POTENTIAL BIOCONTROL OF THE GREATER WAX MOTH, *GALLERIA MELLONELLA* L. (LEPIDOPTERA: PYRALIDAE) AND MASS REARING OF THE PARASITIC MITE, *PYEMOTES SCOLYTI* OUD. (ACARI: PYEMOTIDAE)

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ABSTRACT

The present work was carried out to test the possible biocontrol of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) by the parasitic mite, *Pyemotes scolyti* Oud. (Acari: Pyemotidae) and mass rearing of the mite on this pest. Experiments were carried out at the Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt, during 2018-2019 years. Rearing conditions, *i.e.* 25 ± 1°C and 70 ± 5 % RH were applied. The obtained results showed that duration of immature stages of *P. scolyti* ranged between 6-9 days. Mean count of emerged offspring was 198.22 individuals/ gravid female mite. The male mite lived for 1.20 day, while the female lived for 8.90 days. During bioassay test of different stages of the moth, larvae seemed to be a preferable host for the tested mite compared with pupae or adults. Further investigations should be carried out to clarify the mite-wax moth-honey bee-interactions.

Keywords: Biocontrol, Mass rearing, *Pyemotes scolyti*, *Galleria mellonella*.

1. INTRODUCTION

The greater wax moth, *Galleria mellonella* L. (GWM) is well known harmful pest to deposited and stored honey bees wax. Infection causes great damages in apiaries that lead to annual financial losses. Larval feeding *i.e*. consuming constructed wax cells impairs and spoils beeswax combs and frames or wooden parts of the hive are sometimes corrupted or destroyed during pupation of the larvae. Moreover, adult and larvae of wax moths can also carry pathogens of serious honey bee diseases (Charrière and Imdorf, 1997; Owayss and Abd-Elgayed, 2007). Different, *e.g*. biological, physical and chemical control means were applied to minimize the population of this pest worldwide (Zhu, *et al*., 2016; Michael, *et al*., 2017; Sohail, *et al*., 2017; Jorjao, *et al*., 2018).

The parasitic mites, *Pyemotes* spp. (Acari: Pyemotidae) have relevant characteristics as biocontrol agents, *e.g*. rapid population growth, short life cycle and a wide range of hosts (Tawfik *et al*., 1981; Abdel-Rahman *et al.*,1999; Broce *et al.*, 2006; Cunha *et al*., 2006; Aksit *et al.*,2007). Female mites can tightly attach their hosts and paralyze them by a venom injection. This venom injures humans and causes dermatitis also (Yeruham *et al*., 1997; Rosen *et al*., 2007). Many insect species among the orders; Lepidoptera, Coleoptera, Hymenoptera, Diptera, Homoptera, and Strepspitera have been described as hosts of *Pyemotes* spp. These mites were found to desecrate mass rearing in the laboratory cultures and can defect the populations of some insect pests, *e.g*. larvae of *Tuta absoluta* Meyrick (Cunha *et al*., 2006; De Oliveira *et al*., 2007); scale insects (Tena-Barreda and Garcia-Mari, 2006); stored products insect pests (Hoschele and Tanigoshi, 1993 ; De Oliveira *et al*., 2010);

aphids (Han, 2016; Litao *et al*., 2019); plant stem borers (Zhang *et al*., 2008; He *et al*., 2009) and bark beetles (Yu and Liang, 1996; Khaustov, 1998; Ostovan and Kamali, 2006).

Despite advantages, pyemotid mites can attack beneficial insects, *e.g.* natural enemies (Marei,1992; Mahunka and Mahunka-Papp, 1998; Levie, *et al*., 2005; Obano and Do Anlar, 2006); silkworm, *Bombyx mori* L. (Ikegami and Ebihara, 1980) and honey bee, *Apis mellifera* L. (Morse and Owogrodzki, 1990; Chmielewski, 1991; Eickwort, 1994; José *et al*., 2004; Macías and Otero-Colina, 2004; Menezes *et al*., 2009; Vickruck *et al*., 2010; Klimov *et al*., 2016; Prendergast and Yeates, 2018).

To figure out an impact of a pyemotid mite as biocontrol agent of GWM, the species, *P. scolyti*, was experimented. The present work was focused to test, in vitro, the following parameters: (1) potential biocontrol of GWM by the parasitic mite, *P. scolyti*, and (2) mass rearing of the tested mite on GWM as a probable host.

2. MATERIALS AND METHODS

2.1. Samples collection

The present experiment was conducted during April, 2018 till March, 2019. The parasitoid mite, *P. scolyti*, natrually recorded in samples relevant to the larvae of almond bark beetle, *Scolytus amygdali* Guerin-Meneville was taxonomically identified and kept. These samples were collected while studying population dynamics of apricot, *Prunus armeniaca* L., insect pests and their natural enemies in a field survey at Sanhour village (coordinates: N29°25'10.4592" E30°45'49.428"), Fayoum Governorate, Egypt. Then, specimens were carried to the Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt (coordinates: N29°19'16.4136" E30°50'18.8196") for further investigation. In the lab, movable mite individuals were observed to contaminate and destroy cultures of GWM around.

2.2. Stock cultures

2.2.1. Culturing of GWM

Naturally GWM-infested honey bee, *A. mellifera*, wax combs (ca. 2-years-age and honey-free) were obtained from the apiary of the Faculty of Agriculture, Fayoum University, and then were taken to a rearing chamber in the same Faculty. Infested beeswax combs were cut into strips and initially transferred to clean 10 kg-glass jars covered with muslin cloth. Jars were protected from ant attack by placing them in plastic dishes where suitable amount of tap water was added. To ascertain pure GWM culture, emerged female and male moths were carefully taken to new jars fortified with uninfected beeswax cubes (each of 5×5×2 cm) and then left to copulate and lay eggs. Emerged larvae were monitored to obtain the desired instars for the further assay. Rearing and biological aspects were conducted at room conditions (25±1^{o}C & 70±5% RH) following the procedure mentioned by Owayss and Abd-Elgayed, (2007).

2.2.2. Culturing of P. scolyti

To initiate mite stock culture, newly beetle-infested cutting branches of apricot trees were field-collected, placed in glass jars covered with muslin cloth, then kept under aforementioned rearing conditions. About one month later, these cutting branches were peeled to obtain the full-grown beetle larvae and their probable parasitic mites (Abd-El-Latif *et al*., 2009).

2.3. *Bioassay experiment*

With the aid of a magnification, one *P. scolyti* female was carefully transferred to a clean petri-dish (5-cm diameter) provided with an appropriate filter paper disc and supplied with one GWM larva. A set of such prepared dishes were used. These dishes were then kept at aforementioned temperature and humidity degrees. The dishes were periodically inspected and newly mite nymphs were transferred individually to new petri-dishes prepared in the same way. Every nymph was provided with one GWM larva of the 6^{th} instar (10 replicates were used). Mortality rates of the mite nymphs were recorded until the emergence of adults that were sexed later on. (Tawfik *et al*., 1981; Abdel-Rahman *et al*., 1999)

2.4. *Mass rearing of P. scolyti on larvae of GWM*

In this test, glass jars (2kg capacity) were used, clean honey beeswax combs were cut into small cubes (each of 5×5×2 cm) where five cubes were placed in each jar, holes were covered with musilin colth and tightened by rubber bands. Five pairs of newly GWM moths were placed in each prepared jar and kept for 5 days. During egg hatching and newly-emerged larvae exist (about ten days later) gravid female mites were placed into jars at a rate of 5, 10 and 20 females/ jar. Control jars without mites infection and all the four treatments were replicated ten times. During next 10 days of infection, survived GWM larvae, mite offspring, and gravid female mites were recorded.

2.5. *Evaluating of P. scolyti in control of GWM immature stage*

Under the said laboratory conditions, ten petri-dishes (10-cm diameter) were used, every dish was provided with small beeswax cube (2×2×2 cm). One GWM larvae of the 6^{th} instar and one gravid female of *P. scolyti* mite were transferred to each dish. After ten days, mite offspring individuals associatted with infested GWM larvae were counted. Treatment was replicated for ten times. The aforementioned technique was followed to test either recent pupae or newly-emerged moths.

2.6. Statistical analysis

The obtained data in all experments were statistically analyzed by New LSD test at 5% significance (Senedecor and Cochran, 1980).

3. RESULTS

3.1. Duration of immature stages of P. scolyti reared on GWM larvae

Duration of immature stages (offspring) of the parasitic mite, *P. scolyti, which* reared at (25±1^{o}C and 70±5% RH) on GWM larvae was shown in (table 1). All mite individuals reached the adult stage (0% mortality). Except two emerged males the rest individuals were females (sex ratio was 3:1). The lowest duration period was six days; while the longest one was nine days (mean total duration was 7.80 days).

Table 1 Duration (day) of immature stages of *Pyemotes scolyti* reared on greater wax moth, *Galleria mellonella,* larvae under lab conditions (25±1°C & 70±5% RH).

Parameters	Total duration	Sex ratio (♀: ♂)
Range	(6-9)	(3:1)
Mean± SE	7.80±0.13	

The obtained values are in general accordance with those given in certain previous studies, *e.g.* (Ostovan and Kamali, 2006) mentioned numerous advantages of *P. scolyti* as a biocontrol agent of *Scolytus multistriatus*, *i.e.* this mite is oligophagous, has a short life cycle (8-9 days), and a high reproductive potential with high parasitic rate (63-78%) on larvae and pupae of *S. multistriatus* under natural condition.

Another pymotid species, *P. zhonghuajia*, exhibited high reproductive potential (ca.100 offspring/ female) with an extremely female-biased sex ratio (98%) and a very comparable short life cycle (≈ 8 days at 25 ºC) (Litao *et al*., 2019).

3.2. Duration of adult stages of P. scolyti reared on GWM larvae

Data in (Table 2) explain the duration of adult stages of the tested mite reared on the 6th larval instar of GWM under the said conditions. The males emerged early than females and lived between one to two days, while females lived between seven to ten days (mean 8.90 days). Noticeably, the female body was swollen after coupling. The 1st emerged individual was always a male. The mean number of offspring that emerged from the gravid female ranged between 170 – 275 individuals (mean 198.22 individuals/ female).

Table 2 Duration (day) of adult stages of *P. scolyti* reared on greater wax moth, *Galleria mellonella,* larvae under lab conditions (25±1°C & 70±5% RH).

Parameters	Adult longevity		Count of offspring emerged from one female mite
	Male	Female	
Range	(1-2)	(7-10)	170-275
Mean ±SE	1.20±0.01	8.90±0.22	198.22±6.13

The present values are in general agreement with those of Ostovan and Kamali, (2006) who found that physogastric females are viviparous and births begin eight to ten days after attachment to the host. At 75% RH mean count of offspring per gravid female was 158 (range 135-185) and average sex ratio of male to female was 0.1 (range 0.03-0.26). Births continued for six to seven days with a maximum daily count of emerging mites of 69 individuals.

3.3. Mass rearing of P. scolyti on GWM larvae

Data of mass rearing of *P. scolyti*, intended by feeding this mite at different densities, on GWM larvae are summarized (table 3). In 5-gravid female mites/ jar treatment, the count of GWM larvae decreased to 18.11 larvae after 10 days in relation to 780.10 female mites existed. Meanwhile, the populations of gravid females increased from 500 to 870 females/ jar.

In10-gravid female mites/ jar treatment, all GWM larvae died, however the count of gravid female mites increased to 1390.33 individuals (range 1150-1680 individuals). On the other hand, in 20 gravid female mites/ jar treatment, all GWM larvae died also, regarding low count of mites (179.22 individuals). In zero-gravid female mites/ jar treatment (control), count of GMW larvae reached 86.22 larvae (range 85-96 larvae) with zero mite individuals. Noticeably, 5-gravid female mites/ jar treatment was shown to be insufficient to overcome the population of GWM larvae, *i.e*. population of the existed mites was low coinciding with escaping of certain larvae to be parasitized. While, in 20 gravid female mites/jar treatment super-parasitism was occurred (table 3). Consequently, the 10-gravid female mites/ jar seemed to be the suitable tested treatment, as the lowest host population (0.0 individuals) together with the highest mite population (range 1150-1680 individuals).

Table 3 Impact of treatments of greater wax moth, *Galleria mellonella,* with different densities of the parasitic mite *P. scolyti,* under lab conditions (25±1°C & 70±5% RH)*.

Densities of gravid female mites	Count of moth larvae	Count of mite females
5-individuals	18.11±0.12b (15-21)	780.10±3.13b (500-870)
10-individuals	0.0 c	1390.33±2.17a (1150-1680)
20-individuals	0.0 c	179.22±0.13c (160-210)
Zero-individuals (control)	86.22±0.32a (85-96)	0.00 d

* Mean values ± SE recorded after 10 days of treatment. Varied letters in the same column are significantly (5%) different.

3.4. Biocontrol of different stages of GWM by P. scolyti

Data of parasitized larvae, pupae and adults (moths) of GWM by the mite *P. scolyti* under lab conditions (25±1ºC & 70% RH) were compared (table 4). Larvae, especially of the 6th instar, was relevant to the highest mite count (mean 192.11 individuals/ larva) with a range of 180-263 individuals/ larva, but in newly-formed pupae, less count of mites was recorded being 96.21 mites/ pupa (range 95-110 individuals/ pupa). However, the least mites count (85.86 mites/ moth) was recorded in newly-emerged moths (range 75-96 individuals/ moth).

Table 4 Effect of stage on parasitizing level of greater wax moth, *Galleria mellonella,* with *Pyemotes scolyti* under lab conditions (25±1°C & 70±5% RH)*.

Stages of moth	Counts of mites emerged from one female
Larva	192.11±5.33a (180-263)
Pupa	96.21±0.53b (95-110)
Newly-emerged adult	85.86±0.23b (75-96)

* Mean values ± SE recorded after 10 days of treatment.

Statistically, differences between values of treated pupal and adult stages were insignificant, while were significant between larval and other stages. Accordingly, larva of GWM seemed to be a preferred host to the tested mite compared to pupa or adult. This may be due to escaping of moths by flying away and also to the solidity of pupal puparium.

4. Conclusion and recommendation

According to the obtained results, the biocontrol of *G. mellonella* with the parasitic mite, *P. scolyti* is worthnoting, as the least parasite population controlled the highest pest population treated. In a potential biocontrol of wax moth with *P. scolyti* in stored beeswax combs, any expected mite escaping to honey bee colonies should be avoided. To prevent propable attack of mite stages to adult bees or broods, short chemical treatment, *e.g*. evaporating of stored stock combs with 85% formic acid at a rate of 10 ml/m^3 for at least 15 min should be applied. Aeration of such treated combs in oben air at day light for 6 hours is necessary before adding them to live honey bee colonies. Further work must be carried out to clarify the mite-wax moth-honey bee-interactions.

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المكافحة الإحيائية لدودة الشمع الكبيرة (جاليريا ميلونيلا) والتربية الكمية للمتطفل الأكاروسي (بيموتاس سكوليتاي)

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أُجريت هذه الدراسة حديثا لاختبار المكافحة الإحيائية لدودة الشمع الكبيرة (جاليريا ميلونيلا) بإستخدام المتطفل الأكاروسي (بيموتاس سكوليتاي) مع تجربة التربية الكمية لهذا الأكاروس علي هذه الآفة. أجريت هذه الدراسة في معمل بحوث قسم وقاية النبات بكلية الزراعة، جامعة الفيوم خلال عامي ٢٠١٨/٢٠١٩م في درجات الحرارة ٢٥±١درجة مئوية ورطوبة نسبية ٧٠±٥%. وكان من أهم النتائج التي سجلت أن فترة نمو الاطوار غير الكاملة للمتطفل الأكاروسي المختبر راوحت بين ٦-٩ أيام، وأن الأنثي الواحدة الحاملة للأفراد أنتجت ١٩٨,٢٢ فرداً، كما عاشت الذكور فترة ١,٢ يوماً بينما إمتدت فترة حياة الإناث إلي ٨,٩ يوماً، وبدراسة التقييم الحيوي لهذا لمتطفل الأكاروسي علي جميع أطوار دودة الشمع الكبيرة تبيَّن أن اليرقات هي العائل المفضل لهذا الطفيل مقارنة بالأطوار الأخري، ولابد من إجراء بعض الدراسات التالية علي هذا الطفيل لمعرفة التداخل بين إصابته لديدان الشمع وتأثيره علي نحل العسل.

الكلمات الدالة:- المكافحة الإحيائية- التربية الكمية- بيموتاس سكوليتاي- جاليريا ميلونيلا