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COLD STORAGE OF THE EGG PARASITOID, *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) AND ITS EFFECT ON SOME BIOLOGICAL CHARACTERISTICS.

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ABSTRACT

Nezara viridula (L.) egg masses were parasitized by *Trissolcus basalis* (Wollaston) at 28±1^{o}C then the parasitized egg masses were stored at 4, 8, 12, 16 and 20^{o}C for different periods of storage. Percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* were evaluated.

Results of this study showed that *N. viridula* parasitized eggs could be stored for four weeks at 16 and 20±1^{o}C but only three weeks storage was possible at 12±1^{o}C. Even though there were significant differences between percentage of parasitized eggs, successful parasitism and sex ratio at different storage temperature and different storage periods. High mean emergence showed at 20±^{o}C, ranging from 78.5% to 92.5%, while the lathe number of emergence was at 12^{o}C. Meanwhile, successful parasitism increased with the increase of storage temperature. While, it will be decreased with the increased in storage periods.

Data also revealed that average of egg-larval stages, pupal stage and total developmental time were increased with the increase of storage periods, While they will be decreased with the increased in storage temperature.

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

INTRODUCTION

Cold storage can be a valuable tool for use in rearing of insects for biological control programs. It not only provides a steady supply of insects for research but also yields flexibility and efficiency in mass production, allows synchronization of a desired developmental stage for releases, and lengthens their availability to consumers (Leopold 1998). Storage of some egg parasitoid species has been achieved by inducing dormancy, diapauses, or quiescence as part of the rearing protocol (Pitcher et al. 2002, Foerster *et al*. 2004, Rundle *et al*. 2004 and Chen *et al*. 2008). In temperate and subtropical climates, reproductive diapause is a common adaptation used by insects to survive during winter (Boivin, 1994).

Most egg parasitoids overwinter as immature in their host eggs (Boivin, 1994) Nakama and Foerster (2001) showed that adults of *T. basalis* and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) failed to emerge when the immature stage were kept at 15^{o}C, and survival and emergence of adults after the parasitoids returned to 25^{o}c in the larval stage was significantly reduced after remaining for 20 or more days at the low temperature. However, both *T. basalis* and *T. podisi* were able to reach the pupal stage within the host eggs when the parasitoids were transferred from 25 to 15^{o}C either in the egg stage or as first instars. Foerster and Doetzer (2006) reported that Adult of *T. basalis* and *T. podisi* stored either at 15 or 18^{o}C after their immature developmental had been completed at 18 or 25^{o}C.The fecundity of *T. basalis* females that developed at 18^{o}C and were stored for 120 days at 15 or 18^{o}C was not affected; however, after remaining for 180 days, fecundity was reduced. Little previous studies have been carried out concerning the effect of cold storage of *T. basalis*. Therefore, the objective of this work was to store *N. viridula* egg masses at low temperature for different periods after parasitized by *T. basalis* to study the efficiency of cold storage for *N. viridula* parasitized eggs on the percentage of parasitized eggs, successful parasitism, sex ratio and the developmental time of immature stages of *T. basalis* emerged from the storage egg masses.

MATERIALS AND METHODS

Host cultures:

Pairs of *N. viridula* adults were collected by sweep net from cowpea and soybean plants at the experimental farm of Faculty of Agriculture, Mansoura University during 2011/2012and 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 the parasitized egg masses under low temperature:

Four parasitized egg masses of *N. viridula* were stored at 4, 8 , 12, 16 and 20^{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 treatment and 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, 75.0 ± 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

A-Effect of 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 temperature, storage period and the effect of storage period-temperature interaction in percentage of parasitized eggs. (F=6812, df= 4, P=0.000***, F=182.80, df=3, P=0.000*** and F=64.30, df=12, P=0.000***)

For the effect of temperature and storage period interaction there was a significant variation in percentage of successful parasitism (F=68.91, df=12, P=0.000***). Also there were significant variations for the effect of temperature and storage period in percentage of successful parasitism (F=5306, df=4, P=0.000*** and F= 148.54, df=3, P=0.000***).

Based on 2-way ANOVA, for the effect of temperature, storage period and storage period-temperature interaction there were significant variations in *T. basalis* sex ratio (F=431.51, df=4, P=0.000***, F=27.57, df=3, P=0.000*** and F=9.026, df=12, P= 0.000***)

Developmental times

Based on 2-way ANOVA the data in Table (2) clearly indicated that there were significant variations for the effect of either temperature or storage periods in the duration of egg larval stage of *T. basalis* (F=145.78, df=4, P=0.000*** and F=5.19, df=3, P=0.0025**) Also, there were significant variation for the effect of interaction between storage periods and temperature in the duration of egg-larval stage (F=11.30, df=12, P=0.000***).

As well as, for the effect of either temperature or storage periods there were significant variations in the duration of pupal stage (F=84.84, df=4, P= 0.000*** and F=2.97, df=3, P=0.036*) While, there were significant variation for the effect of interaction between storage periods and temperature in the duration of pupal stage (F= 7.83,df=12, P=0.000***).

In respect of developmental time of immature stages, there were significant variations for the effect of either temperature or storage periods (F=132.55, df= 4, P= 0.000*** and F=4.64, df=3, P= 0.0048**). There was a significant variation for the effect of interaction between storage periods and temperature (F=11.01, df=12, P=0.000***)

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

Source of variation	Degrees of freedom	F	P
Parasitized eggs			
Temperature	4	6812	0.000***
Storage period	3	182.80	0.000***
Temperature × Storage period	12	64.30	0.000***
Successful parasitism			
Temperature	4	5306	0.000***
Storage period	3	148.54	0.000***
Temperature × Storage period	12	68.91	0.000***
Sex ratio			
Temperature	4	431.51	0.000***
Storage period	3	27.57	0.000***
Temperature × Storage period	12	9.026	0.000***

Table (2):2-way ANOVA of *T. basalis* developmental times which reared on *N. viridula* egg masses stored at four temperatures and at four storage periods then reared at 28± 2°C and 70±5 %R.H.

Source of variation	Degrees of freedom	F	P
Egg-larval stage			
Temperature	4	145.7	0.000***
Storage period	3	5.19	0.0025**
Temperature × Storage period	12	11.30	0.000***
Pupal stage			
Temperature	4	84.84	0.000***
Storage period	3	2.97	0036*
Temperature × Storage period	12	7.83	0.000***
Total			
Temperature	4	132.55	0.000***
Storage period	3	4.64	0.0048**
Temperature × Storage period	12	11.01	0.000***

Percentage of parasitized eggs, successful parasitism and sex ratio of *T. basalis* reared at 28±1°C from *N.viridula* parasitized eggs stored at five low temperatures.

1- parasitized eggs.

From the data in Table (3) *N. viridula* parasitized eggs could be stored for four weeks at 16 and 20 ±1°C but only three weeks storage was possible at 12±1°C. Even though there were significant differences between percentages of parasitized eggs at different storage temperature. Meanwhile, there were no significant differences between percentage of parasitized eggs at 4 and 8±1°C in all different storage periods. The data also noted that emerged adults of *T. basalis* from stored host eggs which remained for 7 and 14 days was similar at 12°C.

Table (3):Percentage of parasitized *N. viridula* eggs by the parasitoid *T. basalis* after stored at five low temperature treatments for different periods (reared under 28±1^{o}C and 70±5%R.H.).

Temperatures	Storage periods			
	7 days	14 days	21 days	28 days
4	0.0 b E	0.0 b E	0.0 b E	0.0 b D
8	0.0 b E	0.0 b E	0.0 b E	0.0 b D
12	8.5 b D	7.2 b D	3.5 c D	0.0 d D
16	60.5 b C	55.5 c C	40.0 d C	20.5 e C
20	92.5 b B	90.2 c B	86.4 d B	78.4 e B
Check	98.75 A			

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

2-Successful parasitism

Regarding to the data in Table (4), there were no adults emerged of *T. basalis* from stored host eggs which remained for 7, 14, 21 and 28 days at 4 and 8^{o}C and also from 28 days at 12^{o}C. Furthermore, there were significant differences between percentage of successful parasitism at 12, 16, 20±1^{o}C and check temperature at different storage periods. Throughout the different storage temperature, high mean emergence showed at 20^{o}C, ranging from 78.5% to 92.5%, while the lethal number of emergence was at 12^{o}C. Data in Table (4) revealed that mean successful parasitism increased with the increase of storage temperature, While it will be decreased with the increased in storage periods.

Table (4): Percentage of successful parasitism of *T. basalis* reared at 28^{o}C from *N.viridula* parasitized eggs stored at five low temperatures for different periods.

Temperatures	Storage periods			
	7 days	14 days	21 days	28 days
4	0.0 b E	0.0 b E	0.0 b D	0.0 b D
8	0.0 b E	0.0 b E	0.0 b D	0.0 b D
12	6.2 b D	4.5 b D	1.5 c D	0.0 c D
16	55.0 b C	45.4 c C	35.4 d C	10.4 e C
20	88.4 b B	85.4 c B	80.4 d B	75.2 e B
Check	95.56 A			

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

3-Sex ratio

Throughout the total storage periods, there was reduction in the number of females from parasitized eggs stored for a long time at different storage temperature compared to offspring from fresh eggs (Table 5). Moreover, there were significant differences between sex ratio for emergence parasites at 12, 16, 20^{o}C and check temperature at different storage periods. The results also indicated that at longer storage periods at 12 and 16±1^{o}C, sex ratio was significantly influenced in all periods.

Table(5):Average of sex ratio of *T. basalis* reared at 28±1°C from *N.viridula* parasitized eggs stored at five low temperatures for different periods.

Temperatures	Storage periods 7 days	14 days	21 days	28 days	Check
4	0.0 b E	0.0 b E	0.0 b E	0.0 b D	0.80 a
8	0.0 b E	0.0 b E	0.0 b E	0.0 b D	
12	0.50 b D	0.41 c D	0.25 d D	0.0 e D	
16	0.66 b C	0.62 c C	0.55 d C	0.50 e C	
20	0.75 b B	0.70 c B	0.65 d B	0.60 e B	
Check	0.80 A				

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

Developmental time.

1-Egg-larval stage.

No significant differences were recorded in the average of egg-larval stage between the storage periods for 7 and 14 days and also between 21 and 28 days at 20±1°C Meanwhile, there were no significant differences in the average of egg-larval stage between 7 and 14 days at 12±1°C (Table 6). The data also revealed that average of egg-larval stages increased with the increase of storage periods, While it will be decreased with the increased in storage temperature.

Table (6):Average of Egg-larval stages (mean ± SE) to *T. basalis* reared at 28±1°C from *N.viridula* parasitized eggs stored at five low temperatures for different periods.

Temperatures	Storage periods 7 days	14 days	21 days	28 days
4	0.0±0.0 b E	0.0±0.0 b D	0.0±0.0 b E	0.0±00 b D
8	0.0±0.0 b E	0.0±0.0 b D	0.0±0.0 b E	0.0±0.0 b D
12	15.8±0.81 b A	16.8±1.03 b A	18.8±4.28 a A	0.0±0.0 d D
16	13.6±0.82 c B	15.6±0.82 b A	17.2±0.95 ab B	18.8±1.24 a A
20	10.8±0.86 b C	11.4±0.72 b B	13.2±0.76 a C	14.8±0.76 a B
Check	7.17±0.18 D	7.17±0.18 C	7.17±0.18 D	7.17±0.18 C

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

2- Pupal stage

From the data in (Table 7) there were significant differences between the average of pupal stages at the different storage temperature and different storage periods. The data also revealed that average of pupal stage increased with the increase of storage periods, While it will be decreased with the increased in storage temperature.

Table(7):Average of pupal stages (mean ± SE) of *T. basalis* reared at 28±1°C from *N.viridula* parasitized eggs stored at five low temperatures for different periods.

Temperatures	Storage periods			
	7 days	**14 days**	**21 days**	**28 days**
4	0.0±0.0 b E	0.0±0.0 b E	0.0±0.0 b E	0.0±0.0 b D
8	0.0±0.0 b E	0.0±0.0 b E	0.0±0.0b E	0.0±0.0 b D
12	10.4±0.77 bA	11.6±1.15 ab A	12.8±3.81 a A	0.0±0.0 d D
16	8.4±0.45 c B	9.8±0.76 bc B	10.8±0.17 ab B	12.2±0.59 a A
20	6.2±0.95 c C	7.2±0.86 bc C	8.8±0.76 ab C	10.6±0.82 a B
Check	3.66±0.15 a D	3.66±0.15 a D	3.66±0.15 a D	3.66±0.15 a C

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

3-Total developmental time

Results given in (Table 8) indicated that the total developmental time to *T. basalise* from parasitized host eggs which remained for 21 days at 12±1°C was the highest duration compared with check. There were significant differences between the average of total developmental time at the different storage temperature and different storage periods.

Table (8). Average of total developmental time (mean ± SE) of *T. basalis* reared at 28±1°C from *N.viridula* parasitized eggs stored at five low temperatures for different periods.

Temperatures	Storage periods			
	7 days	**14 days**	**21 days**	**28 days**
4	0.0±0.0 E b	0.0±0.0 E b	0.0±0.0 E b	0.0±0.0 D b
8	0.0±0.0 E b	0.0±0.0 E b	0.0±0.0 E b	0.0±0.0 D b
12	26.2±1.33 A b	28.4±2.05 A ab	31.6±7.51 A a	0.0±0.0 D d
16	22.0±1.20 B c	25.4±1.01 B bc	28.0±1.81 B ab	31.0±0.84 A a
20	17.0±1.72 C c	18.6±1.53 C c	22.0±1.46 C b	25.4±1.53 B a
Check	10.83±0.18 D	10.83±0.18 D	10.83±0.18 D	10.83±0.18 C

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

The results of the current study are similar to those addressed by with Noble (1937) stored *N. viridula* eggs parasite by *T. basalis* for 18 days at 10°C adults developed and emerged only from eggs stored with larvae at first stage. James and warren (1991) noted that a close relative of *T. basalis*, is linear with respect to temperature (growth rang 15 - 35°C). The temperature and duration of storage of the hosts eggs influence the degree of parasitization achieved (Awadalla, 1996). The interaction between *T. basalis* and *N. viridula* depends on the temperature and photoperiod (Jones and Westcot, 2002). Meanwhile, Foerster *et al*. (2004) evaluated that *T. basalis* stored at 15°C in the pupal stage continued to develop and emerged at 18 and 25°C they also noted that emergence of *T. basalis* occurred in all treatments at 15°C. Meanwhile, pupae were transferred from 18°C to 12°C at any of the pupal ages showed that storage at this temperature is lethal to the parasitoid in the pupal stage.

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التخـــزين البـــارد لطفيـــل بـــيض البقـــه الخضـــراء *Trissolcus basalis*(Wollaston) Hymenoptera (sceliondea) علـى درجـات حرارة منخفضه وتأثير ذلك على بعض الخصائص البيولوجيه له

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تم دراسة تأثير تخزين كتل بيض البقه الخضراء *Nezara viridula* (L.) المتطفل عليها بواسطة *Trissolcus basalis* (Wollaston) تحت درجات حرارة منخفضه وهى كالتالى ٤، ٨ ،١٢، ١٦، ٢٠ °م وعلى فترات مختلفه ثم نقل البيض المخزن للتربيه على درجة ٢٨ °م ودراسة تأثير ذلك على فترات النمو والبقاء ومعدل التطفل ونسبة التطفل والنسبه الجنسيه للطفيل .

أظهرت النتائج إمكانية تخزين كتل بيض البقه الخضراء المتطفل عليها لمدة ٤ أسابيع على درجات حرارة ١٦، ٢٠°م ولمدة ٣ أسابيع على درجة ١٢ °م كما أوضحت النتائج وجود فروق معنويه بين كل من البيض المتطفل عليه، نسبة التطفل ، النسبة الجنسية وكل من درجات الحرارة وفترات التخزين .كذلك بينت النتائج أن أعلى معدل للتطفل كان من ٧٨.٥ :٩٢.٥ على درجة ٢٠°م بينما أقل نسبة تطفل كانت على ١٢ °م

وأظهرت النتائج أيضا أن نسبة التطفل تزداد بزيادة درجات الحرارة المخزن عليه كتل البيض المتطفل عليها وتقل بزيادة فترات التخزين كما بينت أن فترات النمو للأطوار غير كامله تزداد بزيادة فترات التخزين وتقل بزيادة درجات الحرارة المخزن عليها كتل البيض المتطفل عليه.

قام بتحكيم البحث:

أ.د / حسن محمد فتحى	**كلية الزراعة – جامعة المنصورة**
أ.د /محمود السيد نور	**كلية الزراعة – جامعة القاهرة**