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DEVELOPMENT, LONGEVITY, FECUNDITY, AND LIFE TABLE PARAMETERS OF *Trissolcus basalis* (WOLLASTON), EGG PARASITOID OF THE STINK GREEN BUG, *Nezara viridula* (L.) IN RELATION TO TEMPERATURE

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ABSTRACT

The developmental time of immature stages, developmental rate, longevity, fecundity and life table parameters of *Trissolcus basalis* (Wollaston) were investigated at three temperatures (24, 28 and 31ºC). The data reveled that there was a significant variation in the developmental time of immature stages between the three tested temperatures. The higher developmental rate was recorded at 31ºC.

There were significant differences between the three temperatures in percentage of parasitized eggs and sex ratio, while there was no significant difference in percentage of successful parasitism. The female sex ratio was 4:1 (females: males) at 28 and 31ºC, while it was 3:1 at 24ºC.

There was a significant difference in longevity of females. It was 14.40±1.43, 13.40 ±0.46 and 9.00±2.08 at 24, 28 and 31ºC, while there was no statistical variation in the fecundity of *T. basalis* females reared at the three tested temperatures. The simple linear regression between temperatures and longevity of *T. basalis* females indicated that there was a highly relationship between temperatures and longevity. In addition, there was a positive relationship between the temperatures and fecundity. The calculated value of (T) and (DT) was higher at 24ºC than 28 and 31ºC. In contrast, GRR, R_o, r_m and λ values were higher at 31ºC than 24 and 28ºC. The survivorship (Lx) for female age was higher (0.82) at 28ºC than at 24 and 31ºC.

There was a significant difference between ages of the host egg masses in choice and non-choice tests. The first age (one day old) was preferred than the other ages. Moreover, the parasitoid was able to parasitize the host eggs in all developmental ages. The present investigation provided useful information of some biological aspects of *T. basalis.* In conclusion, *T. basalis* appears to be a promising biological control option for controlling the green stink bug, *N. viridula*.

Keywords: *Trissolcus basalis*, *Nezara viridula*, developmental time, longevity, fecundity, life table parameters.

INTRODUCTION

The green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), is a highly polyphagous insect that is widely distributed in many temperate and tropical regions of the world (Jones, 1988; Todd, 1989; Odermatt *et al*., 2000 and Panizzi *et al*., 2000). It causes important economic damage to various field crops, including soybean, beans, corn, cotton, tomato, sweet pepper, eggplant, cucurbits, sunflowers and grape (Todd, 1989; Jackal *et al*., 1990; Ehler, 2000; Odermatt *et al*., 2000 and Panizzi *et al*., 2000). Both nymphs and adults feed on developing and mature fruit, often resulting in

either direct loss or unmarketable produce, discoloration, stunting and shriveling of tissues (Clarke, 1990; Clarke and Walter, 1993; Ehler, 2000 and Odermatt *et al*., 2000).This pest is typically either bivoltine or multivoltine, and overwinters as a diapausing adult in above-ground habitats, such as beneath the bark of trees. Females deposit pale yellow eggs in large masses predominantly on the undersides of leaves. There are five nymphal instars (Todd, 1989; Ehler, 2000; Odermatt *et al*., 2000 and Panizzi *et al*., 2000).

Control of this pest is based on the intensive use of chemical pesticides, including carbamates, organophosphates and some pyrethroids. Massive use of insecticides not only increases production cost, it may also affect populations of beneficial insects and trigger pest resurgence problems (Panizzi *et al*., 2000 and Godoy *et al*., 2005).

Biological control, as a component within integrated pest management (IPM) programs, is a powerful pest control option in situations where chemical control is insufficient, impossible, or undesirable. Thus, techniques for mass production of some parasitoids may relatively help in solving the problem of *N. viridula* and reduce the hazard of pesticides to human and environment (Panizzi *et al*., 2000 and Godoy *et al*., 2005). Parasitoids are important biological control agents against *N. viridula* (Jones, 1988; Hoffmann *et al*., 1991 and Ehler, 2002). The egg parasitoid, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) is an important biological control agent of stinkbugs worldwide (Correa-Ferreira and Moscardi, 1995 and 1996; Ehler, 2002; Lenteren and Bueno, 2003 and Catalan and Verdu, 2005). It is well established and adapted in Egypt and is credited with the control of *N. viridula*. Releases of the egg parasitoid, *T. basalis* have successfully suppressed outbreaks of the green stinkbug (Correa-Ferreira and Moscardi, 1996).

The effects of temperature are often critical in determining the rate and success of the development of parasitoids. Typically, the development of parasitoids is prolonged in cooler environments and increased in warmer environments, up to a critical temperature (Orr *et al*., 1985 and Nakama and Foerster, 2001). Temperature is probably the most important physical environmental factor influencing the development, reproduction of insects, and regulates insect population dynamics, and seasonal occurrence. Numerous studies have illustrated the effect of temperature on the biological and population growth of *T. basalis* (Orr *et al*., 1985; Porta, 1992; Nakama and Foerster, 2001 and Catalan and Verdu, 2005).

However, scanty attention has been paid on the effect of temperatures on biological characteristics and life table parameters of the scelionid egg parasitoid, *T. basalis* for mass rearing and release. Therefore, the current investigation was conducted to study the influence of temperatures on biological attributes of *T. basalis*, as well as testing the effect of temperatures on life table parameters of this species.

MATERIAL AND METHODS

Host cultures:

Pairs of *N. viridula* adults were collected by sweeping net from cowpea plants at the experimental farm of Faculty of Agriculture, Mansoura University and caged in 30 plastic containers (0.5 x10 cm) covered with muslin for ventilation. Adults were fed with cowpea leaves. Food was changed daily. Egg masses were collected daily to prevent cannibalism by adults.

Parasitoid culture:

Trissolcus basalis was cultured in the laboratory from *N. viridula* parasitized egg masses which collected from cowpea fields. Parasitoids were maintained in Petri-dishes supplied with sugar solution for food. The culture was kept at 28±2.0ºC and 75.0±5.0 relative humidity with 14 hours light: 10 hours dark photoperiod. A female parasitoid was used only once. The host egg masses were exposed to the parasitoid for 24 h, then removed and placed in another Petri-dish for incubation. After the adult emergence, they were counted and sexed. The remaining eggs were dissected and eggs which perceptibly mature or immature forms of the parasitoid were identified and considered to be parasitized. The developmental times, percentage of parasitized eggs, successful parasitism and sex ratio were calculated.

Effects of two constant temperatures (24 and 28ºC) and fluctuated temperature

The effects of two constant temperatures (24 and 28ºC) and fluctuated temperature (average of room temperature=31.0ºC) on the developmental time and rate (1/developmental time) (Omakar and James, 2004) of immature stages, emergence rate, sex ratio, longevity, and fecundity of females were determined. Five egg masses of *N. viridula* were offered to the females for parasitization. Each temperature treatment was replicated five times. Each female was used as a replicate. After the parasitization, parasitoids were removed and parasitized eggs were cultured under the same temperature condition until the immature developed. Developmental time, percentage of adult emergence, longevity and fecundity of females and sex ratio were recorded. Fecundity was expressed as the sum of emerged parasitoids plus parasitized eggs from which no parasitoids emerged. Parasitized eggs are easily recognized by the dark color assumed by the eggs five days after parasitism. Total of parasitized eggs per female and sex ratio were recorded. The effects of temperature on life table parameters were calculated using a BASIC computer program (Abou-Setta *et al*. 1986) for females reared on 24, 28ºC and fluctuated temperature (31ºC). This computer program is based on Birch's method (1948) for the calculation of an animal's life table. Effect of temperature on percentage of parasitized eggs, number of emerged adults, percentage of successful parasitism, the developmental time for immature stags, longevity of female, sex ratio and the fecundity of females was assessed by constructing a life table, using rates of age-specific (Lx), and fecundity (Mx) for each age interval (x).The mean generation time (T), gross reproductive rate (GRR) (=ΣMx), the net

reproductive increase (R_o) the intrinsic rate of increase (r_m), and the finite rate of increase (λ). The doubling time (DT) was calculated according to Mackauer's method (Mackauer, 1983). The life tables were prepared from data recorded daily on developmental time (egg to first egg laid), sex ratio, the number of deposited eggs, the fraction of eggs reaching maturity, and the survival of females. Interval of one day was chosen as the age classes for constructing the life table.

Choice and non-choice tests

To test the preference for any age of host eggs (choice test), four egg masses of different ages (one day, two, three, and four days old) were offered simultaneously to single mated females. In non-choice test, four egg masses of the same age (one day, two, three, and four days old) were offered simultaneously to single mated females. Each egg mass was considered to be a replicate and placed in a plastic container. Females were removed from the plastic containers after 48 h. The number of parasitized eggs, and the number of emerged adult was determined. All tests were done under natural photoperiod at room conditions during July and August, 2006.

Data analysis

All experimental data concerning the developmental times, successful parasitism, longevity, and fecundity were analyzed with one-way analysis of variance (ANOVA). Comparisons of means of biological characters were made with the Duncan's Multiple Range Test (Costat Software, 1990).

RESULTS AND DISCUSSION

Developmental time of immature stages

Analysis of variance (ANOVA) indicated that there were significant differences between the three temperatures in egg-larval stages, pupal stage and total of immature stages, with exception of egg-larval stages at 28 and 31°C (Table 1). In addition, the data in the same table showed that the average developmental time of the total immature stages was 17.84±0.29, 11.68±0.22 and 9.28±0.16 days at 24, 28 and 31°C, respectively. The developmental rate was better at 31°C (0.108) than at 24 and 28°C.

Table 1. Average developmental time (mean±SE) in days of immature stages of *T. basalis* reared at three temperatures.

Temperature	Immature stages			Developmental Rate
	Egg-larval stages	Pupal stage	Total	
24°C	12.6 ± 0.29 a	5.72 ± 0.27 a	17.84± 0.29 a	0.056
28°C	7.12 ± 0.18 b	4.56 ± 0.21 b	11.68± 0.22 b	0.086
31°C	5.68 ± 0.18 b	3.64 ± 0.15 c	9.28± 0.16 c	0.108

Means followed by the same letter in a column are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

The data in Table (1) clearly indicated that the immature stages took a longer time at 24ºC than at 28ºC. Whereas, it was shortest at 31ºC. Orr *et al* (1985) mentioned that *T. basalis* completed its development at all temperatures ranging from 15 to 36ºC and eight constant relative humidity ranging from 64 to 100% R. H. Meanwhile, Porta (1992) noted that the developmental time for *T. basalis* from egg to adult was 13.5± 1.6 days at 26ºC±3.0, 75.0±10.0% R. H. and L:D (16:8).

The percentage of parasitized eggs, successful parasitism and sex ratio

In Table (2), there were significant differences between the three temperatures in percentage of parasitized eggs and sex ratio, while there were no significant differences between the three temperatures in percentage of successful parasitism. According to the data in Table (2), the percentage of parasitized eggs, and the percentage of successful parasitism of *T. basalis* was greater at 28ºC and 31ºC than 24ºC. The female sex ratio was 4:1 (females: males) at 28 and 31ºC, while it was 3:1 at 24ºC. Similar results were obtained by Orr *et al.* (1985) and Porta (1992) who reported that the percentage of parasitoids reaching the adult stage, and the percentage of adult emergence of *T. basalis* were significantly greater at both upper and lower extreme temperatures, and the sex ratio for *T. basalis* reared in the laboratory at 26± 3.0ºC, 75.0 ± 10.0% R. H. and LD 16:8 was 4:1.

Table 2. Percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* reared at three temperatures.

Parameter	Temperature		
	24ºC	**28ºC**	**31ºC**
Parasitized eggs %	79.47 b	90.25 a	82.29 ab
Successful parasitism %	69.91 a	82.14 a	80.93 a
Sex ratio	75.50 b	80.44 a	80.90 a

Means followed by the same letter in a row between the three temperatures are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

Simple linear regression between temperatures (independent variable X) and percentage of parasitized eggs (dependent variable Y) of *T. basalis* females yielded $R^2 = 0.6683$. The regression equation was derived: the percentage of parasitized eggs (Y) =122.78-7.2147 (X). This equation indicated that there was a relationship between temperatures and percentage of parasitized eggs (Fig. 1). Concerning the simple linear regression between temperatures and percentage of successful parasitism, the value of R^2 was 0.7485 and the regression equation was Y=124.24-7.0865X. This equation indicated that there was a positive relationship between the temperatures and percentage of successful parasitism which means that the percentage of successful parasitism gradually increased as the temperatures increased (Fig. 2) Whereas, the simple linear regression between temperatures and sex ratio: (Y) =1.1763-0.0687(X) (R^2=0.691) (Fig. 3).

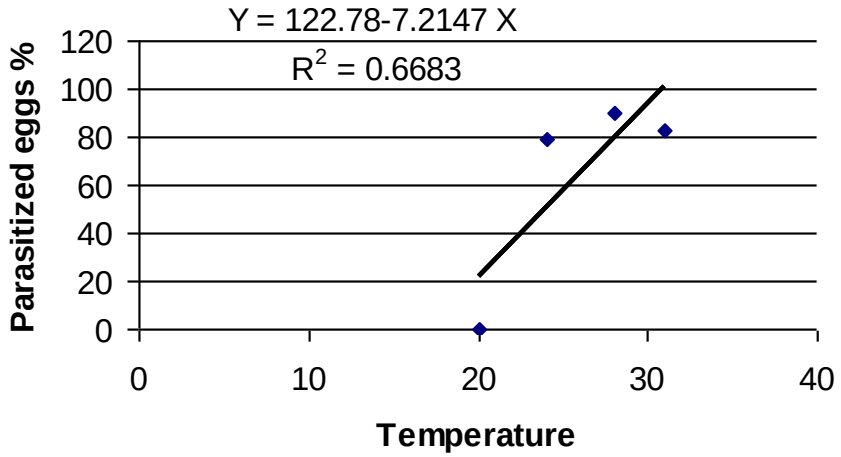

Fig. 1. Simple linear regression between temperatures (X) and % of parasitized eggs (Y) of *T. basalis* at 24, 28 and 31ºC.

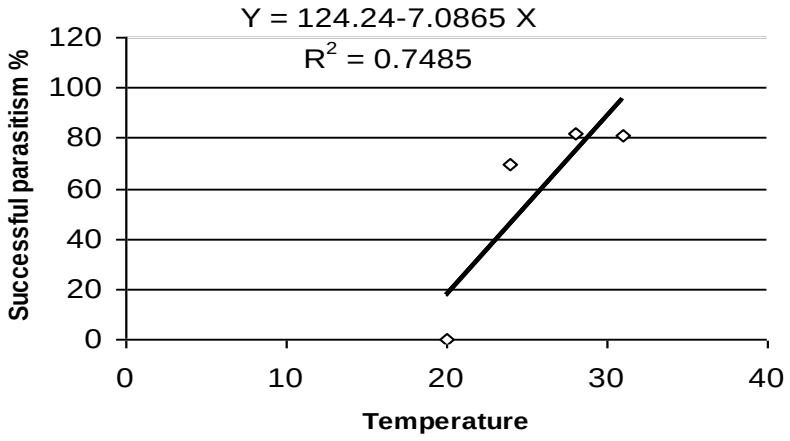

Fig.2. Simple linear regression between temperatures (X) and successful parasitism (Y) of *T. basalis* at 24, 28 and 31ºC.

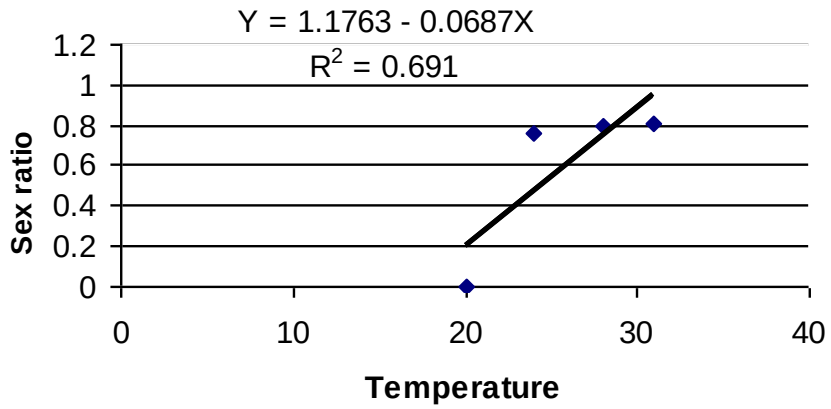

Fig. 3. Simple linear regression between temperatures (X) and sex ratio(Y) of *T. basalis* at 24, 28 and 31°C.

Longevity and fecundity of females

Based on the statistical analysis, there was a significant difference in longevity (Table 3). It was 14.40±1.43, 13.40 ±0.46 and 9.00±2.08 at 24, 28 and 31°C. The simple linear regression between temperatures and longevity (dependent variable Y) of *T. basalis* females reared at 24, 28 and 31°C yielded R^2=0.7913. The regression equation was: Female longevity (Y) = 27.172-1.5076 temperatures (X). This equation indicated that there was a highly relationship between temperatures and longevity (Fig. 4).

The ANOVA indicated that there was no statistical variation in the fecundity for *T. basalis* females reared at the three tested temperatures (Table 3).

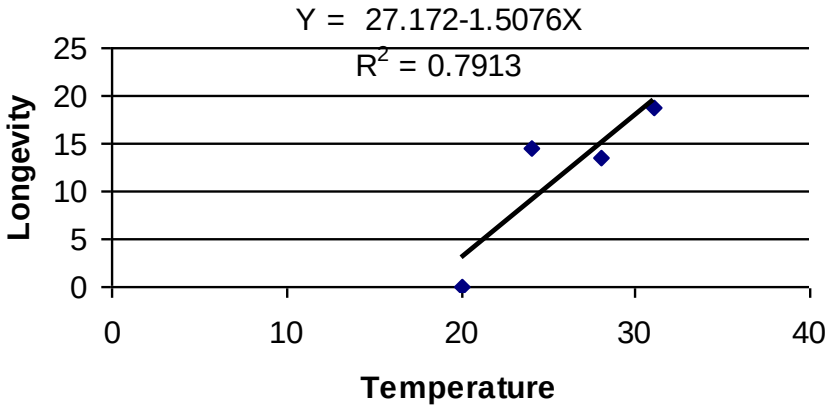

Fig. 4. Simple linear regression between temperatures (X) and longevity (Y) of *T. basalis* at 24, 28 and 31°C.

The following regression equation was calculated for *T. basalis* females between the temperatures and fecundity (Y) =344.3-19.435(X) (R^2=0.777). From this equation, there was a positive relationship (Fig. 5). Catalan and Verdu (2005) noted that the life cycle of *T. basalis* females was 14.48 days and the oviposition of *T. basalis* was recorded early in the life cycle of the parasitoid.

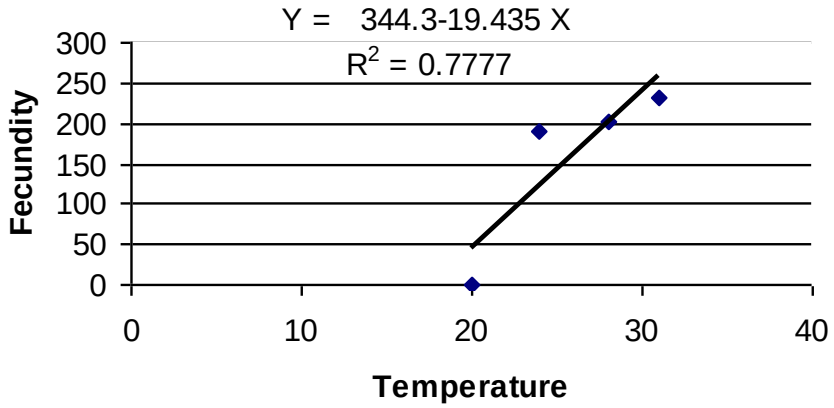

Fig. 5. Simple linear regression between temperatures (X) and fecundity (Y) of *T. basalis* at 24, 28 and 31°C.

Table .3. Longevity and fecundity (mean± SE) of *T. basalis* females reared at three temperatures.

Parameter	Temperature		
	24°C	28°C	31°C
Longevity	14.40±1.43 a	13.40±0.46 ab	9.00± 2.08 b
Fecundity	191.00± 11.09 a	202.00± 6.64 a	231.60± 18.20 a

Means followed by the same letter in a row between the three temperatures for *T. basalis* are not significantly different at the 5% level of probability (Duncan's Multiple Range Test).

Life table parameters

Data presented in Table (4) illustrated the life table parameters of *T. basalis* female reared on *N. viridula* eggs under three temperatures. The mean generation time was 22.26, 15.19 and 12.73 days at 24, 28 and 31°C, respectively. The population could be doubled (DT) every 3.34, 2.15 and 1.76 days at 24, 28 and 31°C, respectively. The values of net reproductive rate (R_o), intrinsic rate of increase (r_m), finite rate of increase (λ) and the gross reproductive rate (GRR) were higher at 31°C than at 24 and 28°C.

From the data illustrated in Figs. 6, 7 and 8, it could be noted that the survivorship (Lx) for female age was higher (0.82) at 28°C than at 24 and 31°C. The maximum reproduction rate per female per day (Mx) was 28.39 on the second day at 24°C. Moreover, it was 36.04 on the third day at 28°C. While, at 31°C, Mx was 36.24 on the first day.

Table 4. Life table parameters of *T. basalis* reared on *N. viridula* eggs at three temperatures.

Life table parameters	Temperature		
	24 ºC	28 ºC	31 ºC
Mean generation time (T) (in days)	22.26	15.19	12.73
Doubling time (DT) (in days)	3.34	2.15	1.76
Net reproductive rate (R_o)	100.81	133.46	151.63
Intrinsic rate of increase (r_m)	0.207	0.322	0.394
Finite rate of increase (λ)	1.230	1.379	1.483
Gross reproductive rate (GRR)	157.57	162.75	169.83

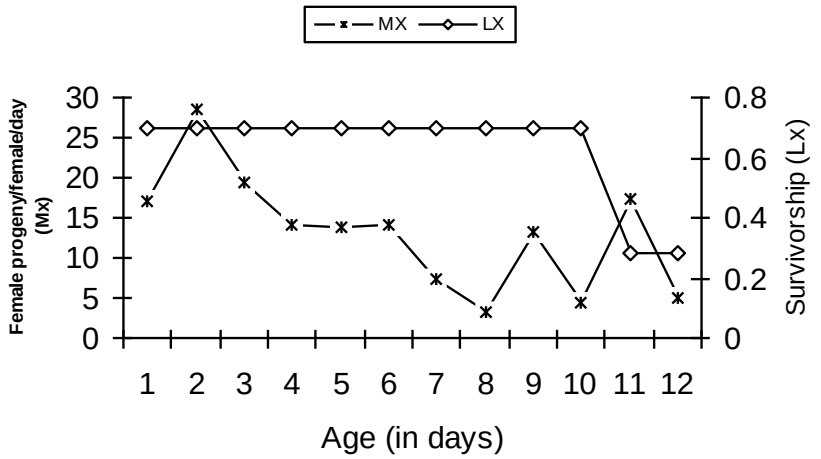

Fig. 6. Age-specific fecundity (Mx) and survivorship (Lx) of *T. basalis* female reared on *N. viridula* eggs at 24ºC.

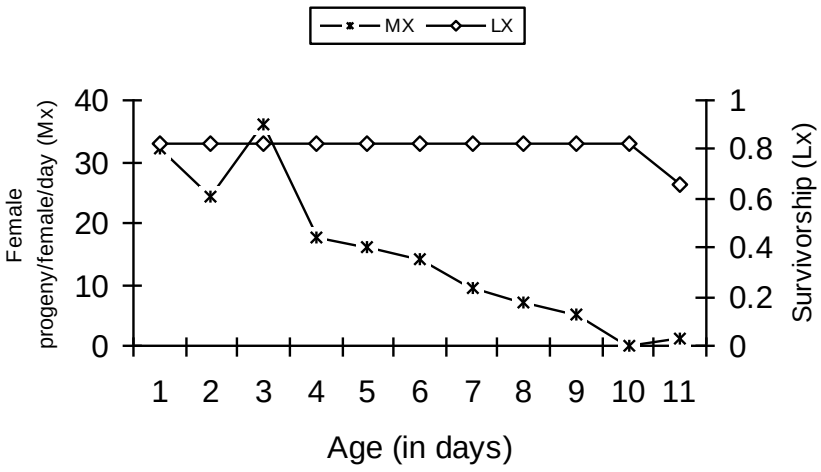

Fig. 7. Age-specific fecundity (Mx) and survivorship (Lx) of *T. basalis* female reared on *N. viridula* eggs at 28ºC.

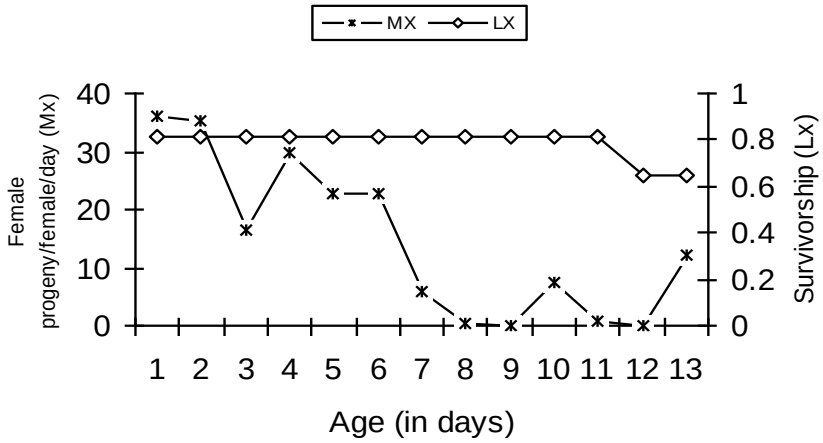

Fig. 8. Age-specific fecundity (Mx) and survivorship (Lx) of *T. basalis* female reared on *N. viridula* eggs at 31ºC.

Choice and non-choice tests

As shown in Figs. 9 and 10, there was a significant difference between eggs ages in choice and non-choice tests. The first age (one day old) was preferred than the other ages. Moreover, the parasitoid was able to parasitize the host eggs in all developmental ages, while the percentage of parasitism on host eggs in earlier ages was higher than in later developmental ages. The parasitism percentages in choice test were 65.80, 54.51, 16.67 and 5.31 % for one day, two, three and four days old, respectively. Moreira and Becker (1986) found that the parasitoids preferred eggs at the earlier stages of development, although later stages were also attacked.

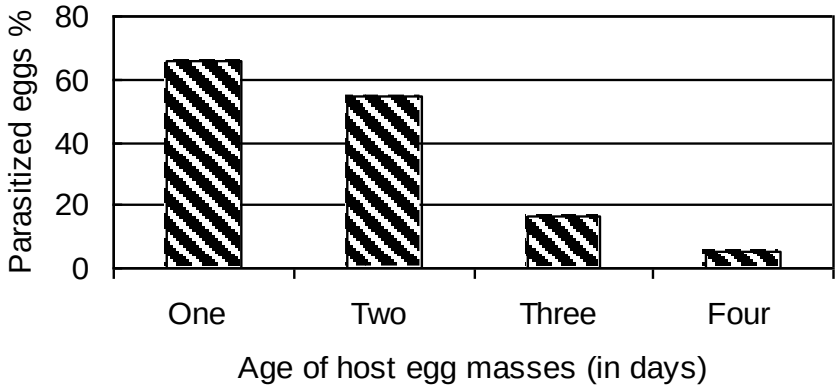

Fig. 9. Effect of age of *N. viridula* egg masses in choice test to parasitism by *T. basalis* under laboratory conditions.

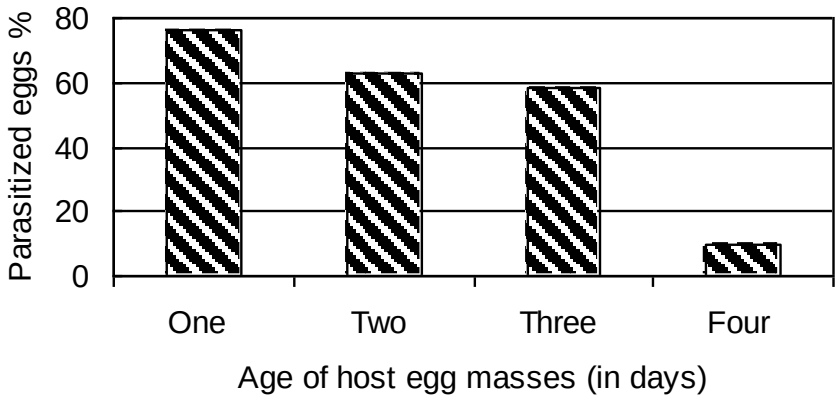

Fig.10. Effect of age of *N. viridula* egg masses in non choice test to parasitism by *T. basalis* under laboratory conditions.

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النمو ، طول العمر ، الخصوبة ومعايير جداول الحياة للطفيل *Trissolcus basalis* "طفيل بيض البقة الخضراء" *Nezara viridula* فى علاقة مع درجات الحرارة

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تم دراسة تأثير درجات الحرارة ٢٤ ، ٢٨ ، ٣١ °م على فترات النمو والبقاء و طول العمرومقاييس جداول الحياة للطفيل *Trissolcus basalis*.

أظهرت النتائج وجود فروق معنوية في طول فترة نمو الأطوارغير الكاملة للطفيل وكذلك فترة طول العمرLongevity. بينما أظهرت نتائج التحليل الإحصائى عدم وجود فروق معنوية في كفاءة الأنثى في وضع البيض fecundity بين الثلاث درجات الحرارة المختبرة . كما أوضحت النتائج أن أعلى معدل للنمو عند درجة الحرارة ٣١ °م . وكذلك بينت النتائج وجود فروق معنوية بين نسبة التطفل ،النسبة الجنسية ودرجات الحرارة المختبرة حيث كانت النسبة الجنسية للإناث بمعدل ١:٤ عند درجتي الحرارة ٢٨ ، ٣١ °م بينما كانت بمعدل ١:٣ عند درجة الحرارة ٢٤ °م .

وأظهرت النتائج أيضا أن قيم جداول الحياة المحسوبة لفترة الجيل (T) ، الزمن اللازم للتضاعف (DT) كانت أعلى على درجة الحرارة ٢٤ °م وأقل في قيمة معدل الزيادة الطبيعي (r_m)، معدل الزيادة النهائي (λ) ، قيم معدل الحياة (Lx) ، قيم معامل التضاعف (R_o) ومعدل التكاثر (GRR) على نفس درجة الحرارة.

كما بينت النتائج أيضا أن الطفيل يفضل التطفل على بيض العائل عمر يوم واحد بقدر أعلى عن باقي الأعمار مع قدرته على التطفل على بيض العائل في الأعمار المتأخرة. ونتائج هذه الدراسة تؤكد أمكانية إستخدام الطفيل كوسيلة مكافحة بيولوجية لمكافحة بيض حشرة البقة الخضراء.