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Article

A practical mass-rearing system for phytoseiid mites: A case study with *Amblyseius swirskii*, *Neoseiulus californicus*, and *Neoseiulus barkeri* (Acari: Phytosiidae)

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ABSTRACT
Phytoseiid mites play a crucial role in biological control programs. Developing efficient mass-rearing techniques for these mites is essential to enhance their applicability in large-scale management programs. This study aimed to develop and validate a simple mass-rearing protocol for the continuous production of generalist phytoseiid mites (*Amblyseius swirskii*, *Neoseiulus californicus*, and *Neoseiulus barkeri*). The rearing system consisted of a Petri dish placed on a water-saturated sponge in a plastic box containing water. Mites were fed a mixture of cattail pollen, rice husk, and *Tyrophagus putrecentiae* mites which were introduced into the rearing units three times a week. The prey mite was maintained in a similar system using yeast granules and rice husk as food. Population growth of the predatory mites was observed over an 8-week period under controlled laboratory conditions (25 ± 1 °C, 65 ± 5% RH, and 16L:8D photoperiod). The results showed that all three species successfully grew and overpopulated in the 6th week, with 380.2 ± 16.7, 211.4 ± 12.9, and 283.9 ± 18.6 adult females of *A. swirskii*, *N. californicus*, and *N. barkeri*, respectively, being produced. These values represent increases of 38, 21.1, and 28.3 times their respective initial population densities. A gradual decline in the phytoseiids population was observed in each unit following the sixth week. This suggests that the sixth week serves as a suitable time frame for subdividing the rearing units to maintain optimal population levels. The proposed rearing protocol turned out to be useful for the continuous production of the three test species, providing optimal conditions for population growth while preventing mites from escaping the box. This protocol offers a practical solution for the commercial mass production of generalist phytoseiids.

KEYWORDS: Alternative food, biocontrol agents, rearing protocol, predatory mites, production.

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INTRODUCTION

Biological control has long been known as the most environmentally safe and economical mode of pest management (van Lenteren 2012). Among biological control agents, phytoseiid mites (Acari: Phytoseiidae) are particularly effective, primarily in controlling spider mites (Acari: Tetranychidae)

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and other pests, including thrips and whiteflies (Bolckmans 2007). This predatory mite family includes about 30 species, commercially produced worldwide (Onzo *et al.* 2012; Yang *et al.* 2019). *Amblyseius swirskii* Athias-Henriot, *Neoseiulus californicus* (McGregor), and *Neoseiulus barkeri* (Hughes) are species that are commercially produced and used in biological control programs (Zhang 2003). The use of phytoseiid mites in biological control and pest management programs requires the availability of sufficient populations of these predatory mites (Overmeer 1985), so it seems necessary to choose an appropriate technique for their mass rearing.

Numerous studies have demonstrated that pollen grains are an effective food source for successfully rearing *A. swirskii* (Nguyen *et al*. 2013; Kumar *et al.* 2014; Hadadi *et al*. 2022), *N. californicus* (Khanamani *et al*. 2017a, b; Soltaniyan *et al*. 2018; Eini *et al*. 2022), and *N. barkeri* (Rezaie and Askarieh 2016). Additionally, various species of astigmatid mites have been used as alternative food sources for rearing *A. swirskii* (Nguyen *et al*. 2013; Asgari *et al*. 2020; San *et al*. 2020; Pirayeshfar *et al*. 2020), *N. californicus* (Barbosa and de Moraes 2015; Elhalawany *et al*. 2023), and *N. barkeri* (Barbosa and de Moraes 2015). Most of these studies employed the Walzer and Schausberger (1999) method for rearing phytoseiid mites, which involves using a green plastic sheet placed on a water-saturated sponge, surrounded by wet paper towels. While effective, this method requires constant cleaning and frequent replacement of the stock culture to prevent mold growth, making it time-consuming and unsuitable for mass-rearing efforts.

Several alternative techniques have been proposed for rearing predatory mites using enclosed systems and alternative food sources (Kamburov 1966; Kostiainen and Hoy 1994; Kishimoto 2005; Hwang *et al*. 2019; Ballal *et al*. 2021). Although various diets suitable for mass-rearing *A. swirskii*, *N. californicus*, and *N. barkeri* have been identified, a practical and efficient mass-rearing protocol that ensures successful mite production without the need for constant cleaning and rearing medium replacement remains a challenge. This study aimed to evaluate a mass-rearing system for these three phytoseiid mite species using a mixture of cattail pollen, *Tyrophagus putrescentiae* (Schrank) (a species of astigmatid mite), and rice husk as a food source.

MATERIALS AND METHODS

Pollen collection

Pollen of the broadleaf cattail, *Typha latifolia* L. was collected from the male inflorescence of cattail plants in Mahallat, Markazi Province, Iran. The pollen grains were dried at 25 ℃ for 36 h, and stored in a refrigerator at 4 °C for daily use or frozen at −20 °C for long-term storage.

Rearing system design

The rearing system was based on the same principles of Hwang *et al*. (2019) double box rearing method with some modifications. It consisted of three basic parts: an outer box, a Petri dish, and a sponge. The outer box includes a clear polypropylene box (16 × 12 × 6 cm) with an opening (4 × 4 cm) made in a plastic lid covered with cloth mesh (200 × 200 μm). The outer box is half-filled with water and contains a water-soaked sponge (5 × 5 × 3 cm) and a plastic Petri dish (8 cm) placed on top of the sponge (Fig. 1).

Rearing of the prey

Storage mites, *T. putrescentiae*, were used as prey to feed the phytoseiids. They were obtained from the Iranian Research Institute of Plant Protection. To establish the colony of mites in the laboratory, the mite individuals were transferred to a Petri dish in the rearing system described above (Fig. 1a, b). Yeast granules and rice husk (1:1) were used as a food source for *T. putrescentiae* (Fig. 1b, c) added (5 ml each) three times a week. The stock colonies were kept at 25 ± 1 °C, 65 ± 5% RH, and a photoperiod of 16L: 8D h.

Figure 1. **a.** Rearing units of the phytoseiid mites and their prey; **b.** Stock culture of *T. putrescentiae*; **c.** Detail of yeast granules and rice husk in stock culture of *T. putrescentiae*.

Rearing of the phytoseiid mites

The stock cultures of predatory mites were obtained from the Iranian Research Institute of Plant Protection. Mites were reared in the system described above (Figs. 1a, 2a, b). A diet for phytoseiid mites consisted of a mixture of *T. putrescentiae*, cattail pollen, and rice husk (1:1:4). Fresh diet was added to the rearing arena three times a week. The stock cultures of the phytoseiid mites were maintained under the same laboratory conditions as the prey culture.

Figure 2. **a.** Stock culture of predatory mites; **b.** Detail of a phytoseiid mite culture.

Evaluation of rearing protocol

To evaluate the suitability of the protocol for mass-rearing of the storage and predatory mites, the harvestable populations (adult females) were quantified weekly for eight weeks. Each mass-rearing unit of the storage mite and each phytoseiid mite started with 10 *T. putrescentiae* (adult female) and approximately 50 *T. putrescentiae* (all developmental stages) and 15 adult predatory mites (10 females and 5 males) per species, respectively. Storage and phytoseiid mites and their diet were placed within a Petri dish in the rearing system. A diet of the mixture of yeast granules and rice husk (1:1; 5 ml each) and 10 *T. putrescentiae* mites plus a quarter of a teaspoon of cattail pollen (1.25 ml) and a teaspoon of rice husk (5 ml) was added to the rearing units every three days for storage and phytoseiid mites, respectively (Fig. 3). Moreover, the diet component per rearing unit was turned upside down every three days. The harvestable population densities of the storage and phytoseiid mites were estimated by counting adult females in one random sample of 0.10 g per rearing unit (the

total amount was 0.50 g at the first week) taken every week during an 8-week period. Before sampling of per rearing arena, the component of diet per rearing units turned upside down, then samples were taken using a digital scale with an accuracy of 0.01 g (Scale model GR 200, Co. Ltd, Japan), and the adult females per isolated sample were counted under a stereomicroscope (Meiji Techno Co. Ltd, Japan). To estimate the number of storage and phytoseiid mites per rearing arena, the total weight per rearing arena was measured too. Storage and each predator mite species had 10 replications, randomly placed in the growth room. All experiments were performed in four growth rooms (Storage and each phytoseiid mite in a separate growth room) at 25 ± 1 °C, 65 ± 5% RH, and 16L:8D photoperiod. In addition, by placing the external sensor of a thermometer and hygrometer (Model Htc-2, Iran) inside the rearing compounds, the temperature and relative humidity inside the rearing containers were measured weekly. Throughout the 8-week monitoring period, the temperature and relative humidity within the rearing unit inside the Petri dish were at approximately 25 ± 2 °C and 75 ± 5%, respectively, as measured by an external sensor of a thermometer and hygrometer (Model Htc-2, Iran).

Figure 3. a. Rearing unit of the predatory mite at the start of the experiment; **b.** Rearing unit of the predatory mite after 8 weeks.

Statistical analysis

Repeated measures ANOVA in SAS version 9.4 TS level 1M6 was used to analyze the harvestable population densities of three predatory mite species during 8 weeks.

RESULTS

The storage mites per rearing unit increased continuously. During the first week, the storage mite population experienced a rapid increase, reaching a count of 484.50 ± 40.47 adult female mites in each rearing unit. This represents a remarkable 48.4-fold growth from their starting population. Following the first week, the storage mite population exhibited an even more significant surge in numbers, making it impractical to accurately quantify their population in subsequent weeks.

The harvestable population densities of all three phytoseiid mites tested, continuously increased until week 6 (Fig. 4). At the first week of rearing, average population densities of *A. swirskii*, *N. barkeri*, and *N. californicus* were 81.9 ± 13.4, 56.4 ± 7.71, and 22.56 ± 5.09 adult females per rearing units. The population densities gradually increased to 380.2 ± 16.7, 283.9 ± 18.6 and 211.4 ± 12.9 (i.e. 38, 28.3, and 21.1 times of initial population densities) adult females of *A. swirskii*, *N. barkeri* and *N. californicus*, respectively, at the 6th week. Then the population densities decreased to 328.4 ± 16.4, 234.3 ± 17.7, and 205.8 ± 14.3 adult females of *A. swirskii*, *N. barkeri*, and *N. californicus*, respectively, in the 8th week. At that time, many individuals of phytoseiid mites were observed to have drowned in the water in the outer box. ANOVA revealed a significant effect of the interaction

between time and phytoseiid species on mite densities (Table 1). A significantly higher population density of *A. swirskii* was harvested per rearing unit compared to the other predatory mites weekly, except the first week (Fig. 4; $F_2 = 154.89$, $P < 0.001$).

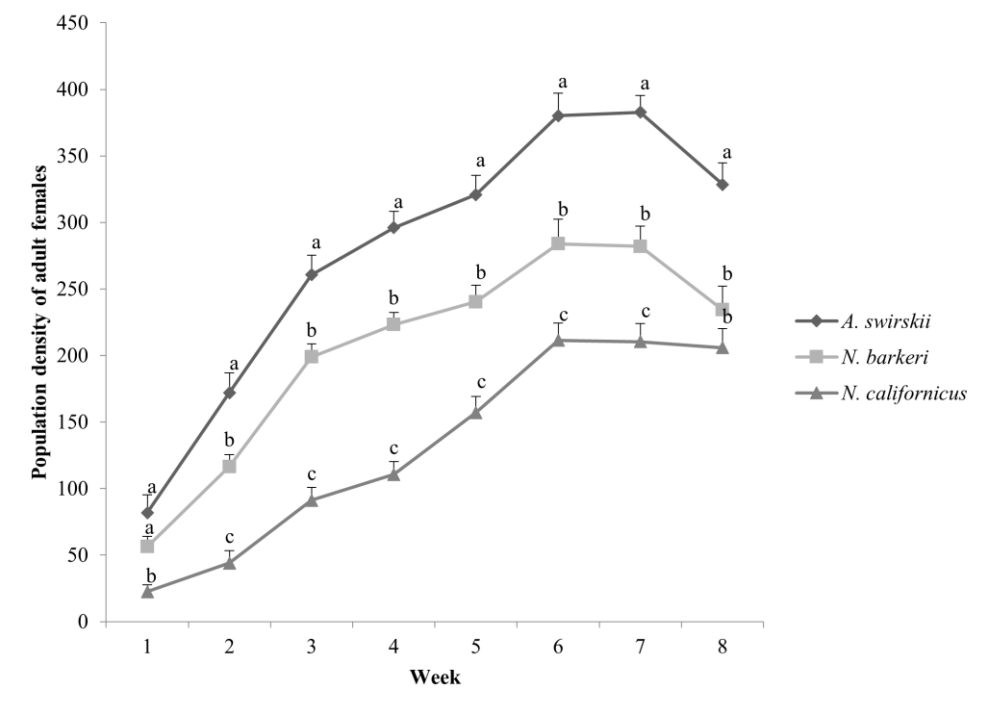

Figure 4. The mean number + standard error of adult females of three phytoseiid mites per rearing unit was harvested weekly. *Different letters per week indicate significant differences among the phytoseiid mites (Tukey test: $P < 0.05$).

Table 1. ANOVA results of the time and phytoseiid species effects on the harvestable population density of phytoseiid mites weekly

Source	DF	Mean square	F value	*P*
Treat (phytoseiid species)	2	427527.80	154.89	< 0.001
Time	7	219061.68	143.46	< 0.001
Time × treat	14	5452.56	3.57	< 0.001
Error	27			
Error (time)	189			

DISCUSSION

The obtained results showed that the proposed rearing system consisting of a Petri dish arena placed on a water-saturated sponge in a plastic box using a fictitious diet of the mixture of *T. putrescentiae*, cattail pollen, and rice husk is an appropriate protocol for the mass-rearing of the generalist phytoseiid mites, such as *A. swirskii*, *N. californicus*, and *N. barkeri*. Moreover, this rearing system is suitable for the rearing of storage mites such as *T. putrescentiae* using a mixture of yeast granules and rice husk. This system provides two advantages for maintaining the conditions necessary for the mass-rearing. First, the water content in the plastic box is beneficial for retaining humidity in the rice husks that are used as a shelter for the predatory mites and their prey *T. putrescentiae* within the Petri dish. Humidity is one of the most important environmental conditions in mite rearing (Freire and de Moraes 2007; Hwang *et al.* 2019). Most phytoseiid mites are susceptible to dry conditions but can absorb water vapor from unsaturated air as long as the relative humidity (RH) is above their critical

equilibrium activity (CEA) (i.e. the point at which water loss equals water gain) (Stenseth 1979; Bakker *et al.* 1993; Yoder 1998). In this line, San *et al.* (2021) indicated that the eggs of *A. swirskii* failed to hatch at 30% RH, and reproduction parameters decreased at low RH%, so this negative effect was eliminated when water was available. It has also been shown that *Phytoseiuls persimilis* Athias-Henriot fails to control *T. urticae* at relative humidities as low as 40% and 27 °C (Stenseth 1979). Although water can be ingested (i.e. drinking), the passive water uptake is a crucial element in maximizing the performance of the predator. In this regard, the predatory mite *Gaeolaelaps aculeifer* (Canestrini) demonstrated favorable population growth when maintained at a relative humidity of 95% within the rearing environment (Hwang *et al.* 2019). Second, this system makes it possible to monitor the density of the mite population within the Petri dish during the rearing period. When the mite density greatly increased, many drowned individuals were observed in the water of the plastic box. Although the presence of water generally prevents most mites from escaping the enclosure, when the mite population within the rearing unit reaches its environmental carrying capacity, they may still attempt to escape in search of additional space and food resources. Additional methods such as applying oily substances to the edges of the Petri dish or introducing detergents or other substances to the surrounding water could be helpful in mite escaping which needs further investigation. The population densities of three phytoseiid mites were highest in the 6th week, then they decreased. We used this as an indirect indicator for overpopulation and time to divide the overpopulated box into two boxes. Therefore, we programmed the division of the predatory mite population into two boxes in the 6th week.

In the current study, using the diet of a mixture of mold mites, *T. putrescentiae* with cattail pollen and rice husk supplied suitable growth for three phytoseiids. Pollen grains may provide important nutrients (including protein, lipids, sugars, and vitamins) for predatory mites, and their nutritional value varies greatly among and even within plant species (Khanamani *et al.* 2017a). In addition, astigmatid mites are known as the main food source in the mass rearing of generalist phytoseiids (Bolckmans and van Houten 2006; Britto *et al.* 2012; Midthassel *et al.* 2013; Pirayeshfar *et al.* 2020). Rice husks are a good source of shelter for predatory mites and they are useful for retaining moisture within the media (Hwang *et al.* 2019). As each diet possesses varying nutrient compositions, combining different diets allows for a more balanced and diverse nutritional intake for predatory arthropods, promoting their overall health and growth (Mayntz *et al.* 2005). In this rearing system, by turning upside down the component of diet per rearing unit every three days and the presence of mold mites (*T. putrescentiae*) in diet compositions, diet components do not mold, and there is no need to remove old food from the culturing environment. The presence of astigmatid mites has a two-fold benefit toward fungal control: direct feeding on the fungal mycelia and indirect via the production of antifungal compounds (Kuwahara 2004). All mites belonging to the cohort of astigmata possess a pair of opisthonotal glands that emit a range of volatile semiochemicals with different functions (Kuwahara 2004). Those volatile compounds may act as alarm, aggregation, or sex pheromones. The behavioral response depends on the mite species and concentration (Kuwahara *et al*. 1980; Kuwahara 2004; Midthassel *et al*. 2015). One such volatile is citral, a mix of its two isomers geranial (trans-citral) and neral (cis-citral), which was shown to have antifungal and antimicrobial properties (Matsumoto *et al*. 1979; Onawunmi 1989; OuYang *et al*. 2018). It is also possible that astigmatid mites in the rearing arena are the source of water for phytoseiids, which needs further investigation.

The mass-rearing protocol presented in this study provided adequate population growth for three phytoseiid mites. The population densities of *A. swirskii*, *N. barkeri*, and *N. californicus* underwent 38, 28.3, and 21.1-fold increases, respectively, within six weeks. Other effective predatory mite-rearing methods have also yielded population growth. For instance, Hwang *et al.* (2019) observed a 15.5-fold increase in the *G. aculeifer* (Canestrini) population within two weeks. Similarly, *Amblyseius eharai* Amitai & Swirski, *Typhlodromus* (*Anthoseius*) *vulgaris* Ehara, and *Amblyseius tsugawai* Ehara populations increased 18.1, 11.9, and 36.1 times their initial populations, respectively, over two weeks (Kishimoto 2004). In our study, the population densities of predatory mites gradually

decreased in the 8th week because many individuals escaped and drowned in water in the plastic box. In a similar double box-rearing system presented by Hwang *et al.* (2019), the population density of *G. aculeifer* increased during the first two weeks and then decreased in the third week.

The rearing system of a Petri dish on a water-saturated sponge within a plastic box using a mixture of mold mites, *T. putrescentiae* with cattail pollen and rice husk presented in this study proved to be a consistent and robust protocol to produce generalist phytoseiid mites, such as *A. swirskii*, *N. californicus*, and *N. barkeri* and could be proposed for commercial production when rearing scale is increased. However, further studies are needed to scale up the rearing system and optimize harvesting rates.

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سیستمی عملی برای پرورش انبوه هرناهای فیتوزئید: مطالعه موردی با *Amblyseius swirskii*، *Neoseiulus californicus* و *Neoseiulus barkeri* (Acari: Phytoseiidae)

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چکیده

هرناهای فیتوزئید نقش مهمی در برنامه‌های مهار زیستی دارند. گسترش تکنیک‌های کارآمد برای پرورش انبوه این هرناها به منظور افزایش کاربرد آن‌ها در برنامه‌های مدیریتی در مقیاس بزرگ ضروری است. این مطالعه با هدف توسعه و ارزیابی روشی ساده برای پرورش انبوه و تولید مداوم کنه‌های فیتوزئید عمومی (*Amblyseius swirskii*، *Neoseiulus californicus* و *Neoseiulus barkeri*) انجام شده است. سیستم پرورش شامل یک ظرف پتری بود که روی یک اسفنج اشباع از آب درون ظرفی پلاستیکی حاوی مقداری آب قرار می‌گرفت. هرناهای شکارگر با مخلوطی از گرده لویی، پوسته برنج و هرنای انباری *Tyrophagus putrescentiae* تغذیه می‌شدند که سه بار در هفته به واحدهای پرورش اضافه می‌شدند. هرناهای طعمه نیز در سیستمی مشابه با استفاده از دانه‌های مخمر و پوسته برنج به عنوان غذا نگهداری شدند. رشد جمعیت هرناهای شکارگر طی یک دوره ۸ هفته‌ای تحت شرایط آزمایشگاهی کنترل‌شده (۱±۲۵ درجهٔ سلسیوس، رطوبت نسبی ۵±۶۵٪، و دوره نوری ۱۶ روشنایی: ۸ تاریکی (ساعت)) ارزیابی شد. نتایج نشان داد که هر سه گونه به طور موفق رشد یافته و در هفته ششم به اوج جمعیت خود رسیدند که به ترتیب ۱۶/۷±۳۸۰/۲، ۱۲/۹±۲۱۱/۴ و ۱۸/۶±۲۸۳/۹ مادهٔ بالغ از کنه های *A. swirskki*، *N. californicus* و *N. barkeri* در هر ظرف پرورش تولید شدند. این مقادیر نشان‌دهنده افزایش ۳۸، ۲۱/۱ و ۲۸/۳ برابری انبوهی جمعیت نخستین آن‌ها بود. پس از هفته ششم، کاهش تدریجی جمعیت فیتوزئیدها در هر واحد پرورش مشاهده شد که نشان دهنده زمان مناسب تقسیم واحدهای پرورشی برای حفظ سطح بهینه جمعیت هرناهای شکارگر است. روش پرورش پیشنهادی با موفقیت امکان تولید مداوم سه گونه فیتوزئید مورد آزمایش را فراهم کرد. این روش شرایط مطلوبی را برای رشد جمعیت این شکارگرها ایجاد کرده و در عین حال از خروج کنه‌ها از ظروف پرورش جلوگیری کرد. بنابراین، این روش یک راهکار عملی برای تولید انبوه فیتوزئیدهای عمومی ارایه می‌دهد.

واژگان کلیدی: غذای جایگزین، عوامل بیوکنترل، شیوه‌نامهٔ پرورش، هرناهای شکارگر، تولید.

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