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Short Communication

Methods for long-term storage of predatory mite *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae)

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Abstract

Neoseiulus californicus (McGregor) (Acari: Phytoseiidae) is one of the most effective predators in controlling various pest species in greenhouses. Cold storage is a common method for preserving natural enemies in insectariums long-term. This study used different food materials (wheat bran, pumice, hollow oat, corn pollen sheath, date pollen sheath, sawdust, and rubber) to store *N. californicus* to examine its survival and predation. In each container, 20 female mated predatory mites (3-4 days old) were placed and kept for 1, 5, 10, and 15 days at four different temperatures (0, 5, 10, and 15°C with 60% relative humidity). The percentage of survival and lifespan were determined and the efficiency of the stored predatory mites (fecundity rate and predatory rate) was also compared with the main colony individuals (as a control treatment). The survival rate in the materials, including corn and date pollen sheaths, was higher than the other materials. The survival rate of individuals 1, 10, 15, and 20 days after storing predatory mites in corn pollen sheaths was 81, 70.62, 54.87, and 37.75%, respectively. Five or 10 °C was the best temperature for storing predatory mites. The efficiency of the predatory mite kept with corn pollen sheaths at 5°C was not significantly different from the control. Therefore, storing and transporting *N. californicus* to the market with corn and date pollen sheaths for a maximum period of 10 days at 5 °C is recommended.

Keywords: Storage, temperatures, cover materials, survival rate, and pollen

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گزارش کوتاه انگلیسی

روش‌های ذخیره‌سازی طولانی‌مدت کنه شکارگر *Neoseiulus californicus* (McGregor) (Acari: Phytoseidae)

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چکیده

کنه *Neoseiulus californicus* (McGregor) از شکارگرهای موثر در کنترل بسیاری از آفات در گلخانه‌ها است. ذخیره سازی در سرما یک روش رایج برای نگهداری طولانی‌مدت دشمنان طبیعی در انسکتاریوم‌ها است. برای ذخیره‌سازی این کنه شکارگر از ظروف کوچک پلاستیکی همراه با یک ماده پوشانده (سبوس گندم، پوکه‌های معدنی، دانه‌های جوپوک، کائوچو،غلاف‌های نگهدارنده گرده‌های ذرت، خرما و خاک اره) استفاده شد. در هر ظرف ۲۰ عدد ماده کنه شکارگر جفت گیری کرده (۳-٤ روزه) را قرار داده و تیمارهای مختلف در مدت زمان‌های ۱، ۵، ۱۰ و ۱۵ روز و دماهای صفر، ۵، ۱۰ و ۱۵ درجه سلسیوس (همگی در رطوبت ٦۰٪) نگهداری شدند و در ادامه درصد تلفات، بقا، طول عمر وکارایی کنه‌های شکارگر ذخیره شده (میزان تخم‌ریزی و میزان شکارگری) با افراد کلنی اصلی (به عنوان شاهد) مقایسه شد. درصد بقا افراد در مواد همراه‌ی شامل غلاف گرده ذرت و غلاف گرده خرما بیشتر از سایر مواد مورد آزمون بود. درصد زنده‌مانی افراد ۱، ۱۰، ۱۵ و ۲۰ روز بعد از انبارکردن کنه شکارگر در غلاف گرده ذرت به‌ترتیب ۸۱، ۷۰/٦۲، ٥٤/۸۷ و ۳۷/۷٥ درصد ثبت شد. پنج و ۱۰ درجه سلسیوس بهترین دما برای ذخیره‌سازی کنه‌های شکارگر بود. کارایی کنه شکارگر نگهداری‌شده با غلاف گرده ذرت در دمای ٥ درجه سلسیوس با شاهد اختلاف معنی‌داری نداشت. ذخیره‌سازی و انتقال کنه شکارگر به بازار فروش با غلاف‌های گرده ذرت و گرده خرما در مدت زمان حداکثری ۱۰ روز در دمای ٥ درجه سلسیوس توصیه می‌گردد که تا حدودی شرایط مناسب را برای زنده بودن کنه‌های شکارگر فراهم می‌کنند.

کلیدواژه‌ها: شکارگر، نگهداری، طولانی مدت، گرده و کارایی

دبیر تخصصی: دکتر آرش راسخ

Introduction

There are various pest control methods; the safest, most economical, and best is the biological control method. Among the biological control agents, predatory mites, especially mites of the Phytoseiidae family are very important. Phytoseiid mites are used in biological control programs to suppress pests of greenhouse plants and field crops worldwide (Mcmurtry & Croft, 1997).

Predatory mite, *Neoseiulus californicus* (McGregor) is one of the most important species of Phytoseiidae. This predatory mite preys on phytophagous mites and small insect pests. The predatory mite is commonly used for biological pest control of spider mites. Although *N. californicus* prefers spider mites, it can also feed on other food sources such as mites, thrips, or pollen. This predatory mite tolerates higher temperatures, lower humidifies, and pesticides (Rezaie, 2019; Azzazy, 2021).

Storage at low temperatures is valuable for increasing the shelf-life of natural enemies such as insect parasitoids or predators. Cold storage is a general technique for long-term preservation of the parasitoids or predators in insectaries (Aliabadi et al., 2019). Cold storage is usually performed under sub-optimal temperatures and is generally associated with major fitness costs. Tolerance to cold storage is a very plastic trait influenced by a wide range of endogenous (biotic) and exogenous (abiotic) factors experienced before, during, or after cold exposure (Colinet & Boivin, 2011), Cold storage is a common technique to prolong the shelf life of the mass-reared natural enemies in insectaries. Cold storage may affect different aspects of the predator's life, such as the survival of different developmental stages (Ricci et al., 2006; Coudron et al., 2007; Aliabadi et al., 2019).

Knowing the factors affecting the efficiency of cold storage to optimize them has always been of interest to researchers. In general, the degree of tolerance of a natural enemy against cold is a complex process that is influenced by a variety of non-living external factors, such as temperature and duration of storage, and internal factors, such as nutritional status, sex, age, and developmental stage (Yanik & Unlu, 2015).

A study on the possibility of storing the predatory mite can help us with IPM principles and be used in pest control in greenhouses. In this study, the possibility of maintaining *N. californicus* was investigated in different materials at different temperatures, and finally, the efficiency of stored predatory mites was investigated.

Materials & Methods

Colony

The storage mite *Tyrophagus putrescentiae* (Schrank) (Acari, Acaridae) was used as prey. Wheat bran (10 gr.) and corn pollen, along with protective sheath (3 gr.) and barley yeast (0.5 gr.), were used to rear *T. putrescentiae*.

The predatory mite, *Neoseiulus californicus* was maintained on all developmental stages of *T. putrescentiae* and corn pollen. The stock culture of *N. californicus* was maintained in a growth chamber at 27 ± 1°C, 70 ± 5% RH, and 16 L: 8 D hours. The tested pollens were collected by hand (corn pollen from Karaj and date pollen from Bam). Pollens were stored in the refrigerator during the experiments.

A plastic sheet was used on a sponge saturated with water inside a plastic container (closed container with a mesh lid) filled with water to rear this predatory mite. To prevent mites from escaping, strips of wet paper towels were placed on the edge of these plastic sheets in such a way that one side of it was immersed in the water of the plastic container, and by sucking the moisture, it provided the water needed by the predatory mite. Different life stages of *T. putrescentiae* and corn pollen were used to rear the predatory mite.

Experiments

The gravid female of the predatory mites of the same age were transferred from the main culture onto a small container (3 cm diameter and 10 cm height) that was used with different cover materials (wheat bran, pumice, hollow oat, corn pollen sheath, date pollen sheath, sawdust, and rubber) at four constant temperatures (0, 5, 10 and 15 °C) and 60% relative humidity with total darkness conditions. In each container, 20 female predatory mites were used with 20 replicates. The numbers of predatory mites were counted after 1, 5, 10, and 15 days. The survival rate was determined, and finally, the efficiency of the stored predatory mites (lifetime longevity, lifetime fecundity, and predation rate) was also compared with the individuals of the original colony.

To investigate the effectiveness of the stored predatory mites, the female mites kept in containers for 5 days were subjected to a supplementary examination. The effectiveness of predatory mites placed in containers with corn or date pollen sheaths and wheat bran for 5 days was investigated. The females were placed in separate containers, and the egg-laying rate of these predatory mites was checked within 24 hours and then the female individuals were placed in separate containers, and their lifespan was determined in 10 repetitions. In each petri dish with a diameter of 6 cm, a bean leaf disk of 2 × 2 cm^2, 30 two-spotted spider mite eggs were provided to the females for 24 hours, and the number of prey consumed was compared. The number of eggs is used as a scale to determine the predation rate.

Statistical Analysis

A completely randomized design with a factorial arrangement was used for data analysis. The repeated measure ANOVA method was used, and the effect of mite counting times was also investigated and used one – way ANOVA analysis for predation rate. The software used was SAS (Ver. 9.4). Tukey's test was used to group and compare the averages. SPSS software (Ver. 26) was used to determine the standard errors of the means.

Results & Discussions:

The survival rate of the predatory mites after one, 5, 10 and 15 days showed significant differences among different materials ($F_{6, 252}$=85.81, P<0.0001) (Table1).

The survival rate of the predatory mite was observed to be highest on corn and date pollen sheath. The survival rate of the predatory mites showed significant differences among temperature conditions ($F_{6, 252}$=312.32, P<0.001). The survival rates of *N. californicus* were 33.92 ±2.48 % at 0°C & 77.42±1.91% at 10°C after a day stored the predatory mites. The survival rates showed no significant differences at 5 °C or 15°C. The highest percentage of survival rate was observed at 5°C and 10°C. The survival rate of the predatory mite was 49.0±1 %, 73.0±1.22%, 61.0±4.30%, and 69.0±1.00 % at 5°C in a sheath of corn pollen after 1, 10, 15 and 20 days, respectively. During this investigation, the storage of this predator for 10 days at a temperature of 0 °C led to the loss of 83.7 % of the adult females. Also, if preliminary acclimatization was carried out for 10 days at a temperature of +5 °C prior to the indicated cooling, the mite loss was only 53.36 %.

The interaction effect of the two investigated factors (type of materials and temperature) was not significant ($F_{18, 756}$=2.99, P=0.06). The effect of sampling time was significant ($F_{30, 756}$=1160.58, P<0.0001). The interaction effect of type of materials, temperature, and duration of store was significant ($F_{18, 756}$=16.56, P<0.01), ($F_{9, 756}$=16.87, P<0.0001). The interactions of store duration, and the two factors studied were not significant ($F_{54, 756}$=1.87, P=0.26).

Zero-degree temperature shows the lowest percentage of survival in all investigated treatments. The survival rate in the second to third sampling does not indicate a significant decrease, and in the fourth sampling (15 days after placing in containers), there was a significant decrease in most treatments.

Female lifespan if stored with wheat bran (4.70 ± 0.36 days) with control (main colony) or female mites kept on corn pollen and date pollen show significant differences ($F_{3, 36}$=8.21, P<0.001) (Fig.1). The fecundity rate of four -day -old females kept on corn, date pollen and wheat bran at 10 ℃ for 5 days and four -day female people in colonies at 27 ℃ were compared.

The fecundity rate and predation rate of mites kept in corn pollen sheath did not show any significant difference with the control (main colony); in terms of lifespan, there was no difference between the individuals stored in date pollen sheath and the control ($F_{3, 28}$=5.57, P<0.001).

The fecundity rate of females was significant differences ($F_{3, 28}$= 5.57, P<0.001) (Fig. 1). The fecundity rate of females in the control treatment was higher than the others (3.25 ±0.18 eggs per female), and the lowest was observed in wheat bran treatments (0.87±0.22 eggs per female) and the fecundity rate of mites kept in corn pollen sheaths did not show any significant difference with control (2.12±0.21 eggs per female).

The predation rate of females kept on different material and females of the main colony show a significant difference ($F_{3, 30}$= 9.21, P<0.001). The predation rate of the predatory mite of the main colony (8.60± 0.18 eggs) and the predatory mite kept on corn pollen sheath (6± 0.43 eggs) were not significantly different, and the lowest was observed on wheat bran (Fig. 1).

Ghazy et al. (2014) reported that low air temperature with high humidity is effective for long-term cold storage of the predatory mite, *N. califonrnicus*, and the pre-storage nutrition affact on quality of the predatory mite. The interspecific variation in the response of these predators to low air temperature might be attributed to their natural habitat and energy requirements. These results may be useful for the long-term storage of these predators, which is required for cost-effective biological control.

N. californicus showed a longer survival time than *P. persimilis* at all the air temperatures. The longest mean survival time of *N. californicus* was 11 weeks at 7.5 ℃, whereas that of *P. persimilis* was 8 weeks at 5.0 ℃. After storage at 7.5 ℃ for 8 weeks, no negative effect on post-storage oviposition was observed in *N. californicus*, whereas the oviposition of *P. persimilis* stored at 5.0 ℃ for 8 weeks was significantly reduced (Ghazy et al., 2014).

Table 1- survival rate (Mean ± SE) of *Neoseiulus californicus* on different materials (wheat bran, pumice, hollow oat, rubber, corn pollen sheath, date pollen sheath and sawdust) in consecutive days (1, 5, 10 and 15 days after treatment).

time	wheat bran	pumice	hollow oat	Corn pollen sheath	Date pollen sheath	Sawdust	Rubber
After 1 day	77.37 ± 3.49 ^{a}	61.62 ± 3.48 ^{b}	61.62 ± 3.48 ^{b}	81.0 ± 3.21 ^{a}	77.62 ± 3.02^{a}	44.50 ± 2.37 ^{c}	46.50 ± 3.75 ^{c}
After 5 days	57.87 ± 3.58 ^{b}	50.87 ± 3.58 ^{c}	53.00± 3.45 ^{bc}	70.62 + 3.09 ^{a}	65.75±2.75^{a}	34.75 ± 2.25 ^{d}	34.87 ± 3.49 ^{d}
After 10 days	32.75 ± 3.30 ^{d}	36.62 ± 2.85 ^{cd}	42.87 ± 3.15^{bc}	54.87 ± 2.86 ^{a}	46.37 ± 2.81^{b}	30.45± 2.25 ^{d}	29.37 ± 3.19 ^{de}
After 15 days	20 ± 3.08^{de}	29.25 ± 2.66^{b}	26.50 ± 2.37^{bcd}	37.75 ± 3.15 ^{a}	27.75± 2.18 ^{bc}	13.75 ± 2.18 ^{e}	18.50± 2.56 ^{de}

Different letters in each row indicate a significant difference between the treatments at the 95% probability level.

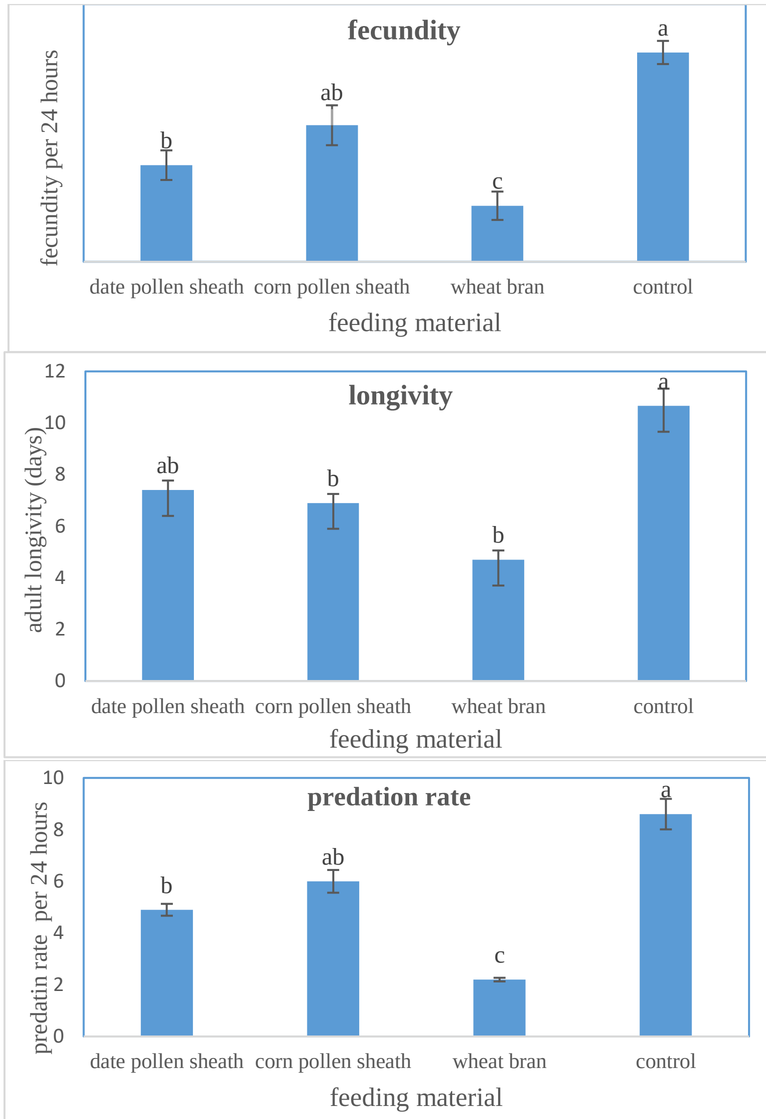

Fig1. Lifespan, fecundity and predation rate of predatory mite *Neoseiulus californicus* kept with date pollen sheath, corn pollen sheath, wheat bran for 5 days at 25 ℃

Conclusions:

Low-temperature storage is a conventional way to increase the shelf life of natural enemies and provide a stable storage for use in biological control programs. According to the results, it is suggested that the sheath of corn pollen and date pollen at 5°C is the best storage condition for this predatory mite. Thus, it is recommended to store and transport predatory mites to the market with corn pollen and date pollen pods for a maximum period of 10 days at a 5°C, which provides suitable conditions for the survival of the predatory mite. The optimum temperature for long-term storage of the predatory mite is 5°C. Temperatures of 10°C or 15 °C increase the proliferation of saprophytic fungi in predatory mite storage containers; the efficiency of mites stored with corn pollen sheath or date pollen sheath is not different from the original colony in terms of predation rate and fecundity rate, and lifespan of individuals.

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