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VIABILITY OF *Nezara viridula* (L.) EGG MASSES FOR PARASITISM By *Trissolcus basalis* (WOLLASTON) AFTER STORAGE UNDER LOW TEMPERATURES.

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ABSTRACT

Adults of *Trissolcus basalis* (Wollaston) were reared on *Nezara viridula* egg masses witch stored at 4, 8, 12 and 16°C for different periods of storage. The percentage of parasitized eggs, successful parasitism, sex ratio, and developmental time of immature stages of *T. basalis* were evaluated.

The results of this study showed that there were a decreased in percentage of parasitized eggs and successful parasitism of *T. basalis* when reared on *N. viridula* egg masses which stored at 12 and 16°C. Moreover, there were a reduction with the percentage of parasitized eggs, successful parasitism and sex ratio generated with increased storage period. Furthermore, there was no effected on the percentage of parasitized eggs, successful parasitism and sex ratio for *N. viridula* egg masses which stored at 4 and 8°C for 7 and 14 days compared with check. The developmental time was decreased with the increased of storage temperatures, it also increased with the increased of storage periods.

Keywords: *Trissolcus basalis* (Wollaston), *Nezara viridula* (L.), egg masses viability, storage periods, low temperatures, developmental time.

INTRODUCTION

The green stink bug, *Nezara viridula* (L.) (Heteroptera: 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).

Trissolcus basalis (Wollaston) is the most important natural enemy of *N. viridula* eggs, as it plays an important role in the regulation of the insect pest population. However, egg parasitism of *N. viridula* is the most effective mortality factor in most affected regions of the world (Awadalla and Shanab, 1993; Awadalla, 1996; Correa-Ferreira and Moscardi, 1995 and 1996; Ehler, 2002; Lenteren and Bueno, 2003; Catalan-Ramos and Verdu, 2005; Khalafalla *et al*., 2005; Canton-Ramos and Callejon-Ferre, 2010; Wright and Diez, 2011 and Liljesthrom *et al*., 2013).

In biological control programs which involve the release of great numbers of parasitoids, it is extremely important to preserve the eggs of the hosts for prolonged periods to make better use of the production from a pre-established colony, and to have great quantities of beneficial agents available when field release is necessary. Egg parasitoids can successfully develop in host eggs that have undergone freezing or heating (Wajnberg and Hassan, 1994). Eggs from various Heteroptera species can be stored at low temperatures and still be parasitized by scelionidae species (Orr, 1988). Powell and Shepard (1982) found that *T. basalis* emergence was not reduced in *N. viridula* eggs stored in a freezer and they could be stored successfully for periods up to seven months. However, scanty attention has been paid on the effect of low temperatures on viability of *N. viridula* egg masses for rearing the scelionid egg parasitoid, *T. basalis*. Therefore, the objective of this work was to store *N. viridula* egg masses at low temperatures for different periods to evaluate the viability of egg masses to parasitize by *T. basalis* and its effect on the percentage of parasitized eggs, successful parasitism, sex ratio and the developmental time of *T. basalis*.

MATERIALS AND METHODS

Host cultures:

Pairs of *N. viridula* adults were collected by sweeping net from cowpea and soybean plants at the experimental farm of Faculty of Agriculture, Mansoura University during 2011/2012 and caged in 30 plastic containers (15 cm x30 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 and soybean fields. Parasitoids were maintained in Petri-dishes supplied with sugar solution for food. The culture was kept at 28±1.0^{o}C and 70.0±5.0 R.H. with 14:10 light: dark photoperiod. A female of 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.

Storage *N. viridula* egg masses under low temperatures:

Four egg masses of *N. viridula* were stored at 4, 8 , 12 and 16±1^{o}C and 60.0 ± 5% R.H. with constant darkness in Petri dishes (9 cm diameter) for 7, 14, 21 and 28 days. There were four replicates for each storage period. After the storage period was completed, all egg masses were transferred to an air conditioned in laboratory at 28.0 ±1.0^{o}C, 70.0 ± 5.0 % R. H. and photoperiod of 14: 10 (light: dark). The egg masses were offered to the females for parasitization. The number of parasitized eggs, percentage of successful parasitism, the developmental time for immature stages and sex ratio was determined.

Data analysis:

All experimental data concerning the parasitized eggs, successful parasitism, sex ratio and developmental stage were analyzed with one or two way analysis of variance (ANOVA). Comparisons of means of biological characters were made with the Duncan's Multiple Range Test (CoStat Software, 2004).

RESULTS AND DISCUSSION

Effect on percentage of parasitized eggs, successful parasitism and sex ratio:

In Table (1), 2- way ANOVA indicated that there were significant variations for the effect of storage period, temperature and the effect of storage period-temperature interaction in percentage of parasitized eggs (F=588.75, df=3, P=0.000*** for storage periods, F= 2162.3, df=3, P=0.000*** for temperatures, F=360.9, df=9, P=0.000*** for interaction between storage periods and temperatures).

For the effect of storage periods, temperatures and the interaction between storage periods and temperatures, there was significant variation in the percentage of successful parasitism (F= 1086, df=3, P=0.000***, F=2385, df=3, P=0000*** and F=89.08, df=9, P=0.000***, respectively).

Based on 2-way ANOVA, for the effect of storage periods, temperatures and the interaction between storage periods and temperature, there were significant variation in *T. basalis* sex ratio (F= 35.94, df=3, P=0.000***, F=27.80, df=3, P=0.000*** and F= 2.23, df=9, P=, 0.035, respectively).

Developmental times

Based on 2-way ANOVA, the data in Table (2) clearly showed that there were significant variations for the effect of either storage periods or temperature in the duration of egg-larval stage of *T. basalis* (F=4.41, df=3, P=0.008** and F=2.97, df=3, P=0.040*). Meanwhile, there were no significant variation for the effect of interaction between storage periods and temperature in the duration of egg-larval stage (F=0.128, df=9, P=0.998 ns).

For the effect of storage periods, temperatures and the interaction between storage periods and temperature there were no significant variation in the duration of pupal stage (F=0.320, df=3, P= 0.81 ns, F=1.555, df=3, P=0.21 ns and F=0.057, df=9, P=1.00 ns).

In respect to developmental time of immature stages, there were no significant variations for the effect of either storage periods or the storage periods-temperatures interaction (F=2.562, df=3, P=0.065 ns and F=0.110, df=9, P=0.999 ns). While, there was a significant variation for temperatures (F=3.096, df=3, P=0.035*).

Table (1):2-way ANOVA for percentage of parasitized eggs, successful parasitism and sex ratio of *Trissolcus basalis* which reared on *N. viridula* egg masses stored at four temperatures and at four storage periods then reared at 28± 1^{o}C and 70±5 %R.H.

Source of variation	**Degrees of freedom**	**F**	**P**
Parasitized eggs			
Storage periods	3	588.75	0.000***
Temperatures	3	2162.3	0.000***
Temperatures × Storage periods	9	360.9	0.000***
Successful parasitism			
Storage periods	3	1086	0.000***
Temperatures	3	2385	0.000***
Temperatures × Storage periods	9	89.08	0.000***
Sex ratio			
Storage periods	3	35.94	0.000***
Temperatures	3	27.80	0.000***
Temperatures × Storage periods	9	2.23	0.035*

Table (2). 2-way ANOVA for *Trissolcus basalis* developmental times which reared on *N. viridula* egg masses stored at four temperatures and at four storage periods then reared at 28± 1^{o}C and 70±5 % R.H.

Source of variation	**Degrees of freedom**	**F**	**P**
Egg-larval stage			
Temperatures	3	4.41	0.008**
storage periods	3	2.97	0.040*
Temperatures × Storage periods	9	0.128	0.998 ns
Pupal stage			
Temperatures	3	0.320	0.81 ns
storage periods	3	1.555	0.21 ns
Temperatures × Storage periods	9	0.057	1.00 ns
Total			
Temperatures	3	2.562	0.065 ns
storage periods	3	3.096	0.035*
Temperatures × Storage periods	9	0.110	0.999 ns

1-*Trissolcus basalis* reared on *N. viridula* egg masses which stored at 4 ^{o}C.:

Percentage of parasitized eggs, the successful parasitism percentage and sex ratio of *T. basalis* reared on *N. viridula* egg masses which stored at 4^{o}C for different periods of storage is presented in Table (3). The data referred that there were significant differences between the storage periods for 21 and 28 days. Meanwhile, there were no significant differences between 7, 14 days and check. According to the date in Table (3), there were significant differences between the different period of storage and the percentage of successful parasitism and sex ratio of *T. basalis*. Moreover, the percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* was greater at 7, 14 days and check.

Table (3):Percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* reared on *N. viridula* eggs at 28±1°C and 70±5%R.H. after stored at 4°C for different periods of storage.

Parameter	Storage periods of *N.viridula* eggs				Check
	7 days	14 days	21 days	28 days	
Parasitized eggs %	98.01 a	97.80 a	95.20 b	86.07 c	98.75 a
Successful parasitism %	95.50 a	94.88 a	85.96 b	56.97 c	95.56 a
Sex ratio (F/F+M)	0.78 b	0.78 b	0.65 c	0.58 d	0.80 a

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

In Table (4), there were significant differences between storage periods for 7, 14, days and check regarding to egg-larval, pupal and total of immature stages. The egg-larval stage, pupal stage and total developmental time of immature stages were higher after 21 and 28 days of storage at 4°C.

Table (4):Developmental times (in days±SEM) of *T. basalis* immature stages which reared on *N.viridula* eggs at 28 ±1°C and 70±5 R.H. after stored at 4°C for different periods of storage.

Developmental Stages	Storage periods of *N.viridula* eggs				Check
	7 days	14 days	21 days	28 days	
Egg-larval Stages	8.00±1.22 c	8.75±0.96bc	10.50±0.55ab	11.50±0.43 a	7.17±0.18 c
Pupal stage	4.50±1.03 ab	5.00±1.17ab	5.50±0.55 a	5.75±1.08 a	3.66±0.15 b
Total	12.50±2.19bc	13.75±1.90b	16.00±0.61 a	17.25±1.51 a	10.83±0.18c

[a] Means followed by same small letter in a row are not significantly different at the 5% level of probability (Duncan's Multiple Rang Test).

2-*Trissolcus basalis* reared on *N. viridula* egg masses which stored at 8 °C.:

Analysis of variance (ANOVA) in Table (5) indicated that there were significant differences between the percentage of parasitized eggs, successful parasitism, and sex ratio for different storage periods. Moreover, the percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* was greater at 7, 14 days and check.

Table (5):Percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* reared on *N. viridula* eggs at 28±1°C and 70±5 R.H.after stored at 8°C for different periods of storage.

Parameter	Storage periods of *N. viridula* eggs				Check
	7 days	14 days	21 days	28 days	
Parasitized eggs %	95.50 b	90.75 c	79.11 d	55.34 e	98.75 a
Successful parasitism %	94.50 a	88.50 b	68.65 c	25.14 d	95.56 a
Sex ratio (F/F+M)	0.75 b	0.75 b	0.60 c	0.55 d	0.80 a

[a] Means followed by same small letter in a row are not significantly different at the 5 % level of probability (Duncan's Multiple Rang Test)

The data in Table (6) showed that there were significant differences in egg-larval stages and total developmental time of immature stages after 7, 14 days storage periods. Moreover, there were no significant differences between the different periods of storage in pupal stage

Table (6): Developmental times (in days±SEM) of *T. basalis* immature stages which reared on *N.viridula* eggs at 28±1^{o}C and 70±5 R.H. after stored at 8^{o}C for different periods of storage.

Developmental Stages	Storage periods				Check
	7 days	14 days	21 days	28 days	
Egg-larval Stages	7.25±1.14 b	8.25±1.13ab	9.25±0.96 a	9.75±1.08 a	7.17±0.18 b
Pupal stage	4.25±0.73 a	4.50±1.03 a	4.50±0.75 a	4.50±0.90 a	3.66±0.15 a
Total	11.50±1.78 bc	12.75±1.24 ab	13.75±1.55 a	14.25±1.13a	10.83±0.18 c

[a] Means followed by same small letter in a row are not significantly different at the 5 % level of probability (Duncan's Multiple Rang Test).

3-*Trissolcus basalis* reared on *N. viridula* egg masses which stored at 12 ^{o}C.

The viability of eggs stored at 12^{o}C as expressed by the emergence rate of *T. basalis* adults showed that there were significant differences between the different periods of storage and parasitized eggs, successful parasitism and sex ratio (Table 7).

Table (7). Percentage of parasitized eggs, successful parasitism and sex ratio to *T. basalis* reared on *N. viridula* eggs at 28±1^{o}C and 70±5 R.H. after stored at 12^{o}C for different periods of storage.

Parameter	**Storage periods of *N.viridula* eggs**				**Check**
	7days	**14 days**	**21 days**	**28 days**	
Parasitized eggs%	75.31 b	70.18 c	45.50 d	15.22 e	98.75 a
Successful parasitism %	70.40 b	68.18 c	30.00 d	10.30 e	95.56 a
Sex ratio (F/F+M)	0.70 b	0.65 c	0.64 c	0.55 d	0.80 a

[a]Means followed by same small letter in a row are not significantly different at the 5 % level of probability (Duncan's Multiple Rang Test).

ANOVA in Table (8) indicated that there were significant differences between the different periods of storage in total developmental stages. Neither mean of developmental times of egg- larval stages nor mean of pupal stage of *T. basalis* reared on *N. viridula* egg masses at 28^{o}C after stored at 12^{o}C were affected by storage periods at 12^{o}C compared with check.

Table (8). Developmental times (in days±SEM) of *T. basalis* immature stages which reared on *N.viridula* eggs at 28±1°C and 70±5 R.H. after stored at12°C for different periods of storage.

Developmental Stages	Storage periods of *N.viridula* eggs				Check
	7 days	14 days	21 days	28 days	
Egg-larval Stages	7.25±1.24 b	8.00±0.93 ab	9.00±0.70 ab	9.50±0.90 a	7.17±0.18 b
Pupal stage	3.75±0.73 a	3.75±0.96 a	4.25±1.29 a	4.50±0.55 a	3.66±0.15 a
Total	11.00±1.96 c	11.75±1.84 bc	13.25±1.84 ab	14.00±1.36 a	10.83 ±0.18c

[a] Means followed by same small letter in a row are not significantly different at the 5 % level of probability (Duncan's Multiple Rang Test).

4-*Trissolcus basalis* reared on *N. viridula* egg masses which stored at 16 °C.:

The data in Table (9) clearly indicated that there were significant differences between the different periods of storage with respect to percentage of parasitized eggs, successful parasitism and sex ratio.

Table (9) Percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* reared on *N. viridula* eggs at 28±1°C and 70±5 R.H. after stored at 16°C for different periods of storage.

Parameter	Storage periods of *N.viridula* eggs				Check
	7 days	14 days	21 days	28 days	
Parasitized eggs%	40.60 b	30.40 c	23.50 d	10.00 e	98.75 a
Successful parasitism %	25.20 b	10.65 c	4.00 d	3.00 d	95.56 a
Sex ratio (F/F+M)	0.62 b	0.55 c	0.50 d	0.29 e	0.80 a

[a] Means followed by same small letter in a row are not significantly different at the 5 % level of probability (Duncan's Multiple Rang Test).

Regarding to pupal stage reared at 28°C after egg masses of *N. viridula* stored at 16°C, there were no significant differences between the different storage periods and check. While, there were significant differences between the egg-larval stage, total developmental time of immature stages after different of storage periods (Table 10).

Table (10):Developmental times (in days±SEM) of *T. basalis* immature stages which reared on *N.viridula* eggs at 28±1°C and 70±5 R.H. after stored at 16°C for different periods of storage.

Developmental Stages	Storage periods of *N.viridula* eggs				Check
	7 days	14 days	21 days	28 days	
Egg-larval Stages	6.50±0.55 b	7.25±0.73 ab	7.50±0.24 ab	8.50±0.82 a	7.17± ab
Pupal stage	3.50±0.25 a	3.75±0.89 a	3.90±0.42 a	4.00±0.61 a	3.66± a
Total	10.00±0.79 b	11.00±1.58 ab	11.40±0.64ab	12.50±1.25 a	10.83± ab

[a] Means followed by same small letter in a row are not significantly different at the 5 % level of probability (Duncan's Multiple Rang Test).

These results are in agreement with those of Orr (1988) who reported that eggs from various Heteroptera species can be stored at low temperatures and still be parasitized by scelionidae species. In addition, Correa-Ferreira and Moscardi (1993) reported that the quality of *T. basalis* emerged adults from stored host egg masses were similar to that of adults emerging from fresh eggs. However, developmental time was longer with stored eggs. Wajnberg and Hassan (1994) noted that egg parasitoids can successfully develop in host eggs that have undergone freezing or heating. Correa-Ferreira and Oliveira (1998) mentioned that mean sex ratios was higher than those from eggs stored at low temperatures, although real differences were only found for some storage periods and in certain techniques. Twafik (2007) reported that there were significant differences between the percentage of parasitized eggs and successful parasitism after 21 and 28 days storage periods. While, the emerged adults of *T. basalis* which reared at 28^{o}C after parasitized on *N.viridula* egg masses stored at 6^{o}C for 7 and 14 days was similar to that of adults emerging from fresh eggs. Meanwhile, the egg-larval stage, pupal stage and total developmental time of immature stages were higher after 14, 21 and 28 days of storage at 6^{o}C. Doetzer and Foerster (2013) reported that eggs of *N. viridula* and *Acrosternum pengue* (Rolston) were successfully parasitized by *T. basalis* after storage in liquid nitrogen. For *T. basalis*, storage in liquid nitrogen did not affect the fecundity quiescent females

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قابلية كتل بيض البقه الخضراء ***Nezara viridula*** **(L.)** للتطفل بواسطة طفيل البيض ***Trissolcus basalis*** **(Wollaston)** بعد تخزينها تحت درجات حرارة منخفضه.

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تم دراسة تأثير تخزين كتل بيض البقه الخضراء *Nezara viridula* (L.) تحت درجات حرارة منخفضه وهى كالتالى 4, 8 ، 16،12 ^{o}م وعلى فترات مختلفه ثم نقل البيض المخزن للتطفل عليه بواسطة *T. basalis* وللتربيه على درجة 28 ^{o}م ودراسة تأثير ذلك على فترات النمو والبقاء ومعدل التطفل ونسبة التطفل والنسبه الجنسيه للطفيل .
أظهرت النتائج وجود إنخفاض فى عدد البيض المتطفل عليه و نسبة التطفل لكتل البيض المتطفل عليها والمربى على درجة 12 و 16 ^{o}م . كما بينت النتائج وجود علاقه عكسيه بين فترات التخزين وكل من معدل التطفل ، نسبة التطفل و النسبة الجنسيه. كذلك أوضحت النتائج عدم تأثر كل من معدل التطفل، نسبة التطفل والنسبه الجنسيه لكتل بيض البقه الخضراء المخزنه على درجتى 4، 8 ^{o}م لمدة 7-14 يوم مقارنة بالكنترول , كما أظهرت النتائج أن فترات النمو والبقاء تتناقص بزيادة درجات الحرارة.

قام بتحكيم البحث

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