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Some Biological and Morphometric Aspects of *Xylocoris galactinus* (Fieber) (Hemiptera: Anthocoridae) Reared on the Mite, *Tyrophagus putriscentiae* Schr. as an Alternative Prey

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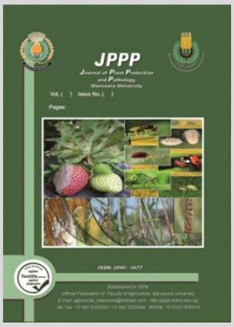

ABSTRACT

Successful rearing of alternative preys for the predatory bugs on synthetic diets facilitate the mass production of such predators. In this study, the saprophytic mite, *Tyrophagus putriscentiae* was reared on a simple semi-synthetic diet, as prey, that enabled rearing of the predatory anthocorid bug, *Xylocoris galactinus* under laboratory conditions of 25.5 ^{0}C and 65% R.H. Egg incubation period, nymphal durations of both predator male and female, and preadult survival rates were estimated as well as dimensions of the five nymphal instars were measured. Further, measurements and longevity of both adult male and female were determined. Female fecundity and daily female reproductive were estimated. Survival rate of the nymphal stage of predatory bug was 90.24% with the lowest rate (74.8%) was recorded for first instar. The nymphal stage duration took 16±1.76 days for males and 21±1.05 for females. The female fecundity was 46.74±7.43 eggs with longevity averaged 25.91±4.79 days. Male longevity averaged 25.91±2.11 days. As each nymphal instar moulted, its dimensions increased. Males were longer (2.907 mm), however it were slimmer (0.727mm) than females (2.538mm). Successful development of *X. galactinus* on *T. putriscentiae* with high nymphal survival rates is acceptable. Such easy rearing of *T. putriscentiae* on the semi-synthetic diet as a new alternative prey is in favor of the mass rearing not only of *X. galactinus*, but also for other anthocorid predators that used for biocontrol of phytophagous pests in greenhouses.

Keywords: Anthocoridae, Biocontrol, reproduction, oviposition, semi-synthetic diet

INTRODUCTION

The minute pirate bug, *Xylocoris galactinus* (Fieber) (Hemiptera: Anthocoridae) is first recorded, as a predatory species, in Egypt by Priesner and Alfieri (1953) followed by Attia and Kamel (1965) and Tawfik and El Husseini (1971). While, it was recorded for the first time in Thailand (Yamada *et al.* 2013), Slovenia (Gogala *et al.* 2014), and Romania (Virteiu *et al.*2014). It is a cosmopolitan generalist species that widely distributed in Europe, Northern Asia, North America, South West Pacific and Africa (Lattin 2005 and 2007, Henry and Froeschner 1988, Swanson 2016 and Moulet 2017). It inhabits warm habitats, *e.g.*, manure heaps, hot-beds, stable straw and grain stores (Attia and Kamal 1965, Yamada *et al.* 2013, Moulet 2017, Henry and Froeschner 2019), where it feeds on mites and small insect larvae. Chu (1969) studied the biology of *X. galactinus* when fed on 43 insect species of 22 families scattered in nine orders and gave a trial for rearing the predator on an artificial diet. Tawfik and El Husseini (1971) studied some biological aspects of *X. galactinus* feeding on mixed preys of the mite *Tyrophagus* sp., eggs and young larvae of the housefly *Musca domestica* L. Schöller *et al.* (2006) suggested *X. galactinus* to be used for the biocontrol of stored product pests. Further, in 2013, the European Plant Protection Organization (EPPO) also recommended the use of *X. galactinus* for augmentative biological control of stored product pests in organic agriculture and coded this predator as *XYOCGA* in its Global Database of 2013. It is known that most of the anthocorid predators are highly polyphagous and many species were mass reared on factitious (i.e. alternative) preys, mostly eggs of many lepidopteran pests *e.g.*, *Sitotroga cerealella* (Olivier), *Corcyra cephalonica* Stainton and *Plodia interpunctella* (Hubner). These eggs are mostly used as frozen, irradiated or lyophilized eggs to improve their storage ability (Riddick 2009). The EPPO's decision (2013) encouraged the survey of insect fauna searching for this beneficial predator. Thus, mass rearing of this predator becomes of great importance for the biological control companies. The present study is an attempt to rear the predator *X. galactinus* on the mite prey *Tyrophagus putriscentiae* Schr. using a simple artificial diet for facilitating rearing of the prey under laboratory conditions. Under this system, some biological and morphometric aspects of *X. galactinus* were estimated.

MATERIAL AND METHODS

Prey culture

The mite *T. putriscentiae* was reared on a semi-synthetic diet consisted of wheat bran as described by El Husseini and Sermann (1992) and El Husseini *et al.* (1993) to be used further, as an alternative prey, for rearing the anthocorid predator, *X. galactinus* under laboratory conditions of 25.5 °C and 65% R.H. The mite colony was initiated on fermented wheat bran left one week on a manure heap at the Agricultural Experiment Station,

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Faculty of Agriculture, Cairo University in Giza. The field collected mite was transferred to the laboratory and placed on the semi-synthetic diet in 1 x 3-inch glass tubes plugged with cotton wool. After four weeks, the mite rearing tubes became filled by masses of the mite in different stages (eggs, immature and mature stages).

Predator culture

Mature and immature feeding stages of *X. galactinus* were collected by a rubber bladder aspirator from manure heaps located beside the Animal Production Unit at the Agricultural Experiment Station. The collected materials were placed in 5.5 cm glass Petri-dishes furnished with a moistened disc of filter paper, a corrugated piece of paper served as shelter and then the dishes were provided with surplus of the mite in different stages. Newly emerged adults were sexed and each pair (n = 20) was confined in a similar Petri-dish that provisioned daily with sufficient numbers of the mite prey. The adult females insert their eggs in the filter paper disc which facilitated the inspection and counting the eggs laid. Discs with inserted eggs were removed daily, kept in Petri-dishes and lightly moistened with water to avoid dryness which affect the hatching. Once eggs showing signs of hatching that recognized by the two red spots under operculum representing compound eyes of the first nymphal instar inside the egg, the Petri-dished were provided with eggs and newly hatched individuals of the mite as a food for newly hatched bug nymphs; an accordion-folded piece of paper was placed in the dishes as a shelter to facilitate distribution of predators and the preys, as a way to avoid cannibalism. The newly hatched nymphs were transferred individually in similar Petri-dishes using fine moistened camel hair brush and supplied daily with the mite prey. The filter paper enables to identify the nymphal instars through localizing the nymphs' exuvia . The dishes were inspected daily for recoding the molted individuals of the next stadium. Durations and survival rates of the different immature stages of the predator were recorded. Female fecundity and adult longevity were also estimated. Morphometric study was also measured by killing the either the newly molted nymphal instars or emerged adults in cyanide glass container. These morphometric measurements included head width, rostrum length, antennal length, pronotum width and length, abdomen width and body length of the five nymphal instars and male and female adults.

RESULTS AND DISCUSSION

Immature development and survival of *X. galactinus*

As presented in Table (1), incubation period lasted between 3 and 4 days with an average of 3.6 ± 0.48 days under laboratory conditions of 25.5 C and 65% R.H. The first and second nymphal instars ranged between 2 and 3 days with an average of 2.4 ± 0.49 and 2.7 ± 0.40 days, respectively. Meanwhile, the third and fourth nymphal instars ranged between 3 and 4 days and between 3 and 5 days with an average of 3.7 ± 0.46 and 4.3 ± 0.77 days, respectively. The fifth nymphal instar was the longest in duration and its duration ranged from 4 and 8 days with an average of 6.2 ±1.43 days. The nymphal stage duration ranged between 14 and 19 days for males and between 20 and 23 days for females with an average of 16 ± 1.76 and 21 ± 1.05 days, respectively. The egg-adult duration lasted 19.6 ± 2.24 and 24.6 ± 1.53 days for predator male and female, respectively. It was obviously that predator male had shorter development than female.

Table 1. Preadult durations of *X. galactinus* reared on the mite *T. putriscentiae* using semi-synthetic diet under laboratory conditions of 25.5 ˚C and 65% R.H

Values	Egg incubation (day)	Nymphal instars (day)					Total nymphal stage (day)		Egg-adult period	
		1st	2nd	3rd	4th	5th	Male	Female	Male	Female
Minimum	3	2	2	3	3	4	14	20	17	23
Maximum	4	3	3	4	5	8	19	23	23	27
Mean	3.6	2.4	2.7	3.7	4.3	6.2	16	21	19.6	24.6
S.D.	0.48	0.49	0.40	0.46	0.77	1.43	1.76	1.05	2.24	1.53

As presented in Table (1), incubation period lasted between 3 and 4 days with an average of 3.6 ± 0.48 days under laboratory conditions of 25.5 C and 65% R.H. The first and second nymphal instars ranged between 2 and 3 days with an average of 2.4 ± 0.49 and 2.7 ± 0.40 days, respectively. Meanwhile, the third and fourth nymphal instars ranged between 3 and 4 days and between 3 and 5 days with an average of 3.7 ± 0.46 and 4.3 ± 0.77 days, respectively. The fifth nymphal instar was the longest in duration and its duration ranged from 4 and 8 days with an average of 6.2 ±1.43 days. The nymphal stage duration ranged between 14 and 19 days for males and between 20 and 23 days for females with an average of 16 ± 1.76 and 21 ± 1.05 days, respectively. The egg-adult duration lasted 19.6 ± 2.24 and 24.6 ± 1.53 days for predator male and female, respectively. It was obviously that predator male had shorter development than female.

Nymphal mortality of the predator *X. galactinus* decreased as each nymphal instar molted to next instar with the highest nymphal survival rate was for the fifth nymphal instar of the predator (Table 2).

Table 2. Survival rates (%) of *X. galactinus* reared on the mite *T. putriscentiae* using semi-synthetic diet under laboratory conditions of 25.5 ˚C and 65% R.H

Values	Nymphal instars					Total
	1st	2nd	3rd	4th	5th	
Min.	70	82	95	96	96	87.8
Max.	80	91	97	98	98	92.8
Mean	74.8	88.2	95.6	97.3	97.1	90.24
S.D.	6.06	5.09	0.84	0.82	0.87	9.45

Morphometric measurements of *X. galactinus* nymphal instars

The newly hatched first nymphal instar showed a head width of 0.213 ± 0.008 mm, the rostrum of 0.394 ± 0.009 mm long, antennae length of 0.385 ± 0.007 mm, pronota width and length of 0.385 ± 0.009 and 0.202 ± 0.009 mm, respectively, abdomen width of 0.371 ± 0.008 mm, body length of 1.098 ± 0.037 mm (Table 3). By moulting to the next instars, these measurements increased gradually as shown in Table (3). The present measurements are somewhat slightly shorter than those measured by Tawfik and El Husseini (1971), probably due to the mixed diet (mites, fly eggs and young larvae) they offered to this predator in contrast to the present study that used only the mite *T. putriscentiae*. Moreover, their morphometrics were measured on specimens mounted in Canada Balsam on glass slides, whereas in the present study the specimens were measured directly after killing in cyanide glass container.

Table 3. Morphometric measurements (mm) of the fifth nymphal instars of *X. galactinus* reared on the mite *T. putriscentiae* using semi-synthetic diet under laboratory conditions of 25.5 °C and 65% R.H

Instars	Head width	Rostrum length	Antennal length	Pronotum width	Pronotum length	Abdomen width	Body length
				First nymphal instar			
Min.	0.20	0.38	0.38	0.26	0.19	0.36	1.05
Max.	0.22	0.40	0.40	0.28	0.21	0.38	1.20
Mean	0.213	0.394	0.385	0.272	0.202	0.371	1.098
S.D.	0.008	0.009	0.007	0.009	0.009	0.008	0.037
				Second nymphal instar			
Min.	0.25	0.46	0.55	0.34	0.25	0.45	1.40
Max.	0.29	0.50	0.60	0.38	0.30	0.50	1.50
Mean	0.271	0.479	0.581	0.363	0.276	0.482	1.438
S.D.	0.017	0.020	0.20	0.017	0.018	0.020	0.026
				Third nymphal instar			
Min.	0.30	0.72	0.65	0.44	0.27	0.55	1.78
Max.	0.36	0.78	0.73	0.46	0.34	0.60	1.89
Mean	0.331	0.752	0.691	0.439	0.297	0.578	1.826
S.D.	0.026	0.021	0.027	0.016	0.023	0.042	0.049
				Fourth nymphal instar			
Min.	0.33	0.78	0.87	0.51	0.30	0.66	1.88
Max.	0.37	0.84	0.90	0.58	0.37	0.69	2.00
Mean	0.314	0.811	0.879	0.547	0.337	0.678	1.971
S.D.	0.016	0.20	0.020	0.027	0.030	0.009	0.039
				Fifth nymphal instar			
Min.	0.39	0.88	1.05	0.75	0.41	0.99	2.91
Max.	0.44	0.96	1.10	0.80	0.49	1.09	3.11
Mean	0.409	0.917	1.082	0.785	0.453	1.030	2.986
S.D.	0.021	0.030	0.017	0.025	0.037	0.042	0.073

Morphometric measurements of *X. galactinus* adults

Head width in *X. galactinus* adult male was wider (0.411 ± 0.008 mm) than that of the female (0.429 ± 0.005 mm) as presented in Table (4). The length of male rostrum was shorter (0.850 ± 0.008 mm) than that of the female (0.949 ± 0.007 mm). Length of the antenna in both sexes ranged between 1.10 – 1.12 mm with an average was slightly longer in female. Pronotum width averaged 0.919 ± 0.005 and 0.925 ± 0.008 mm in male and female, respectively. However, pronotum length was shorter (0.446 ± 0.007 mm) in males than females (0.479 ± 0.010 mm). Abdomen width was larger in females (1.089 ± 0.007 mm) than in males (0.727 ± 0.008 mm); apparently due to eggs filling female abdomen. But the opposite was measured for body length where it was longer in males (2.907 ± 0.004 mm) than in females (2.538 ± 0.006 mm); apparently to facilitate twisting male abdomen during copulation to enable its genitalia reaching the Ribaga organ located underneath the females' wings between the fourth and fifth abdominal tergum sclerites to insert its penis inside for hemocoelic insemination (Chapman 1998; Tawfik and El Husseini 1971). The general description and morphometric measurements of *X galactinus* adults are in agreement with those of Moulet (2017) and Henry and Froeschner (2019).

Table 4. Morphometric measurements (mm) of female and male adults of *X. galactinus* reared on the mite *T. putriscentiae* using semi-synthetic diet under laboratory conditions of 25.5 °C and 65% R.H

Sex	Head width	Rostrum length	Antennal length	Pronotum width	Pronotum length	Abdomen width	Body length
				Male			
Min.	0.40	0.84	1.10	0.92	0.46	0.72	2.90
Max.	0.42	0.86	1.12	0.93	0.48	0.74	2.92
Mean	0.411	0.850	1.109	0.919	0.446	0.727	2.907
S.D.	0.008	0.008	0.007	0.005	0.007	0.008	0.004
				Female			
Min.	0.42	0.94	1.10	0.91	0.47	1.08	2.53
Max.	0.44	0.96	1.12	0.93	0.49	1.10	2.55
Mean	0.429	0.949	1.118	0.925	0.479	1.089	2.538
S.D.	0.005	0.007	0.007	0.008	0.010	0.007	0.006

Fecundity and adult longevity of *X. galactinus*

Females of *X. galactinus* showed a pre-oviposition period averaged 5.75 ± 1.97 days (Table5). The female oviposition and post-oviposition periods averaged 21.80 ± 3.75 days and 2.33 ± 1.10 days , respectively. Female fecundity averaged 46.74 ±7.43 eggs with a daily reproductive rate averaged 13.5 ± 1.95 eggs. The female longevity averaged 25.91± 4.79 days while that of male averaged 12.14 ± 2.11 days (Table5). The present results are closed to those documented by Tawfik and El Husseini (1971) and Afifi and Ibrahim (1991). Due to the warm climate of Egypt, *X. galactinus* is present active in manure heaps and grain stores through the entire year (Afifi and Ibrahim 1991). Meanwhile, it enters a winter diapause in other cold regions as in Russia (Saulich and Musolin 2009). The latter authors pointed the importance of biological studies of Anthocoridae on alternative preys to optimize mass rearing of these predators and their application in IPM programs.

Finally, the mite *T. putriscentiae* was successfully used as an alternative prey for rearing the predatory bug *Xylocoris galactinus* depending on a semi-synthetic diet for mite rearing. Such easy rearing of this mite on semi-synthetic diet could serve the biological control companies and biological labs for mass production not only of *X. galactinus,* but also for other anthocorid predators that widely used for controlling spider mite, aphid, thrip and whitefly pests especially in greenhouses.

Table 5. Fecundity and adult longevity *X. galactinus* reared on the mite *T. putriscentiae* using semi-synthetic diet under laboratory conditions of 25.5 °C and 65% R.H

Values	Ovipositional periods (/days)			Fecundity/female		Longevity (/days)	
	Pre-oviposition	Oviposition	Post-oviposition	Total	day	Female	Male
Min.	4	15	0	42	11	19	9
Max.	7	27	4	64	16	33	15
Mean	5.75	21.80	2.33	46.74	13.5	25.91	12.41
S.D.	1.97	3.75	1.10	7.43	1.95	4.79	2.11

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بعض النواحي البيولوجية والقياسات المورفوموترية للبق المفترس *Xylocoris galactinus* عند تربيتة علي الحلم *Tyrophagus putriscentiae* كإحدى الفرائس البديلة

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التربية الناجحة للفرائس البديلة للبق المفترس علي البيئات نصف الصناعية يسهل الانتاج الكمي لهذه المفترسات. في الدراسة الحالية تم تغذية الحلم المترمم *Tyrophagus putriscentiae* علي بيئة غذائية شبه طبيعية، ثم تربية المفترس *Xylocoris galactinus* علي الحلم في المعمل علي درجة حرارة ٢٥,٥° , ٦٥ % رطوبة نسبية. تم تسجيل فترة حضانة بيض المفترس, وكذا فترة نمو الحوريات الذكور والإناث ومعدل البقاء, علاوة علي بعض القياسات المورفوموترية لخمسة أعمار للحوريات. كما تم تسجيل طول مدة بقاء الأكاروس الكامل لكل من الذكور والإناث. هذا بالإضافة إلي حساب عمر الاناث وخصوبتها وكذلك معدل وضع البيض لكل أنثى. كان معدل البقاء للبق المفترس في طور الحورية بصفة عامة % 24 .90 وكان أقل معدل بقاء هو في طور الحورية الأول (% 80 .74) واكتمل طور الحوريات الإناث في ١٦± ١,٠٥ يوما. بلغ متوسط عدد البيض ٤٦,٤٧ ± ٧,٧٣ بيضة للانثي الواحدة، و ترواحت مدة بقاء الطور الكامل للمفترس بين ١٤, ١٩ يوما (بمتوسط ٢٥,٩١ ± ٤,٧٩ يوما). عندما عاشت الإناث لفترة ١٩ ± ٣٣ يوما أمكنها وضع أكبر عدد من البيض (٦٤ بيضة). وكان متوسط العمر للطور الكامل من الذكور ٢٥,٩١ ± ٢,١١ يوما. أظهرت القياسيات الموفوموترية أن الذكور كانت أطول (٢,٩٠٧مم) و أدق في العرض (0.٧٢٧ مم) من الإناث (٢,٥٣٨ , ١,٠٨٩ مم علي التوالي). كان معدل البقاء للبق المفترس في طور الحورية بصفة عامة % 24 .90 وكان أقل معدل بقاء هو في طور الحورية الأول (% 80 .74) واكتمل طور الحوريات الإناث في ١٦ ± ١,٠٥ يوما. بلغ متوسط عدد البيض ٤٦,٤٧ ± ٧,٧٣ بيضة للانثي الواحدة, و ترواحت مدة بقاء الطور الكامل للمفترس بين ١٤, ١٩ يوما (بمتوسط ٢٥,٩١ ± ٤,٧٩ يوما) .عندما عاشت الإناث لفترة ١٩ - ٣٣ يوما أمكنها وضع أكبر عدد من البيض (٦٤ بيضة). وكان متوسط العمر للطور الكامل من الذكور ٢٥,٩١ ± ٢,١١ يوما. أظهرت القياسيات الموفوموترية أن الذكور كانت أطول (٢,٩٠٧مم) و أدق في العرض (0.٧٢٧ مم) من الإناث (٢,٥٣٨ , ١,٠٨٩ مم علي التوالي).