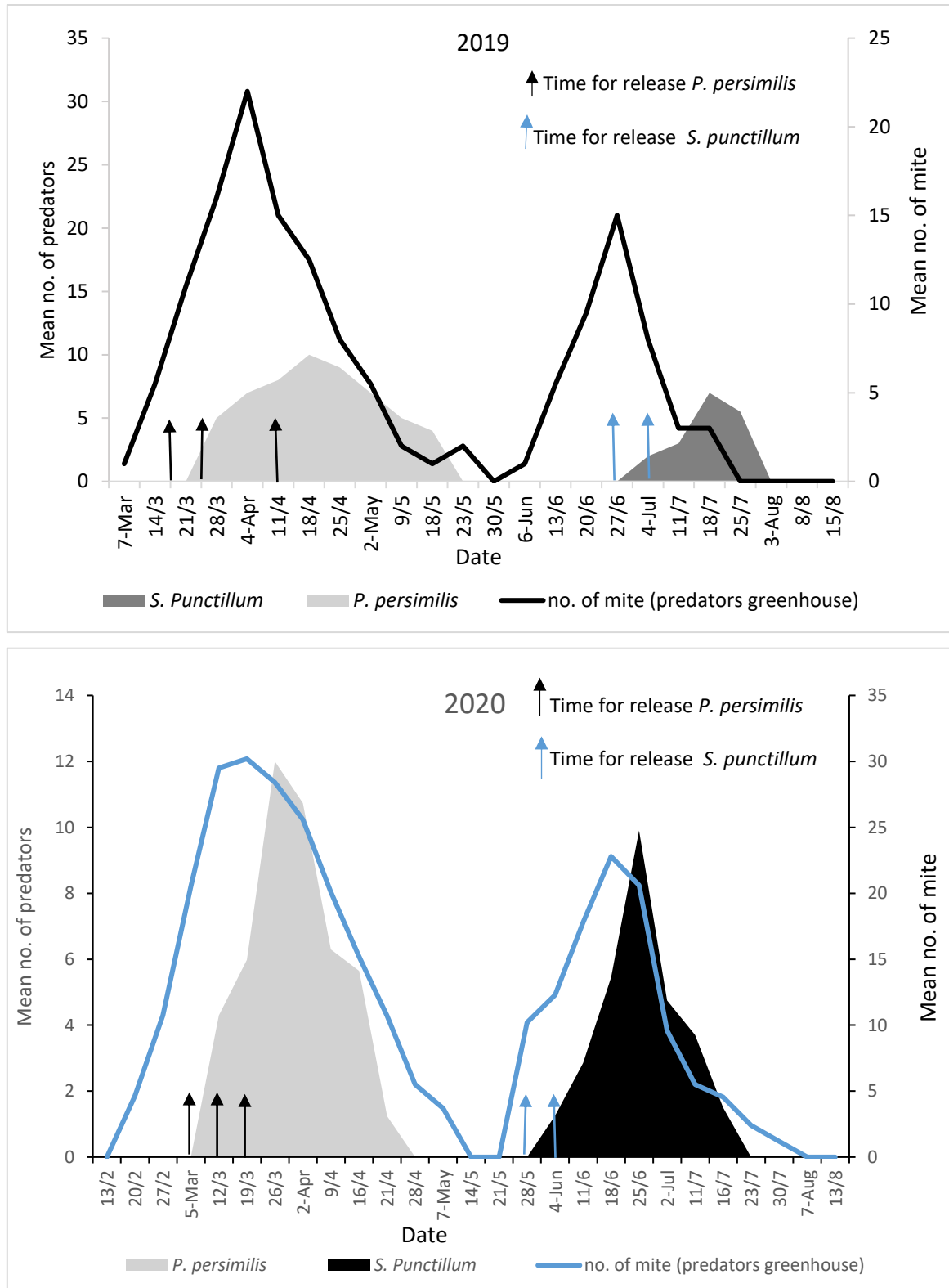


Figure 2. The population density of *T. urticae* and the released predators, *P. persimilis* and *S. punctillum* in croton greenhouse in 2019 and 2020.

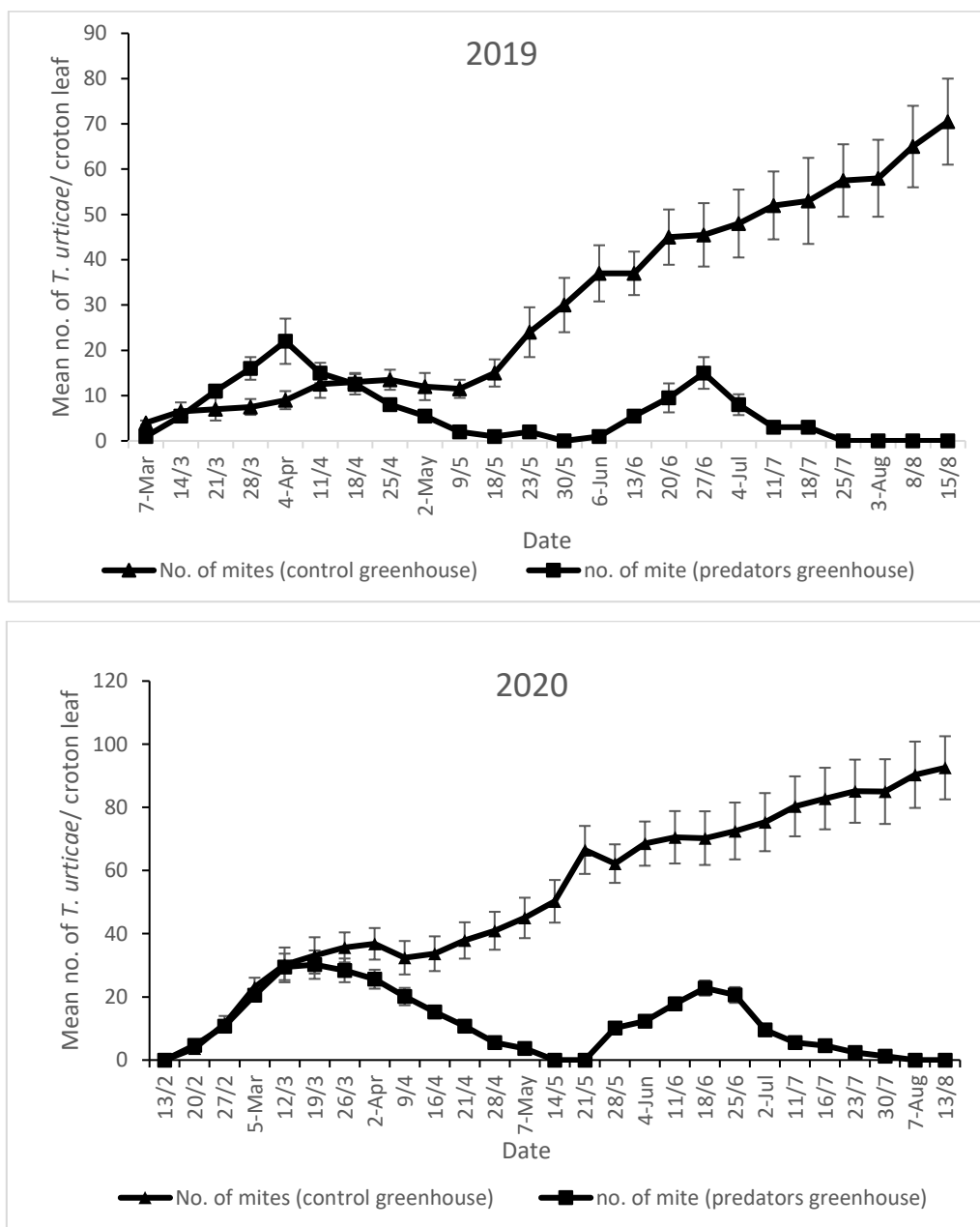


Figure 3. The comparison between the population of *T. urticae* in predators' greenhouse and control greenhouse.

From May 21st to July 23rd, the temperature and humidity ranged between 31.8–35 °C and 65–77% RH, respectively. This range of temperature and humidity was suitable for the development of the predator, *S. punctillum*.

The population of *T. urticae* decreased significantly ($P < 0.001$) in the predators' greenhouse and increased significantly ($P < 0.001$) in the control greenhouse. There was a significant interaction between the predators and control greenhouses ($F = 2813.6$, $df = 1$, $p < 0.001$). There was also a significant interaction between the inspection date and treatments ($p < 0.001$).

The correlation coefficient of T. urticae population with abiotic factors

A significant positive correlation was found between *T. urticae* and temperature ($r = 0.707$ and $r = 0.784$) in 2019 and 2020, respectively, while the mite population had a negative correlation ($r = -0.638$ and $r = -0.669$) with relative humidity in 2019 and 2020, respectively.

The correlation coefficient of the predators' population with abiotic factors

A negative correlation was found between *P. persimilis* and temperature ($r = -0.613$ and -0.608) in 2019 and 2020, respectively, while the same predator had a positive correlation with relative humidity ($r = 0.577$ and 0.569) in 2019 and 2020, respectively.

A positive correlation was found between *S. punctillum* and temperature ($r = 0.581$ and 0.514) in 2019 and 2020, respectively, while the same predator had a negative correlation with relative humidity ($r = -0.580$ and -0.644) in 2019 and 2020, respectively.

DISCUSSION

The present study showed that the release of the two predators, *P. persimilis* and *S. punctillum*, depending on the suitable temperature of each predator, was successful in controlling the population of *T. urticae* in croton greenhouses. The results of this study showed that *T. urticae* populations occurred throughout the year, and there was a positive correlation with temperature and a negative correlation with relative humidity.

These findings agree with Sarkar and Somchowdhary (1989), Rajakumar *et al.* (2005), Yadavbabu and Manjunatha (2007), Mahato *et al.* (2008) and Meena *et al.* (2013) who reported that *T. urticae* incidence was recorded on orchids all year round, indicative of overlapping generations; however, two distinct population peaks were recorded during April and May. There was a significant positive correlation between mite incidence and maximum and minimum temperatures, daylight had a non-significant positive correlation and rainfall and relative humidity had a non-significant negative correlation with mite incidence.

In this study, once the mite infestation was detected, *P. persimilis* and *S. punctillum* were released. Parvin and Haque (2008), reported that the early release of the three predators; *Scolothrips sexmaculatus* Pergande, *P. persimilis* and *S. punctillum* has been shown to keep the mite population lower than their late release. All three predators may be used as bio-control agents against the two-spotted spider mite.

In the present study, the population density of *P. persimilis* increased when the temperature and humidity ranged between 25.5–29 °C and 72.8–86.4% RH, respectively, while it disappeared when the temperature increased above 30 °C and the humidity decreased.

According to Pruszyński (1976), *P. persimilis* stopped feeding at about 35 °C (95 °F), and it was more sensitive to temperatures over 30 °C (86 °F) than the prey. The predator development rates were optimum at 27 °C (at 60 to 85% RH). Rojas *et al.* (2013) reported that *P. persimilis* could develop and reproduce at all tested temperatures from 21 to 31 °C and survived much better at temperatures below 29 °C.

The abiotic conditions for optimal performance of *P. persimilis* are temperatures between 15 °C and 27 °C, and relative humidity of 60–90%. To avoid economic damage of *T. urticae*, a lower initial predator: prey ratio can be accepted at 27 °C than at 15 °C (Stenseth 1979). While this mite is an excellent predator, its efficacy under hot and dry conditions is often insufficient (Force 1967; Nihoul 1992; Skirvin and Fenlon 2003; Escudero and Ferragut 2005; Weintraub and Palevsky 2008).

In the present study, the population density of *S. punctillum* increased when the temperature and humidity ranged between 31.8–35 °C and 65–77% RH, respectively. There was a positive correlation between the predator, *S. punctillum*, and temperature, but a negative correlation with relative humidity.

Rott and Ponsonby (2000) found that the activity of *S. punctillum* increased significantly at higher temperatures but had no effect on relative humidity.

Studies investigating the effect of temperature on coccinellid species have shown that fecundity and oviposition increased with increasing temperatures up to 30 °C (Ponsonby and Copland 1998)

and an increase in larval developmental rate was found at 28 °C (Ponsonby and Copland 1996; Lamana and Miller 1998). *Stethorus punctillum* developed successfully to an adult stage between 14 and 34 °C, but survival was low at both ends of the temperature range. The optimal temperature for immature survival ranged between 16 to 32 °C; eggs did not hatch at 12 °C or 36 °C (Roy *et al.* 2002).

This indicates that temperature level variations can influence the predation, reproduction, and development of coccinellids and that the thermal requirements of each coccinellid species have to be considered in the biological control programs (Obrycki and Kring 1998).

In conclusion, the present study shows that the population of *T. urticae* in croton greenhouses could be successfully controlled by releasing the two predators, *P. persimilis* and *S. punctillum*, depending on the suitable temperature for each.

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ارزیابی کارایی رهاسازی دو گونه شکارگر در دمای بهینه آنها برای کنترل کنه تارتن دولکه‌ای *Tetranychus urticae* (Acari: Tetranychidae) در گلخانه کروتون

دالیا عدلی

گروه تحقیقات کنترل بیولوژیک، مؤسسه تحقیقات گیاهپزشکی، مرکز تحقیقات کشاورزی، غزه، مصر؛ رایانامه: dalia.adly@arc.sci.eg

چکیده

کروتون، *Codiaeum variegatum* L.، گیاه استوایی زیبایی است. کنه تارتن دو لکه‌ای *Tetranychus urticae* Koch باعث زیان اقتصادی به کروتون می‌شود. مطالعه حاضر با هدف کنترل *T. urticae* با رهاسازی دو گونه شکارگر *Phytoseiulus persimilis* Athias-Henriot و *Stethorus punctillum* Weise بسته به دما و رطوبت بهینه هر شکارگر انجام شد. آزمایش‌ها در گلخانه‌های پلاستیکی تجاری به مدت دو فصل انجام شد. هنگامی که آلودگی *T. urticae* آشکار شد، شکارگران رها شدند. گونه *Phytoseiulus persimilis* سه بار با تعداد نه کنه در متر مربع رهاسازی شد. جمعیت آن زمانی که دما بین ۲۹-۲۵/۵ درجه سلسیوس و رطوبت نسبی بین ۷۲/۸-۸۶/۴٪ متغیر بود افزایش یافت، در حالی که وقتی دما به بالای ۳۰ درجه سلسیوس افزایش یافت ناپدید شد. بین *P. persimilis* و دما همبستگی منفی و با رطوبت نسبی همبستگی مثبت وجود داشت. گونه *Stethorus punctillum* دو بار به میزان ۰/۵ بر متر مربع رهاسازی شد. جمعیت آن زمانی افزایش یافت که دما بین ۳۱/۸-۳۵ درجه سلسیوس و رطوبت نسبی بین ۶۵-۷۷٪ متغیر بود. بین *S. punctillum* و دما همبستگی مثبت و با رطوبت نسبی همبستگی منفی وجود داشت. در دو فصل، جمعیت *T. urticae* در گلخانه بیولوژیکی به تدریج به صفر در هر برگ کروتون کاهش یافت در حالی که در گلخانه شاهد در پایان فصل به ترتیب به $۶/۳۴ \pm ۵۹/۴۷$ و $۱۰/۴۵ \pm ۸۵/۶۳$ کنه/برگ کروتون در سال‌های ۲۰۱۹ و ۲۰۲۰ افزایش یافت. ترکیب این دو شکارگر زمانی که با توجه به دمای بهینه برای هر شکارگر در گلخانه‌های کروتون استفاده شود، می‌تواند به کنترل مؤثر *T. urticae* دست یابد.

واژگان کلیدی: مهار زیستی، کنه‌ها، *Phytoseiulus persimilis*، *Stethorus punctillum*، دما.

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