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Biological Control of Two-Spotted Spider Mite, *Tetranychus urticae* with Predatory Phytoseiid Mites *Neoseiulus californicus* and *Amblyseius swirskii* in Strawberry (Acari: Tetranychidae: Phytoseiidae)

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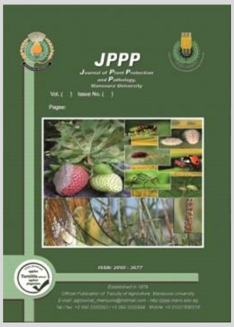

ABSTRACT

The two-spotted spider mite *Tetranychus urticae* Koch (TSSM), considered as a key arthropod pest affecting strawberries and other crops worldwide. Extensive use of pesticides negatively impacts the human environment and beneficial microorganisms. Therefore, the approach for using biological control should be applied to suppress the population of spider mites in fruits and other crops. This study evaluated the influence of releasing the two predatory phytoseiid mites *Neoseiulus californicus* (Mc Gregor) and *Amblyseius swirskii* A.-H. to control *T. urticae* on strawberry plants growing in the greenhouse. The results suggested that releasing *N. californicus* and *A. swirskii* significantly decreased the number of *T. urticae* eggs, nymphs and adults compared to untreated (control) plants. The highest overall mean number of survived nymphs and adults was observed on untreated leaflets, while the lowest survived ones were found on leaflets exposed to *N. californicus*. The release of *A. swirskii* on leaflets decreased the number of nymphs and adults, but significantly less than *N. californicus* treatment. In addition, the highest overall mean consumption rate for nymphs and adults of spider mites were noticed on *N. californicus* treatment, reaching 100% at the end of the evaluation. The present research proposes that releasing *N. californicus* could be a promising strategy for *T. urticae* suppression in strawberries.

Keywords: Two-spotted spider mite, strawberry plants, biological control, *Neoseiulus californicus*, *Amblyseius swirskii*

INTRODUCTION

The strawberry (*Fragaria×ananassa* Duchesne) (Family: Rosaceae) considered one of the most excellent consumed fruits worldwide (Albendin et al., 2015). Strawberry fruits are the primary source of a lot of vitamins and minerals (Khunte et al., 2020). The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a crucial pest that attacks a wide range of economic crops widespread (van Leeuwen et al., 2010 and 2015 and Farazmand et al., 2012). One of those crops is the strawberry which is observed to be infested with pests in several countries like New Zealand (Butcher et al., 1987), Spain (Garcia-Mari and GonzalezZamora, 1999), Argentina (Greco et al., 1999), USA (Dara et al., 2018) and Egypt. Larvae, nymphs, and adults of *T. urticae* feeding behavior damages the chlorophyll, which have a mesophyll cell within the leaf tissue, causing decrease of photosynthetic capacities of infested leaves (Sances et al., 1982), yield loss and economic damage (Akyazi and Liburd, 2019) and fruit production reduction by up to 80% (Çobanoğlu and Güldali, 2017).

The massive use of pesticides drives pest resistance and severely impacts human health, the environment, and beneficial organisms. So, recent tendency to improve using a biological control approach (Matson et al., 1997; Margni et al., 2002; UN Human Rights Council, 2017 and Wyckhuys et al., 2019). Augmentative biological control is one of the most applied strategies to control harmful pests. It involves seasonally releasing many natural enemies to prevent pest population outbreaks (Hajek, 2004 and van Lenteren et al., 2018).

Predatory mites, belonging to the family Phytoseiidae, are biological control agents in many agricultural ecosystems, especially in suppressing phytophagous mites and small insects (van Lenteren et al., 2018). These predatory mites are fundamental natural enemies for the two-spotted spider mite, which is considered one of the most polyphagous destructive pests that attacks over 1100 plant species and can easily develop pesticide resistance (Van Leeuwen et al., 2010 and Migeon and Dorkeld, 2015). *Neoseiulus californicus* can preys on various phytophagous mite species (McMurtry and Croft, 1997) and pollen (Pascua et al., 2020). *N. californicus* showed efficient control for *T. urticae* in different countries (Barber et al., 2003 and Elmoghazy et al., 2011). Also, the predatory mite, *Amblyseius swirskii* is one of the most effective biocontrol agents used in over 50 countries worldwide (Calvo et al., 2015). This predatory mite is a polyphagous phytoseiid preys on thrips, whiteflies (Calvo et al. 2011) and spider mites (Xu and Enkegaard, 2010).

The present study aims to evaluate the efficacy of releasing both predatory phytoseiid mites, *N. californicus* and *A. swirskii* for controlling *T. urticae* infesting strawberry plants under greenhouse conditions.

MATERIALS AND METHODS

Spider mite and predatory mites colonies

The cultures of two-spotted spider mite and predatory mites were started with individuals, which previously obtained from infested strawberry plants growing in the greenhouse. Infested strawberry leaflets with TSSM and the two predatory mites were sampled and transferred to the laboratory for segregation. Spider mite colony was reared on strawberry plants, maintained in the laboratory in a mesh cage, and provided with fresh plant leaves ones a week. The

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colony was maintained for many generations before using in the experiments.

The two phytoseiid mites, *N. californicus* and *A. swirskii* were maintained after segregation and reared on infested strawberry leaflets and provided with TSSM under laboratory conditions. Infested leaflets were placed on large trays with moist cotton pads in the center of the tray, leaving a space provided with water to prevent mites from escaping. Infested leaves with TSSM culture were provided to the phytoseiids cultures as a food. The trays were kept in an incubator at 28±2 °C and 70±5% RH and 16 h L: 8 h D photoperiod.

Greenhouse experimental design

This experiment was carried out to evaluate the impact of the two predatory mites release in TSSM suppression on strawberry plants compared to untreated plants. Bare root strawberry cultivar (Fortona) was planted on 6.7 inch pots filled with 60: 40 mixes of basic soil to peat moss in a randomized complete block design at the greenhouse. Plants were fertilized with slow-release fertilizer (Osmocote) and watered three times weekly. Thirty pots of strawberry plants were naturally infested with TSSM adults. Infested plants were divided into three treatments: (1) release of *N. californicus,* (2) release of *A. swirskii* and (3) untreated control free of predatory mites. Each treatment was represented by 10 plants and kept inside a bug dorm covered with fine mesh to prevent TSSM from escaping. Each of the two predatory mites were released separately with a 1:5 ratio (1 predatory mite: 5 TSSM) in each bug dorm. To release the predatory mites, a plastic bottle (250 mL with 5-mm openings in the covers) was gently rotated and slightly shook the bottle over strawberry plants. After 48 hours (about two days) of predatory mite release, the number of TSSM eggs, nymphs, and adults in all treatments was determined, where mortality percentage of nymph and adult was calculated and compared with control.

Sampling

Strawberry leaflets were sampled after two months of cultivation, starting from July 1st and continued to the end of September (almost 12 weeks). One leaflet from each plant replicate per treatment was randomly collected (10 leaflets/ treatment) one time weekly from the three treatments until the end of the study. Samples were kept in Ziplock bags, then transferred to the laboratory for inspection. The number of TSSM eggs, nymphs, and adults (live and dead) were counted using a stereomicroscope and recorded for each leaflet, and each sampling date and mortality percentage was estimated for nymphs and adults of spider mite.

Data Analysis

All data were presented as mean± standard error and analyzed using JMP Pro 16.0. Numbers of spider mite eggs, nymphs, and adults were log-transformed. Measured parameters, including the number of eggs, survived nymphs and adults, and mortality percentage for each stage per leaflet, were compared to each week across treatments using an analysis of variance (ANOVA). Means were compared using Tukey's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Results

***Tetranychus. urticae* eggs**

The number of TSSM eggs was counted and recorded weekly (for 12 weeks) after the release of the two predators *N. californicus* and *A. swirskii* on treated and untreated plants (Figure 1). Results showed that the number of spider mite eggs was significantly different among treatments (ANOVA: $F=66.5$, $P <0.0001$) and among weeks (ANOVA: $F=4.7$, $P <0.0001$). In all treatments, the mean number of eggs in the first week (July 1st) was very low < 5.0 eggs/ leaflet. Then the number of eggs increased in the 2nd and 3rd weeks of July with significantly a greater number of eggs on untreated leaflets (24.3 eggs), but <10 eggs on treated leaflets. The number of prey eggs decreased from the 4th week until the 6th week in all treatments, then peaked on the 7th week on untreated leaflets, reached to 43.8 eggs. The decrease in eggs continued in treated leaflets until the 12th week. No eggs were found on treated leaflets with both predators in the 12th week. The number of eggs on untreated leaflets decreased in two weeks, then peaked again in the 10th week (September 2nd) (25.2 eggs) and dropped for the rest of weeks after that.

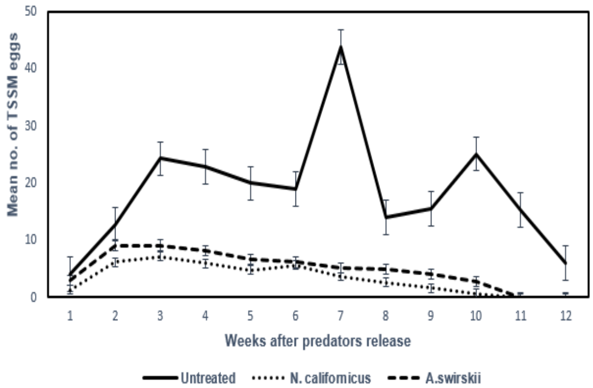

Figure 1. Mean number of two-spotted spider mites egg counted weekly on untreated leaflets (control) and treated leaflets of strawberry plants subjected to release of *N. californicus* and *A. swirskii*

Survived nymphs and mortality percentage of *Tetranychus urticae*

The number of survived nymphs and mortality % were recorded on untreated and treated leaflets after release of *N. californicus* and *A.swirskii* were represented in Figure (2). The number of survived nymphs was (30.2 nymphs) on untreated leaflets and that was higher than those found on leaflets treated with *N. californicus* and *A.swirskii* (20.3 and 22.2 nymphs, respectively). On untreated treatment, the number of surviving nymphs started gradually to increase. It reached its peak in the 6th week (200.6 nymphs), otherwise, in the same period, the number of surviving nymphs decreased on leaflets treated with *N. californicus* and *A. swirskii* through the time of evaluation. No nymphs stayed at week 11th and 12th on leaflets treated with *N. californicus*, while a few nymphs (<7 individuals) were detected on leaflets treated with *A. swirskii.*

The mortality percentage in nymphal stage was estimated through the study for all treatments as figured in Figure (2). Generally, the average number of nymph mortality (%) was significantly higher at leaflets exposed to *N. californicus* than leaflets exposed to *A. swirskii* and untreated leaflets. No dead nymphs were observed on untreated treatment from 1st to 3rd week, while nymph mortality in *N. californicus* treatment ranged between 50-66% and 33-40% for *A. swirskii* at this sampling time. Low and slow nymph mortality was started in untreated treatment from the 4th week

(2%) and gradually reached 43% in 12th week. On the other hand, nymph mortality was significantly increased in *N. californicus* treatment, coming to a maximum mortality rate of 100% at the end of experiment. Similar results were noticed in *A. swirskii* treatment but significantly lower than *N. californicus* treatment, where nymph mortality elevated to 90% in the 12th week of study.

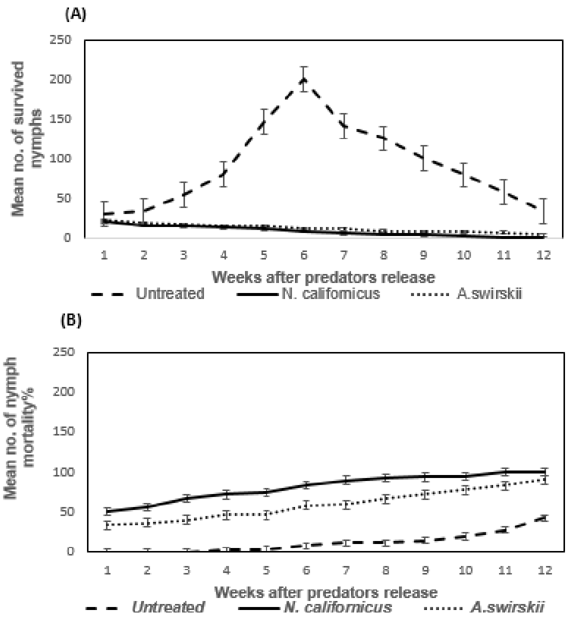

Figure 2. Mean number of (A) survived nymphs and (B) mortality (%) of two-spotted spider mites counted weekly on untreated leaflets (control) and treated leaflets of strawberry plants subjected to release of *N. californicus* and *A. swirskii*

Survived adult and mortality percentage of *Tetranychus urticae*

This study recorded the average number of survived adults and mortality (%) for untreated *N. californicus*, and *A.swirskii* treatments (Figure 3). There was a significant difference among treatments in the number of survived adults. The average number of survived adults initially was approximately in all treatment through the 1st week ranged between 20-24 adults. In untreated treatment, the number of adults raised from the 2nd week and peaked in the 8th week (150.5 adults), then lowered in the 9th week and peaked again in the10th week, reaching 144.8 adults, then decreased again at the end of sampling. However, the average number of adults continued to decrease in *N. californicus* and *A. swirskii* treatments for the whole weeks of evaluation. No surviving adults were observed on the 11th and the 12th weeks on leaflets exposed to *N. californicus* and 3.8 and 6,3 adults were recorded during those two weeks on *A. swirskii* treatment. The number of survived adults decreased gradually from 3rd week until the end of evaluation on *N. californicus* and *A. swirskii* treatments compared to untreated treatment.

The percentage of adult mortality in all treatments was also considered and illustrated in Figure (3). Although treatments differed in adult mortality, distinctly adult mortality is significantly higher in *N. californicus* treatment started with 42% in 1st week of study and continued to elevated until reaching 100% in the 12th week at the end of the experiment. Likewise, the result of *A. swirskii* treatment ended up with 90% for adult mortality. In contrast, the average adult mortality was low at the beginning of sampling time; after that, mortality raised in the 11th and 12th weeks and reached almost 38%.

Overall survived *Tetranychus urticae* nymphs and adults

The overall mean of survived nymphs and adults during the study was calculated and illustrated in Figure (4a and 4b). Visibly, the lowest overall mean number of survived nymphs and adults significantly occurred on *N. californicus* treatment < 20 nymphs and 21.2 adults. Likewise, *A. swirskii* treatment had a lower number of nymphs and adults (50% less than untreated treatment) but higher than *N. californicus* treatment. Otherwise, untreated treatment had significantly the highest overall mean number of survived nymphs (88.3 nymphs) and adults (79.6 adults).

Overall mortality (%) of *Tetranycus urticae* nymphs and adults

The overall mean of nymphs and adults mortality (%) was figured out and illustrated in Figure (4c and d). The most significant overall mean number of nymphs (81.2%) and adult mortality (78.8%) were notably observed in *N. californicus* treatment. At the same time, the nymph (59.2%) adult (63.4%) mortality was significantly less in leaflets exposed to *A.swirskii*. Conversely, untreated treatment had the lowest overall mean number of nymphs (11.7%) and adults (20.4%) mortality than other treatments.

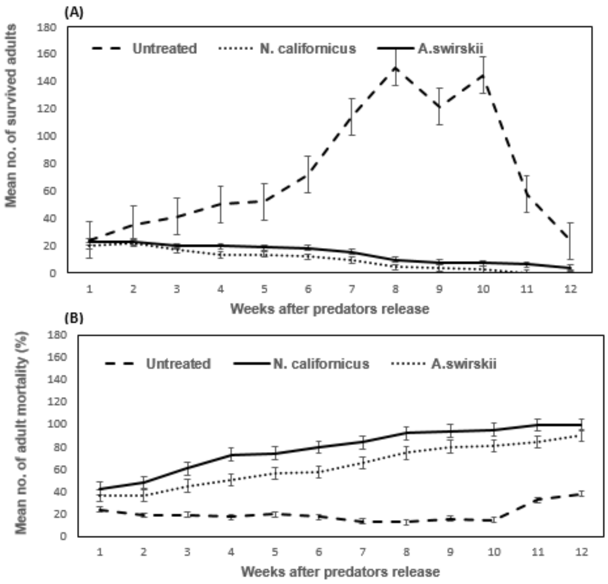

Figure 3. Mean number of (A) survived adults and (B) mortality (%) of two-spotted spider mites counted weekly on untreated leaflets (control) and treated leaflets of strawberry plants subjected to release of *N. californicus* and *A. swirskii*

Additionally, the overall mean number of TSSM eggs, nymphs, and adults on untreated leaflets and treated leaflets with predators (*N. californicus and A.swirskii)* through twelve months is presented in (Table 1). Results showed a significant difference among treatments (ANOVA: F= 49.5, P <0.0001) in the number of eggs, nymphs, and adults. The overall number of eggs was significantly higher on untreated leaflets (18.6 eggs) than on treated leaflets with *N. californicus* and *A.swirskii* (3.3 and 4..8 eggs, respectively). Likewise, the overall number of nymphs and adults had the most significant number of nymphs and adults

(90.2 nymphs and 74.1 adults) than treated leaflets, with no significant differences between the two treated leaflets *californicus* and *A.swirskii* (8.4 and 12.0 nymphs, respectively) and (9.9 and 14.5 adults, respectively).

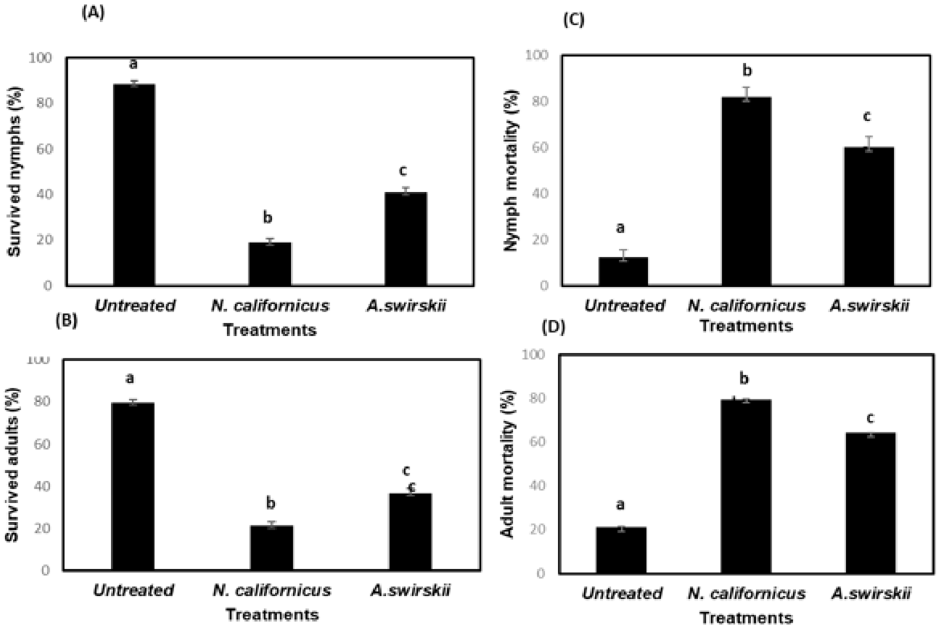

Figure 4. Overall mean± SE number of A) survived nymphs, B) adult of two-spotted spider mites (%), C) number of two-spotted spider mites nymphs and D) adult mortality (%) in untreated leaflets and treated leaflets of strawberry plants subjected to release of *N. californicus* and *A. swirskii*

Table 1. Overall mean± SE number of eggs, nymphs, and adult of two-spotted spider mites monitored for 12 weeks on untreated and treated strawberry plants subjected to release of *N. californicus* and *A. swirskii*

Treatment	Overall mean ± SE		
	Eggs	Nymphs	Adults
Untreated	18.6± 1.9a	90.2± 7.1a	74.1± 5.5a
N. californicus	3.3± 0.4b	8.4± 0.8b	9.9± 0.9b
A. swirskii	4.8± 0.5b	12.0± 0.7b	14.5± 0.8b
P-value	F= 49.5 <0.0001	F= 123.6 <0.0001	F= 122.7 <0.0001

Discussion

Intensive use of pesticides by growers to control pest on crops harm the human environment and non-target organisms. Biological control is an economically and environmentally perfect alternative method to pesticides in several agricultural systems (van Lenteren and Bueno, 2003). Accordingly, the predatory phytoseiid mites are a successful biological control agent for *T. urticae* especially on strawberry (Easterbrook et al., 2001; Fitzgerald and Easterbrook, 2003; Tuovinen and Lindquist, 2014). Our study elucidated that releases of *N. californicus* and *A. swirskii* at a 1:5 predator: prey ratio maintained low *T. urticae* populations for the duration (12 weeks) of the experiment. The present results showed that the two phytoseiids could suppress *T. urticae* egg, nymph, and adult populations below the control. These results are in harmony with those obtained by Xiao et al. (2012); Farazmand and Amirs-Maafi (2020) who mentioned that *A. swirskii* could successfully prey on *T. urticae.* After the releases of the predatory mites, *N. californicus* and *A.swirskii* suppressed the number of two spider mites eggs in the treated plants started from the 3rd week of release < 10 eggs comparing with the increasing number in untreated plants (24.3 eggs) (Figure 1). The number of eggs not exceeded than 10 eggs in the rest of sampling period for treated plants, while was four double times more in untreated plants. The number of survived *T. urticae* nymphs greatly decreased in treated plants with *N. californicus* and *A. swirskii* < 25 nymphs through the first six weeks, then no survived nymphs were observed at the end of the season. Meanwhile, higher numbers of nymphs were recorded on untreated plants reached to 200 nymphs in the 6th week of evaluation. In addition, the average number of nymph mortality (%) was significantly higher at leaflets exposed to *N. californicus* (100%) than leaflets exposed to *A. swirskii* (90%) and untreated leaflets (43%) in the end of the season (Figure 2).

The same results were observed in number of survived *T. urticae* adults, the number of adults were significantly higher in untreated plants reached to 140 adults in the 8th week, while number of adults was less than 20 adults in treated plants with predatory mites. On the other hand, highly *T. urticae* adult mortality percentage for treated plant with *N. californicus* (100%) and *A. swirskii* (90%) compared with untreated plants (38%) (Figure 3). These results suggest that the sharp decline in the nymph and adult populations of *T. urticae* referred to higher release of each predatory mites; *N. californicus* and *A.swirskii* with 1 predator: 5 prey enable predatory mites to efficient control for *T. urticae* population. Our suggestion agrees with Hassel et al. (1976) who mentioned that *N. californicus* is an effective and voracious predator at high prey density if the 1:10 predator: prey ratio stays unmodified. Waheeb (2016) revealed that releasing *N. californicus* at 1:10 level represents a useful management strategy of *T. urticae* Koch on both soybean and cotton plants. Moreover, El-Moghazy et al. (2012) reported that *N.californicus* gave the highest reduction percentage than *Typhlodromips swirskii* to *T. urticae* on two cultivars of faba bean in open field with a release ratio of 1 predator:7 *T. urticae.* Also, high rates of *N. californicus* development and prey consumption will enable this predator to achieve and maintain control over TSSM populations (Sabelis and Janssen 1994). Our findings showed that the *T. urticae* nymph and adult populations were sustained at the initial low numbers after the introduction of *N. californicus* and *A.swirskii* than the untreated plants. McMurtry and Croft (1997) noticed that *N. californicus* can maintain *T. urticae* populations at low densities.

However, the results also presented that the overall mean number of nymphs and adults mortality percentage was significantly higher in *N. californicus* than *A. swirskii*, suggesting that *N. californicus* could be a better bioagent against *T. urticae*. This could interpret that *N. californicus* has a food specialization, which prefers to prey the tetranychid spider mites such as *T. urticae* that can produce heavy webbing (McMurtry and Croft, 1997). While *A. swirskiii* is capable to prey on other preys such as thrips or whitefly, when compared with *T. urticae* (Xu and Enkegaard, 2010). The present findings also agree with van Houten et al., (2007) who mentioned that *A. swirskii* can slow down *T. urticae* populations but cannot suppress it in hot spots because the spider mite thick webbing hinders *A. swirskiii* entrance. In agreement with our results, Elmoghazy et al. (2011) observed that *N. californicus* decreased *T. urticae* by 87.22%, while A. *swirskii* showed a 57.49% reduction. Furthermore, *N. californicus* could be adapted to low R.H.% (Bakker et al. 1993) and a wide temperature range of 15–35 °C (Gotoh et al. 2004). Otherwise, *N. californicus* may move to areas of high prey density within the plant, possibly due to olfactory response to kairmones emitted from infested leaves with *T. urticae* (Dicke and Sabelis 1988; Liburd et al. 2007). Additionally, *N. californicus* has a slower metabolism and lower searching efficiency compared with other phytoseiids, but it has a high rate of spatial coincidence with TSSM and can tolerate starvation (Greco et al. 2004). Consequently, the potential of this predatory mite as a biocontrol agent may supply long-term control for *T. urticae* on strawberries (Rhodes et al. 2006; Fraulo et al. 2008).

CONCLUSION

In conclusion, releasing the two predatory mites *N. californicus* and *A. swirskii* significantly decreased the number of *T. urticae* eggs, nymphs, and adults infesting strawberry plants under greenhouse conditions with a significant impact of *N. californicus* to control *T. urticae* population. These findings pointed out that predators as a part of biological control are a promising approach for *T. urticae* suppression.

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المكافحة الحيوية لاكاروس العنكبوت الاحمر بالعناكب المفترسة *Neoseiulus californicus* و *Amblyseius swirskii* في الفراولة

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الملخص

يعتبر اكاروس العنكبوت الاحمر أفة رئيسية من مفصليات الارجل التي تؤثر علي الفراولة وغيرها من المحاصيل في جميع انحاء العالم. يؤثر الاستخدام المفرط للمبيدات سلبيا علي بيئة الانسان والكائنات الحية الدقيقة. من المهم استخدام وتطبيق نهج المكافحة الحيوية لقمع اعداد اكاروس العنكبوت الاحمر في الفراولة والمحاصيل الاخري. نفذت هذه الدراسة لتقييم تأثير اطلاق اثنان من العناكب المفترسة *Neoseiulus californicus* و*Amblyseius swirskii* لمكافحة اكاروس العنكبوت الاحمر في الصوب. اقترحت النتائج ان اطلاق *N. californicus* و*A. swirskii* يخفض معنويا اعداد البيض والحوريات والحشرات الكاملة مقارنة بالنباتات الغير معاملة. لوحظ اعلي اجمالي متوسط الحوريات والحشرات الكاملة الحية في الوريقات الغير معاملة. بينما وجدت اقل علي الاوراق التي تم تعريضها ل *N. californicus*. اطلاق *A. swirskii* علي الوريقات ادي الي خفض اعداد الحوريات والحشرات الكاملة ولكن معنويا بشكل اقل من معاملة *N. californicus*. بالاضافة الي انه لوحظ ان اعلي اجمالي متوسط النسبة المئوية لموت الحوريات والحشرات الكاملة في معاملة *N. californicus* ووصلت الي 100% في نهاية التقييم. يفترض البحث الحالي ان اطلاق *N. californicus* من الممكن ان يكون من الاستراتيجيات الواعدة في مكافحة اكاروس العنكبوت الاحمر في الفراولة.