



## Predaciousness Efficiency of Three Manure-Inhabiting Mite Species Against The Eggs of *Callosobruchus Chinensis* and *Sitotroga Cerealella*

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**ABSTRACT:** Individuals of three manure-inhabiting predaceous mite species were chosen to evaluate their efficiency as biological control agents against the eggs of two stored-product insects. The mite species were *Cheyletus malaccensis* (Acari: Cheyletidae), *Macrocheles robustulus* (Acari: Macrochelidae) and *Gamasholaspis variabilis* (Acari: Parholaspididae). Meanwhile, the tested insects were *Sitotroga cerealella* (Lepidoptera: Gelechiidae), and *Callosobruchus chinensis* (Coleoptera: Bruchidae). For each insect, twenty eggs were exposed to five individuals of the predaceous mite. The eggs and mites were kept in a dark room in the laboratory. After treatment, inspections were performed 24, 48 and 72 hrs later. In most inspection intervals, the average percentages of the consumed eggs by these predaceous mite species were significantly different from those of control. All three mite species consumed a lot of eggs, especially after 72-hour post treatment. The average percentages of consumed *S. cerealella* eggs by either mite species were always higher than the average percentages of *C. chinensis* eggs consumed by the same mite species. *Macrocheles robustulus* was found to be the most effective predator against both insects' eggs where the average percentages of *S. cerealella* and *C. chinensis* consumed eggs after 72 hrs were 91.67 and 53.33%, respectively. *Gamasholaspis variabilis* was found to be the least effective predator against the eggs of both insects where the average percentages of the consumed eggs of *S. cerealella* and *C. chinensis* after 72 hrs were 86.67% and 38.33%, respectively. The consumption of the eggs of each of the two targeted insects by the individuals of the three evaluated mite species increased as the exposure time increased. Cannibalism between the individuals of each of the three evaluated mites wasn't observed while performing the experiment.

**Keywords:** Manure, predatory mites, eggs consumption, *Callosobruchus chinensis*, *Sitotroga cerealella*

### INTRODUCTION

Food grains are durable items that can be stored for extended periods under safe storage conditions. Various biotic and abiotic factors influence the quality of stored grains. Pests attacking and infesting stored foods are serious (Bharathi *et al.*, 2017).

Stored-product insects are very difficult to control. They can break out and be transported from one place to another. To obtain high-quality foods, these pests must be controlled and eradicated.

The biological management of insect and mite pests is a common practice as part of the IPM method (Pilkington *et al.*, 2010). Our understanding and prevention of postharvest losses are critical if we are to feed a growing global population. In most cases, insect infestation-related losses of stored commodities are only measured in terms of quantitative and physical weight losses. Insect infestation affects the nutritional value; some nutritional components

are impacted more severely than others (Stathers *et al.*, 2020).

Biological manipulation is the use of non-chemical and environmentally pleasant strategies for controlling insect pests, diseases and other harmful agents. In recent decades, the amplified usage of biological manipulation has been due to its safety and long-term action on the target pests. On the contrary, the chemical-based methods kill non-target species and have hazardous consequences for humans and the environment (Sanda and Sunusi, 2014). Native natural enemies are favored for biological control because they are not expected to cause negative effect against non-target organisms (Schöller and Prozell, 2014). Biological management is a key ecosystem service and an underlying pillar of integrated pest management (IPM) (Naranjo *et al.*, 2015).

Predaceous mites are important in commercial augmentation biological control. They are mainly used in protected vegetable and ornamental cultivation systems to control

phytophagous pests like mites, thrips, and whiteflies. Mesostigmatid mites are mostly predators that help to regulate the density of saprophages oribatid mites in ecosystems (Walter and Proctor, 2013).

As regards mass production of natural enemies, selecting cost-effective food sources is of critical importance for reducing costs. Several species of predaceous mites are now produced by astigmatid mites (Midthassel *et al.*, 2013).

Predatory mites are known to be available in manure. The density of Mesostigmata ranged from 2000 individuals/m<sup>2</sup> in a cattle pasture to 10000 individuals/m<sup>2</sup> in a goose pasture (Pacek *et al.*, 2020). Hence, the aim of the present study was to evaluate and estimate the efficiency of three manure-inhabiting predaceous mite species against two stored-product insect species.

The objective of the present study is to find out the most effective predators, which can be easily obtained from manure and can be used as biological control agents against the targeted insects in the grains used as seeds for agriculture. In future, mass production of those mites could be achieved to protect the grains specialized as food.

## MATERIALS AND METHODS

Three manure-inhabiting predatory mite species were considered to evaluate their efficiency as biological control agents against the eggs of two stored-product insects. The evaluated mites were *Cheyletus malaccensis* (Acari: Cheyletidae), *Macrocheles robustulus* (Acari: Macrochelidae) and *Gamasholaspis variabilis* (Acari: Parholaspididae). Meanwhile, the insects were *Sitotroga cerealella* (Lepidoptera: Gelechiidae) and *Callosobruchus chiensis* (Coleoptera: Bruchidae).

### 1. Predaceous mites

#### 1.1. Obtaining the predatory mites

Manure was the source of mites and therefore three types of manure (sheep, cattle and poultry) were obtained from certain locations in Abohomous, Behera governorate, Egypt. Manure samples were obtained with the aid of an iron sampler. Each sample, about half a kilogram, was placed in a plastic bag, which was marked with a label denoting the manure type. Manure bags were transferred to the laboratory of the Plant Protection Department, Faculty of Agriculture (Saba Basha), Alexandria University.

#### 1.2. Mite extraction

Tullgrn funnels (20 cm in diameter) were utilized to extract the mite individuals from the manure samples. A 40-watt light bulb was placed above the manure sample in each funnel and served as a source of heat and light. Each funnel was used to drive the arthropods downwards into a tight 200-ml glass jar. Samples were left in the funnels for about 72 hours. After the extraction

process, the glass jars below the funnels contained different types of arthropods. Predatory mites were picked up from the glass content by using a very fine camel hair brush with the aid of a binocular microscope. After extraction, the mites were presented to the eggs immediately without starving them.

#### 1.3. Mite mounting for identification

The mite individuals that were used in the experiments for evaluating their predaciousness efficiency were subjected to taxonomical identification to be sure of the mite species. More than 100 specimens were mounted and identified using keys of Zaher (1986).

#### 1.4. Mite preparation

According to Zaki (1983), the mites were treated with two types of preparations: temporary and permanent. As regards the temporary preparation, a slide with a medium concave area and a thin glass cover of 20 mm were used. A small drop of lactic acid was placed in the center of the concave area and the thin cover was placed on the concave area of the slide, leaving a space to transfer the mite specimens. Mites were examined taxonomically under the 640x magnification of a research microscope.

The second type of preparation (the permanent one) was carried out by placing a very small drop of Berlese's fluid in the center of a clean glass slide. The glass cover of 20 × 20 mm was cut into four equal quarters using a glass cutter. Using a dissecting needle, the small cover was laid on the surface of the animal immediately and before the Berlese's fluid dried. These permanently mounted specimens were dried at 50 degrees for 3–4 weeks, and then they were ready for identification.

#### 1.5. Identification

The identification process was carried out at first by using the temporary mounted preparation (open preparation). The specimens were easy to study by changing their orientations as desired and using the maximum magnification force of a binocular microscope. The open preparations were subjected to the initial routine identification tasks. On the other hand, the permanent mounts were easily handled, ready for an immediate study of different parts of the mite. Taxonomic characters were obvious by using a research microscope under a magnification force of 640 or more.

### 2. Tested insects

#### 2.1. Insect rearing

The eggs of the two tested insect species (Angoumois grain moth *Sitotroga cerealella* and the Adzuki bean beetle *Callosobruchus chinensis*) were obtained from the Agricultural Research Center, Eldokki, Egypt. Large numbers of the two

insect species were produced in the laboratory of Plant Protection Department, Faculty of Agriculture (Saba Basha), Alexandria University.

### 2.1.1. *Sitotroga cerealella*

One kilogram of wheat grain was cleaned and sterilized in an oven at 40° C. Afterwards, the wheat grains were kept out of the oven to lose heat and were transferred into a plastic container. Heat disinfestation requires only heating all the particles in a batch of infested grains to the appropriate lethal temperature (Kitch *et al.*, 1992). Different stages of mite and insect species have different susceptibilities to heat treatment, but most species don't survive more than 12 hrs at 45° C, 5 min at 50° C and 30 s at 60° C (Fields, 1992). Mortality is obviously related to the temperature to which the pests are exposed and also related to the exposure period (Crooker, 1985). Wheat grains were placed in a plastic container. The eggs of *S. cerealella* were added to the wheat grains. The container was covered with muslin. A month later, the container was full of moth adults. Adults were transferred gently to a small glass container. The opening of it was covered tightly with a piece of white paper, on which the adults laid eggs. Some eggs were used for evaluating the predation efficiency of the mites.

### 2.1. 2. *Callosobrucus chinensis*

One kilogram of cowpea grains was cleaned and serialized in an oven at 40° C. Afterwards, the cowpea grains were kept out of the oven to lose heat and were transferred into a plastic container. The eggs of *C. chinensis* were added to the cowpea grains. The container was covered with muslin. A month later, the container was full of beetle adults. Some of the grains on which the eggs were laid were used for evaluating the predation efficiency of the mites.

## 3. Evaluating the predation efficiency

### 3. 1. Exposing the insect eggs to the predatory mites

Twenty eggs of *S. cerealella* were put in a Petri dish (5 cm in diameter). Five individuals of

the predatory mite were placed in the same Petri dish. To prevent mite individuals from escaping, the Petri dish was covered with a plastic shrink film and stored in a dark place at a temperature of  $28 \pm 5^\circ \text{C}$  and a relative humidity of  $60 \pm 5\%$ . Three replicates of that unit, *i.e.*, a Petri dishes containing the eggs and the mites were used for evaluating the predation efficiency of each of the three evaluated mite species.

Likewise, eggs of *C. chinensis* were examined as prey for each of the three mite species. Herein, the cowpea grains that contain the eggs of *C. chinensis* were used and placed inside the Petri dishes to make the eggs available for the mite individuals. For control, twenty eggs of each insect species were kept at the same above mentioned conditions without the predators.

### 3.2. Detecting the eggs

The eggs in the Petri dishes were examined after 24, 48 and 72 hrs. Dented eggs were counted in each inspection interval to estimate the mortality percentage; those eggs are considered to be consumed by the mites. Moreover, the hatchability of the rest of the eggs was considered as an indicator of their being alive.

## 4. Statistical analysis

The data were analyzed using ANOVA and "F" test, with 3 replicates for each treatment. The least significant differences (L.S.D.) at the  $0.05 \leq$  level were determined according to the computer program COSTAT software and Duncan's Multiple Range.

## RESULTS

### 1. Comparison between the average percentages of the consumed eggs and the control

Table (1) demonstrates the average percentages of the insect eggs that have been consumed by the three species of the evaluated predatory mites. After the 72-hrs interval, inspection detected that all of the eggs used as controls were viable and still alive.

**Table 1:** Average percentages of the detected insect eggs that have been consumed by the predatory mites in different periods

Predatory mites	Insects	Average percentages of the lifeless eggs detected at different intervals (hrs)					
		24		48		72	
		Treated	Control	Treated	Control	Treated	Control
<i>C. malaccensis</i>	<i>S. cerealella</i>	55.00 bc*	0.00 h	78.33 a	0.00 h	90.00 a	0.00 h
	<i>C. chinensis</i>	11.67 gh	0.00 h	13.33 gh	0.00 h	40.00 cde	0.00 h
<i>M. robustulus</i>	<i>S. cerealella</i>	26.67 efg	0.00 h	51.67 bc	0.00 h	91.67 a	0.00 h
	<i>C. chinensis</i>	20.00 fg	0.00 h	30.00 def	0.00 h	53.33 bc	0.00 h
<i>G. variabilis</i>	<i>S. cerealella</i>	45.00 bcd	0.00 h	60.00 b	0.00 h	86.67 a	0.00 h
	<i>C. chinensis</i>	15.00 fgh	0.00 h	30.00 def	0.00 h	38.33 bc	0.00 h

L.S.D. (0.05) = 16.39

\* Averages followed by the same letter(s) are not significantly different at  $P < 0.05$  level.

In most inspections at the different intervals, the average percentages of the consumed eggs were significantly different from the average percentages of the lifeless eggs in the control. Exceptionally, the average percentages of *C. chinensis* eggs consumed by *C. malaccensis* showed no significant differences with controls in those inspections performed after 24 and 48 hrs. Similarly, in the 24-hour inspection, the average percentages of *C. chinensis* eggs consumed by *G. variabilis* showed no significant difference from the control's lifeless eggs.

It was proved that the consumption of the eggs of each of the two targeted insects by the individuals of the three mite species increased as the time of exposure increased.

## 2. Comparison between the efficiency of three mite species for controlling the eggs of the two insect species

The inspection showed that all the three evaluated mite species consumed a lot of eggs, especially after the 72-hrs interval. The average percentages of *S. cerealella* eggs consumed by either mite species were always higher than the average percentages of *C. chinensis* eggs consumed by the same mite species. *Macrocheles robustulus* was found to be the most effective predator against both insect's eggs where the average percentages of *S. cerealella* and *C. chinensis* eggs consumed after 72 hrs were 91.67 and 53.33 %, respectively. On the other hand, *Gamasholaspis variabilis* was found to be the least effective predator against the eggs of both tested insects where the average percentages of *S. cerealella* and *C. chinensis* eggs consumed after 72 hrs were 86.67 and 38.33%, respectively. It is worth stating that cannibalism between the individuals of each mite wasn't observed while performing the experiment.

## DISCUSSION

In the present study, insect adults that are able to lay eggs were not used. It's worth stating that Petri dishes didn't include these adult insects. The adult insects weren't involved for two reasons. First, if the adult laying eggs were present, they would lay more eggs frequently, and predation would be difficult to be assessed and measured. Second, the studied insects aren't known to protect their eggs against predators. **Saitoh et al. (2021)** reported that adult female predatory mites *Gynaeseius liturivorus* Ehara (Acari: Phytoseiidae) reduce intraguild predation on their eggs by remaining at oviposition sites, thus deterring the egg predators.

Because the thermal limits of insects and mites typically fall between 0.0 and 50° C, and temperatures within these limits determine the rates of population growth, the mites and insect

eggs were kept in a dark place at a temperature of 28± 5° C. More extreme temperatures have an acute influence. At their upper limit, the high temperatures destabilize phospholipid membranes and affect intracellular proteins adversely (**Bligh et al., 1976**). Petri dishes were kept in a dark place to be similar to the normal circumstances of storing grains. Moreover, some species of predatory mites are known to prefer predation at night. **Abd El-Tawab et al. (1982)** found that predation by *A. swirskii* was greater under complete darkness or a short photophase (8 hrs).

It was noticed that a few of the initial inspections revealed no significant differences between the consumed eggs and the control's lifeless eggs during the first 24 or 48 hrs post exposing the eggs to mites; this could be attributed to the fact that the mites extracted from the manure were presented to the eggs immediately without starving them for a short period of time. Thereupon, some mites are expected to not be hungry.

The two targeted insects belong to two different orders, *i.e.*, Coleoptera and Lepidoptera. Thereupon, the eggs of the two insects have different morphological and physical properties. Egg consumption may refer to variations in egg properties; that is why the average percentages of *S. cerealella* eggs consumed by either mite species were always higher than the average percentages of *C. chinensis* eggs consumed by the same mite species.

On the other hand, *M. robustulus* was proven to be the most effective predator against the eggs of both insects; that may refer to the mite's predatory efficacy. Predatory mites of the family macrochelidae are often associated with the manure of domestic animals and other sites where there are accumulations of dung and organic matter (**Cicolani, 1992**). Previous studies proved that Mesostigmata or Gamasida can live in a wide range of habitats. **Koehler (1997)** stated that most Gamasid mites are common predators in soil and litter, on the soil surface, or on plants. Some of them disperse quickly due to phoresy. They feed on different small invertebrates, such as collembola and nematodes. **Coja and Bruchner (2003)** found that the soil microhabitat diversity of a temperate Norway spruce (*Picea abies*) forest does not influence the community composition of gamasid mites (Gamasida, Acari). Hence, gamasid mites are expected to survive in grain stores. *Cheyletus malaccensis* represented the second rank among the three evaluated mites. It is a beneficial predator of stored product pests (**Wu et al., 2016**).

Cannibalism between mite individuals wasn't observed while performing the experiment, which is considered to prove that the mites were not hungry as long as eggs are available. Cannibalism is usual for *C. malaccensis* (Pulpan and Verner, 1959) but because the abundant presence of the prey (e.g., insect eggs), *C. malaccensis* may not be engaged in cannibalism.

## CONCLUSION

Different predatory mites can be obtained easily in large numbers from manure. The above-mentioned three mite species could be utilized for controlling the eggs of *Callosobruchus chinensis* and *Sitotroga cerealella* in grain stores. The store and the laboratory have similar conditions of light, temperature, and humidity; thereupon, the three mites are expected to be successful biological control agents for stored-product insects.

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## الملخص العربي

الكفاءة الإفتراضية لثلاثة أنواع من الأكاروسات المتواجدة بالروث ضد بيض كل من خنفساء اللوبيا و فراشة الحبوب

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أجريت هذه الدراسة بمعمل قسم وقاية النبات - كلية الزراعة - (سابا باشا) - جامعة الإسكندرية لدراسة الكفاءة الافتراضية لثلاثة أنواع من الأكاروسات المفترسة المتواجدة بالسماد البلدي الطبيعي ضد بيض نوعين من آفات الحبوب المخزونة الحشرية. كانت الأكاروسات المختبرة هي: *Cheyletus malaccensis*, *Macrocheles robustulus*, *Gamasholaspis variabilis* حيث تم دراسة كفاءة افتراس كل منها لبيض كل من خنفساء اللوبيا وفراشة الحبوب.

تم وضع عشرون بيضة من بيض الحشرة في طبق بتري وتعريضها لخمسة أفراد من الأكاروس المفترس وسجلت النتائج بعد 24 ، 48 و 72 ساعة. وقد أظهرت النتائج أن الثلاثة أنواع الأكاروسية لها كفاءة افتراضية عالية لبيض الحشرتين المختبرتين خاصة بعد 72 ساعة من المعاملة. كانت النسب المئوية لافتراس بيض فراشة الحبوب أعلى من النسب المئوية لإفتراس بيض خنفساء اللوبيا وذلك عند التعرض لأفراد أى نوع مفترس من أنواع الأكاروسات الثلاثة المختبرة. وكان المفترس *Macrocheles robustulus* الأعلى في كفاءته الإفتراضية حيث كانت نسب افتراسه لبيض كل من فراشة الحبوب وخنفساء اللوبيا عند الفحص بعد 72 ساعة هي 91.67 و 53.33 % على الترتيب. وعلي الجانب الآخر كان المفترس *Gamasholaspis variabilis* الأقل في كفاءته الإفتراضية حيث كانت نسب افتراسه لبيض فراشة الحبوب وخنفساء اللوبيا عند الفحص بعد 72 ساعة 86.67 و 38.33 % على الترتيب ولم تظهر ظاهرة الإفتراس بين أفراد الأكاروسات المختبرة.